ISSN 0004-2803
ISSN 1678-4219 on-line
Coden ARQGA

# ARQUIVOS DE GASTROENTEROLOGIA

Número 4 | Outubro/Dezembro 2021 | Volume 58

## ARCHIVES OF GASTROENTEROLOGY

Publication of the Brazilian Institute for Studies and Research in Gastroenterology and others Specialities - IBEPEGE

Founded in 1963 by Prof. Dr. José Fernandes Pontes

IBEPEGE
Instituto Brasileiro de Estudos e Pesquisas de Gastroenterologia e Outras Especialidades

ORGÃO DE DIVULGAÇÃO
Publication

**CBCD**
Colégio Brasileiro de Cirurgia Digestiva

**FBG**
Federação Brasileira de Gastroenterologia

**SOBED**
Sociedade Brasileira de Endoscopia Digestiva

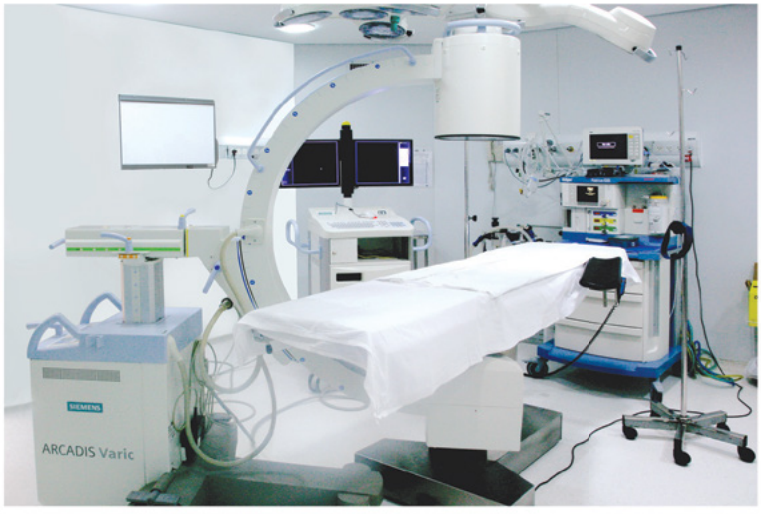
**SBNPE**
Sociedade Brasileira de Nutrição Parenteral e Enteral

**SBMDN**
Sociedade Brasileira de Motilidade Digestiva e Neurogastroenterologia

**SBH**
Sociedade Brasileira de Hepatologia

**ABD**
Academia Brasileira de Disfagia

**GEDIIB**
Grupo de Estudos da Doença Inflamatória Intestinal do Brasil




O IGESP, um hospital geral com perfil cirúrgico que é referência no atendimento de alta complexidade, agora também vai ser referência em modernidade.

Além de especialistas renomados e do seu centro cirúrgico de última geração, o IGESP agora também conta com novas instalações e serviços, garantindo maior segurança aos médicos e maior conforto para pacientes.

HOSPITAL IGESP

*O melhor pra você*

R. Silvia 276 I Bela Vista
São Paulo I SP I 11 3147.6200
www.hospitaligesp.com.br


MKTONE

# UMA ESTRUTURA MODERNA QUE REFLETE A NOSSA VOCAÇÃO: O CUIDADO COM A VIDA.


*Uma empresa do grupo*

Trasmontano
Saúde

# ARQUIVOS DE GASTROENTEROLOGIA

## ARCHIVES OF GASTROENTEROLOGY

IS THE OFFICIAL PUBLICATION OF:

**Brazilian Institute for Studies and Research in Gastroenterology and Other Specialities (IBEPEGE)**
Alcides Felix Terrivel (Representative)
**Brazilian College of Digestive Surgery (CBCD)**
Luiz Augusto Carneiro D´Albuquerque (President)
**Brazilian Federation of Gastroenterology (FBG)**
Décio Chinzon (President)
**Brazilian Society of Digestive Endoscopy (SOBED)**
Ricardo Anuar Dib (President)
**Brazilian Society of Parenteral and Enteral Nutrition (SBNPE)**
Melina Gouveia Castro (President)
**Brazilian Digestive Motility & Neurogastroenterology Society (SBMDN)**
Ricardo Guilherme Viebig (President)
**Brazilian Society of Hepatology (SBH)**
Carlos Eduardo Brandão (President)
**Brazilian Academy of Dysphagia (ABD)**
Ana Furkim (President)
**Brazilian Intestinal Inflammatory Disease Study Group (GEDIIB)**
Rogerio Saad-Hossne (President)





**Expediente / Editorial Office**
Mariana Rodovalho
Isabella Coelho

**Redação e Administração / Correspondence**
Rua Dr. Seng, 320 – Bela Vista – CEP 01331-020 – São Paulo, SP – Brasil – Tel.: (11) 3147-6227
E-mail: secretariaarqgastr@hospitaligesp.com.br

Quarterly publication. The journal ARCHIVES OF GASTROENTEROLOGY is abstracted and/or indexed in: EMBASE / Excerpta Medica, Hygiene and Communicable Diseases (CAB Abstracts), LILACS, PUBMED / MEDLINE, Periódica (Índice de Revistas Latinoamericanas en Ciencias), Tropical Diseases Bulletin (CAB Abstracts). Online: http://www.scielo.br/ag

ISSN 0004-2803
ISSN 1678-4219 - on-line
**Coden ARQGA**

## CONTENTS

# ARQUIVOS DE GASTROENTEROLOGIA
ARCHIVES OF GASTROENTEROLOGY

v. 58 Nº 4 Out/Dez 2021

## EDITORIAL



## ORIGINAL ARTICLE

## REVIEW

## BRIEF COMMUNICATION



## E-VIDEO

# INSTRUCTIONS TO AUTHORS

## Scope and policy

The **Archives of Gastroenterology** publishes s and unseen contributions, from national and foreign researchers, compatible with the goals of the journal and suited to the scientific and editorials standards.

The submission of the manuscript implies that the work in full or part it has not been published in another source or means of communication and not under review in another journal for publication.

Only studies, from clinical or surgical nature, new techniques, epidemiology studies and Review article are accepted. Case reports are not published. The Article sections are: Endoscopy, Surgery, Hepatology, Digestive Motility, Clinical Gastroenterology, Experimental Surgery, Pediatric Gastroenterology, Gastroenterological Clinical Pathology, and Nutrition. It also publishes Editorials, Letter to the Editor, Consensus, Brief Communication, Supplements and E-video.

The assessment work is done impartially and anonymous, that is, omitting to the reviewers, any identification of its origin. Articles are evaluated by at least two reviewers (peer review). The estimated time process is 90 days from submission. The decision about acceptance for publication is taken by the Editorial Board.

No fee is required from authors for submission, evaluation and publication of articles. The **Archives of Gastroenterology** is available online with an open and free access. It is not necessary to ask the journal for permission for electronic copy, provided that the proper credit is given to the source.

Submissions only through the ScholarOne interface, on SciELO Portal: http://mc04.manuscriptcentral.com/ag-scielo

**Archives of Gastroenterology** is available online with an open and free access: http://www.scielo.br/scielo.php?script=sci_issues&pid=0004-2803&lng=en&nrm=iso

## General rules

The text must be in English language. The number of authors is limited to six for Articles and three for Brief Communication. Exceptions can be made in the case of multicentric studies.

The word limit for Brief Communication recommended is no more than 2500; it may contain a figure and a table and the references do not exceed 15.

Articles of research involving human subjects must be marked in Methods section, expressly agreed with the ethical standards and with due informed consent of the participants. Research with human must bring the title page the number of the opinion of the Committee's approval of Research Ethics. Brazilian studies should be in accordance with Resolution 466/2012 of the National Health Council of the Ministry of Health (Brazil), which deals with the Code of Ethics for Human Research, and for studies outside Brazil, shall be in accordance with the Declaration of Helsinki.

Studies involving animals should state the agreement with international ethical principles (e.g., *Committee for Research and Ethical Issues of the International Association for the Study of Pain*, published in PAIN, 16: 109-110, 1983) and national instructions (Laws 6638 / 79, 9605/98, 24665/34 Decree) governing animal research and bring the number of the opinion approved by the Ethics Committee on Animal Research.

For clinical trials, the presentation of the clinical trial registration number on the Methods is mandatory. The complete list of all clinical trials registries can be found at: http://www.who.int/ictrp/network/primary/en/index.html.

It is recommended a cover letter with the intention in publish on the **Archives of Gastroenterology**, highlighting the importance of this publication and research. This letter must be written in the "Author's Cover Letter" field in the online submission.

By determination of SciELO, the adoption of orcid as an identifier of the authors will become mandatory from January 2019.

## Format

The submitted manuscript must be sent in Microsoft Word format and organized as follows:

1) Title in English and Portuguese; for foreign authors the translation will be done.
2) Authors names; do not insert staff positions or similar adjectives.
3) For each author should be described his participation in the study. (e.g. data collection, survey execution, writing of text, statistical analysis and so on).
4) The department and institution where the work was performed.
5) Orcid from all authors.
6) Acknowledgement of grants and other financial support. Interest of conflicts must be declared or not if so. If so, sponsors must be declared.
7) Structured Abstract (Background, Objective, Methods, Results, Conclusion) - The papers should be sent in English and Portuguese (200–600 words); abbreviations, footnotes and references should be avoided; for foreign authors the translation will be done.
8) Headings (3 to 10). Always use terms of Medical Subject Headings (MeSH) list from MEDLINE. Available from: http://www.nlm.nih.gov/mesh/meshhome.html
9) We strongly recommend this paper division: Introduction; Methods; Results; Discussion; Conclusion; Acknowledgements.
10) All contributors who do not meet the criteria for authorship may be mentioned in Acknowledgments.
11) References - **Archives of Gastroenterology** adopts the Vancouver format. Complete text in: https://www.nlm.nih.gov/bsd/uniform_requirements.html Cite references in the text using Arabic numerals in the order of appearance, within parentheses. Do not arrange the list alphabetically. For up to six authors, list all authors. For more than six authors, list first six authors followed by "et al.".
12) Tables and Figures should be cited in the text in Arabic numerals. Preferably, attached separately in JPG or PNG. If they are inside the article, they should after the references. Please do not insert tables and figures in the middle of the text.
13) Tables (in Microsoft Word or Excel format) - Is called Table only when there are numeric results. Explanations and abbreviations should be placed in the footer of the table.
14) Figures - photographs, graphics and drawings must be sent in high resolution digital format (2 mb). Photos can be colored, being left to editors to decide if the publication will be in color or not. The Figures should contain a short text on the subject.

### Authorship

- E-Videos may have a maximum of six authors.
- Authors names: do not insert staff positions or similar adjectives. Include the department and institution where the work was performed.
- The name, telephone number and electronic address of author to whom galley proofs and requests for reprints should be sent.

### Main text

- Title in English and Portuguese; for foreign authors the translation will be done.
- Please include the following in the main text:
  - Text: no more than 400 words.
  - A video legend must be insert after the main text and must be as short as possible (maximum 40 words).

### Video

- Only one video is allowed for each submission.
- Note for not appear any identification from the patient (name or institutional number for example).
- Only AVI or MP4 formats are acceptable.
- Video time should not exceed 4 minutes.
- Make sure that steps and/or main findings explained and highlighted in the video must have overlay titles.

### Figures

- A maximum of six images can be submitted: Upload it separately in JPG or JPG format with at least 300 dpi. Each one must have a number and a legend.

### References

- The reference rules are the same as those of articles. Please read above. No more than six and numbered and cited at the main text.

doi.org/10.1590/S0004-2803.202100000-75

# Publications in inflammatory bowel diseases in the Archives of Gastroenterology

Saad-Hossne R. Publications in inflammatory bowel diseases in the Archives of Gastroenterology. Archives of Gastroenterology. 2021;58(4):417-8.

A brief historical analysis of inflammatory bowel diseases (IBD) allows us to visualize a new scenario, either in incidence and prevalence, combined with new concepts, new strategies and consequently new challenges, which are more defying in children and adolescents in terms of diagnosis and treatment. As we know, IBD still has no cure and its treatment and control will be permanent, associated with that there is the real necessity for surgical treatment, whether it is due to complications, such as failure of clinical treatment or due to the more aggressive course of the pediatric disease. Likewise, delayed puberty and compromised growth have definite and devastating impacts on this population.

We have observed an increase in the number of publications with international and national data about the epidemiology of IBD. In a deeper and more detailed reading, we observe that in the international scenario, especially in developed countries, there is a certain trend towards the stabilization of its incidence[1]. In developing countries, such as Brazil, there is an increase in incidence and prevalence in young adults, adolescents and children; when comparing the two diseases, ulcerative colitis (UC) seems to be more common in preschool children, while CD is more prevalent in older children[1].

The largest epidemiological study ever carried out on national data, in which more than 210,000 patients were analyzed, revealed an increase in the prevalence of both IBD, but the increase in incidence was only observed in UC, and in the same way there was a predominance of UC when compared to Crohn's disease (CD)[2]. These data differ from other national studies, in which there is a predominance of CD in relation to UC, as in one of the articles in this issue[3], which was carried out in a reference center for IBD in the city of Campinas (São Paulo). The authors emphasize that the increase in incidence and prevalence rates is more evident in recently industrialized countries, specifically in Asia, Africa, Eastern Europe and Latin America. They observed that the patients were young, with no gender predominance, and that there was a higher frequency of patients with CD (66.6%). Most of them (85.4%) were undergoing pharmacological treatment and a significant percentage of patients with CD had undergone surgery. This fact might be explained, in part, by the greater complexity of CD, its management and, consequently, the more frequent referral to reference centers, when compared to UC.

Also, regarding epidemiology, the article reinforces the increase of incidence in the young population in parallel with global data on the increase in the pediatric and adolescent populations, possible causes can be attributed to genetic, hygienic, dietary, environmental facts; intestinal microbiota and other causes. The impact on quality of life, school absenteeism, emotional development is evident in all these populations.

These data are shown in another article in this issue[4], specifically in adolescents and young adults in yet another reference center for IBD in Brazil, in the city of São Paulo. In conclusion, the authors observed that adolescents and young adults with IBD activity reported low quality of life related to health at school and at work and in the general health perception domains, which highlights a criterion of disability in these vulnerable populations.

Also in the pediatric population, another article in this issue[5] analyzes the classic serological markers in pediatric inflammatory bowel disease in Brazil in a multicenter study. Its conclusions were that in pediatric patients with IBD in São Paulo, Brazil, serological tests were highly specific, although not very sensitive, for the diagnosis of IBD. However, serological markers were positively correlated to disease severity. In this scenario, the development and search for diagnostic, prognostic and therapeutic markers, play an extremely important role in IBD.

This subject has also been studied and published in another issue of this magazine. Neto et al.[6] performed the metabolic analysis on stool samples from patients with IBD to perform a differential diagnosis between CD and UC, through the analysis of stool metabolomics by high resolution nuclear magnetic resonance (HI-NMR). They concluded that there was a difference in the metabolomic profile in feces between volunteers and patients

* Universidade Estadual Paulista, Botucatu, SP, Brasil. ORCID: 0000-0002-8166-0304.

with IBD, being possible to discriminate between patients with CD and those with UC. Metabolomic analysis is a promising new technique for the recognition and surveillance of patients with IBD. This technique may be useful for diagnosis in adults, but also in the pediatric population, whose diagnosis at an early stage may be more difficult.

Thus, we observe that over the years, **Archives of Gastroenterology** has been the journal of choice for the publication of articles related to IBD, whether by Brazilian or international authors (Portugal, Argentina, Chile, China and the United Kingdom). Analyzing the number of publications in IBD between 2013 and 2018, there was an annual average of 5.2 articles/year, and from 2019 to 2020 this average practically multiplied, reaching 10.5/year. I believe that this growth is due to the quality of its editorial board and publications, growing impact factor, indexation, and the fact that it became the official journal of publications of the Brazilian Organization of Crohn and colitis (GEDIIB) with other scientific societies in the field of gastroenterology.

**Rogerio SAAD-HOSSNE***

Saad-Hossne R. Publicações sobre doenças inflamatórias na Arquivos de Gastroenterologia. Archives of Gastroenterology. 2021;58(4):417-8.

## REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2018;390:2769-78.
2. Quaresma AB, Damiao AOMC, Coy CSR, Magro DO, Valverde DA, et al. Poster Session 5 - Recent advances in epidemiology DOP41 Temporal trends in epidemiology of IBD in the public healthcare system in Brazil. Journal of Crohn's and Colitis. 2021;15:S079-S080. doi.org/10.1093/ecco-jcc/jjab073.080.
3. Fucilini LMP, Genaro LM, Sousa DC, Coy CSR, Leal RF, Ayrizono MLS. Epidemiological profile and clinical characteristics of inflammatory bowel diseases in a Brazilian referral center. Arq Gastroenterol. 2021;58:483-90.
4. Oba J, Sobrado C, Damião A, Azevedo M, Carlos A, Queiroz N, et al. Health-related quality of life in adolescents and young adults with inflammatory bowel disease is associated with reduction in school and work productivity rather than physical impairment: a multidisciplinary study. Arq Gastroenterol. 2021;58:541-7.
5. Rodrigues M, Bueno C, Lomazi EA, Fernandes MIM, Neufeld CB, D'Amico MFM, Patino MF. Classical serological markers in pediatric inflammatory bowel disease in Brazil. Arq Gastroenterol. 2021;58:495-503.
6. Lins Neto MÁF, Verdi GMX, Veras AO, Veras MO, Caetano LC, Ursulino JS. Use of metabolomics to the diagnosis of inflammatory bowel disease. Arq Gastroenterol. 2020;57:311-5.

ORIGINAL ARTICLE

AG-2020-228
doi.org/10.1590/S0004-2803.202100000-76

# B-Raf protein immunoexpression in hepatocellular carcinoma due to hepatitis C virus related cirrhosis

Paula Piedade **GARCIA**[1], Ronniel Morais **ALBUQUERQUE**[1], Fernanda Maria Farage **OSÓRIO**[2], Cláudia Alves **COUTO**[2], Agnaldo Soares **LIMA**[3] and Paula Vieira Teixeira **VIDIGAL**[1]

Received: 22 October 2020
Accepted: 15 June 2021

**ABSTRACT** – **Background** – Hepatocarcinogenesis is a multistep process that lead to genetic changes in hepatocytes resulting in neoplasia. However, the mechanisms of malignant transformation seem to differ widely. To know carcinogenesis mechanisms is essential to develop new treatment and prevention methods. **Objective** – The aim of this study is to analyze B-Raf protein immunoexpression in explants with hepatocellular carcinoma (HCC) related to hepatitis C (HCV), in adjacent cirrhotic tissue and in normal livers. We also associated the immunoexpression with known HCC related histopathogical prognostic features. **Methods** – Livers from 35 patients with HCV related cirrhosis and HCC that underwent liver transplantation or hepatectomy at Clinical Hospital – UFMG and 25 normal livers from necropsy archives were studied. Tumors were classified according to: tumor size, vascular invasion and differentiation grade. B-Raf protein expression was determined by immunohistochemistry. **Results** – B-Raf was strongly expressed in the HCV cirrhotic parenchyma cytoplasm of 17.1% cases and in 62.9% of HCC samples. Strong B-Raf protein staining was associated with tumor tissue ($P$<0.0001; OR=8.18 (2.62–26.63)). All normal livers showed weak or negative expression for B-Raf. There was no significant association among B-Raf scores and tumor differentiation grade ($P$=0.9485), tumor size ($P$=0.4427) or with vascular invasion ($P$=0.2666). **Conclusion** – We found B-Raf protein immunostaining difference in normal livers, in the areas of HCV cirrhosis and in the hepatocarcinoma. We did not find association between B-Raf expression and histopathological markers of tumor progression. Our data suggests that B-Raf may play an important role in initial HCC carcinogenesis. Larger studies are needed to validate these observations.

**Keywords** – Hepatocellular carcinoma; B-Raf; hepatitis C virus.

## INTRODUCTION

Primay liver cancer is the sixth most common cancer worldwide and the fourth main cause of death from cancer[1]. Hepatocellular carcinoma (HCC) accounts for 85 to 90% of primary liver cancers[2]. The prominent agents associated with HCC include chronic hepatitis B and hepatitis C virus infection (HCV), chronic alcohol consumption, dietary exposure to aflatoxin-B1 and virtually all cirrhosis-inducing diseases[3]. In face of a still poorly understood etiopathogenesis, signaling pathways related to hepatocarcinogenesis have been the subject of constant studies[4]. Multiple signaling pathways that regulate cell proliferation, angiogenesis and vascular invasion may be altered in HCC. Among them, we can highlight those with the participation of BRAF gene.

BRAF is a proto-oncogene that encodes a serine/threonine kinase that transduces regulatory signals through Ras/Raf/MEK/ERK cascade[5]. This pathway mediates cellular response to growth signals. Somatic mutations of BRAF provide an alternative mode of aberrant activation of the MAPK signaling pathway that is implicated in many human cancers[6]. Up regulation of this signaling pathway has been well documented in HCC and correlates with advanced stage[7].

HCV infection is the most frequent risk factor of HCC in Latin America, representing 48% of the cases[8]. HCV contributions to hepatocarcinogenesis are supposed to be related with the viral proteins, such as core, NS3 and NS5A proteins[9,10]. It is believed that simultaneous evaluation of multiple genes and regulatory pathways in HCC should help to identify causative factors, markers for early detection and prognosis prediction, as well as new therapeutic approaches[11]. As BRAF has been associated with hepatocarcinogenesis[12], the aim of this study was to analyze the immune expression of its encoded protein, B-Raf, in explants with HCC due to hepatitis C. As long as we know, this is the first study that analyses these signaling pathway in HCV hepatocarcinogenesis. We also evaluated B-Raf protein expression with known anatomopathological features of worse post-transplant or hepatectomy patients outcomes: the size of HCC tumoral lesion, the tumoral differentiation grade and the presence of tumoral vascular invasion[13,14].

## METHODS

### Approval

The study was submitted and approved by the Research Ethics Committee of the Clinical Hospital of Federal University of Minas Gerais (COEP ETIC 278/08).

Declared conflict of interest of all authors: none
Disclosure of funding: this work was supported by *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG).
[1] Universidade Federal de Minas Gerais, Faculdade de Medicina, Departamento de Anatomia patológica e Medicina Legal, Belo Horizonte, MG, Brasil. [2] Universidade Federal de Minas Gerais, Hospital das Clínicas - EBSERH, Instituto Alfa de Gastroenterologia, Belo Horizonte, MG, Brasil. [3] Universidade Federal de Minas Gerais, Faculdade de Medicina, Departamento de Cirurgia, Belo Horizonte, MG, Brasil.
Corresponding author: Paula Vieira Teixeira Vidigal. E-mail: pvidigal@medicina.ufmg.br

### Study population

Liver explants from 35 patients that underwent liver transplantation or hepatectomy in the Clinics Hospital (HC–UFMG–EBSERH) from January/2002 to December/2010 for HCC related cirrhosis were reviewed. Inclusion criteria were as follows: 1) diagnosis of HCV infection by PCR in sera, 2) liver specimens from liver explant available for review and 3) confirmation of histological features of HCC and cirrhosis. Exclusion criteria included any other form of associated liver disease. Twenty-five cases of normal livers obtained from the institution necropsy archives were selected and used as control group. Appropriate institutional review board approved the study. All patients were submitted to a protocol that includes diagnosis of etiology of liver disease and macroscopic evaluation of the explant to evaluated size of tumor nodules.

### Preparation of tissues and histological analysis

Sections of 4 μm thickness were performed in paraffin blocks. These sections were fixed later on slides, deparaffinized and stained with Hematoxylin & Eosin technique to choose the most representatives specimens of HCC and cirrhosis. Samples of the cirrhotic liver and of the tumor came from the same patients.

### Immunohistochemistry

Additional paraffin sections were made and submitted to immunohistochemical technique to investigate protein expression of B-Raf in HCC, cirrhotic and normal hepatocytes. For the application of the technique, the sections were dewaxed in xylene and hydrated with graded ethanol. They were then immersed in a solution of 1 mM EDTA (pH 8.0) and heated to 96ºC in vaporizer steamer to antigen retrieval. After cooling and washing the samples with buffer TRIS, 0.05 M Tris-HCL (pH 7.6), endogenous peroxidase activity was blocked with 3% $H_2O_2$ in water for 10 minutes. Another washing with TRIS was made. Then, the sections were incubated with primary antibodies: anti-B-Raf (Santa Cruz Biotechnology Inc., USA) at a 1:100 dilution. This was followed by incubation with the labeled streptavidin–biotin (LSAB) Kit (DakoCytomation California, Carpinteria, CA). Peroxidase activity was developed with 3.3 diaminobenzidine (DAB) (Sigma, St. Louis, MO) with timed monitoring using a positive control sample. The sections were then counterstained with hematoxylin, dehydrated, and mounted.

### Histological analysis

Histological evaluation of all samples was performed by a single expert liver pathologist. HCC was characterized according the following histological features: Predominant Edmonson and Steiner's[15] grade was classified and grouped, I and II as low grade, and III and IV as high grade and the presence of microvascular invasion, defined as the presence of tumor cells in the portal vein, the large capsular vessels or in a vascular space limited by endothelium. Tumor size was obtained by macroscopic evaluation of the liver explant. Immunostaining was evaluated on a scale of 0–3 for intensity as: 0: negative; 1: weak; 2: moderate and 3: strong and 0–4 for the extent of positive staining among hepatocytes as: 0:<5%; 1:5–25%; 2:26–50%; 3:51–75% and 4:>75%. Final score was obtained by multiplying the two individual scores, yielding a range from 0 to 12. Scores of 9–12 were considered strong staining, 6–8 as weak staining and 0–4 as markedly reduced or negative expression. For the purpose of statistical analyses the groups were classified in two and considered strong if scored 9–12 and not strong ≤8.

### Statistical analyses

The statistical analysis was performed using the SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The data correlation was performed using the chi-square association test. Values of $P<0.05$ were considered statistically significant. For data with significance, the odds ratio was calculated in order to quantify this association.

## RESULTS

This study included 35 liver explants from liver transplantation or hepatectomy for hepatocellular carcinoma treatment. All cases of HCC in the present study were obtained from cirrhotic liver with HCV as underlying cause. The majority of patients were male (80.6%) and the mean age was 56.4 years (32–79 years). As a control group, we included 25 samples of normal liver. The same way, majority of patients were male (52%) and the mean age was 42.5 years (11–73 years). Demographic and morphological data are summarized in TABLE 1.

**TABLE 1.** Demographic and morphological data from hepatocellular carcinoma/hepatitis C cirrhosis and normal samples.

| | HCC/HCV cirrhosis n (%) | Normal samples n (%) |
|---|---|---|
| Gender | | |
| Male | 28 (80.0) | 13 (52.0) |
| Female | 7 (20.0) | 12 (48.0) |
| Morphological characterization | | |
| Tumor size* | | |
| <20 mm | 8 (24.24) | |
| ≥20 mm | 25 (74.76) | |
| Tumor differentiation | | |
| Low grade | 24 (68.8) | |
| High grade | 11 (31.4) | |
| Vascular invasion | | |
| Present | 15 (42.9) | |
| Absent | 20 (57.1) | |

*n=33 cases. HCC: hepatocellular carcinoma; HCV: hepatitis C.

### B-Raf expression in normal, HCV cirrhotic liver and HCC parenchyma

All normal livers showed weak or negative expression for B-Raf. B-Raf was strongly expressed in the HCV cirrhotic parenchyma cytoplasm of 17.1% cases and in 62.9% of HCC samples. Strong B-Raf protein staining was also associated with tumor parenchyma ($P<0.0001$; OR=8.18 (2.62–26.63)). When comparing the strongly positive scores proportion B-Raf proteins in normal livers, in the areas of HCV cirrhosis and in the hepatocarcinoma, there was a statistically significant difference between the groups ($P<0.0001$), as shown in TABLE 2. FIGURE 1 shows an example of cytoplasmic labeling by immunohistochemistry for B-Raf protein in normal liver, HCV cirrhosis and in the HCC.

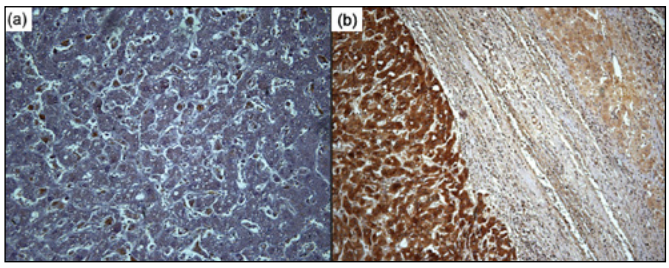



**FIGURE 1.** B-Raf negative/weak expression in normal liver (a), in HCV cirrhosis (b, up-right) and strong expression in the HCC (b, left). Magnification of 100x.

**TABLE 2.** Immunostaining score of B-Raf expression in normal liver and hepatocellular carcinoma/hepatitis C cirrhotic parenchyma.

| | Normal | Cirrhotic | HCC | *P* |
|---|---|---|---|---|
| | n (%) | n (%) | n (%) | OR (CI 95%) |
| B-Raf | | | | *P**<0.0001 |
| Negative/weak staining | 25 (100) | 29 (82.9) | 13 (37.1) | *P***<0.0001 |
| Strong staining | 0 (0) | 6 (17.1) | 22 (62.9) | OR**=8.18 (2.62–26.63) |

*Chi-square test performed for normal vs cirrhotic vs hepatocellular carcinoma. **Chi-square test performed for cirrhotic vs hepatocellular carcinoma (HCC).

### Association among B-Raf expression and anatomopathological data

We evaluate the correlation between B-Raf protein expression and morphological markers for HCC. B-Raf scores was not associated with the following predictors of patients' outcome after liver transplantation or hepatectomy: tumor size (*P*=0.4427), tumor differentiation (*P*=0.9485) and vascular invasion (*P*=0.2666), as shown in TABLE 3.

**TABLE 3.** Correlation between B-Raf protein score in the tumor parenchyma and morphological markers for hepatocellular carcinoma from to hepatitis C liver explant.

| Morphological variable | Immunostaining score | | *P* |
|---|---|---|---|
| | Strong staining n (%) | Negative or weak staining n (%) | |
| B-Raf | | | |
| Tumor size* | | | 0.4427 |
| <20 mm | 6 (18.18) | 2 (6.06) | |
| >20 mm | 15 (45.45) | 10 (30.30) | |
| Tumor differentiation | | | 0.9485 |
| Low grade | 15 (42.86) | 9 (25.71) | |
| High grade | 7 (20) | 4 (11.43) | |
| Vascular invasion | | | 0.2666 |
| Present | 11 (31.43) | 4 (11.43) | |
| Absent | 11 (31.43) | 9 (25.71) | |

*n=33 cases.

## DISCUSSION

Hepatocarcinogenesis is a multistep process initiated by external stimuli that lead to genetic changes is hepatocytes or stem cells, resulting in proliferation, apoptosis, dysplasia and neoplasia. While etiologic factors, including environmental ones, may interfere in carcinogenesis, the mechanism by which each of them induces malignant transformation seems to differ. In the present study, we evaluated the expression of B-Raf protein in surgical specimens with HCC in patients with HCV undergoing liver transplantation or hepatectomy. We found statistically significant difference in immunostaining for B-Raf protein in normal livers, in the areas of HCV cirrhosis and in the hepatocarcinoma in a Brazilian population.

We have shown a progressive enhance of B-Raf expression from normal liver to HCV cirrhotic liver and to HCC samples. Considering that HCV cirrhosis is a known risk factor for HCC, this progressive expression of B-Raf suggests that this protein may be an important factor in the process of hepatocarcinogenesis. Some previous studies have examined the frequency of BRAF gene mutations in HCC samples, with controversial results. Our results are in agreement with Colombino et al. who, in a Italian cohort, demonstrated somatic BRAF mutations in 23% of the HCC samples and a positive correlation of those mutations with the presence of multiple HCC nodules and higher proliferation rates[16]. These same authors did not observe this change in the normal areas adjacent to the tumor. Newell et al. also found an overexpression of BRAF gene in hepatocarcinoma[17]. On the other hand, Tannapfel et al. and Zuo et al. did not observe a BRAF mutation in their studies that analyzed patients with HCC[18,19]. These results indicates that populations with genetic differences may also present differences in the mechanisms and pathways of hepatocarcinogenesis.

Although the mutation in the BRAF gene is not found in some studies, the change with consequent abnormal activation of the RAF/MAPK/ERK pathway, in which the B-Raf protein participates, is reported as a common phenomenon in hepatocellular carcinoma, being associated with the stage of the tumor[19]. According to our findings, changes in the BRAF gene may play an important role in hepatocarcinogenesis in patients with hepatitis C and cirrhosis in this population.

We did not find association between B-Raf protein expression and histopathological markers of tumor progression such as tumor size, tumor differentiation and vascular invasion. Such a find suggest that BRAF gene is more important in tumor initiation than in the differentiation process. Colombino et al. demonstrated an association between gene mutation and the presence of multiple tumor nodules[16]. However, the same authors did not find any statistically significant association when comparing the mutation of the gene with the degree of differentiation or size of the tumor, which is in accordance with our findings.

In our study we used immunohistochemistry for B-Raf protein, a fast, inexpensive technique that can be used in the routine of most surgical pathology services. This choice was made because immunohistochemistry could be used as a marker of prognosis if any association between B-Raf protein expression and histopathological markers of tumor malignance was found. We did not find any association. The fact that the gene sequencing was not carried out, as well as the sample consisting only of patients with HCC due to cirrhosis associated with virus C are the main limiting factors of these result. However, we have shown a pro-

gressive enhance of B-Raf expression from normal liver to HCV cirrhotic liver and to HCC samples. Future studies are needed to validate the role BRAF gene in liver carcinogenesis in a larger group of patients with different cirrhosis etiologies and environmental exposures. Analyzing genetic and epigenetic alteration as well as different molecular pathways involved in the development of HCC is a critical process toward identifying potential new therapies[20] and also making a genome-based classification of risk factors and prognosis.

## CONCLUSION

We found B-Raf protein immunostaining difference in normal livers, in the areas of HCV cirrhosis and in the hepatocarcinoma. We did not find association between B-Raf protein expression and histopathological markers of tumor progression. Our data suggests that BRAF gene pathway may play an important role in initial HCC carcinogenesis. Larger studies are needed to validate these observations.

## ACKNOWLEDGMENTS

The authors express sincere thanks to Fernando Henrique Pereira, Ivone Marinho and Fernanda Césari.

### Authors' contribution

Methodology: Garcia PP, Vidigal PVT. Formal analysis: Garcia PP, Albuquerque RM, Vidigal PVT. Funding acquisition: Vidigal PVT. Project administration: Vidigal PVT. Writing original draft: Garcia PP, Albuquerque RM. Writing review, conceptualization, editing and approval of final manuscript: all authors.

### Orcid

Paula Piedade Garcia: 0000-0003-3343-6946.
Ronniel Morais Albuquerque: 0000-0001-5499-1602.
Fernanda Maria Farage Osório: 0000-0002-1030-3828.
Cláudia Alves Couto: 0000-0002-9776-4757.
Agnaldo Soares Lima: 0000-0001-6421-3062.
Paula Vieira Teixeira Vidigal: 0000-0002-7003-5159.

Garcia PP, Albuquerque RM, Osório FMF, Couto CA, Lima AS, Vidigal PVT. Expressão da proteína B-Raf em carcinomas hepatocelulares relacionados à cirrose por hepatite C. Arq Gastroenterol. 2021;58(4):419-23.

**RESUMO** – **Contexto** – A hepatocarcinogênese é um processo de múltiplas etapas que leva a alterações genéticas nos hepatócitos, resultando em neoplasia. No entanto, os mecanismos da transformação maligna parecem diferir amplamente. Conhecer os mecanismos da carcinogênese é fundamental para o desenvolvimento de novos métodos de tratamento e prevenção. **Objetivo** – O objetivo deste estudo é analisar a imunoexpressão da proteína B-Raf em explantes de carcinoma hepatocelular (CHC), em tecido cirrótico relacionado à hepatite C adjacente e em fígados normais. Também analisamos a imunoexpressão com características histopatológicas prognósticas relacionadas ao CHC. **Métodos** – Foram estudados fígados de 35 pacientes com CHC relacionado à cirrose por vírus C submetidos a transplante hepático ou hepatectomia no Hospital das Clínicas – UFMG e 25 fígados normais de arquivos de necropsia. Os tumores foram classificados de acordo com tamanho do tumor, invasão vascular e grau de diferenciação. A expressão de B-Raf foi determinada por imunohistoquímica. **Resultados** – B-Raf foi fortemente expresso no citoplasma do parênquima cirrótico em 17,1% dos casos e em 62,9% das amostras de CHC. A forte expressão da proteína B-Raf foi associada ao tecido tumoral ($P$<0,0001; OR=8,18 (2,62–26,63)). Todos os fígados normais apresentaram expressão fraca ou negativa para B-Raf. Não houve associação significativa entre os escores B-Raf e o grau de diferenciação do tumor ($P$=0,9485), tamanho do tumor ($P$=0,4427) ou invasão vascular ($P$=0,26666). **Conclusão** – Encontramos diferença na imunoexpressão da proteína B-Raf em fígados normais, nas áreas de cirrose por HCV e no hepatocarcinoma. Não encontramos associação entre a expressão de B-Raf e marcadores histopatológicos de progressão tumoral. Nossos dados sugerem que o B-Raf pode desempenhar um papel importante na carcinogênese inicial do CHC. Estudos maiores são necessários para validar essas observações.

**Palavras-chave** – Carcinoma hepatocelular; B-Raf; vírus da hepatite C.

## REFERENCES

1. Dasgupta P, Henshaw C, Youlden DR, Clark PJ, Aitken JF, Baade PD. Global Trends in Incidence Rates of Primary Adult Liver Cancers: A Systematic Review and Meta-Analysis. Front Oncol. 2020;10:171.
2. Goodarzi E, Beiranvand R, Mosavi-Jarrah A, Naemi H, Khazaei Z. Epidemiology Incidence and Mortality Worldwide Common cancers in males and Their Relationship with the Human Development Index (HDI): An Ecological Study Updated in the World. Journal of Contemporary Medical Sciences. 2019;5:6.
3. Ghouri YA, Mian I, Rowe JH. Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. J Carcinog. 2017;16:1. DOI: 10.4103/jcar.JCar_9_16. eCollection 2017.
4. Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. Semin Liver Dis. 2010;30:35-51.
5. Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J. 2000;351 Pt 2:289-305.
6. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949-54.
7. Huynh H, Nguyen TT, Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol. 2003;3:19.
8. Debes JD, Chan AJ, Balderramo D, Kikuchi L, Gonzalez Ballerga E, Prieto JE, et al. Hepatocellular carcinoma in South America: Evaluation of risk factors, demographics and therapy. Liver Int. 2018;38:136-43.
9. Yamanaka T, Kodama T, Doi T. Subcellular localization of HCV core protein regulates its ability for p53 activation and p21 suppression. Biochem Biophys Res Commun. 2002;294:528-34.
10. Florese RH, Nagano-Fujii M, Iwanaga Y, Hidajat R, Hotta H. Inhibition of protein synthesis by the nonstructural proteins NS4A and NS4B of hepatitis C virus. Virus Res. 2002;90:119-31.
11. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis. 2007;27:55-76.
12. Sia D, Villanueva A. Signaling pathways in hepatocellular carcinoma. Oncology. 2011;81(Suppl 1):18-23.
13. El Jabbour T, Lagana SM, Lee H. Update on hepatocellular carcinoma: Pathologists' review. World J Gastroenterol. 2019;25:1653-65.

14. Yilmaz C, Karaca CA, Iakobadze Z, Farajov R, Kilic K, Doganay L, et al. Factors Affecting Recurrence and Survival After Liver Transplantation for Hepatocellular Carcinoma. Transplant Proc. 2018;50:3571-6.
15. Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer. 1954;7:462-503.
16. Colombino M, Sperlongano P, Izzo F, Tatangelo F, Botti G, Lombardi A, et al. BRAF and PIK3CA genes are somatically mutated in hepatocellular carcinoma among patients from South Italy. Cell Death Dis. 2012;3:e259.
17. Newell P, Toffanin S, Villanueva A, Chiang DY, Minguez B, Cabellos L, et al. Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. J Hepatol. 2009;51:725-33.
18. Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut. 2003;52:706-12.
19. Zuo Q, Huang H, Shi M, Zhang F, Sun J, Bin J, et al. Multivariate analysis of several molecular markers and clinicopathological features in postoperative prognosis of hepatocellular carcinoma. Anat Rec (Hoboken). 2012; 295:423-31.
20. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378-90.

ORIGINAL ARTICLE

AG-2020-255
doi.org/10.1590/S0004-2803.202100000-77

# Gastroesophageal reflux disease in infants who presented Brief Resolved Unexplained Event (BRUE)

Maria Angela **BELLOMO-BRANDÃO**[1,2], Fernanda Maso **STRANGUETTI**[1], Iara Ferreira **LOPES**[1], Andressa Oliveira **PEIXOTO**[1,2,3], Fernando Augusto Lima **MARSON**[1,2,3,4,5] and Elizete Aparecida **LOMAZI**[1]

Received: 11 December 2020
Accepted: 15 June 2021

**ABSTRACT** – **Background** – The term brief resolved unexplained events (BRUE) is a description of the acute event occurring in infants less than 1-year-old that includes at least one of the following characteristics: cyanosis or pallor; absent, decreased, or irregular breathing; marked change in tone or altered level of responsiveness. An investigative proceeding is required to identify the triggering phenomenon in those who are at high risk of complications. Prolonged esophageal pHmetry has been used as a tool in searching for gastroesophageal reflux disease (GERD) as one of the underlying etiologies. **Objective** – The study aims to verify the frequency of GERD in infants up to 1-year-old, when pHmetry has been performed for investigating high-risk BRUE (HR-BRUE) and to analyze if clinical characteristics or any particular symptom related by caregivers during BRUE could be correlated to GERD. **Methods** – It was performed a cross-sectional study. The data was collected retrospectively of patients less than 1-year-old, who had performed pHmetry in a tertiary hospital for investigating HR-BRUE between October 2008 and January 2018. For the analysis of medical records, a data collection protocol included: gender, age at the first HR-BRUE episode, age at the time of the pHmetry, gestational age, type of delivery (normal or caesarean) and birth weight and symptoms associated to HR-BRUE related by caregivers. Relation between variables were assessed using Fisher's exact test and Mann-Whitney test. The significance level was set at 0.05. **Results** – A total of 54 infants were included (preterm 25, term 29), 62.9% males, median age at the HR-BRUE was 36 days, 53.7% HR-BRUE episodes had occurred during or right after feeding. According to pHmetry results: nine pHmetry results were considered inconclusive, physiological reflux (n=30) and GERD (n=15). The frequency of GERD diagnosed by pHmetry was 33%. GERD was not statistically related to gender (*P*-value=0.757), age at first HR-BRUE episode (*P*-value=0.960), age at the time of the pHmetry (*P*-value=0.720), prematurity (*P*-value=0.120) or type of delivery (*P*-value=0.738). GERD was statistically related to low birth weight (*P*-value=0.023). There was no association between symptoms reported by caregivers during HR-BRUE and GERD. **Conclusion** – GERD diagnosed by the pHmetry was found in one third of infants that experiencing a HR-BRUE, showing the importance of properly investigation. In half of infants BRUE occurred during or right after feeding. Besides low birth weight, it was not possible to select other data from the clinical history that suggest that these patients would be more likely to have GERD.

**Keywords** – Brief resolved unexplained events; apparent life-threatening events; gastroesophageal reflux disease; infants; neonates.

## INTRODUCTION

Initially, infants who were evaluated after a sudden and apparently fatal event were classified as having sudden infant death syndrome (SIDS), subsequently, as apparent life-threatening event (ALTE), defined as an alarming episode characterized as a combination of apnea; color change, usually cyanotic or pallid; marked change in muscle tone (usually marked limpness); choking or gagging[1]. In 2016, the American Academy of Pediatrics introduced the term brief resolved unexplained events (BRUE) aiming to facilitate diagnosis and to reduce the episode's impact on caregivers. The definition of BRUE includes at least one of the following characteristics: cyanosis or pallor; absent, decreased, or irregular breathing, marked change in tone (hypertonia or hypotonia); or altered level of responsiveness, occurring in infants under 1-year-old. During BRUE, caregivers report a sudden, brief episode (less than one minute) but then resolved. Infants with BRUE have been categorized as low-risk patients, who require brief monitoring, or as high-risk patients, who need further investigation and treatment[2-5], including investigation of gastroesophageal reflux disease (GERD)[6]. Physical examination and a detailed clinical history are essential in determining the possible causes of BRUE, including child abuse, arrhythmias and others cardiac conditions, metabolic diseases, and other factors.

Gastroesophageal reflux consists in the involuntary passage of gastric content into the esophagus, with or without externalization by mouth in the form of regurgitation and/or vomiting, it is a physiological event that occurs in all individuals – especially

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade Estadual de Campinas, Faculdade de Ciências Médicas, Departamento de Pediatria, Campinas, SP, Brasil. [2] Universidade Estadual de Campinas, Faculdade de Ciências Médicas, Centro de Investigação em Pediatria, Campinas, SP, Brasil. [3] Universidade Estadual de Campinas, Faculdade de Ciências Médicas, Hospital Universitário, Unidade de Pronto Atendimento de Urgência e Emergência, Campinas, SP, Brasil. [4] Universidade Estadual de Campinas, Faculdade de Ciências Médicas, Departamento de Genética e Medicina Genômica, Campinas, SP, Brasil. [5] Universidade São Francisco, Programa de Pós-Graduação em Ciências da Saúde, Bragança Paulista, SP, Brasil.
Corresponding author: Maria Angela Bellomo-Brandão. E-mail: angbell@unicamp.br

infants. Some children may develop GERD, which occurs when reflux causes symptoms or complications[7-9]. According to North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines, published in 2018, pH-metry and multichannel intraluminal impedance coupled to a pH-meter sensor (pH-MII) are both recommended methods for the diagnosis of GERD. Such guidelines consider anti-reflux surgery in infants with GERD and life-threatening complications such as apneas or BRUE[10].

Although there are studies that have analyzed GERD and life-threatening events relation[11-13], the issue is still controversial, since reflux assessment methods vary widely. This study aims to verify the frequency of GERD in infants up to one-year-old, when pHmetry has been performed for investigating currently classified as high-risk BRUE (HR-BRUE). The secondary aim was to analyze if any particular symptom of HR-BRUE could be correlated to GERD.

## METHODS

It was done a cross-sectional study and the clinical data was collected retrospectively of patients less than 1-year-old, who had performed pHmetry in a tertiary hospital for investigating HR-BRUE or retrospective application of the HR-BRUE criteria, defined as: less than 60 days of life, or gestational age less than 32 weeks and corrected gestational age less than 45 weeks, or more than one event, or needed of resuscitation maneuvers[2], between October 2008 and January 2018.

For the analysis of medical records, a data collection protocol included: gender, age at the first HR-BRUE episode, age at the time of the pHmetry, gestational age, type of delivery (normal or caesarean) and birth weight. Prematurity and low birth weight were defined by World Health Organization (WHO):

- prematurity[14]: preterm <37 weeks of gestational age;
- low birth weight[15]: weight at birth less than 2,500 g (5.5 lb).

Symptoms associated to HR-BRUE: HR-BRUE episode during or right after feeding, vomiting, choking, hypertonia, hypotonia, cyanosis, apnea, and pallor were presented as positive (presence) by the description in the medical records. In this context, the absence of information was considered as a negative result.

Exclusion criteria: patients diagnosed with genetic syndrome or malformations, or those not classified as HR-BRUE.

Prolonged esophageal pHmetry: the ALACER III® (Alacer biomédica Ltda.) portable recorder was used, connected to a single-channel antimony electrode, calibrated prior to each examination using pH standard solutions of 7 and 1. The electrode was introduced in one of the nostrils, positioned using the Strobel's formula [Height (cm) x 0,252] and position was confirmed by X-ray[16]. No gastric acid suppressors (hydrogen potassium blocker or proton pump inhibitor) were used in the week prior to the examination. Routine diets and physical activities were maintained. In esophageal pHmetry, the diagnosis of GERD was made through the reflux index (RI), defined as the percentage of time that pH <4. Then, GERD was defined as pH <4 for >10% for infants <1 year and physiological reflux was established for all infants who did not reach a 10% RI; in the absence of BRUE episodes[16].

The study was approved by the local Research Ethics Committee of the University – number #92692718.0.0000.5404.

Relation between variables were assessed using Fisher's exact test and Mann-Whitney test. The significance level was set at 0.05.

## RESULTS

The study included 54 infants with HR-BRUE, 34/54 males; 29 term; 25 preterm; 20 normal delivery, 34 caesarean delivery; 36 normal birth weight, 18 low birth weight. Clinical data are summarized in TABLE 1.

**TABLE 1.** Clinical characteristics of infants investigated by high-risk brief resolved unexplained events (HR-BRUE) (n=54).

| Variables | Frequency | Percentage |
|---|---|---|
| Gender | | |
| Male | 34 | 62.96% |
| Female | 20 | 37.04% |
| Type of delivery | | |
| Normal | 20 | 37.03% |
| Caesareans | 34 | 62.29% |
| Prematurity | | |
| Pre-term[a] | 25 | 42.29% |
| Term[a] | 29 | 53.71% |
| Birth weight | | |
| Low weight[b] | 18 | 33.33% |
| Normal weight[b] | 36 | 66.67% |

[a]Classification of gestational age: term ≥37 weeks; preterm <37 weeks; [b]Classification of birth weight: less than 2,500 g – low weight; above 2,500 g – normal weight.

First HR-BRUE episode occurred at mean of 42.98±40.82 days, median age of 36 days, ranging from 1 to 180 days of life. Regarding age at the time of the pHmetry, mean: 76.67±49.65 days was identified, with a minimum of 13 days, a median of 65 days and a maximum of 222 days.

Results of frequency of symptoms associated to HR-BRUE were showed in in TABLE 2; 29/54 (53.7%) HR-BRUE episodes were reported as having occurred during or right after feeding.

Nine pHmetry results were considered inconclusive by lack/poor information about posture/feeding or detachment of the external pHmetry sensor with consequent insufficient analysis time. Such exams were not repeated, as other etiologies had been identified. No BRUE episodes occurred during pHmetry monitoring.

**TABLE 2.** Distribution of frequency and percentage of symptoms recorded in medical file as related to the high-risk brief resolved unexplained events (HR-BRUE).

| Symptom | Frequency | Percentage |
|---|---|---|
| Episode during or right after feeding | 29 | 53.7% |
| Vomiting | 19 | 35.19% |
| Choking | 12 | 22.22% |
| Hypertonia | 5 | 9.26% |
| Hypotonia | 18 | 33.33% |
| Cyanosis | 45 | 83.33% |
| Apnea | 17 | 31.48% |
| Pallor | 3 | 5.56% |

According to pHmetry results [physiological reflux (n=30) and GERD (n=15)], distribution of symptoms in 45 remained patients was showed in TABLE 3; where it were HR-BRUE episodes reported as having occurred during or right after feeding, cyanosis, vomiting, apnea, choking, pallor, hypertonia and hypotonia. Also, in the TABLE 3 it was shown the association between the pHmetry results with prematurity (*P*-value=0.120), type of delivery (natural birth or caesarean birth) (*P*-value=0.738) and birth weight (*P*-value=0.023). Curiously, the group with GERD presented 9/15 (60%) cases of low birth weight versus 7/30 (23.33%) cases in the groups with physiological reflux (odds ratio – conditional maximum-likelihood estimate (cMLE)=4.73; 95% confidence interval=1.08 to 23.11).

The pHmetry variables were compared between term and preterm patients. We found that number of reflux acid episodes lasting longer than 5 min was statistically significant (*P*-value=0.028) being higher in the preterm individuals [preterm: 7.55±4.77 events vs term: 4.90±4.13 (TABLE 4).

Finally, GERD diagnosed by pHmetry was not statistically related to gender (*P*-value=0.757), age at first HR-BRUE episode (*P*-value=0.960), and age at the time of the pHmetry (*P*-value=0.720).

## DISCUSSION

GERD diagnosed by prolonged esophageal pHmetry in HR-BRUE infants occurred in one third of our patients. Frequency of GERD is closely related to the method used for its investigation. In 1991, a study in infants with a history of near-miss sudden infant death, used continuous esophageal pH monitoring and polygraphic recording in 49 infants showed pathologic gastroesophageal reflux in 34 (69%) patients[17], using the Boix-Ochoa parameters[18]. GERD investigated by the radioisotope milk scan method occurring in 26% of infants diagnosed with ALTE[11] and 8/33 (24%) in HR-BRUE, but there were no reports on the methods used to diagnose GERD[12]. A study conducted by Macchini et al. found 80% of patients with ALTE were diagnosed with GERD

**TABLE 3.** Distribution of clinical characteristics/symptoms and pHmetry results in infants with high-risk Brief Resolved Unexplained Event.

| Clinical characteristics and Symptoms/ pHmetry results | Physiological reflux (n=30) | Gastroesophageal reflux disease (n=15) | *P-value |
|---|---|---|---|
| Prematurity | | | |
| Preterm birth | 12 (40%) | 10 (66.7%) | 0.120 |
| Term birth | 18 (60%) | 5 (33.3%) | |
| Type of delivery | | | |
| Caesarean birth | 19 (63.3%) | 11 (73.3%) | 0.738 |
| Natural birth | 11 (36.7%) | 4 (26.7%) | |
| Birth weight | | | |
| Low birth weight | 7 (23.3%) | 9 (60%)[b] | **0.023** |
| Normal birth weight | 23 (76.7%) | 6 (40%) | |
| Episode during or right after feeding | 18 (60%) | 7 (46.67%) | 0.595 |
| Vomiting | 12 (40%) | 6 (40%) | 1 |
| Choking | 5 (16.67%) | 5 (33.33%) | 0.33 |
| Cyanosis | 24 (80%) | 12 (80%) | 1 |
| Apnea | 11 (36.67%) | 3 (20%) | 0.43 |
| Pallor | 1 (3.33%) | 1 (6.67%) | 1 |
| Hypertonia | 4 (13.33%) | 1 (6.67%) | 0.92 |
| Hypotonia | 10 (33.33%) | 4 (26.67%) | 0.90 |

*Fisher's exact test. Positive *P*-value is shown as presented bold-type. Alpha: 0.05. [b]Odds Ratio CMLE: 4.73 and 95% confidence interval: 1.078 to 23.11. The odds ratio was calculated based on low birth weight in the group with gastroesophageal reflux.

**TABLE 4.** pHmetry variables compared between term and preterm infants with high-risk Brief Resolved Unexplained Event.

| pHmetry variables | Preterm | Term | *P-value |
|---|---|---|---|
| Number of acid reflux episodes[#] (mean±SD) | 42.30±22.34 | 36.65±20.08 | 0.289 |
| Number of reflux acid episodes lasting longer than 5 min (mean±SD) | 7.55±4.77 | 4.90±4.13 | **0.028** |
| Esophageal Clearance-min/refl (mean±SD) | 3.60±1.89 | 2.72±1.21 | 0.201 |

[#]Acid Reflux episodes: a decrease in pH below four that lasts for more than 10 sec. *Mann-Whitney Test. Positive *P*-value is shown as presented bold-type. Alpha: 0.05.

by pHmetry. The authors used RI: <3%, 3–7%, and >7% and conclude that severity of GERD influenced on normalization of cardiorespiratory monitoring, suggesting that treat reflux could prevent recurrence of ALTE[13]. However, pHmetry has reference values identified in healthy infants, being able to identify physiological reflux. The diagnosis of pathological reflux in children under 1 year of age was RI >10%[16] in our study. We had used these criteria because our concern that diagnosis of GERD may lead to unnecessary treatment, and perhaps delay investigation of other disorders. BRUE episodes did not occur during pHmetry monitoring although 33% of those who reported having a HR-BRUE demonstrated having GERD due to the 24-hour esophageal pH monitoring parameters.

MII-pH monitoring revealed acid or nonacid reflux in 2/3 of 53 HR-BRUE infants, non-acid reflux events were the most common findings (66%)[19]. A study performed in 25 infants presenting with BRUE who underwent concurrent MII-pH and video-polysomnography showed severe esophageal acid exposure (RI>7%) is associated with increased reflux-associated symptoms in wake state[20].

The median age at which the patients presented the first symptoms was 36 days and the median age at which the pHmetry test was performed was 65 days. This delay of approximately one month possibly could be justified by a delay in referral to hospitals which have necessary resources for a complete investigation.

Despite not being related to gender, age at first HR-BRUE episode, age at the time of the pHmetry, prematurity or type of delivery; GERD was statistically related to low birth weight, as described in the literature[21]. Comparing pHmetry of preterm and term infants, we found higher number of longest acid reflux in preterm, compared to that of term infants, what was also found in a MII-pH monitoring study[19].

It is known that pHmetry has some limitations for the diagnosis of GERD in infants and young children, because it is not sensitive to detect non-acid reflux[2,10]. Notably, pH-MII is not available in health care units in resource-limited countries and a recent consensus published by NASPGHAN and ESPGHAN recommend both methods for properly investigation of GERD[10].

The symptoms reported as HR-BRUE occurred during or right after feeding in half of infants, and there was no symptom significantly involved in HR-BRUE and GERD. A recent systematic review demonstrates that the reported prevalence of GERD symptoms may vary considerably in infants and children[22]. Given this outcome, it was not possible to select clinical history that could assist in selecting patients who would be more likely to have GERD.

Weir et al. (2009) reported that neither the clinical history nor observed feeding sessions can accurately predict which patients have oropharyngeal dysphagia versus GERD[23]. During the neonatal period, a proven relation occurs through such symptoms and the triggering of laryngeal reflex caused by stimulus of laryngeal chemoreceptors, promoting glottal closure or spasm as a protective reflex of the airways, preventing any foreign body from entering the lower respiratory system[24-27]. Considering that caregivers had reported symptoms of BRUE as occurring during or right after feeding in half of the studied infants, we reinforce the importance of investigating and treating appropriately aerodigestive dysfunctions in the management of BRUE[28].

Although GERD is one of the most remembered etiologies of BRUE by caregivers and physicians, American Academy of Pediatrics recommends that evaluation of a baby after BRUE is driven by a complete clinical history and a careful physical examination. In most cases, the child is free of symptoms at the time the medical evaluation takes place, allowing a systematic approach to anamnesis and physical examination. The complementary tests that should be evaluated in a child after an episode of BRUE include venous blood gases, toxicological studies, bacterial or viral cultures, electroencephalogram, pHmetry or MII-pH monitoring, neuroimaging, and other features[2].

As a limitation of our study, we were not able to change the formula to extensive hydrolyzed formula or 100% amino-acid formula after GERD diagnosis, because GERD guideline from NASPGHAN 2018 was published after the cases had been included in our study. Nowadays, infants suspected of having GERD are systematically tested for allergic etiology using hypoallergenic formula in our clinics[10].

## CONCLUSION

GERD and life-threatening events relation is still controversial, since reflux assessment methods vary widely. GERD diagnosed by pHmetry was found in 33% of infants who presented HR-BRUE. The symptoms reported as HR-BRUE occurred during or right after feeding in half of infants, and there was no symptom significantly involved in HR-BRUE and GERD. Future studies are needed to understand the role of GERD in BRUE etiopathogenesis. Curiously, this is the first report from Brazil that associate the BRUE or ALTE with esophageal pHmetry or pH-MII in infants or preterm.

### Authors' contribution

Bellomo-Brandão MA: conceived the project, collected participants' data, wrote and critically reviewed the study. Stranguetti FM: validated the results. Peixoto AO: accounted for supervision, performance and validation using reproducibility criteria. Stranguetti FM, Lopes IF, Lomazi EA, Peixoto AO: evaluated medical records of the study participants and validated findings using reproducibility criteria. Marson FAL: analyzed an interpreted the data. All authors have read and approved the last version of the manuscript prior to submission.

### Orcid

Maria Angela Bellomo-Brandão: 0000-0002-1145-2606.
Fernanda Maso Stranguetti: 0000-0002-2975-8187.
Iara Ferreira Lopes: 0000-0001-8810-5218.
Andressa Oliveira Peixoto: 0000-0002-8407-4087.
Fernando Augusto Lima Marson: 0000-0003-4955-4234.
Elizete Aparecida Lomazi: 0000-0001-5504-4746.

Bellomo-Brandão MA, Stranguetti FM, Lopes IF, Peixoto AO, Marson FAL, Lomazi EA. Doença do refluxo gastroesofágico em lactentes que apresentaram Eventos Resolvidos Breves Não Explicados – *Brief Resolved Unexplained Event* (BRUE). Arq Gastroenterol. 2021;58(4):424-8.

**RESUMO** – **Contexto** – O termo Eventos Resolvidos Breves Não Explicados (*Brief Resolved Unexplained Event* – BRUE) é uma descrição do evento agudo que ocorre em lactentes menores de 1 ano de idade que inclui pelo menos uma das seguintes características: cianose ou palidez; respiração ausente, diminuída ou irregular, alteração acentuada no tônus ou nível alterado de responsividade. É necessário um procedimento investigativo para identificar o fenômeno desencadeante naqueles que apresentam alto risco de complicações. A pHmetria esofágica prolongada tem sido usada como uma ferramenta na pesquisa de doença do refluxo gastroesofágico (DRGE) como uma das etiologias subjacentes. **Objetivo** – Este estudo tem como objetivo verificar a frequência da DRGE em lactentes de até 1 ano de idade, quando a pHmetria foi realizada para investigação da BRUE de alto risco, e analisar se alguma característica clínica ou sintoma particular relatado pelos cuidadores durante a BRUE poderia estar correlacionado a DRGE. **Métodos** – Foi realizado um estudo observacional, transversal, cujos dados foram coletados retrospectivamente de pacientes menores de 1 ano de idade, que realizaram pHmetria em hospital terciário para investigação de BRUE de alto risco de outubro de 2008 e janeiro de 2018. Para a análise dos prontuários, um protocolo de coleta de dados incluiu: sexo, idade no primeiro episódio de BRUE de alto risco, idade no momento da pHmetria, idade gestacional, tipo de parto (normal ou cesárea), peso ao nascer e sintomas associados a alto risco-BRUE relatado por cuidadores. A relação entre as variáveis foi avaliada por meio do teste exato de Fisher, qui-quadrado e teste de Mann-Whitney. O nível de significância foi estabelecido em 0,05. **Resultados** – Foram incluídos 54 lactentes (pré-termo 25, termo 29), 62,9% do sexo masculino, idade mediana na BRUE de alto risco foi de 36 dias. De acordo com o relatório do cuidador, 53,7% dos episódios de BRUE de alto risco ocorreram durante ou logo após a alimentação. Resultados da pHmetria: nove resultados da pHmetria foram considerados inconclusivos, refluxo fisiológico (n=30) e DRGE (n=15). A frequência de DRGE diagnosticada por pHmetria foi de 33%. A DRGE não foi estatisticamente relacionada ao sexo (*P*=0,757), idade no primeiro episódio de BRUE de alto risco (*P*=0,96), idade no momento da pHmetria (*P*=0,72) prematuridade (*P*=0,321) ou tipo de parto (*P*=0,738). A DRGE foi estatisticamente relacionada ao baixo peso ao nascer (*P*=0,023). Não houve associação entre os sintomas relatados pelos cuidadores durante BRUE de alto risco e o diagnóstico de DRGE. **Conclusão** – A DRGE diagnosticada pela pHmetria foi encontrada em um terço dos lactentes que vivenciaram BRUE de alto risco, mostrando a importância da investigação adequada. Em metade das crianças, o evento ocorreu durante ou logo após a alimentação. Além do baixo peso ao nascer, não foi possível selecionar outros dados da história clínica que sugiram que esses pacientes terão maior probabilidade de apresentar DRGE.

**Palavras-chave** – Eventos inexplicáveis breves e resolvidos; eventos de aparente risco de vida; doença do refluxo gastroesofágico; lactentes; neonatos.

## REFERENCES

1. American Academy of Pediatrics. National Institutes of Health Consensus Development Conference on Infantile Apnea and Home Monitoring. Pediatrics. 1987;79:292-9.
2. Tieder JS, Bonkowsky JL, Etzel RA, Franklin WH, Gremse DA, Herman B, et al. Clinical practice guideline: brief resolved unexplained events (formerly apparent life-threatening events) and evaluation of lower-risk infants: executive summary. Pediatrics. 2016;137:e20160591.
3. Tate C, Sunley R. Brief resolved unexplained events (formerly apparent life-threatening events) and evaluation of lower-risk infants. Arch Dis Child Educ Pract Ed. 2018;103:95-8.
4. McFarlin A. What to do when babies turn blue: beyond the basic brief resolved unexplained event. Emerg Med Clin North Am. 2018;36:335-47.
5. Gatto A, Paglietti MG, Bocchi MB, Lazzareschi I, Cutrera R, Valentini P. Brief resolved unexplained events and apparent life-threatening events: the wall between guidelines and clinical practice. Acta Paediatr. 2020;109:1267-68
6. Baird DC, Harker DJ, Karmes AS. Diagnosis and treatment of gastroesophageal reflux in infants and children. Am Fam Physician. 2015;92:705-14.
7. Barfield E, Parker MW. Management of pediatric gastroesophageal reflux disease. JAMA Pediatr. 2019;173:485-6.
8. Colletti RB, Di Lorenzo C. Overview of pediatric gastroesophageal reflux disease and proton pump inhibitor therapy. J Pediatr Gastroenterol Nutr. 2003;37:S7-s11.
9. Czinn SJ, Blanchard S. Gastroesophageal reflux disease in neonates and infants: when and how to treat. Paediatr Drugs. 2013;15:19-27.
10. Rosen R, Vandenplas Y, Singendonk M, Cabana M, DiLorenzo C, Gottrand F, et al. Pediatric Gastroesophageal Reflux Clinical Practice Guidelines: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2018;66:516-54.
11. Davies F, Gupta R. Apparent life threatening events in infants presenting to an emergency department. Emerg Med J. 2002;19:11-6.
12. Colombo M, Katz ES, Bosco A, Melzi ML, Nosetti L. Brief resolved unexplained events: Retrospective validation of diagnostic criteria and risk stratification. Pediatr Pulmonol. 2019;54:61-5.
13. Macchini F, Morandi A, Cognizzoli P, Farris G, Gentilino V, Zanini A, et al. Acid gastroesophageal reflux disease and apparent life-threatening events: simultaneous pH-metry and cardiorespiratory monitoring. Pediatr Neonatol. 2017;58:43-7.
14. World Health Organization (WHO). [Internet]. Available from: www.who.int/news-room/fact-sheets/detail/preterm-birth
15. World Health Organization (WHO). Newborns with low birth weight (%). [Internet]. Available from: https://www.who.int/whosis/whostat2006NewbornsLowBirthWeight.pdf
16. Vandenplas Y, Sacré-Smits L. Continuous 24-hour esophageal pH monitoring in 285 asymptomatic infants 0-15 months old. J Pediatr Gastroenterol Nutr. 1987;6:220-4.
17. Veereman-Wauters G, Bochner A, Van Caillie-Bertrand M. Gastroesophageal reflux in infants with a history of near-miss sudden infant death. J Pediatr Gastroenterol Nutr. 1991;12:319-23.
18. Boix-Ochoa J, Lafuenta JM, Gil-Vernet JM. Twenty-four-hour exophageal pH monitoring in gastroesophageal reflux. J Pediatr Surg. 1980;15:74-8.
19. Jarasvaraparn C, Belen Rojas Gallego M, Wang B, Crissinger KD, Gremse DA. The Characteristics of Esophageal Multichannel Intraluminal Impedance-PH Measurements in Infants Experiencing Brief Resolved Unexplained Events. Ann Gastroenterol Dig Disord. 2018;1:1-8.
20. Sankaran J, Qureshi AH, Woodley F. Effect of Severity of Esophageal Acidification on Sleep vs Wake Periods in Infants Presenting with Brief Resolved Unexplained Events. J Pediatr. 2016,179:42-8.
21. Dutta S, Singh B, Chessell L, Wilson J, Janes M, McDonald K, et al. Guidelines for feeding very low birth weight infants. Nutrients. 20158;7:423-42.
22. Singendonk M, Goudswaard E, Langendam M, van Wijk M, van Etten-Jamaludin F, Benninga M, et al. Prevalence of gastroesophageal reflux disease symptoms in infants and children: a systematic review. J Pediatr Gastroenterol Nutr. 2019;68:811-7.
23. Weir K, McMahon S, Barry L, Masters IB, Chang AB. Clinical signs and symptoms of oropharyngeal aspiration and dysphagia in children. Eur Respir J. 2009;33:604-11.
24. Bauman NM, Sandler AD, Schmidt C, Maher JW, Smith RJ. Reflex laryngospasm induced by stimulation of distal esophageal afferents. Laryngoscope. 1994;104:209-14.
25. Thach BT. Reflux associated apnea in infants: evidence for a laryngeal chemoreflex. Am J Med. 1997;103:120s-4s.
26. Hernández-Cortez E. Old laryngospasm complication: new treatments. Anest. Méx. 2017;8:00327.
27. Jadcherla SR. Pathophysiology of aerodigestive pulmonary disorders in the neonate. Clin Perinatol. 2012;39:639-54.
28. Duncan DR, Amirault J, Mitchell PD, Larson K, Rosen RL. Oropharyngeal dysphagia is strongly correlated with apparent life-threatening events. J Pediatr Gastroenterol Nutr. 2017;65:168-72.

ORIGINAL ARTICLE

AG-2021-10
doi.org/10.1590/S0004-2803.202100000-78

# The management of dermatitis herpetiformis by the gastroenterologist. A series of cases

Lorete Maria da Silva **KOTZE**[1], Luiz Roberto **KOTZE**[1], Katia Sheylla Malta **PURIM**[2] and Renato **NISIHARA**[1,2]

Received: 15 January 2021
Accepted: 22 June 2021

**ABSTRACT** – **Background** – Dermatitis herpetiformis (DH) is considered a skin celiac disease (CD). The individuals can be seen by primary care professionals or by dermatologists that could refer the patient to a gastroenterologist. **Objective** – The study aimed to investigate the clinical profile of patients diagnosed with DH and referred to a gastroenterologist and evaluate the treatment response. **Methods** – We retrospectively studied patients with DH referred to the same gastroenterologist at a private office in Curitiba, Brazil, between January 2010 to December 2019. We included adult patients with a confirmed DH diagnosis. Symptoms, clinical signs, laboratory and histological data, as well as treatment response, were collected. **Results** – Thirty-three patients were studied (60.6% women, mean age at diagnosis 40.8±12.61 years). The median delay for DH diagnosis was four years. Skin involvement was mild in 33.3%, moderate in 18.2%, and severe in 48.5%. The more frequent gastrointestinal complaints were abdominal distension (78.8%), flatulence (75.7%), and gastroesophageal reflux (51.5%). Depression and anxiety were observed in 81.8% and anemia in 51.1%. A higher prevalence of bone disorders was associated with higher age at DH diagnosis ($P$=0.035). Duodenal biopsy showed changes in all patients. Improvement after treatment only with a gluten-free diet (GFD) plus dapsone was verified in 81.2%. **Conclusion** – Patients with DH referred to a gastroenterologist showed a high frequency of gluten intolerance and systemic complaints. Duodenal histological alterations were found in all the cases. The treatment based on GFD plus dapsone was effective in most patients.

**Keywords** – Dermatitis herpetiformis; treatment; complaints.

## INTRODUCTION

Dermatitis herpetiformis (DH) is an immune-mediated chronic recurrent disorder caused by gluten intolerance (GI)[1] and is considered a skin celiac disease (CD)[2,3]. The pathophysiology of DH is complex and involves genetic factors (HLA predisposition), environment trigger (gluten), and dysregulation of the immune system[4]. In most cases, patients with DH are attended by primary care physicians or by dermatologists and then referred to a gastroenterologist to ensure better management of the disease and related complications and control by a gluten-free diet (GFD) that is essential for treatment[5]. There are few studies stressing the importance of GI and systemic symptoms in patients with DH and complications consequent to malabsorption of nutrients, as anemia and bone disease. The treatment of choice is a life-long GFD which resolves the rash and enteropathy and offers a good prognosis[6]. The relief of symptoms results in humor benefits and better quality of life[2].

The study aimed to investigate the broad clinical profile of patients diagnosed with DH and referred to a gastroenterologist and evaluate treatment response.

## METHODS

Approval was obtained from the Ethics Committee of Mackenzie Evangelical School of Medicine. The procedures used in this study adhere to the tenets of the Declaration of Helsinki, and all participants signed consent.

This retrospective study is a series of cases studied on patients with a confirmed diagnosis of DH by skin biopsies. Noteworthy, all the cases were attended by the same gastroenterologist at a referred private office in Curitiba, Brazil, from January 2010 to December 2019. These patients were referred to this gastroenterologist for clinical approach and management with a GFD. Patients with other concomitant skin diseases and incomplete medical charts were excluded.

All data were collected from the medical charts of the patients. Information on gender, age, period of the onset of the symptoms, degree of skin involvement, gastrointestinal (GI) complaints, humor distress, presence of anemia, bone disease evaluation by dual-energy X-ray absorptiometry (DEXA) was collected at the first consultation with the gastroenterologist. The presence of autoantibodies IgA anti-endomysium was investigated by indirect immunofluorescence or IgA anti-tissue transglutaminase (ELISA) and HLA-DQ2/DQ8.

All patients underwent upper GI endoscopy with two or three biopsies from the bulb and four to five fragments of the second portion of duodenal mucosa while on a gluten-containing diet[7]. The histological findings were reported according to Marsh classification[8]. The response to a GFD and the combination of GFD plus drugs were investigated. Treatment of DH was done following several authors' guidelines[2,3,6]. Firstly, a strict GFD was recommended to all patients for one month. If there was no improvement of the skin

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade Federal do Paraná, Setor de Ciências da Saúde, Curitiba, PR, Brasil. [2] Universidade Positivo, Departamento de Medicina, Curitiba, PR, Brasil.
Corresponding author: Renato Nisihara. E-mail: renatonisihara@gmail.com

lesions, dapsone was prescribed, with posology determined by the severity of the clinical manifestation and patient weight. Patients with severe skin symptoms at the time of diagnosis were immediately given GFD and dapsone. The time of use and the dose were adapted according to the drug efficacy response[2,9]. Dapsone was discontinued as early as possible if the skin symptoms disappeared. GFD was recommended for all life. Azathioprine was prescribed if GFD plus a high dose of dapsone did not improve the lesions (refractory cases)[10,11]. In addition, reports of other immune-mediated diseases (IMDs) previously to the diagnosis of DH and the family history of these affections were asked to patients.

The data was summarized in frequency and contingency tables. The Fisher test was used to compare nominal data. Unpaired *t*-tests or Mann Whitney tests were used to compare numerical data. Data distribution was evaluated by the Shapiro Wilkin's test. The adopted significance level was 5%.

## RESULTS

Thirty three patients were studied, being 20/33 (60.6%) women and 13/33 (39.4%) men ($P$=0.08). All were Caucasians, with a mean age at diagnosis 40.8±12.61 years, similar between the genders. The diagnostic delay showed a median age of 4 years (IQR=1–12), between 0 to 42 years. Age at diagnosis and severity of DH was not associated with delay in diagnosis ($P$=0.19 and $P$=0.35, respectively). Autoantibodies (IgA EmA or IgA tTG) were positive in 59.3% (16/27) patients. HLA DQ2/DQ8 was determined in 13/33 cases, of which 86.7% were had HLA-DQ2 and 13.3% DQ8 positive.

At the first consultation, the degree of skin involvement evaluated was considered mild in 33.3% (11/33), moderate in 18.2% (6/33), and severe in 48.5% (16/33). TABLE 1 shows frequencies of GI symptoms and clinical signs at the first gastroenterological consultation. The more frequent were abdominal distension (78.8%), flatulence (75.7%), and gastroesophageal reflux (51.5%). Anemia was present in 51.1% of the cases, the iron deficiency being more frequent than vitamin B12 deficiency. Regarding bone disorder, osteopenia was detected in 48% of the cases and osteoporosis in 8%. A higher prevalence of bone disorders was associated with higher age of DH at diagnosis ($P$=0.035). No occurrence of fracture was informed. In addition, a high prevalence of humor disorders (81.8%) revealed more depression than anxiety, without significant difference comparing gender, age, or disease severity.

Intestinal biopsy showed changes similar to the histologic findings observed in CD patients in all of the cases. According to Marsh classification[8], Grade I was detected in 24.4% (8/33), Grade II in 21.1% (7/33), and Grade III in 54.5% (18/33). FIGURE 1 shows that Marsh III was more frequent in patients with severe forms of DH, however, without significant difference ($P$=0.14).

Only female patients reported other IMD 10/33 = (30.3%) being found hypothyroidism (seven cases), endometriosis (two cases), and type 1 diabetes mellitus (one case). About familial history, 45.4% (15/33) of the patients informed other relatives with a gluten-related disorder, being DH in 24.2% (8/33) and CD in 18.2% (6/33) of the cases.

The treatment preconized before the reference to the gastroenterologist was adjusted. Strict GFD was recommended for all patients, and 11/33 (33.3%) with mild/moderate disease referred had significant improvement of the skin lesions only with diet. Patients without improvement (22/33) remained on GFD and dapsone, with dose ranges from 25 to 400 mg (average dose of 100 mg per day) adjusted individually. The drugs were interrupted according to the evolution of each case as soon as possible, and GFD was maintained. Therefore, after 30 days, 81.8% of patients that used this association had improvement. One patient showed jaundice 2 weeks after starting dapsone, so this drug was excluded, and sulfasalazine was prescribed. Two patients did not improve until intentional denning or accidental ingestion of gluten and a high dose of dapsone, so azathioprine was recommended. For one male patient, this drug caused rapid improvement and, after 12 months, could he stop the medication. Another female patient is using azathioprine until now with an adjusted dose. As expected, the GI complaints disappeared with GFD. Anemia and bone disorders were treated with repositions in cases where they were necessary together with a GFD. Humor distresses were reviewed in the follow-up.

**TABLE 1.** Frequencies of gastrointestinal symptoms and clinical signs, detection of anemia, bone disease, and humor disorder in the studied patients with dermatitis herpetiformis (n=33).

| Symptoms and clinical signs | n | % |
|---|---|---|
| Abdominal distension | 26 | 78.8 |
| Flatulence | 25 | 75.7 |
| Gastroesophageal reflux | 17 | 51.5 |
| Diarrhea | 13 | 39.3 |
| Epigastric pain | 11 | 33.3 |
| Constipation | 10 | 30.3 |
| Mouth ulcers | 10 | 30.3 |
| Mal digestion | 8 | 24.2 |
| Nausea | 8 | 24.2 |
| Weight loss | 6 | 18.2 |
| Vomitus | 3 | 9.1 |
| Anemia n=33 | 17 | 30.3 |
| Iron deficiency | 14 | 42.3 |
| B12 vitamin deficiency | 3 | 9.1 |
| Both | 4 | 12.1 |
| Bone disorder n=25 | 14 | 56.0 |
| Ostheopenia | 12 | 48.0 |
| Ostheoporosis | 2 | 8.0 |
| Humor disorder n=33 | 27 | 81.8 |
| Depression | 17 | 51.5 |
| Anxiety | 10 | 30.3 |

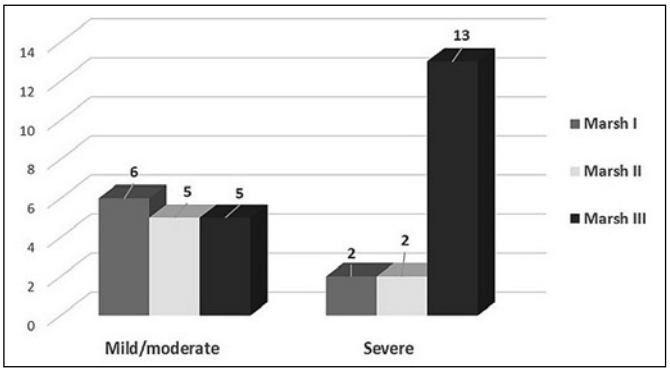



**FIGURE 1.** Severity of clinical presentation of dermatitir herpetiformis and findings in duodenal biopsy according to Marsh's classification.
**Marsh I:** infiltrative lesion, normal villous architeture and mucosa, IEL increase (>30-40 lymphocytes/enterocytes counted). **Marsh II:** hyperplasctic lesion; similar to Marsh I, but it al also presents crypt hyperplasia. **Marsh III:** destrutive lesion, subdivided in IIIa - partial villous atrophy; IIIb - subtotal villous atrophy, and IIIc - total villous atrophy.

## DISCUSSION

This study presents data showing a high prevalence of digestive and systemic symptoms CD-like related and changes in duodenal mucosa in Brazilian patients firstly diagnosed with DH who were referred to a gastroenterologist. In addition, they presented a good response to GFD and dapsone.

In our study, a slight preponderance of women was verified, and the mean age was very similar between genders as referred to in the literature. Some studies from Europe[4] and North America[12] report a 2:1 prevalence of DH between male and female patients and the onset of symptoms at 40.1 years for men and 36.2 years for women.

In this study, independently of the severity of DH or age of the patients, the delay in diagnosis ranged from 1 to 42 years. Only in three cases, the diagnosis was done in less time than one year. Considering the natural history of the DH that shows periods of spontaneous remission and exacerbations, doctors cannot be aware of diagnosing this affection. Physical examination alone may suggest DH due to the morphology and distribution of the lesions being very characteristic. So, why delay in the diagnosis of DH in patients with skin lesions? Clinical presentation of DH is not easy to recognize by general practitioners or other professionals due to the variation in the presentation of skin lesions due to the intense itch and scratching, delaying the diagnosis[13]. In addition, the cutaneous symptoms are troublesome and decrease the QoL of the patients[13]. Serology could be used for DH screening; however, in this study, 59.3% of patients had positive serology to IgA EmA or IgA anti-tTG. In Italy, Schiepatti et al. reported positivity in 70–75%[14] and Antiga et al. 50–95%[15]. Adult patients with DH commonly can exhibit a negative serology, making duodenal biopsy mandatory[16].

Regarding digestive complaints, abdominal distention, flatulence, and gastroesophageal reflux were the most prevalent, as reported in other gluten-related diseases[17]. On opposite sides, Holmes et al. referred that 90% of the patients with DH do not present GI symptoms[18], and Reunalla et al. described symptoms in only 20% of cases in Finland[3]. Rose et al., in Germany, in 32 patients retrospectively studied, reported that none of the patients reported GI symptoms although 29 cases presented CD-like changes at histology[19]. Interestingly, oral lesions were described as extremely rare in DH[20]. However, 30.3% of our patients reported mouth ulcers. These findings could result from gluten action or deficiencies of iron, folic acid, or vitamin B12[20]. The presence of GI symptoms and signs were found independently of the severity of the skin lesions, regardless of histologic findings.

We found a high frequency of depression (51.5%) and anxiety (30.3%), higher than those pointed out by Mansikka et al. in Finland (28.7% of anxiety and 18.8% of depression)[16]. Thus, the skin disease can cause psychological problems to patients, low self-esteem, and interfering with the QoL[6,7].

Anemia was detected in 51.5% of the cases due to iron or vitamin B12 deficiencies, or both, as pointed out by other authors[2,18]. In addition, bone disorders were found in 56% of studied patients, mainly osteopenia. There are scarce reports about bone disorders in patients with DH, and the results are conflicting. Abuzakouk et al. reported no evidence of bone disorders despite evidence of enteropathy[21]. On the other hand, Di Stefano et al., alert to significant changes in bone mass, similar to observed in patients with CD[22]. Garcia-Manzanares et al. pointed out the relation between bone disorders and higher grades of villous atrophy, a fact observed in some patients in the present study[23].

Among our cases, all showed enteropathy on duodenal biopsy, and the severity of the intestinal changes did not differ according to the age or gender of the patients. However, in patients with a severe presentation of DH, there was a predominance of Marsh III. There are some controversies about the indication of duodenal biopsy for patients with DH. According to Holmes et al.[18], a small bowel biopsy is required in all patients. Salmi et al. reported that 100% of the patients with DH have some intestinal disease, varying the extent of the lesions[6]. Mansikka et al. related, in 181 patients with DH, 72% of partial or total villous atrophy[16]. Schiepatti et al. informed that there is an increased number of intraepithelial lymphocytes in almost 100%, but true villous atrophy in only 70–75% of patients[14]. The authors of the present research suggest biopsy in all the cases of DH at diagnosis because if in the follow-up some evidence of GI complication or malignancy is suspected, there are elements for comparison.

According to Holmes et al.[18], family studies indicate that 5% of first-degree relatives also have DH or CD. In this study, 1/3 of first-degree relatives had a diagnosis of DH, DC, or both. These findings reinforce the close monitoring of first-degree relatives of patients with DH.

A strict GFD is the first line of treatment in DH and can improve skin lesions and intestinal injuries. The consultation with a dietitian is recommended for help in the adherence to a GFD. The dermatological condition can respond slowly to a GFD (1/3 of the patients of this study) but undergoes prompt resolution with the addition of oral dapsone. Dapsone relieves the DH rash and itch effectively but does not affect enteropathy[10,11]. Other drugs as azathioprine can be used in patients with "refractory DH," like for CD, for a subgroup of patients in whom clinical symptoms and small bowel villous atrophy do not recover[10,11]. This study has limitations related to retrospective research. Despite a reasonable sample for the period evaluated, this is a single-center study.

DH is a treatable condition with a favorable prognosis[7]. However, independently of the physician responsible for the management, a celiac disease-like protocol for which comprehensive investigation is highly recommended. The present study's findings reinforce the need for an interprofessional team to better manage DH cases, even in asymptomatic enteropathy. The ideal is the involvement of a dermatologist, gastroenterologist, dietician, and psychologist.

In conclusion, patients with DH referred to a gastroenterologist showed a high frequency of GI and systemic symptoms independently of the severity of the skin lesions. Duodenal mucosa showed histological alterations in all the cases. The treatment based on GFD or dapsone was effective in most patients.

### Authors' contribution

All authors contributed to the study conception and design. Material preparation, data collection were performed by: Kotze LMS, Kotze LR, Purim KSM. The data analysis and the first draft of the manuscript was done by: Kotze LMS and Nisihara R and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### Orcid

Lorete Maria da Silva Kotze: 0000-0003-2683-6132.
Luiz Roberto Kotze: 0000-0001-8456-4361.
Katia Sheylla Malta Purim: 0000-0001-9982-6408.
Renato Nisihara: 0000-0002-1234-8093.

Kotze LMS, Kotze LR, Purim KSM, Nisihara R. O manejo de dermatitis herpetiformis pelo gastroenterologista. Uma série de casos. Arq Gastroenterol. 2021;58(4):429-32.

**RESUMO** – **Contexto** – A dermatite herpetiforme (DH) é considerada como a doença celíaca (DC) da pele. Os pacientes podem ser atendidos por profissionais do atendimento primário ou por dermatologistas que podem encaminhar o paciente a um gastroenterologista. **Objetivo** – Os objetivos do estudo foram investigar o perfil clínico dos pacientes com diagnóstico de DH encaminhados a um gastroenterologista e avaliar a resposta ao tratamento. **Métodos** – Foram investigados retrospectivamente pacientes com DH encaminhados ao mesmo gastroenterologista em consultório particular em Curitiba, Brasil, entre janeiro de 2010 a dezembro de 2019. Foram incluídos pacientes adultos com diagnóstico confirmado de DH. Dados sobre sintomas e sinais clínicos, dados laboratoriais, histológicos e resposta ao tratamento foram coletados. **Resultados** – Foram estudados 33 pacientes (60,6% mulheres, média de idade 40,8±12,61 anos). O atraso médio para o diagnóstico de DH foi de 4 anos. O envolvimento cutâneo foi considerado leve em 33,3%, moderado em 18,2% e grave em 48,5%. As queixas gastrointestinais mais frequentes foram distensão abdominal (78,8%), flatulência (75,7%) e refluxo gastroesofágico (51,5%). Depressão e ansiedade foram observadas em 81,8% e anemia em 51,1%. Maior prevalência de alterações ósseas foi associada à maior idade ao diagnóstico de DH (*P*=0,035). A biópsia duodenal mostrou alterações em todos os pacientes. A melhora após o tratamento apenas com dieta sem glúten e/ou dapsona foi verificada em 81,2%. **Conclusão** – Pacientes com DH encaminhados ao gastroenterologista apresentaram alta frequência de queixas gastrointestinais e sistêmicas. Alterações histológicas duodenais foram encontradas em todos os casos. O tratamento à base de dieta sem glúten e/ou dapsona foi eficaz na maioria dos pacientes.

**Palavras-chave** – Dermatite herpetiforme; queixas; tratamento.

## REFERENCES

1. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH, et al. Sanders DS, Walker MM, Zingone F, Ciacci C. The Oslo definitions for coeliac disease and related terms. Gut. 2013;62:43-52.
2. Kotze LMS. Dermatitis herpetiformis, the celiac disease of the skin. Arq Gastroenterol. 2013;50:321-5.
3. Reunala T, Salmi TT, Hervonen K, Kaukinen K, Collin P. Dermatitis herpetiformis: A common extraintestinal manifestation of coeliac disease. Nutrients. 2018;10:602-10.
4. Bolotin D, Petronic-Rosic V. Dermatitis herpetiformis. Part I. Epidemiology, pathogenesis, and clinical presentation. J Am Acad Dermatol. 2011;64:1017-24.
5. Plotnikova N, Miller JL. Dermatitis herpetiformis. Skin Therapy Lett. 2013; 18:1-3.
6. Salmi TT, Hervonen K. Current concepts in dermatitis herpetiformis. Acta Derm Venereol. 2020; 100:adv00056.
7. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. American College of Gastroenterology Clinical Guideline: Diagnosis and Management of Celiac Disease. Am J Gastroenterol. 2013;108:656-77.
8. Marsh MN. Mucosal pathology in gluten sensitivity. In: Marsh MN. Coeliac disease. Oxford, Blackwell Scientific Publications; 1992, pp. 136-91.
9. Vale ECSD, Dimatos OC, Porro AM, Santi CG, Consensus on the treatment of autoimmune bullous dermatoses: dermatitis herpetiformis and linear IgA bullous dermatosis – Brazilian Society of Dermatology. Ann Bras Dermatol. 2019;94:48-55.
10. Schmidt E. Optimizing therapy in patients with severe autoimmune blistering skin disease. Hautarz. 2009;60: 633-40.
11. Hervonen K, Salmi TT, Ilus T, Paasikivi K, Vornanen M, Laurila K, et al. Dermatitis herpetiformis refractory to gluten-free dietary treatment. Acta Derm Venereol. 2016;96:82-6.
12. Zone JJ. Skin manifestations of celiac disease. Gastroenterology. 2005;128: S87-S91.
13. Mansikka E, Salmi T, Kaukinen K, Collin P, Huhtala H, Reunala T, Hervonen K. Diagnostic delay in dermatitis herpetiformis in a high-prevalence area. Acta Derm Venereol. 2018;98:195-9.
14. Schiepatti A, Savioli J, Vernero M, de Andreis FB, Perfetti L, Meriggi A, Biagi F. Pitfalls in the diagnosis of coeliac disease and gluten-related disorders. Nutrients. 2020;12:1711-20.
15. Antiga E, Maglie R, Quintarelli L, Verdelli A, Bonciani D, Bonciolini V, Caproni M. Dermatitis herpetiformis: Novel perspectives. Front Immunol. 2019;10:1290. eCollection 2019.
16. Mansikka E, Hervonen K, Kaukinen K, Collin P, Huhtala H, Reunala T, Salmi T. Prognosis of dermatitis herpetiformis patients with and without villous atrophy at diagnosis. Nutrients. 2018;10:641.
17. Lima RF, Kotze LM, Kotze R, Chrisostomo KR, Nisihara R. Gender-related differences in celiac patients at diagnosis. Arch Med Res. 2019;50:437-41.
18. Holmes G, Catassi C, Fasano A. Fast Facts: Celiac Disease. 2009. Dermatitis herpetiformis. P 83-90.
19. Rose C, Bröcker EB, Zillikens D. Clinical, histological and immunopathological findings in 32 patients with dermatitis herpetiformis Duhring. J German Society Dermatol. 2010;8:265-71.
20. Rodrigo L, Beteta-Gorriti V, Alvarez N, Gómez de Castro C, de Dios A, Palacios L, Santos-Juanes J. Cutaneous and mucosal manifestations associated with celiac disease. Nutrients. 2018;10:800.
21. Abuzakouk M, Barnes L, O'GormAN n, o'Grady A, Mohamed B, McKenna MJ, et al. Dermatitis herpetiformis: No evidence of bone disease despite evidence of enteropathy. Dig Dis Sci. 2007;52:659-64.
22. Di Stefano M, Jorizzo RA, Veneto G, Cechetti L, Gasbarrini G, Corazza GR. Bone mass and metabolism in dermatitis herpetiformis. Dig Dis Sci. 1999;44:2139-43.
23. Garcia-Manzanares A, Tenias JM, Lucendo AJ. Bone mineral density correlates with duodenal Marsh stage in newly diagnosed adult celiac patients. Scand J Gastroenterol. 2012;47:927-36.

ORIGINAL ARTICLE

AG-2021-23
doi.org/10.1590/S0004-2803.202100000-79

# Synergistic immunomodulatory activity of probiotics *Bifidobacterium animalis* and *Lactobacillus casei* in Enteroaggregative *Escherichia coli* (EAEC)-infected Caco-2 cells

Andréa Fonseca **FERREIRA**, Ricardo Luís Lopes **BRAGA**, Maysa Ferreira **ANDRADE**, Ana Claudia de Paula **ROSA** and Wânia Ferraz **PEREIRA-MANFRO**

Received: 3 February 2021
Accepted: 3 May 2021

**ABSTRACT** – **Background** – Enteroaggregative *Escherichia coli* (EAEC) is an *E. coli* pathotype that presents aggregative adhesion patterns on in vitro cultivated cells, mainly related to persistent diarrhea cases in children. EAEC virulence factors are important for host colonization and pathogenicity. Intestinal epithelial cells (IECs) recognize pathogen-associated molecular patterns (PAMPs) and initiate an immune response. Several studies using in vivo and in vitro models emphasize the probiotic activity and immunomodulatory capacity of *Lactobacillus* and *Bifidobacterium* species. **Objective** – To evaluate the modulation of cytokine production by probiotics *Bifidobacterium animalis* and *Lactobacillus casei* in human intestinal Caco-2 cells exposed to different strains of EAEC. **Methods** – Caco-2 cells were incubated with EAEC strains in the presence or absence of probiotics. The production of cytokines IL-8, TNF-α, IL-1β and IL-10 was evaluated in the supernatants by a sandwich enzyme-linked immunosorbent assay (ELISA). **Results** – Cytokine production did not change when uninfected and EAEC-infected Caco-2 cells were exposed to probiotics separately. All EAEC induced a significant increase in IL-8 production by Caco-2 cells, but the probiotics, even together, could not reduce its production. On the other hand, the synergic activity of probiotic strains significantly increased TNF-α production but decreased the basal production of IL-1ß. Also, probiotics induced a significant increase in the production of the anti-inflammatory cytokine IL-10 during EAEC infection. **Conclusion** – Our results reinforce the synergistic immunomodulatory activity of probiotics during EAEC infection.

**Keywords** – *Escherichia coli*; probiotics; *Bifidobacterium animalis*; *Lactobacillus casei*; cytokines; epithelial cells; immunity.

## INTRODUCTION

Intestinal infections caused by *Escherichia coli* are set due to infection by pathotypes of diarrheagenic *E. coli* (DEC) strains, which includes Enteroaggregative *Escherichia coli* (EAEC)[1]. This pathotype is recognized as the cause of persistent diarrhea in children, foodborne outbreaks, traveler's disease and intestinal infections of people infected with human immunodeficiency virus (HIV)[2,3].

EAEC strains are characterized by manifesting aggregative adherence (AA) pattern ("stacked bricks") on HEp-2 cells and may present the aggregative adherence plasmid (pAA)[1,4]. The pathogenesis of infection follows up three stages: (i) initial adherence to the mucosa and early colonization with biofilm formation; (ii) toxins secretion; and (iii) inflammatory response induction, cytokine secretion and mucosal damage[2]. Virulence genes, such as aggregative adherence fimbriae (AAF) located on pAA and cytotoxins are essential for EAEC colonization, which assure adherence and invasion, mediating inflammatory responses as well[5-7]. The inflammatory response includes increased secretion of pro-inflammatory cytokines, leukocyte migration to the infected site and tissue damage, being close to diarrheal disease symptoms[8,9]. EAEC strains can promote IL-8, IL-1β, IL-6, and TNF-α production by intestinal epithelial cells (IECs) as shown in studies using in vitro models and infected patients' analysis. However, the production of anti-inflammatory cytokines such as IL-10 has not been detected[6,10,11].

IECs play an essential role in the recognition of microorganisms in the intestinal lumen through pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs), such as molecules expressed on the bacterial surface. Toll-like receptors (TLRs), which can be expressed on cell membranes, are important proteins included in the PRRs group. PAMPs are recognized by PRRs located on IECs membrane, resulting in intestinal cell activation. This process leads to different immune responses, managing colonization or infection progress[12,13].

Probiotics are live non-pathogenic microorganisms found on different surfaces such as the urogenital and gastrointestinal mucosa that confer health benefits to the host as protection against

Declared conflict of interest of all authors: none
Disclosure of funding: this work was supported by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* – Brasil (CAPES) – Finance Code 001; *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ) and *Sub Reitoria de Pós Graduação e Pesquisa* (SR-2/UERJ).
Universidade do Estado do Rio de Janeiro, Faculdade de Ciências Médicas, Departamento de Microbiologia, Imunologia e Parasitologia, Rio de Janeiro, RJ, Brasil.
Corresponding author: Wânia Ferraz Pereira Manfro. E-mail: waniafpm@gmail.com

colonization by pathogens[14]. Probiotics are also found in fermented foods such as dairy products[15,16]. The group of lactic acid bacteria (LAB) includes *Lactobacillus* and *Bifidobacterium* genera and their species. These microorganisms are widely used in probiotic activity studies[17].

Several studies report LAB probiotic activity against foodborne pathogens such as DEC, including their ability to reduce bacterial biofilm formation, reduce adherence, inhibit damage to in vitro cultured cells and prevent diarrhea on in vivo models. LAB also present antimicrobial properties and immunomodulatory capacity[17,18]. This study investigated cytokines' production by Caco-2 cells exposed to different EAEC strains and their modulation by probiotics *Bifidobacterium animalis* and *Lactobacillus casei.*

## METHODS

### Bacterial strains

Four EAEC strains were included in this study. The prototype EAEC 042, EAEC I49 and EAEC H92/3 strains were previously isolated and characterized for putative virulence factors and adherence pattern[11,19-21]. EAEC 1500 strain was isolated from faeces of hospitalized HIV$^+$ patients with persistent diarrhea in Rio de Janeiro. All EAEC strains were stored at -80°C in Luria Broth (LB; Difco Laboratories) supplemented with 20% glycerol (Merck).

Probiotic strains *Bifidobacterium animalis* DN 173 010 (Activia – Danone®) and *Lactobacillus casei* Shirota (Yakult®), respectively identified as *Ba* and *Lc*, were kindly provided by Paulo de Góes Microbiology Institute (Rio de Janeiro Federal University – UFRJ) or obtained from commercial products. Probiotic strains were stored at -80°C in Man, Rogosa and Sharpe broth (MRS; Difco Laboratories) supplemented with 20% glycerol (Merck) and maintained on MRS agar (Difco Laboratories) at 4°C.

### Cell culture and infection

The human intestinal colon adenocarcinoma Caco-2 cell line (ATCC HTB37) was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2% Fetal Bovine Serum (FBS) and antibiotics in 24-well plates for 14 days, when cell monolayers were polarized and differentiated. Infection with EAEC and probiotic strains was performed as previously described by Rosa et al.[22] and Braga et al.[11] with modifications.

Probiotic strains were cultured in 3 mL of MRS broth (Difco) and incubated for 48 hours at 37°C under anaerobic conditions. Bacterial EAEC strains were cultured in 3 mL of LB (Difco) and incubated for 18 hours at 37°C. Aliquots of 100 µL of EAEC and probiotics standardized suspensions (approximately $10^7$ CFU.mL$^{-1}$) were inoculated on the cell monolayers in triplicates. Caco-2 cells were exposed to EAEC strains, probiotics only, and EAEC+probiotics (each probiotic and both probiotics together). The plates were centrifuged at 2500 rpm for five minutes at 15°C for synchronization and incubated for three hours. After incubation, the wells were washed and added DMEM medium supplemented with 2% of FBS, 1% D-mannose and 100 µg/mL amikacin (Teuto, Brazil). Plates were then cultured for additional 21 hours. Following a total of 24 hours of incubation, supernatants were collected, centrifuged, transferred to microtubes and stored at -20°C.

The production of cytokines IL-8, IL-1β, TNF-α and IL-10 was measured in the supernatants by a sandwich enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (R&D Systems – Wiesbaden, Germany).

### Statistical analysis

Cytokine concentrations were expressed as mean and standard error of mean (S.E.M) from three experiments performed independently. Statistical analyses were performed using Graph-Pad Prism version 7.0. Mann-Whitney test was performed to compare differences between different culture conditions and $P<0.05$ was considered statistically significant.

## RESULTS

There was no significant change in the production of the cytokines IL-8, IL-1β, IL-10 and TNF-α by Caco-2 cells exposed to each probiotic alone or to EAEC in co-cultivation with probiotics *Ba* and *Lc* separately when compared to basal production by non-infected cells (data not shown).

IL-8 production by Caco-2 was significantly increased by EAEC strains 042 ($P$=0.0004, FIGURE 1.A), I49 ($P$=0.0008, FIGURE 1.B), H92/3 ($P$<0.0001, FIGURE 1.C) and 1500 ($P$=0.0004, FIGURE 1.D) but not by the probiotics *Ba+Lc* (FIGURE 1). The EAEC 042 strain (FIGURE 1.A) co-cultivated with probiotics also significantly increased IL-8 production when compared to non-infected cells ($P$=0.0004) and cells exposed to both probiotics ($P$=0.0496). Similar results were observed to EAEC I49 strain ($P$=0.0008 and $P$=0.0319, respectively, FIGURE 1.B) and EAEC 1500 ($P$=0.0004 and $P$=0.039, respectively, FIGURE 1.D), but the amounts of IL-8 induced by EAEC H92/3 in the presence of both probiotics were higher only when compared to basal production ($P$=0.0003, FIGURE 1.C). Interestingly, both probiotics, together with each EAEC strain, did not reduce the IL-8 production induced by the EAEC strains (FIGURE 1).

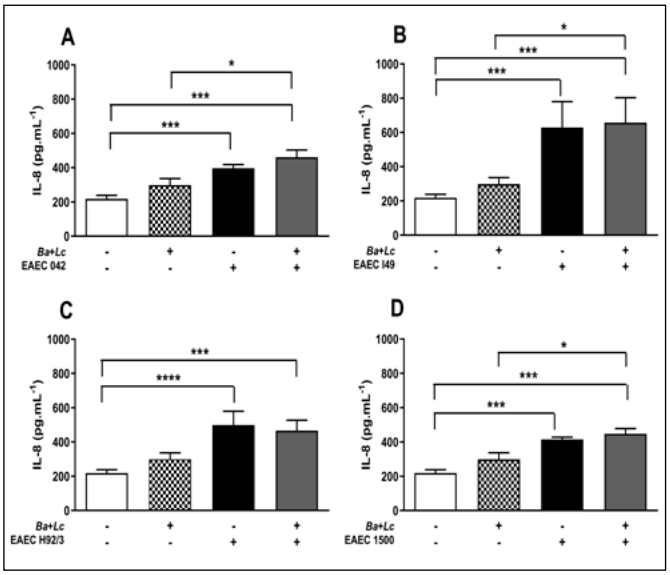



**FIGURE 1.** IL-8 production by uninfected Caco-2 cells (basal production), exposed to both probiotic strains (*Ba+Lc*) and to EAEC 042 (A), I49 (B) H92/3 (C) and 1500 (D) strains in the absence and in the presence of both probiotics. Co-cultivation lasted for three hours and cells were cultured for additional 21 hours. Results are expressed as mean and S.E.M from three experiments performed independently. EAEC: Enteroaggregative *Escherichia coli*; *Ba: Bifidobacterium animalis*; Lc: *Lactobacillus casei*. Differences between culture conditions were calculated using Mann-Whitney test. *$P$<0.05; ***$P$<0.001; ****$P$<0.0001.

The infection of Caco-2 cells by EAEC strains used in this study did not induce IL-1β production (FIGURE 2). Importantly, Caco-2 cells exposed only to *Ba+Lc* probiotic strains reduced IL-1β production significantly compared to basal levels ($P$=0.0411, FIGURE 2). Moreover, co-cultivation of the EAEC I49 (FIGURE 2.B) with both probiotics reduced significantly IL-1β production compared to cells infected only ($P$=0.0095) and compared to basal production ($P$=0.0216). Besides, probiotics, even in the presence of H92/3 (FIGURE 2.C) or 1500 strain (FIGURE 2.D), also reduced the production of IL-1β compared to basal levels ($P$=0.0238 and $P$=0.0303, respectively).

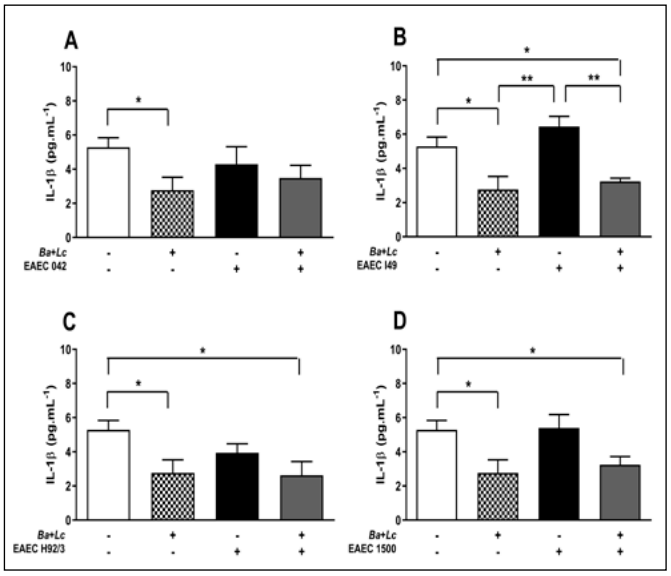



**FIGURE 2.** IL-1β production by uninfected Caco-2 cells (basal production), exposed to both probiotic strains (*Ba+Lc*) and to EAEC 042 (A), I49 (B) H92/3 (C) and 1500 (D) strains in the absence and in the presence of both probiotics. Co-cultivation lasted for three hours and cells were cultured for additional 21 hours. Results are expressed as mean and S.E.M from three experiments performed independently. EAEC: Enteroaggregative *Escherichia coli*; *Ba: Bifidobacterium animalis*; Lc: *Lactobacillus casei*. Differences between culture conditions were calculated using Mann-Whitney test. *$P$<0.05; **$P$<0.01.

As observed to IL-1β, none of EAEC strains significantly modified TNF-α production (FIGURE 3). However, Caco-2 cells exposed to both probiotics showed higher levels of TNF-α production compared to basal ones ($P$=0.026) and to EAEC strains 042 ($P$=0.026, FIGURE 3.A), H92/3 ($P$=0.0087, FIGURE 3.C) and 1500 ($P$=0.0087, FIGURE 3.D). Furthermore, co-cultivation of EAEC strains 042 (FIGURE 3.A), I49 (FIGURE 3.B), H92/3 (FIGURE 3.C) and 1500 (FIGURE 3.D) with both probiotics induced significantly higher levels of TNF-α compared to the production by Caco-2 cells alone ($P$=0.0043, $P$=0.0022, $P$=0.0411 and $P$=0.011, respectively) or to the EAEC infected ones ($P$=0.0043, $P$=0.0022, $P$=0.0108 and $P$=0.0087, respectively).

The production of anti-inflammatory cytokine IL-10 was not altered by exposition to both probiotics only. However, except for the H92/3 strain, EAEC strains' infection reduced IL-10 levels compared to baseline production, although with no statistical difference (FIGURE 4). The presence of both probiotics held IL-

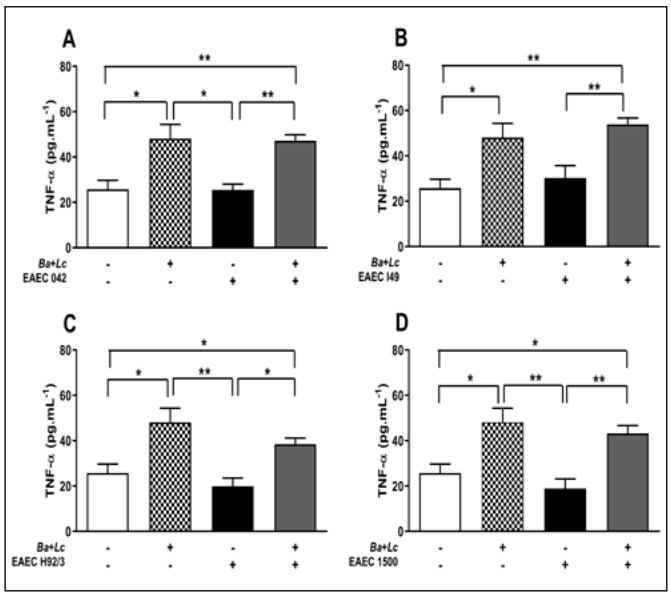



**FIGURE 3.** TNF-α production by uninfected Caco-2 cells (basal production), exposed to both probiotic strains (*Ba+Lc*) and to EAEC 042 (A), I49 (B) H92/3 (C) and 1500 (D) strains in the absence and in the presence of both probiotics. Co-cultivation lasted for three hours and cells were cultured for additional 21 hours. Results are expressed as mean and S.E.M from three experiments performed independently. EAEC: Enteroaggregative *Escherichia coli*; *Ba: Bifidobacterium animalis*; Lc: *Lactobacillus casei*. Differences between culture conditions were calculated using Mann-Whitney test. *$P$<0.05; **$P$<0.01.

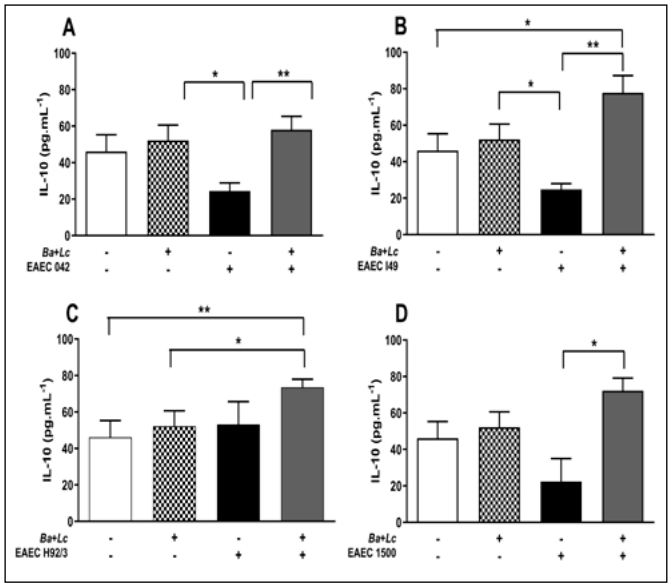



**FIGURE 4.** IL-10 production by uninfected Caco-2 cells (basal production), exposed to both probiotic strains (*Ba+Lc*) and to EAEC 042 (A), I49 (B) H92/3 (C) and 1500 (D) strains in the absence and in the presence of both probiotics. Co-cultivation lasted for three hours and cells were cultured for additional 21 hours. Results are expressed as mean and S.E.M from three experiments performed independently. EAEC: Enteroaggregative *Escherichia coli*; *Ba: Bifidobacterium animalis*; Lc: *Lactobacillus casei*. Differences between culture conditions were calculated using Mann-Whitney test. *$P$<0.05; **$P$<0.01.

10 levels similar to basal levels but statistically higher than that induced by the infection with 042 strain ($P$=0.0152, FIGURE 4.A) and I49 strain ($P$=0.0346, FIGURE 4.B). Notably, co-cultivation of both probiotics in the presence of EAEC strains induced a significant increase in the amount of IL-10 compared to that produced only in the presence of 042 ($P$=0.0065, FIGURE 4.A), I49 ($P$=0.0043, FIGURE 4.B) and 1500 ($P$=0.0216, FIGURE 4.D) EAEC strains. The association of both probiotics and EAEC H92/3 strain increased IL-10 levels compared to basal production and cells exposed to both probiotics only ($P$=0.0065 and $P$=0.026, respectively, FIGURE 4.C).

## DISCUSSION

Diarrhoeagenic *Escherichia coli* (DEC) strains are highlighted as the most common intestinal infections cause among enteric pathogens. As a common characteristic, *E. coli* pathotypes included in DEC group colonize mucosa by adhering to IECs, often evading host defenses and causing tissue damage. These events are often associated with alteration in water absorption and electrolytes, causing diarrhea. EAEC is the most frequently detected pathotype in acute and persistent diarrhea in children cases worldwide[1,23,24].

The mucosa is an essential component of natural immunity, acting as a barrier to microorganisms. When an enteric pathogen overcomes this host defense, IECs can induce an inflammatory response, resulting in intestinal damage and symptoms associated with the infection[9,12,13]. Studies have shown the association between virulence factors and stimulation of cytokine production in EAEC infection. Increased levels of the cytokines IL-1β, IL-8, IFN-γ and other inflammatory markers in stool samples, such as lactoferrin, gross mucus, leukocytes and occult blood, are related to some virulence factors, including AggR, AAFs, dispersin and flagellin[2,11,25-28].

Several studies have adopted in vitro cell infection model to investigate cytokine production induced by EAEC infection. Braga et al.[11] studied three of the four strains used in this work (042, I49 and H92/3). The authors observed that all EAEC strains induced IL-8 production by differentiated T84 cells. Other studies also show that Caco-2 cells produce IL-8 in vitro after EAEC infection[2,26]. IL-8 is associated with the inflammatory response of EAEC infection and is a central interleukin involved in polymorphonuclear (PMN) leukocyte chemotaxis[2]. In our study, EAEC strains were evaluated for inducing cytokine production in polarized and differentiated Caco-2 cells. Our results corroborate previous studies, once all EAEC strains induced IL-8 production and EAEC 042, I49 and H92/3 strains present AAFs and aggR transcriptional activator. These virulence factors are related to inflammatory response, which could explain the high IL-8 production by infected Caco-2 cells[11,25,26]. We will further perform the genotypic characterization of EAEC 1500 strain.

The production of other pro-inflammatory cytokines besides IL-8 has been reported. Using the cell line HCT-8 derived from colonic adenocarcinoma, Cennimo et al.[27] detected increased IL-8, IL-6 and TNF-α production induced by EAECs expressing AggR. Using the same cell line and measuring mRNA expression, Medeiros et al.[29] observed that the strain EAEC 042 induced the expression of IL-8 and TNF-α, which did not occur with IL-6. Also, this strain reduced the expression of TGF-β. Braga et al.[11] also observed the stimulation of IL-6 and TNF-α production by T84 cells infected with EAEC 042 and I49 strains. Although we observed an increase in IL-8 levels, we did not detect an increase in the production of IL-1β and TNF-α by Caco-2 cells infected with EAEC strains, which may be associated with low TLR expression at IECs apical membrane under homeostatic conditions[30] or due to the different IEC used in our system.

Probiotics are microorganisms that colonize surfaces such as mucosa and confer benefits to the host and their prophylactic or therapeutic use is related to action against intestinal dysbiosis, especially antibiotic-associated diarrhea and infectious diarrhea[31,32]. Probiotic bacteria, especially *Bifidobacterium* and *Lactobacillus* species, may inhibit growth, toxin production and other virulence factors expression of pathogenic microorganisms[33].

Probiotics also play an important role in the development of mucosal immunity. Ogawa et al.[34] identified high levels of anti-Stx 1 and 2 IgA in Shiga toxin-producing *E. coli*-infected rabbits after *L. casei* treatment, decreasing intestine toxin concentration of the animals. Ashraf and Shah[35] also associated immunological mechanisms, including higher numbers of IgA+ cells in animals' intestines, to the administration of probiotic bacteria *B. animalis*, *L. johnsonii*, *B. lactis* and the yeast *Saccharomyces boulardii*. The immunomodulatory properties of probiotics have been linked to TLR and NF-ϰB activation pathways. Karlsson et al.[36] found that a *L. rhamnosus* strain increases NF-ϰB expression of uropathogenic *E. coli*-infected bladder cells by TLR-4 activation, with increased TNF-α production. Jung et al.[37] observed that a *L. sakei* strain increased phagocytic activity and induced nitric oxide (NO) production, IL-6 and TNF-α by probiotic-exposed macrophages through activation of NF-ϰB. This inflammatory response was related to TLR-2 activation since the authors observed that the blockage of TLR-2 inhibited NO production. Rocha-Ramírez et al.[38] also obtained similar results in which lactobacilli strains led to NF-ϰB activation and the production of activated macrophage-related cytokines as a TLR-2-dependent inflammatory response.

Multispecies synergism is an essential factor in the action of probiotics against pathogens[39]. Lee et al.[16] demonstrated that yogurt consumption containing *B. animalis*, *L. paracasei* and *L. plantarum* together is related to increased NK cell action, suggesting that multiple probiotic sources may have a positive effect on host immune response. In this study, *B. animalis* and *L. casei* separately were unable to modulate cytokine production by Caco-2 cells exposed to different EAECs strains, suggesting that these probiotics strains better act synergistically.

In our study, EAEC strains induced IL-8 production, and probiotics could not reduce its production. Probiotics induced a reduction in IL-1β production when compared to non-infected Caco-2 cells. Additionally, probiotics were able to reduce IL-1β levels induced by EAEC I49 strain. Interestingly, EAEC infected and non-infected Caco-2 cells exposed to probiotics produced higher amounts of TNF-α.

The absence of the modulation in IL-8 production and the induction of increased TNF-α levels by probiotic-exposed Caco-2 cells conflict with some results of previous studies using co-cultivation in vitro models[40,41]. Jiang et al.[40] demonstrated that *L. plantarum* decreased the expression of pro-inflammatory cytokines induced by *Salmonella typhimurium* in Caco-2 cells but had no effect on IL-10 expression. Recently, Kim et al.[41] have shown that *L. acidophilus* reduced the expression of TNF-α while increasing IL-10 expression in RAW-macrophages stimulated with LPS. On the other hand, different studies have shown that TNF-α production is at least in part dependent on NF-ϰB activation and that TLR expression is induced in IECs exposed to probiotics. In this way, the increase in TNF-α production by probiotic-exposed

and infected Caco-2 cells observed in our system may be related to the signaling pathway of TLR responsive to probiotics and surface molecules present in enterobacteria, such as LPS[30,36-38,42].

IL-10 has a regulatory function of the immune response by inhibiting the inflammatory response. We found that Caco-2 cells infected with three of the four EAEC strains and treated with probiotics produced higher levels of IL-10 than untreated cells, highlighting the immunomodulatory properties of probiotics as previously described[40,41].

This study demonstrates the synergistic activity of probiotics *B. animalis* and *L. casei* in modulating inflammation induced by EAEC in Caco-2 cells.

## Authors' contribution

Ferreira AF: survey execution, data analysis, writing of text. Braga RLL: survey execution. Andrade MF: survey execution. Rosa ACP: designed the research, data analysis, writing of text. Pereira-Manfro WF: designed the research, data analysis, writing of text.

## Orcid

Andréa Fonseca Ferreira: 0000-0001-7490-8546.
Ricardo Luís Lopes Braga: 0000-0002-1896-0144.
Maysa Ferreira Andrade: 0000-0002-6613-5800.
Ana Claudia de Paula Rosa: 0000-0002-3308-1922.
Wânia Ferraz Pereira Manfro: 0000-0003-0166-8144.

Ferreira AF, Braga RLL, Andrade MF, Rosa ACP, Pereira-Manfro WF. Atividade imunomoduladora sinérgica de probióticos *Bifidobacterium animalis* e *Lactobacillus casei* em células Caco-2 infectadas com *Escherichia coli* enteroagregativa (EAEC). Arq Gastroenterol. 2021;58(4):433-8.

**RESUMO** – **Contexto** – *Escherichia coli* enteroagregativa (EAEC) é um patotipo de *E. coli* que apresenta o padrão de aderência agregativa em células cultivadas in vitro, sendo comumente relacionada a casos de diarreia persistente em crianças. Fatores de virulência presentes em EAEC são importantes para a colonização do hospedeiro e patogenicidade. As células epiteliais intestinais (IECs) reconhecem padrões moleculares associados a patógenos (PAMPs) e iniciam uma resposta imune. Vários estudos usando modelos in vivo e in vitro enfatizam a atividade probiótica e a capacidade imunomoduladora de espécies de *Lactobacillus* e *Bifidobacterium*. **Objetivo** – Este estudo avaliou a modulação da produção de citocinas pelos probióticos *Bifidobacterium animalis* and *Lactobacillus casei* em células intestinais humanas Caco-2 expostas a diferentes cepas de EAEC. **Métodos** – As células Caco-2 foram incubadas com as cepas de EAEC na presença ou ausência dos probióticos. A produção das citocinas IL-8, TNF-α, IL-1β e IL-10 foi avaliada nos sobrenadantes por ELISA sanduíche. **Resultados** – Não houve alteração na produção de citocinas quando as células não infectadas e as células infectadas com EAEC foram expostas aos probióticos separadamente. Todas as cepas de EAEC induziram aumento significativo na produção de IL-8 pelas células Caco-2, mas os probióticos, ainda que em conjunto, não foram capazes de reduzir a produção desta citocina. Por outro lado, as cepas de probióticos aumentaram significativamente a produção de TNF-α mas diminuíram a produção basal de IL-1ß. Além disso, os probióticos induziram um aumento significativo na produção da citocina anti-inflamatória IL-10 durante a infecção por EAEC. **Conclusão** – Nossos resultados reforçam a atividade imunomodulatória sinérgica dos probióticos durante a infecção de EAEC.

**Palavras-chave** – *Escherichia coli*; probióticos; *Bifidobacterium animalis*; *Lactobacillus casei*; citocinas; células epiteliais; imunidade.

## REFERENCES

1. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998;11:142-201.
2. Estrada-Garcia T, Perez-Martinez I, Bernal-Reynaga R, Zaidi MB. Enteroaggregative Escherichia coli: A Pathogen Bridging the North and South. Curr Trop Med Reports. 2014;1:88-96.
3. Lima AAM, Medeiros PHQS, Havt A. Enteroaggregative Escherichia coli subclinical and clinical infections. Curr Opin Infect Dis. 2018;31:433-9.
4. Clements A, Young JC, Constantinou N, Frankel G. Infection strategies of enteric pathogenic Escherichia coli. Gut Microbes. 2012;3:71-87.
5. Chattaway MA, Harris R, Jenkins C, Tam C, Coia JE, Gray J, et al. Investigating the link between the presence of enteroaggregative Escherichia coli and infectious intestinal disease in the United kingdom, 1993 to 1996 and 2008 to 2009. Euro Surveill. 2013;18:20582.
6. Gupta D, Sharma M, Sarkar S, Thapa BR, Chakraborti A. Virulence determinants in enteroaggregative Escherichia coli from North India and their interaction in in vitro organ culture system. FEMS Microbiol Lett. 2016;363:fnw189.
7. Havt A, Lima IF, Medeiros PH, Clementino MA, Santos AK, Amaral MS, et al. Prevalence and virulence gene profiling of enteroaggregative Escherichia coli in malnourished and nourished Brazilian children. Diagn Microbiol Infect Dis. 2017;89:98-105.
8. Abe CM, Knutton S, Pedroso MZ, Freymüller E, Gomes TAT. An enteroaggregative Escherichia coli strain of serotype O111:H12 damages and invades cultured T84 cells and human colonic mucosa. FEMS Microbiol Lett. 2001;203:199-205.
9. Hebbelstrup Jensen B, Olsen KEP, Struve C, Krogfelt KA, Petersen AM. Epidemiology and clinical manifestations of enteroaggregative Escherichia coli. Clin Microbiol Rev. 2014;27:614-30.
10. Khan K, Konar M, Goyal A, Ghosh S. Enteroaggregative Escherichia coli infection induces IL-8 production via activation of mitogen-activated protein kinases and the transcription factors NF-ϰB and AP-1 in INT-407 cells. Mol Cell Biochem. 2010;337:17-24.
11. Braga RLL, Pereira ACM, Ferreira AF, Rosa AC de P, Pereira-Manfro WF. Intracellular persistence of enteroaggregative Escherichia coli induces a proinflammatory cytokines secretion in intestinal epithelial T84 cells. Arq Gastroenterol. 2018;55:133-7.
12. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;34:637-50.
13. Kinnebrew MA, Pamer EG. Innate immune signaling in defense against intestinal microbes. Immunol Rev. 2012;245:113-31.
14. Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev. 2003;16:658-72.
15. Liévin-Le Moal V, Servin AL. Anti-infective activities of Lactobacillus strains in the human intestinal microbiota: from probiotics to gastrointestinal anti- infectious biotherapeutic agents. Clin Microbiol Rev. 2014;27:167-99.
16. Lee A, Lee YJ, Yoo HJ, Kim M, Chang Y, Lee DS, et al. Consumption of dairy yogurt containing Lactobacillus paracasei ssp. paracasei, Bifidobacterium animalis ssp. lactis and Heat-Treated Lactobacillus plantarum improves immune function including natural killer cell activity. Nutrients. 2017;9:558-66.
17. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, et al. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci U S A. 2006;103:15611-6.
18. Ferreira AF, Pereira-Manfro WF, Rosa AC de P. Diarrheagenic Escherichia coli and Probiotic Activity against Foodborne Pathogens: A Brief Review. Gastroenterol Hepatol Open Access. 2017;7:00248. DOI: 10.15406/ghoa.2017.07.00248.
19. Nataro JP, Scalestsky ICA, Kaper JB, Levine MM, Trabulsi LR. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic Escherichia coli. Infect Immun. 1985;48:378-83.
20. Rosa ACP, Mariano AT, Pereira AMS, Tibana A, Gomes TAT, Andrade JRC. Enteropathogenicity markers in Escherichia coli isolated from infants with acute diarrhoea and healthy controls in Rio de Janeiro, Brazil. J Med Microbiol. 1998;47:781-90.

21. França FLS, Wells TJ, Browning DF, Nogueira RT, Sarges FS, Pereira AC, et al. Genotypic and Phenotypic Characterisation of Enteroaggregative Escherichia coli from Children in Rio de Janeiro, Brazil. PLoS One. 2013;8:1-9.
22. Rosa ACP, Vieira MAM, Tibana A, Gomes TAT, Andrade JRC. Interactions of Escherichia coli strains of non-EPEC serogroups that carry eae and lack the EAF and stx gene sequences with undifferentiated and differentiated intestinal human Caco-2 cells. FEMS Microbiol Lett. 2001;200:117-22.
23. Edwards LA, Bajaj-Elliott M, Klein NJ, Murch SH, Phillips AD. Bacterial-epithelial contact is a key determinant of host innate immune responses to enteropathogenic and enteroaggregative Escherichia coli. PLoS One. 2011;6: e27030.
24. Boisen N, Krogfelt KA, Nataro JP. Enteroaggregative Escherichia coli. In: Escherichia coli: Pathotypes and Principles of Pathogenesis: Second Edition. 2013. p. 247-73.
25. Steiner TS, Lima AAM, Nataro JP, Guerrant RL. Enteroaggregative Escherichia coli produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. J Infect Dis. 1998;177:88-96.
26. Steiner TS, Nataro JP, Poteet-Smith CE, Smith JA, Guerrant RL. Enteroaggregative Escherichia coli expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. J Clin Invest. 2000;105:1769-77.
27. Cennimo D, Abbas A, Huang DB, Chiang T. The prevalence and virulence characteristics of enteroaggregative Escherichia coli at an urgentcare clinic in the USA: A case-control study. J Med Microbiol. 2009;58:403-7.
28. Mercado EH, Ochoa TJ, Ecker L, Cabello M, Durand D, Barletta F, et al. Fecal leukocytes in children infected with diarrheagenic Escherichia coli. J Clin Microbiol. 2011;49:1376-81.
29. Medeiros P, Bolick DT, Roche JK, Noronha F, Pinheiro C, Kolling GL, et al. The micronutrient zinc inhibits EAEC strain 042 adherence, biofilm formation, virulence gene expression, and epithelial cytokine responses benefiting the infected host. Virulence. 2013;4:624-33.
30. de Kivit S, Tobin MC, Forsyth CB, Keshavarzian A, Landay AL. Regulation of intestinal immune responses through TLR activation: Implications for pro- and prebiotics. Front Immunol. 2014;5:60.
31. Goldin BR. Health benefits of probiotics. Br J Nutr. 1998;80:203-7.
32. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS One. 2012;7:e34938.
33. Abd El-Moez SI, Ahmed FY, Samy AA, Ali AR. Probiotic activity of L. acidophilus against major food-borne pathogens isolated from broiler carcasses. Nat Sci. 2010;8:69-78.
34. Ogawa M, Shimizu K, Nomoto K, Takahashi M, Watanuki M, Tanaka R, et al. Protective effect of Lactobacillus casei strain Shirota on Shiga toxin-producing Escherichia coli O157:H7 infection in infant rabbits. Infect Immun. 2001;69:1101-8.
35. Ashraf R, Shah NP. Immune System Stimulation by Probiotic Microorganisms. Crit Rev Food Sci Nutr. 2014;54:938-56.
36. Karlsson M, Scherbak N, Reid G, Jass J. Lactobacillus rhamnosus GR-1 enhances NF-kappaB activation in Escherichia coli-stimulated urinary bladder cells through TLR4. BMC Microbiol. 2012;12:15.
37. Jung JY, Shin JS, Lee SG, Rhee YK, Cho CW, Hong H Do, et al. Lactobacillus sakei K040706 evokes immunostimulatory effects on macrophages through TLR 2-mediated activation. Int Immunopharmacol. 2015;28:88-96.
38. Rocha-Ramírez LM, Pérez-Solano RA, Castañón-Alonso SL, Moreno Guerrero SS, Ramírez Pacheco A, García Garibay M, et al. Probiotic Lactobacillus Strains Stimulate the Inflammatory Response and Activate Human Macrophages. J Immunol Res. 2017;2017:4607491.
39. Kumar M, Dhaka P, Vijay D, Vergis J, Mohan V, Kumar A, et al. Antimicrobial effects of Lactobacillus plantarum and Lactobacillus acidophilus against multidrug-resistant enteroaggregative Escherichia coli. Int J Antimicrob Agents. 2016;48:265-70.
40. Jiang M, Zhang F, Wan C, Xiong Y, Shah NP, Wei H, et al. Evaluation of probiotic properties of Lactobacillus plantarum WLPL04 isolated from human breast milk. J Dairy Sci. 2016;99:1736-46.
41. Kim DH, Kim S, Lee JH, Kim JH, Che X, Ma HW, et al. Lactobacillus acidophilus suppresses intestinal inflammation by inhibiting endoplasmic reticulum stress. J Gastroenterol Hepatol. 2019;34:178-85.
42. Vizoso Pinto MG, Rodriguez Gómez M, Seifert S, Watzl B, Holzapfel WH, Franz CMAP. Lactobacilli stimulate the innate immune response and modulate the TLR expression of HT29 intestinal epithelial cells in vitro. Int J Food Microbiol. 2009;133:86-93.

ORIGINAL ARTICLE

AG-2021-24
doi.org/10.1590/S0004-2803.202100000-80

# Monocyte/HDL ratio in non-alcoholic hepatic steatosis

Ahmet **YOZGAT**[1], Nergis **EKMEN**[2], Benan **KASAPOGLU**[3], Yasemin **UNSAL**[4], Fevzi Coskun **SOKMEN**[5] and Murat **KEKİLLİ**[2]

Received: 3 February 2021
Accepted: 15 June 2021

**ABSTRACT** – **Background** – Non-alcoholic hepatic steatosis (NAS) is characterized by excess fat accumulation in hepatocytes, causing portal and lobular inflammation and hepatocyte injury. **Objective** – We aimed to evaluate the alterations in monocyte count to high-density lipoprotein cholesterol ratio (MHR) in patients with grade 2 or 3 fatty liver disease and the association of this marker with liver function tests and insulin resistance. **Methods** – In this retrospective analysis; patients diagnosed and followed for the grade 2 or 3 fatty liver disease were included in the patient group and the patients who had undergone abdominal ultrasound for any reason and who were not having any fatty liver disease were included in the control group. **Results** – Totally 409 cases were included in the study. Among participants, 201 were in the control group, and 208 were in the NAS group (111 were having grade 2 and 97 were having grade 3 steatosis). The monocyte/HDL ratio was significantly higher in the NAS group compared with the healthy controls ($P$=0.001). There was a significant positive correlation between the monocyte/HDL ratio and age (r=0.109; $P$=0.028), ALT (r=0.123, $P$=0.014) and HOMA-IR (r=0.325, $P$=0.001) values. **Conclusion** – In conclusion, the monocyte to high-density lipoprotein ratio significantly increases in fatty liver disease and correlates with insulin resistance. Since it was suggested as a prognostic marker in atherosclerotic diseases, elevated MHR values in fatty liver disease should be evaluated cautiously.

**Keywords** – Monocyte/HDL ratio; hepatosteatosis; non-alcoholic hepatic steatosis; insulin resistance.

## INTRODUCTION

Nonalcoholic hepatic steatosis (NAS) is characterized by excess fat accumulation in hepatocytes, causing portal and lobular inflammation and hepatocyte injury. Its prevalence is continuously increasing in developed countries. Cardiovascular complications are the main life-threatening co-morbidities associated with the NAS. NAS is closely associated with insulin resistance; and metabolic syndrome[1-3]. Very recently the name of the NAS has been suggested to be changed as metabolic associated fatty liver disease (MAFLD). In diagnosis of NAS, the exclusion of concomitant liver diseases and alcohol consumption was the main point. However, the diagnosis of MAFLD depends mainly on the intrahepatic triglyceride content ≥5% with the presence of overweight/obesity, diabetes, or two other metabolic risk factors, in the presence or absence of concomitant liver diseases[4,5].

Macrophages and monocytes are the most important cell types that mainly secrete proinflammatory and pro-oxidant cytokines. On the other hand, high-density lipoprotein cholesterol (HDL-C) has been shown to have both anti-inflammatory and antioxidant actions, by protecting endothelial cells against the unfavorable effects of low-density lipoprotein (LDL) and preventing LDL oxidation. Recently, monocyte count to HDL-C ratio (MHR) has been shown to be a new prognostic marker in atherosclerotic cardiovascular diseases[6-8].

Based on this information, we aimed to evaluate the alterations in MHR in patients with grade 2 or 3 fatty liver disease and the association of this marker with liver function tests and insulin resistance.

## METHODS

In this multi-centric retrospective study, patients who were admitted to the gastroenterology outpatient clinic of the Gazi University and Health Sciences University, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, between January 2020 and June 2020 were evaluated. Patients diagnosed and followed for the grade 2 or 3 fatty liver disease were included in the patient group. The patients who had undergone abdominal ultrasound for any reason (for non-specific abdominal pain mainly) and who were not having any fatty liver disease, or any chronic metabolic diseases such as diabetes mellitus or hypertension were included in the control group. Patients younger than 18 years and older than 65 years of age, patients under treatment for hyperlipidemia or hypertriglyceridemia, patients having any other known liver diseases, hyperthyroidism, hypothyroidism, pregnant women, patients diagnosed or treated for any kind of infections in last month, malignancy, any kind of rheumatologic diseases or inflammatory bowel disease were excluded from the study. The study was approved by local Ethics Committee.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Ufuk University, Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey. [2] Gazi University, Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey. [3] Lokman Hekim University, Faculty of Medicine, Department of Gastroenterology, Ankara, Turkey. [4] Gazi University, Faculty of Medicine, Department of Internal Medicine, Ankara, Turkey. [5] Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Department of Internal Medicine, Ankara, Turkey.
Corresponding author: Ahmet Yozgat. E-mail: a_yozgat@yahoo.com

Demographic features, and any medications used were recorded. All patients underwent a hepatobiliary ultrasound to determine the presence of fatty liver disease[9]. Patients with grade 1 hepatosteatosis were not included in the study.

Blood samples were obtained from the patients in the morning, after 8 hours of fasting. Laboratory data including complete blood count, plasma glucose level, fasting insulin level, total serum cholesterol, triglyceride, and HDL and LDL cholesterols were recorded. HOMA-IR was calculated. HOMA-IR was calculated according to the formula: fasting insulin (μU/L) x fasting glucose (nmol/L)/22.5[10].

Monocyte count to HDL-C ratio was defined as blood monocyte count to high-density lipoprotein cholesterol concentration ratio[11].

### Statistical analysis

The normality of the distribution of the data was analyzed with the Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation, or count, percentage. Two independent sample *t*-test was performed to compare the continuous data between groups. Chi-square test was used to compare the categorical data between groups. Pearson correlation analysis was performed to determine the association of MHR with the liver function tests, age and the insulin resistance. Statistical analyzes were performed using SPSS 21.0 (IBM SPSS Statistics 19, SPSS Inc, an IBM Co, Somers, New York). The significance level was set at $P<0.05$.

## RESULTS

Totally 409 cases were included in the study. Among participants, 201 were in the control group, and 208 were in the NAS group (111 were having grade 2 and 97 were having grade 3 steatosis). The mean age of the study participants was 51.79±14.49 years in the control group and 53.74±12.42 years in the NAS group ($P$=0.23). The mean ALT levels were 27.97±15.85 and 38.03±18.24 ($P$=0.012) in the control group and in the NAS group respectively. Comparison of demographic features of study participants are summarized in TABLE 1. Monocyte/HDL ratio was significantly higher in the NAS group compared with the healthy controls ($P$=0.001) (FIGURE 1).

**TABLE 1.** Comparison of demographic features and laboratory data of the study participants.

| | Control group (n=201) | NAS group (n=208) | *P* |
|---|---|---|---|
| Gender (female/male) | 117/84 | 120/88 | 0.92 |
| Age (years) | 51.79±14.49 | 53.74±12.42 | 0.23 |
| AST | 24.56±16.77 | 28.74±17.84 | 0.091 |
| ALT | 27.97±15.85 | 38.03±18.24 | 0.012 |
| GGT | 38.36±44.68 | 48.23±44.87 | 0.13 |
| ALP | 92.98±44.31 | 97.01±45.71 | 0.57 |
| HDL | 51.38±11.245 | 35.27±6.274 | 0.001 |
| LDL | 118.82±51.173 | 125.10±44.567 | 0.24 |
| Monocyte/HDL ratio | 9.94±4.63 | 15.10±5.92 | 0.001 |
| HOMA-IR | 2.07±0.44 | 3.30±0.59 | 0.001 |

NAS: non-alcoholic steatohepatitis; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: Homeostasis Model Assessment for Insulin Resistance.

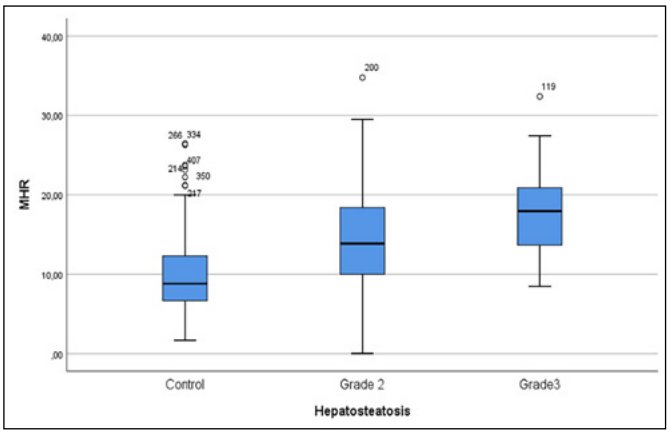



**FIGURE 1.** Distribution of monocyte/HDL-C ratio (MHR) between hepatosteatosis groups and controls.

The results of correlation analysis performed between the monocyte/HDL ratio and liver function tests, HOMA-IR and NLR values are summarized in TABLE 2. There was a significant positive correlation between the monocyte/HDL ratio and age (r=0.109; $P$=0.028), ALT (r=0.123, $P$=0.014) and HOMA-IR (r=0.325, $P$=0.001) values.

**TABLE 2.** The results of correlation analysis.

| | Monocyte / HDL ratio | |
|---|---|---|
| | r | *P* |
| Age | 0.109 | 0.028 |
| AST | 0.062 | 0.217 |
| ALT | 0.123 | 0.014 |
| GGT | 0.074 | 0.144 |
| HOMA-IR | 0.325 | 0.001 |

HDL: high-density lipoprotein; r: correlation coefficient; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HOMA-IR: Homeostasis Model Assessment for Insulin Resistance.

## DISCUSSION

In this study, we determined a significant increase in monocyte/HDL ratio in NAS patients compared with the healthy controls which correlated with the age, HOMA-IR and ALT levels, significantly. To the best of our knowledge, this is the first study in literature evaluating the monocyte/HDL ratio, which is shown to be a predictor of cardiovascular diseases, in NAS patients.

In atherosclerotic diseases, the main pathological mechanisms were shown to be the systemic inflammation and lipid accumulation[12,13]. Monocytes are the major source of proinflammatory cytokines. Monocytes differentiate into the macrophages that ingest oxidized LDL cholesterol. High-density lipoprotein-cholesterol is known with its anti-inflammatory and anti-oxidant effects by inhibiting the activation of monocytes, migration of macrophages and LDL oxidation. Moreover, HDL molecules also increase endothelial nitric oxide expression and improve vasorelaxation[14-16].

In recent literature, monocyte to HDL ratio was studied in many atherosclerotic diseases and defied as a prognostic marker. Bolayir et al. reported that the MHR was significantly higher in patients with

acute ischemic stroke and moreover, it was a significant independent variable of 30-day mortality in these patients[17]. Karatas et al. reported that the MHR in patients with diabetic nephropathy was significantly higher than that of both the normoalbuminuric diabetic patients and the healthy controls and this ratio was independently correlated with urine albumin to creatinine ratio. The authors suggested this ratio as a biomarker for diabetic nephropathy[18]. In a large cohort, Wang et al. reported a linear relation between MHR levels and the ischemic stroke[19]. The role of MHR in predicting the outcomes of cardiovascular diseases is highly important in clinical practice. NAS was defined to be independently associated with both prevalent and incident cardiovascular diseases[20] and in that aspect; MHR may be suggested as a helpful marker in determining the cardiovascular outcomes in NAS patients.

We determined a significant association of MHR with the age and insulin resistance. Insulin resistance is known to be the main pathogenetic mechanism in NAS and some other metabolic diseases. In a cross-sectional study, Dincgez Cakmak et al. reported significantly higher MHR values in patients with polycystic ovary syndrome, which was also determined as an independent predictor of metabolic syndrome[21]. Similarly, Uslu et al. also reported higher MHR values in patients with metabolic syndrome and a significant correlation between the severity of metabolic syndrome and MHR[22]. Battaglia et al. defined MHR as a determinant of metabolic syndrome, which significantly correlated with the body mass index, waist circumference, C-reactive protein, and erythrocyte sedimentation rate[23]. All these data also support our findings reporting the association of MHR with the insulin resistance.

In recent literature, elevated serum gamma-glutamyl transferase (GGT) levels have been associated with increased risk of cardiovascular disease[24,25]. However, some studies did not define any relationship of serum GGT levels with the cardiovascular diseases' presence or outcomes[26]. In this study, though we defined a significant correlation between MHR and ALT levels, there was not any association between MHR and GGT levels.

There are some limitations of this study. First is that NAS was diagnosed with the ultrasound, not with the biopsy. For that reason, this study may be regarded as the preliminary evaluation of MHR in NAS and further studies with biopsy proven NAS patients may give more information regarding this association. Secondly, in this retrospective study, we did not analyze the association of body mass index with MHR, since this was a retrospective study and the data regarding the body mass index was not present in the patient records.

In conclusion, monocyte to HDL ratio significantly increases in fatty liver disease and correlates with the insulin resistance. Since it is suggested as a prognostic marker in atherosclerotic diseases, elevated MHR values in fatty liver disease should be evaluated cautiously. Since this is a non-invasive, inexpensive and simple calculation, further studies are warranted to determine the role of this ratio in predicting outcomes of fatty liver disease and to promptly treat patients with high cardiovascular disease risk.

## Authors' contribution

Study design: Yozgat A, Kasapoglu B, Kekilli M. Data collection: Yozgat A, Ekmen N, Unsal Y, Sokmen FC. Data analysis: Yozgat A, Ekmen N, Unsal Y, Sokmen FC. Manuscript preparation: Yozgat A, Kasapoglu B, Kekilli M.

## Orcid

Ahmet Yozgat: 0000-0002-4414-9929.
Nergis Ekmen: 0000-0002-7291-3169.
Benan Kasapoglu: 0000-0003-3858-0103.
Yasemin Unsal: 0000-0003-2413-7733.
Fevzi Coskun Sokmen: 0000-0002-5621-8274.
Murat Kekilli: 0000-0002-3063-8748.

Yozgat A, Ekmen N, Kasapoglu B, Unsal Y, Sokmen FC, Kekilli M. Relação monócito/HDL em esteatose não hepática. Arq Gastroenterol. 2021;58(4):439-42.

**RESUMO** – **Contexto** – A esteatose não hepática (ENH) é caracterizada pelo acúmulo de gordura nos hepatócitos, causando inflamação portal e lobular e lesões ao hepatócito. **Objetivo** – Avaliar as alterações na contagem de monócitos em relação à proporção de lipoproteína de colesterol de alta densidade (MHR) em doentes com doença hepática gordurosa de grau 2 ou 3 e a associação deste marcador com testes de função hepática e de resistência à insulina. **Métodos** – Nesta análise retrospectiva os pacientes diagnosticados e seguidos para a doença hepática gordurosa de grau 2 ou 3, foram incluídos no grupo de doentes e os indivíduos que tinham sido submetidos a ecografia abdominal por qualquer motivo e que não tinham qualquer doença hepática gordurosa foram incluídos no de controle. **Resultados** – Foram incluídos 409 pacientes no estudo. Entre os participantes, 201 estavam no grupo controle e 208 estavam no grupo ENH (111 caracterizados como grau 2 e 97 com esteatose de grau 3). A relação monócito/HDL foi significativamente maior no grupo ENH em comparação com os controles saudáveis (*P*=0,001). Verificou-se correlação positiva significativa entre a relação monócitos/HDL e a idade (r=0,109; *P*=0,028), e valores de ALT (r=0,123; *P*=0,014) e HOMA-IR (r=0,325; *P*=0,001). **Conclusão** – A razão entre monócitos e a lipoproteína de alta densidade aumenta significativamente na doença hepática gordurosa e correlaciona-se com a resistência à insulina. Uma vez que foi sugerido como um marcador prognóstico em doenças ateroscleróticas, os valores elevados de MHR na doença hepática gordurosa devem ser avaliados com cautela.

**Palavras-chave –** Relação monocito/HDL; hepatosteatose; esteatose não hepática; resistência à insulina.

## REFERENCES

1. Brunt EM, Wong VW, Nobili V, Day CP, Sookoian S, Maher JJ, et al. Non-alcoholic fatty liver disease. Nat Rev Dis Primers. 2015;1:15080. doi:10.1038/nrdp.2015.80.
2. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014;59:713-23. doi:10.1002/hep.26672.
3. Salgado AL, Carvalho L, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol. 2010;47:165-9. doi:10.1590/s0004-28032010000200009.
4. Wai-Sun Wong V, Lai-Hung Wong G, Woo J, Abrigo JM, Ka-Man Chan C, She-Ting Shu S, et al. Impact of the New Definition of Metabolic Associated Fatty Liver Disease on the Epidemiology of the Disease. Clin Gastroenterol Hepatol. 2020;S1542-3565:31504-4. doi:10.1016/j.cgh.2020.10.046.
5. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73:202-9. doi:10.1016/j.jhep.2020.03.039.
6. Kundi H, Kiziltunc E, Cetin M, Cicekcioglu H, Cetin ZG, Cicek G, et al. Association of monocyte/HDL-C ratio with SYNTAX scores in patients with stable coronary artery disease. Herz. 2016;41:523-9. doi:10.1007/s00059-015-4393-1.
7. Cagdas M, Karakoyun S, Yesin M, Rencuzogullari I, Karabag Y, Uluganyan M, et al. The Association between Monocyte HDL-C Ratio and SYNTAX Score and SYNTAX Score II in STEMI Patients Treated with Primary PCI. Acta Cardiol Sin. 2018;34:23-30. doi:10.6515/ACS.201801_34(1).20170823A.
8. Chen JW, Li C, Liu ZH, Shen Y, Ding FH, Shu XY, et al. The Role of Monocyte to High-Density Lipoprotein Cholesterol Ratio in Prediction of Carotid Intima-Media Thickness in Patients With Type 2 Diabetes. Front Endocrinol (Lausanne). 2019;10:191. doi:10.3389/fendo.2019.00191.
9. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. World J Gastroenterol. 2019;25:6053-62. doi:10.3748/wjg.v25.i40.6053.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9. doi:10.1007/BF00280883.
11. Kanbay M, Solak Y, Unal HU, Kurt YG, Gok M, Cetinkaya H, et al. Monocyte count/HDL cholesterol ratio and cardiovascular events in patients with chronic kidney disease. Int Urol Nephrol. 2014;46:1619-25. doi:10.1007/s11255-014-0730-1.
12. Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol. 2010;7:77-86. doi:10.1038/nrcardio.2009.228.
13. Libby P. Inflammation in atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2012;32:2045-51. doi:10.1161/ATVBAHA.108.179705.
14. Ganjali S, Gotto AM, Jr., Ruscica M, Atkin SL, Butler AE, Banach M, et al. Monocyte-to-HDL-cholesterol ratio as a prognostic marker in cardiovascular diseases. J Cell Physiol. Dec 2018;233:9237-46. doi:10.1002/jcp.27028.
15. Ghattas A, Griffiths HR, Devitt A, Lip GY, Shantsila E. Monocytes in coronary artery disease and atherosclerosis: where are we now? J Am Coll Cardiol. 2013;62:1541-51. doi:10.1016/j.jacc.2013.07.043.
16. Murphy AJ, Woollard KJ. High-density lipoprotein: a potent inhibitor of inflammation. Clin Exp Pharmacol Physiol.2010;37:710-8. doi:10.1111/j.1440-1681.2009.05338.x.
17. Bolayir A, Gokce SF, Cigdem B, Bolayir HA, Yildiz OK, Bolayir E, et al. Monocyte/high-density lipoprotein ratio predicts the mortality in ischemic stroke patients. Neurol Neurochir Pol. 2018;52:150-5. doi:10.1016/j.pjnns.2017.08.011.
18. Karatas A, Turkmen E, Erdem E, Dugeroglu H, Kaya Y. Monocyte to high-density lipoprotein cholesterol ratio in patients with diabetes mellitus and diabetic nephropathy. Biomark Med. 2018;12:953-9. doi:10.2217/bmm-2018-0048.
19. Wang HY, Shi WR, Yi X, Zhou YP, Wang ZQ, Sun YX. Assessing the performance of monocyte to high-density lipoprotein ratio for predicting ischemic stroke: insights from a population-based Chinese cohort. Lipids Health Dis. 2019;18:127. doi:10.1186/s12944-019-1076-6.
20. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017;66:1138-53. doi:10.1136/gutjnl-2017-313884.
21. Dincgez Cakmak B, Dundar B, Ketenci Gencer F, Aydin BB, Yildiz DE. TWEAK and monocyte to HDL ratio as a predictor of metabolic syndrome in patients with polycystic ovary syndrome. Gynecol Endocrinol. 2019;35:66-71. doi:10.1080/09513590.2018.1490401.
22. Uslu AU, Sekin Y, Tarhan G, Canakci N, Gunduz M, Karagulle M. Evaluation of Monocyte to High-Density Lipoprotein Cholesterol Ratio in the Presence and Severity of Metabolic Syndrome. Clin Appl Thromb Hemost. 2018;24:828-33. doi:10.1177/1076029617741362.
23. Battaglia S, Scialpi N, Berardi E, Antonica G, Suppressa P, Diella FA, et al. Gender, BMI and fasting hyperglycaemia influence Monocyte to-HDL ratio (MHR) index in metabolic subjects. PloS one. 2020;15:e0231927. doi:10.1371/journal.pone.0231927.
24. Ndrepepa G, Colleran R, Kastrati A. Gamma-glutamyl transferase and the risk of atherosclerosis and coronary heart disease. Clinica chimica acta; international journal of clinical chemistry. 2018;476:130-8. doi:10.1016/j.cca.2017.11.026.
25. Tu WJ, Liu Q, Cao JL, Zhao SJ, Zeng XW, Deng AJ. gamma-Glutamyl Transferase as a Risk Factor for All-Cause or Cardiovascular Disease Mortality Among 5912 Ischemic Stroke. Stroke. 2017;48:2888-91. doi:10.1161/STROKEAHA.117.017776.
26. Ndrepepa G, Kastrati A. Gamma-glutamyl transferase and cardiovascular disease. Ann Transl Med. 2016;4:481. doi:10.21037/atm.2016.12.27.

ORIGINAL ARTICLE

AG-2021-40
doi.org/10.1590/S0004-2803.202100000-81

# Energy and nutrient intake in ostomy patients and correlations with anthropometric variables: results from a reference hospital in the State of Pernambuco, Brazil

Ivanildo Ribeiro **DOMINGOS JÚNIOR**[1], Maria Izabel Siqueira de **ANDRADE**[1], Emerson Rogério Costa **SANTIAGO**[1], Laís Sousa **BARBOSA**[1,2] and Keila Fernandes **DOURADO**[1,2]

Received: 17 February 2021
Accepted: 15 June 2021

**ABSTRACT** – **Background** – Studies that assess the food intake and nutritional status of ostomy patients are scarce in the literature. However, such individuals have symptoms in the postoperative period that determine changes in the intake of calories and nutrients as well as anthropometric variables. **Objective** – Estimate the energy and nutrient intake of ostomy patients and determine correlations with anthropometric variables. **Methods** – A cross-sectional study was conducted with ostomy individuals in outpatient follow-up at a reference hospital for postoperative ostomy surgery in the city of Recife, Brazil. Demographic, socioeconomic, clinical, anthropometric, and dietary data were collected through interviews and from patient charts. Statistical analyses were performed with the aid of the Statistical Package for the Social Sciences, version 13.0 for Windows, with the level of significance set at 5% ($P \leq 0.05$). **Results** – The sample was composed of 100 individuals (54% males) with a mean age of 55.1±15.4 years. Colostomy patients predominated (82%) and had a greater frequency of excess weight compared to ileostomy patients (86.36% versus 13.64%). Median intake was below the Estimated Average Requirements, especially for vitamins A, C, and E. Significant inverse correlations were found between carbohydrate intake and both arm circumference and triceps skinfold ($P=0.0302$ for each) and a positive correlation was found between protein intake and arm muscle circumference ($P=0.0158$) in male patients. **Conclusion** – The present study found significant correlations between macronutrient intake and anthropometric variables indicative of reserves of lean and adipose mass. Moreover, intake was below the recommended values according to sex and age group, especially with regards to vitamins.

**Keywords** – Ostomy; food intake; nutrients; anthropometry.

## INTRODUCTION

The word ostomy has a Greek origin (*stóma*) and is a temporary or definitive opening connecting the bowels to the external environment. Intestinal ostomies are performed for the clinical control of pathological conditions that interfere with normal intestinal transit, such as neoplasms, trauma, congenital abnormalities, inflammatory diseases, and obstructions[1,2]. There are two types of intestinal ostomies (ileostomy and colostomy), which respectively consist of an anastomosis of the ileal or colic segment to the anterior abdominal wall[1].

According to the Brazilian Healthcare Guide for Ostomy Patients[3], which has been available for public consultation since May of 2019, there are few epidemiological data on the number of individuals with ostomies. An estimated 207 thousand individuals had intestinal or urinary collector bags in Brazil in 2018[3]. The northeastern region of the country has approximately 17 thousand ostomy patients and the state of Pernambuco has the support of the Pernambuco Ostomy Patient Association, with approximately 2000 active patients registered[4].

Nutritional follow-up of ostomy patients is fundamental, as changes in eating habits may occur imposed by symptoms secondary to the surgical intervention and there may be important changes in the digestion and absorption of specific nutrients, with a consequent effect on the nutritional status of these individuals[1,2]. Moreover, inflammation associated with the base disease contributes to the loss of nutrients, especially antioxidants, which are important to metabolic modulation and organic homeostasis[5].

Adequate food intake and meeting the individual nutritional needs of ostomy patients can prevent the nutrient deficits often found in this population. The main deficiencies are related to the loss of proteins, carbohydrates, fats, vitamins A, C, D, E, and B12, folic acid, zinc, magnesium, calcium, selenium, iron, and some electrolytes, such as sodium and potassium[6,1,2].

Studies that assess the food intake and nutritional status of ostomy patients are scarce in the literature. Therefore, the aim of the present investigation was to estimate the energy and nutrient intake in ostomy patients and determine associations with anthropometric variables.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Vitória de Santo Antão, PE, Brasil. [2] Hospital Barão de Lucena, Recife, PE, Brasil.
Corresponding author: Ivanildo Ribeiro Domingos Júnior. E-mail: ivanildoj30@gmail.com

## METHODS

A cross-sectional study was conducted between April and October 2017 at the ostomy outpatient clinic of Barão de Lucena Hospital, which is a reference center for the postoperative period of ostomies in the city of Recife, Brazil.

The sample size was calculated using the Epi-Info program, version 7.2, considering a population of 2000 ostomy patients registered at the hospital, an 80% confidence interval, and a maximum acceptable error of ten percentage points. In the pilot study, the prevalence of irregular calorie consumption in ostomy patients was 43.3%, considering, as a reference, the recommendations proposed by the ACERTO Project (acceleration of total post-operative recovery)[7]. Thus, the minimum sample size was determined to be 90 patients, to which 10% was added to compensate for possible dropouts. Sampling was non-probabilistic but involved the random selection of patients for the study.

The following were the inclusion criteria for participation in the study: having undergone intestinal ostomy more than 30 days earlier; age 18 years or older; and physical capacity to undergo the anthropometric evaluations. Patients with edema, anasarca, amputated limbs, neurological disease, genetic syndromes, or metabolic disease and those not able to provide information were excluded.

This study received approval from the institutional review board of the Center for Health Sciences of the Federal University of Pernambuco (certificate number: 65856117.60000.5282) in accordance with Resolution 466/12 of the National Board of Health. All volunteers received clarifications regarding the objectives and procedures and agreed to participate by signing a statement of informed consent.

Demographic (sex and age), socioeconomic, clinical, anthropometric, and dietary data were collected using a questionnaire administered in interview form as well as direct collection from patient charts.

Socioeconomic status was determined using the Brazilian Economic Classification Criteria recommended by the *Associação Brasileira de Empresas de Pesquisa* (ABEP) [Brazilian Association of Research Firms][8], which are used to classify individuals in Classes A to E. For the purposes of analysis, this variable was dichotomized as high/middle class (categories A1, A2, B1, B2, and C1) and low class (categories C2, D, and E).

Data on clinical conditions were collected either from patient charts or self-reports. Information was obtained on the type of ostomy (ileostomy or colostomy), time of stoma-forming surgery, reason for surgery, and category of ostomy bag (temporary or permanent).

The anthropometric characteristics of interest were weight, height, body mass index (BMI), arm circumference (AC), triceps skinfold (TSF), and arm muscle circumference (AMC). Weight and height were measured using the method proposed by Lohman et al.[9]. Nutritional status based on the BMI was categorized using the values indicated by the World Health Organization (WHO) for adults[10] and the Pan American Health Organization (PAHO) for older people[11]. For statistical purposes, this variable was dichotomized as without excess weight (BMI ≤24.9 kg/m$^2$ for adults and <28 kg/m$^2$ for older people) or with excess weight (BMI ≥25 kg/m$^2$ for adults and ≥28 kg/m$^2$ for older people).

AC was measured using a non-elastic metric tape on the dominant arm with the volunteer in the standing position, arm relaxed, and the measurement made at the midpoint between the most distal point of the acromion and the most distal part of the olecranon. AC was measured with the arm flexed toward the thorax, forming a 90º angle[9]. TSF was measured using a scientific adipometer (CESCORF®) on the non-dominant arm following the methods described by Lohman et al.[9] AC and TSF were used to calculate AMC using the following formula (Blackburn, 1977): AMC (cm) = AC (cm) – π x [TSF (mm) ÷ 10]. AC, TSF, and AMC were compared to the reference standards recommended by Frisancho[12].

Food intake was obtained using the 24-hour recall method. The intake of calories, macronutrients (carbohydrates, proteins, and lipids) and micronutrients (vitamins A, C, E, zinc, and selenium) was estimated using the Brazilian Food Composition Table[13]. The percentage distribution of nutrient intake was determined and the values were compared to the estimated average requirements (EAR) for sex and age group[14].

Statistical analysis was conducted with the aid of the Statistical Package for the Social Sciences (SPSS, version 13.0 for Windows). The Kolmogorov-Smirnov test was used to determine the normality of continuous variables. The description of proportions was followed by 95% confidence intervals (CI) and the overlapping of respective 95% CIs was considered indicative of significant differences. The macronutrient and micronutrient intake were analyzed as continuous variables. The intake of the nutrients of interest was adjusted for total calorie intake based on the residuals of the regression model. For such, the absolute intake of nutrients was considered the dependent variable and total calorie intake was considered the independent variable[15]. The Student's *t*-test was used to determine differences between means. Spearman's correlation coefficients were calculated for the determination of correlations between nutrient intake adjusted for calorie intake and the anthropometric variables. The level of significance was set at 5% ($P \leq 0.05$).

## RESULTS

The sample was composed of 100 individuals. Mean age was 55.1±15.4 years. A total of 54% (n=54; 95%CI: 43.7–64.0) were men and 58% (n=58; 95%CI: 47.7–67.8) belonged to the low socioeconomic class.

Regarding aspects of the ostomies, there was a predominance of individuals with colostomy (82%; n=82; 95%CI: 73.0–88.9) and a permanent bag (52%; n=52; 95%CI: 41.7–62.1). The main reasons for having undergone the surgical procedure were colorectal cancer (66%; n=66; 95%CI: 55.8–75.2), trauma and obstruction (24%; n=24; 95%CI: 16.0–33.5), and diverticulitis (10%; n=10; 95%CI: 4.9–17.6). TABLE 1 displays the sociodemographic and clinical characteristics of the sample according to type of ostomy.

Colostomy patients had a higher frequency of excess weight based on BMI than ileostomy patients (86.36% vs 13.64%). The majority of colostomy patients had important percentages of different nutritional diagnoses according to the other anthropometric variables (TABLE 2).

Mean calorie intake was 1651.6±416.7 kcal and 1754.1±510.0 kcal among the ileostomy and colostomy patients, respectively ($P$=0.428). The percentages regarding the intake of macronutrients as well as the vitamins and minerals of interest (adjusted for calorie intake) and EAR reference values are displayed in TABLE 3 stratified by sex. The main nutrients with median intake below the EAR values were vitamins A and E in both sexes and vitamin C in the male sex.

**TABLE 1.** Characterization of sample according to type of ostomy. Recife, Brazil, 2017.

| Variables | Ileostomy (N=18) | | | Colostomy (N=82) | | |
|---|---|---|---|---|---|---|
| | N | % | 95%CI | N | % | 95%CI |
| Sex | | | | | | |
| Male | 10 | 18.52 | 10.1–31.4 | 44 | 81.48 | 68.5–89.8 |
| Female | 8 | 17.39 | 8.7–31.4 | 38 | 82.61 | 68.5–91.2 |
| Age group | | | | | | |
| ≥18 years | 10 | 18.52 | 10.1–31.4 | 44 | 81.48 | 68.5–98.8 |
| ≥60 years | 8 | 17.39 | 8.7–31.4 | 38 | 82.61 | 68.5–91.2 |
| Economic status | | | | | | |
| High/Middle | 8 | 19.05 | 9.6–34.1 | 34 | 80.95 | 65.8–90.3 |
| Low | 10 | 17.24 | 9.4–29.4 | 48 | 82.76 | 70.5–90.5 |
| Ostomy status | | | | | | |
| Temporary | 7 | 14.58 | 6.9–27.93 | 41 | 85.42 | 72.0–93.0 |
| Permanent | 11 | 21.15 | 11.9–34.6 | 41 | 78.85 | 65.3–88.0 |
| Time of ostomy | | | | | | |
| <2 years | 10 | 20.8 | 10.5–35.0 | 38 | 79.2 | 65.0–89.5 |
| 2–5 years | 5 | 17.2 | 5.8–35.8 | 24 | 82.8 | 64.2–94.2 |
| >5 years | 3 | 13.0 | 2.8–33.6 | 20 | 87.0 | 66.4–97.2 |

CI: confidence interval.

**TABLE 2.** Classification of anthropometric indicators according to type of ostomy, Recife, Brazil, 2017.

| Variables | Ileostomy | | | Colostomy | | |
|---|---|---|---|---|---|---|
| | N | % | 95%CI | N | % | 95%CI |
| BMI | | | | | | |
| Without excess weight | 12 | 21.43 | 12.4–34.3 | 44 | 78.57 | 65.6–87.5 |
| With excess weight | 6 | 13.64 | 6.1–27.6 | 38 | 86.36 | 72.3–93.8 |
| AC | | | | | | |
| Malnourished | 4 | 12.9 | 4.7–30.3 | 27 | 87.1 | 69.6–95.2 |
| Eutrophic | 12 | 22.22 | 12.9–35.4 | 42 | 77.78 | 64.5–87.0 |
| Excess weight | 2 | 13.33 | 3.1–42.2 | 13 | 86.67 | 57.7–96.8 |
| AMC | | | | | | |
| Malnourished | 8 | 18.6 | 9.4–33.4 | 35 | 81.4 | 66.5–90.5 |
| Eutrophic | 10 | 17.54 | 9.5–29.9 | 47 | 82.46 | 70.0–90.4 |
| TSF | | | | | | |
| Malnourished | 7 | 24.14 | 11.6–43.3 | 22 | 75.86 | 56.6–88.3 |
| Eutrophic | 2 | 9.52 | 2.2–32.3 | 19 | 90.48 | 67.6–97.7 |
| Excess weight | 9 | 18 | 9.4–31.4 | 41 | 82 | 68.5–90.5 |

CI: confidence interval; BMI: body mass index; AC: arm circumference; AMC: arm muscle circumference; TSF: triceps skinfold.

**TABLE 3.** Reference values and percentiles of intake of macronutrients and micronutrients adjusted for calorie intake in ostomy patients. Recife, Brazil, 2017.

| Nutrients | EAR | Percentiles | | |
|---|---|---|---|---|
| | | 25 | 50 | 75 |
| Male sex | | | | |
| Carbohydrates | 100 (g/day) | 205.5 | 234.3 | 255.0 |
| Proteins* | 0.66 (g/kg/day) | 83.4 | 93.6 | 104.2 |
| Lipids | – | 35.3 | 46.1 | 55.4 |
| Zinc | 9.4 (mg/day) | 6.1 | 8.8 | 13.0 |
| Selenium | 45 (µg/day) | 9.7 | 31.6 | 50.3 |
| Vitamin A | 625 (µg/day) | 59.5 | 135.7 | 489.0 |
| Vitamin C | 75 (mg/day) | 19.4 | 30.7 | 67.4 |
| Vitamin E | 12 (mg/day) | 3.1 | 6.2 | 13.2 |
| Female sex | | | | |
| Carbohydrates | 100 (g/day) | 222.6 | 238.2 | 262.5 |
| Proteins* | 0.66 (g/kg/day) | 74.2 | 86.2 | 96.5 |
| Lipids | – | 35.7 | 45.7 | 50.1 |
| Zinc | 6.8 (mg/day) | 6.4 | 8.2 | 10.5 |
| Selenium | 45 (µg/day) | 15.2 | 30.4 | 48.8 |
| Vitamin A | 500 (µg/day) | 93.1 | 166.8 | 339.2 |
| Vitamin C | 60 (mg/day) | 18.9 | 68.2 | 97.8 |
| Vitamin E | 12 (mg/day) | 5.2 | 6.8 | 13.8 |

EAR: estimated average requirements/institute of medicine (Padovani, 2006).
*Protein requirements (EAR) presented in g/kg. Protein percentiles values presented in g/day.

In the investigation of correlations between macronutrients and anthropometric variables, significant negative correlations were found between carbohydrate intake and both AC and TSF and a positive correlation was found between protein intake and AMC among the men (TABLES 4 and 5). No significant correlations were found between micronutrient intake (adjusted for calorie intake) and the anthropometric variables analyzed (data not shown in tables).

## DISCUSSION

The scientific literature offers little evidence on the food intake and nutritional status of ostomy patients. However, this population is reported to reduce the consumption of essential foods in terms of energy and nutrients and often practices fasting in order to participate in social situations with fewer symptoms. Therefore, nutritional guidance ensuring empowerment and independence in food choices is essential for these patients[16,17].

The ostomy patients in the present study had median intake values below the EAR according to sex and age group, especially with regards to vitamins A, C, and E. In contrast, Barbosa et al.[18] found that ostomy patients had a diet involving the regular consumption of fruits and vegetables, which are the main sources of micronutrients.

**TABLE 4.** Correlation between anthropometric variables and intake of macronutrients adjusted for calorie intake in male ostomy patients. Recife, Brazil, 2017.

| Macronutrients | Anthropometric indicators | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BMI | | AC | | AMC | | TSF | |
| | rho | *P* | rho | *P* | rho | *P* | rho | *P* |
| Ileostomy | | | | | | | | |
| Carbohydrates | -0.2848 | 0.4250 | -0.6809 | 0.0302* | -0.3939 | 0.2600 | -0.6809 | 0.0302* |
| Proteins | 0.1758 | 0.6272 | 0.4012 | 0.2505 | 0.7333 | 0.0158* | -0.4268 | 0.2186 |
| Fats | 0.4303 | 0.2145 | 0.1824 | 0.6141 | 0.2000 | 0.5796 | -0.0427 | 0.9068 |
| Colostomy | | | | | | | | |
| Carbohydrates | -0.0643 | 0.6782 | -0.0670 | 0.6658 | 0.0509 | 0.7429 | -0.2298 | 0.1335 |
| Proteins | -0.0679 | 0.6613 | -0.0136 | 0.9301 | 0.0574 | 0.7111 | -0.2374 | 0.1208 |
| Fats | -0.0047 | 0.9757 | 0.1228 | 0.4271 | 0.0034 | 0.9826 | 0.2552 | 0.0945 |

Spearman's correlation. *$P \leq 0.05$; BMI: body mass index; AC: arm circumference; AMC: arm muscle circumference; TSF: triceps skinfold.

**TABLE 5.** Correlation between anthropometric variables and intake of macronutrients adjusted for calorie intake in female ostomy patients. Recife, Brazil, 2017.

| Macronutrients | Anthropometric indicators | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BMI | | AC | | AMC | | TSF | |
| | rho | *P* | rho | *P* | rho | *P* | rho | *P* |
| Ileostomy | | | | | | | | |
| Carbohydrates | 0.3810 | 0.3518 | -0.1667 | 0.6932 | -0.4286 | 0.2894 | 0.0000 | 1.0000 |
| Proteins | 0.3571 | 0.3851 | -0.3810 | 0.3518 | -0.5000 | 0.2070 | -0.1464 | 0.7294 |
| Fats | 0.2619 | 0.5309 | 0.4286 | 0.2894 | 0.3095 | 0.4556 | 0.4392 | 0.2763 |
| Colostomy | | | | | | | | |
| Carbohydrates | 0.1522 | 0.3616 | 0.1336 | 0.4239 | 0.1749 | 0.2937 | -0.0195 | 0.9073 |
| Proteins | -0.2209 | 0.1825 | -0.2752 | 0.0945 | -0.1748 | 0.2940 | -0.2175 | 0.1897 |
| Fats | 0.0620 | 0.1865 | 0.1407 | 0.3994 | 0.0402 | 0.8108 | 0.1865 | 0.2622 |

Spearman's correlation. *$P \leq 0.05$; BMI: body mass index; AC: arm circumference; AMC: arm muscle circumference; TSF: triceps skinfold.

The reduction in the intake of fat-soluble vitamins, such as vitamins A and E, may occur as a response to the reduction in the consumption of dietary fat in order to control the consistency and frequency of bowel movements. Barbosa et al.[18] found similar behavior in a group of ostomy patients, reporting the non-inclusion of foods rich in fats in their daily meals.

The low intake of micronutrients is one of the most reported public health problems in the Brazilian population as a whole[19-21]. In the study conducted by Tureck et al.[21] with data from 33,459 participants of the National Dietary Survey (INA), which was a module of the 2008–2009 Family Budget Survey[22] the intake of vitamins A, C, and especially E was below the dietary recommendations in 72 to 95% of individuals.

The inverse correlations found between carbohydrate intake and both AC and TSF in the present study may be explained by the occurrence of reverse causality, which is common in cross-sectional studies, in which a single moment in the natural history of an outcome is analyzed. Dietary composition, use of some medications and misreporting of food consumption or portion sizes due to memory lapses of respondent can also interfere in this relationship. On the other hand, the correlation between AMC and protein intake in men was an interesting finding, given the importance of the use of this anthropometric variable as a simple, fast indicator of lean mass[23,24] which is associated with the adequate intake of proteins, especially in older people[25]. One should bear in mind that the mean age of the individuals analyzed in the present study approaches the minimum age established for the older population. Aging is a risk factor for the development of cancer, which is one of the main reasons for undergoing an ostomy procedure[26]. Moreover, older people account for the majority of new cases and deaths due to cancer, which underscores the need for special attention for this group and its particular characteristics, such as the loss of lean mass[25-27].

Ferreira et al.[28] described a similar result to the findings of the present investigation with regards to the distribution of sex among the participants, reporting a predominance of the male sex (55.6%) among ostomy patients. The sex of the ostomy patients can exert an influence on adaptation to the postoperative period. Women tend to require a shorter rehabilitation period, but exhibit higher levels of depression and fear in the period prior to the surgical intervention[29]. Men, especially those who develop sexual impotence, require a longer period before returning to normal activities and experiencing an improvement in quality of life and have greater difficulties in terms of self-care due to physical, psycho-emotional, and/or social problems in the postoperative period[30].

With regards to socioeconomic status, there is evidence that social and economic inequalities exert considerable influence on the living conditions of individuals and constitute risk factors for a number of diseases, including different types of cancer[31]. In a study conducted by Moraes et al.[1] 47.1% of ileostomy patients earned less than two times the Brazilian monthly minimum wage. The authors state that low income can directly interfere with care for the stoma and hinder the clinical and nutritional follow-up of these patients. In the present study, low income was identified in approximately half of the sample. This may be related to the setting of the study, as public hospitals attract a larger proportion of low-income patients[1].

Colostomy patients predominated in the present sample. Integrative reviews conducted by Cunha, Ferreira, and Backes[32] and Miranda et al.[33] confirmed that colostomy procedures are more common than ileostomies (84.1 vs 15.7%) due to the high prevalence of individuals with colorectal cancer, who often need colostomy bags as part of clinical management for the control of intestinal symptoms. According to the National Cancer Institute[34], colorectal cancer was the second most common type of cancer in Brazil in 2020, with 40,990 new cases described, affecting 20,520 men and 20,470 women.

Diverse risk factors are involved in the development of colorectal cancer, such as an advanced age, genetics, a lifestyle involving a diet rich in fat, refined carbohydrates, and animal protein, a low level of physical activity, and obesity[35]. In a study by Sousa, Santos, and Graça[36], colorectal cancer was one of the main reasons for having undergone the colostomy procedure, which is in agreement with the findings of the present investigation.

No significant difference was found in the proportion of temporary and permanent bags in the present study. Likewise, Silva et al.[37] reported a 51.24% rate of permanent ostomy bags and a 48.76% rate of temporary bags. The main determinant of this aspect is the clinical diagnosis. Permanent bags are associated with colorectal and urogenital cancer and temporary bags are associated with trauma[37].

The analysis of the anthropometric data in the present study revealed a high frequency of overweight among the colostomy patients. This may be explained by the fact that obesity is an independent predictor of colorectal cancer, which is the main reason for the need for a colostomy[38]. Excess weight in these patients is a worrisome factor, as it is related to complications, such as retraction, prolapse, and parastomal hernia[39].

Nonetheless, individuals in the ideal range according to the AC and AMC predominated in the present sample. A similar finding was described in the study by Attolini and Gallon[40], who reported that adequacy in terms of these indictors may have been due to late adaptations of the ostomies, leading to less impairment in terms of food intake as well as the absence of nutritional and absorptive disorders, minimal metabolic changes, and the absence of obstructive factors or a hormonal effect. Such aspects may also explain the large number of individuals with excess weight according to BMI in the present investigation. Furthermore, it is important to emphasize that time of stoma-forming surgery can lead to possible organic adaptations and contribute to maintenance or weight gain.

This study has limitations that should be considered when interpreting the results. The cross-sectional design does not enable the establishment of cause-and-effect relationships. The use of only one 24-hour recall does not take into account intrapersonal variation in nutrient intake. However, this bias was minimized by the adjustment for energy intake and the simple comparison of intake with the EAR references. The sample size may be a limiting factor for the extrapolation of the findings. Nonetheless, the present study was able to demonstrate the clinical, nutritional, and dietary profile of a sample of ostomy outpatients at a reference hospital in the state of Pernambuco, Brazil and could contribute to decision making in the clinical setting for this population.

## CONCLUSION

In the present study, the intake of carbohydrates and proteins was significantly correlated with anthropometric indicators (AC, TSF, and AMC) in a sample of ostomy patients at a reference hospital. The nutritional status of the majority of individual was indicative of excess weight, especially the colostomy patients, and nutrient intake was below dietary recommendations, especially for vitamins A, C, and E. Further studies are needed with other designs and representative samples correlating nutritional status and nutrient intake to assist in improving the clinical-nutritional status and quality of life of patients following ostomy procedures.

### Authors' contribution

Domingos Júnior IR: study design, execution of study, interpretation of data, and drafting of manuscript. Andrade MIS: study design, data analysis, interpretation of data, editing of manuscript, and approval of final version. Santiago ERC: study design, data analysis, interpretation of data, editing of manuscript, and approval of final version. Barbosa LS: study design, data collection, execution of study, and approval of final version. Dourado KF: study design, interpretation of data, editing of manuscript, and approval of final version.

### Orcid

Ivanildo Ribeiro Domingos Júnior: 0000-0001-5232-0012.
Maria Izabel Siqueira de Andrade: 0000-0003-1087-1320.
Emerson Rogério Costa Santiago: 0000-0001-8501-0096.
Laís Sousa Barbosa: 0000-0002-8320-6180.
Keila Fernandes Dourado: 0000-0003-1346-8940.

Domingos Júnior IR, Andrade MIS, Santiago ERC, Barbosa LS, Dourado KF. Ingestão de energia e nutrientes em pacientes ostomizados e sua correlação com variáveis antropométricos: resultados de um hospital de referência em Pernambuco, Brasil. Arq Gastroenterol. 2021;58(4):443-9.

**RESUMO** – **Contexto** – Estudos que avaliam o consumo alimentar e o estado nutricional de pacientes ostomizados são escassos na literatura, entretanto, sabe-se que tais indivíduos cursam com sintomatologia que determinam modificações na ingestão calórica e de nutrientes, bem como nos parâmetros antropométricos durante o pós-operatório. **Objetivo** – Estimar a ingestão de energia e nutrientes em pacientes ostomizados e verificar sua relação com variáveis antropométricas. **Métodos** – Estudo transversal, realizado com grupo de indivíduos ostomizados em acompanhamento ambulatorial em um hospital de referência para pós-operatório de ostomias em Recife – Pernambuco. Foram obtidos dados demográficos, socioeconômicos, clínicos, antropométricos e dietéticos por meio de entrevistas e coleta direta nos prontuários. As análises estatísticas foram realizadas no software *Statistical Package for the Social Sciences* versão 13.0 para Windows, adotando-se o valor de 5% para verificação de significância estatística ($P \leq 0,05$). **Resultados** – A amostra foi composta por 100 indivíduos, sendo 54% do sexo masculino, com média de idade de 55,1±15,4 anos. O grupo foi caracterizado por um predomínio de pacientes colostomizados (82%; n=82), os quais apresentaram maiores frequências de excesso de peso, quando comparados àqueles com ileostomia (86,36% vs 13,64%). Foram identificadas medianas de ingestão abaixo dos valores recomendados pelos requisitos médios estimados principalmente para as vitaminas A, C e E. Houve correlação inversa significante entre o consumo de carboidratos com a circunferência do braço e a prega cutânea tricipital ($P=0,0302$), e correlação positiva entre o consumo de proteínas e a circunferência muscular do braço ($P=0,0158$) nos pacientes do sexo masculino. **Conclusão** – O presente estudo encontrou relação significante entre o consumo de macronutrientes e variáveis antropométricas indicativas de reservas de massa magra e adiposa. A ingestão, principalmente de vitaminas, foi abaixo dos valores preconizados segundo o sexo e a faixa etária.

**Palavras-chave** – Ostomia; consumo alimentar; nutrientes; antropometria.

## REFERENCES

1. Moraes JT, Melo AFF, Araújo C, Faria RGS, Ferreira NR, Belo VS. Anthropometric and Dietetic evaluation of people with ileostomies. Arq. Gastroenterol. 2019;56:34-40.
2. Oliveira AL, Boroni Moreira AP, Pereira Netto M, Gonçalves Leite IC. A Cross-sectional Study of Nutritional Status, Diet, and Dietary Restrictions Among Persons With an Ileostomy or Colostomy. Ostomy Wound Manage. 2018;64:18-29.
3. Brasil. Ministério da Saúde. Secretaria de Atenção Especializada em Saúde. Departamento de Atenção Especializada em Temática. Guia de Atenção à Saúde da Pessoa com Estomia. 1. ed. Brasília: Ministério da Saúde, 2019.
4. Instituto Nacional de Câncer. Coordenação Geral de Gestão Assistencial. Hospital do Câncer I. Serviço de Nutrição e Dietética. Consenso Nacional de Nutrição Oncológica. Rio de Janeiro: INCA, 2014.
5. Santos DR, Claiza B, Henschel MC, Tortato PB, Lorenzo SB, Fernando ME, et al . Nutritional status and consumption of inflammatory and anti-inflammatory foods by patients with inflammatory bowel diseases. J. Coloproctol. (Rio J.). 2020;40:099-104. DOI: 10.1016/j.jcol.2019.10.006.
6. Vasilopoulos G, Makrigianni P, Polikandrioti M, Tsiampouris I, Karayiannis D, Margari N, et al. Pre- and Post-Operative Nutrition Assessment in Patients with Colon Cancer Undergoing Ileostomy. Int J Environ Res Public Health. 2020;17:6124.
7. AGUILAR-NASCIMENTO, J. E. ACERTO–Acelerando a recuperação total pósoperatória. 3. Edição. Rio de Janeiro. Rubio, 2016.
8. Associação Nacional de Empresas de Pesquisa. Critério de classificação econômica. São Paulo: ABEP, 2015. [Access 2020 October 16]. Available from: http://www.abep.org.br/mural/anep/04- 1297-cceb.htm.
9. Lohman TG, Roche A, F Martorell, R. Anthropometric standardization reference manual. Champaign, llinois, Human Kinetics, Inc, 1988.
10. World Health Organization (WHO). Report of a WHO Expert Committee. Physical status: the use and interpretation of anthropometry. Geneva. 1995;854.
11. Organização Pan-americana Da Saúde (OPAS). SABE - saúde, bem-estar e envelhecimento: o projeto SABE no município de São Paulo: uma abordagem inicial. Brasília; OPAS;2002. 255.
12. Frisancho AR. New standards of weight and body composition by frame size and height for assessment of nutritional status of adults and the elderly. Am J Clin Nutr. 1984;40:808-19.
13. Tabela brasileira de Composição de Alimentos-TACO/ NEPA. UNICAMP. 4ª edição. rev. e ampl. NEPA- UNICAMP. 2011.
14. Padovani RM, Jaime A-F, Colugnati Basile FA, Álvares DSM. Dietary reference intakes: aplicabilidade das tabelas em estudos nutricionais. Rev. Nutr. 2006;16:741-60. doi.org/10.1590/S1415-52732006000600010.
15. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65(Suppl:4):1220S-1228S. doi: 10.1093/ajcn/65.4.1220S.
16. Cronin E. Dietary advice for patients with a stoma. Br J Nurs. 2012;21:S32-4, S36-8, S40. doi: 10.12968/bjon.2012.21.Sup16.S32.
17. Oliveira AL. Qualidade de vida relacionada à saúde e perfil nutricional de portadores de derivação intestinal – colostomia e ileostomia. Tese (Saúde Brasileira) – Universidade Federal de Juiz de Fora. Juiz de Fora, MG, 2017.
18. Barbosa MH, Alves PIC, Silva R, Luiz RB, Poggetto MT, Barichello E. Nutritional Aspects Of Intestinal Ostomy Patients From A City Of Minas Gerais State (BRAZIL). Revista de Enfermagem e Atenção à Saúde REAS, Uberaba-MG, v. 2, n. 3, p.77-87, 2013. [Internet]. Availble from: http://seer.uftm.edu.br/revistaeletronica/index.php/enfer/article/view/614.
19. Leão ALM, Santos LC. Consumo de micronutrientes e excesso de peso: existe relação? Rev Bras Epidemiol 2012; 15(1):85-95.
20. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Cadernos de Atenção Básica. Carência de Micronutrientes. 1. ed. Brasília: Ministério da Saúde, 2007.
21. Tureck Camila, Locateli Gelvani, Corrêa Vanesa Gesser, Koehnlein Eloá Angélica. Avaliação da ingestão de nutrientes antioxidantes pela população brasileira e sua relação com o estado nutricional. Rev. bras. epidemiol. [Internet]. 2017 Mar [cited 2021 Jan 13] ; 20( 1 ): 30-42.
22. IBGE – Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008-2009 – POF. Rio de Janeiro, 2011.
23. Wu LW, Lin YY, Kao TW, Lin CM, Liaw FY, Wang CC, et al. (2017) Mid-arm muscle circumference as a significant predictor of all- cause mortality in male individuals. PLoS ONE. 12(2): e0171707. doi:10.1371/journal.pone.0171707
24. Noori N, Kopple JD, Kovesdy CP, Feroze U, Sim JJ, Murali SB, et al. Mid-arm muscle circumference and quality of life and survival in maintenance hemodialysis patients. Clin J Am Soc Nephrol. 2010;5:2258-68.
25. Geirsdottir OG, Arnarson A, Ramel A, Jonsson PV, Thorsdottir I. Dietary protein intake is associated with lean body mass in community-dwelling older adults. Nutr Res. 2013;33:608-12.
26. World Health Organization. 2018 [Internet]. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/
27. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, et al. SEER Cancer Statistics Review, 1975-2014, National Cancer Institute [Internet]. Bethesda.Availble from: https://seer.cancer.gov/csr/1975_2014/
28. Ferreira E, Barbosa M, Sonobe H, Barichello E. Self-esteem and health-related quality of life in ostomized patients. Rev. Bras. Enferm. 2017;70:271-8.

29. Freitas JPC, Borges EL, Bodevan EC. Caracterização da clientela e avaliação de serviço de atenção à saúde da pessoa com estomia de eliminação. ESTIMA, Braz. J. Enterostomal Ther. 2018;16: e0918. doi:10.30886/estima.v16.402_PT.
30. Meira IFA, Silva FR, Sousa AR, Carvalho ESS, Santa Rosa DO, Pereira A. Repercussions of intestinal ostomy on male sexuality: an integrative review. Rev Bras Enferm. Brasília, 2020;73:e20190245.
31. Buss PM, Pellegrini Filho A. Health and its social determinants. Physis: Rev Saude Coletiva. 2007;17:77-93. https://doi.org/10.1590/S0103-73312007000100006.
32. Cunha RR, Ferreira AB, Backes VMS. Revisão - Características Sócio-Demográficas e Clínicas de Pessoas Estomizadas: Revisão de Literatura. Rev Estima. [Internet]. 2013;11:210-30. Available from: https://www.revistaestima.com.br/estima/article/view/327
33. Miranda SM, Luz MHBA, Sonobe HM, Andrade EMLR, Moura ECC. Caracterização Sociodemográfica e Clínica de Pessoas com Estomia em Teresina. ESTIMA [Internet]. 2016;14:7. Available from: https://www.revistaestima.com.br/estima/article/view/117
34. Instituto Nacional de Câncer José Alencar Gomes da Silva. Rio de Janeiro: INCA; c1996-2020. [Internet]. Câncer de intestino. Available from: https://www.inca.gov.br/tipos-de-cancer/cancer-de-intestino
35. Libutti SK, Salz LB, Willett CG, Levine RA. Chapter 57: Cancer of the colon. In: DeVita VT, Lawrence TS, Rosenberg SA, eds. DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. 10th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2015.
36. Sousa CF, Santos C, Graça LCC. Construção e validação de uma escala de adaptação a ostomia de eliminação. Rev Enf Ref. 2015;4:21-30. http://dx.doi.org/10.12707/RIV14021.
37. Silva AC., Silva GNS, Cunha RR. Caracterização de pessoas estomizadas atendidas em consulta de enfermagem do serviço de estomoterapia do município de Belém-PA. Estima. 2012;10:20-27.
38. Sena JF , Medeiros LP, Melo MDM, Souza AJG, Freitas LS, Costa IKF. Perfi l de estomizados com diagnóstico de neoplasias cadastrados em uma associação. Rev Enferm UFPE on line. 2017;11(Suppl 2):873-80.
39. Pinto IES, Queirós SMM, Queirós CDR, Silva CRR, Santos CSVB, Brito MAC. Fatores de risco associados ao desenvolvimento de complicações do estoma de eliminação e da pele periestomal. Rev Enf Ref. 2017;15:155-66. https://doi.org/10.12707/RIV17071.
40. Attolini RC, Gallon CW. Life quality and nutritional profile of colostomized colorectal câncer patients. Revista Brasileira de Coloproctologia. Rio de Janeiro, 2010;30:289-98.

ORIGINAL ARTICLE

AG-2021-47
doi.org/10.1590/S0004-2803.202100000-82

# The impact of colorectal chromendoscopy with enhanced mucosal imaging on adenoma miss rate in screening colonoscopy

Bruna Suelen Raymundo **LUZ**, Juliana Carneiro Cabral Dourado **CANTERAS**, Karen de Carvalho **GON**, Maria Luisa de Deus **BATISTA**, Thomy Jun **AHN** and Fauze **MALUF-FILHO**

Received: 3 March 2021
Accepted: 15 June 2021

**ABSTRACT** – **Background** – Colonoscopy is the gold standard for the diagnosis and treatment of adenomas. It is related with decreased colorectal cancer incidence and mortality. However, an important problem is missed colorectal adenoma. All efforts should be undertaken to reduce this rate. Enhancing imaging technologies including electronic chromoendoscopy and magnification has been increasingly adopted for improving the colorectal neoplasia detection rate and the detailed study of its surface, as well. I-scan images (Pentax, Tokyo, Japan) provides virtual chromoendoscopy in real-time during the examination to view the surface pattern, highlighting the microvasculature of the neoplastic lesion. The evidence on the impact of the use of I-scan on the colorectal adenoma detection rate is scarce. **Objective** – To evaluate whether the use of I-scan has impact on the adenoma miss rate (AMR) of screening colonoscopy exams. **Methods** – Observational and prospective study conducted by monitoring patients over 50 years undergoing colonoscopy. There were two groups: Group 1 – first inspection with standard high-definition white-light (HDWL) followed by a second inspection with I-scan 1; Group 2 – first inspection with I-scan 1 followed by a second inspection with standard HDWL. The primary outcome was the AMR from the first exam, calculated with the number of adenomas detected in the second exam, divided by the total number of adenomas detected in both exams. **Results** – A total of 85 patients participated in the study. 14 were excluded, with a final sample of 71 patients, in the Group 1, 34 patients, and the Group 2, 37. A total of 58 adenomas were detected, 40 in the first inspection (20 in each group) and 18 in the second inspection in group 1. The overall AMR was higher for the Group 1 than the Group 2 (47.4% vs 0% $P$=0.0002). **Conclusion** – The use of I-scan 1 during colonoscopy exam reduces the AMR.

**Keywords** – Adenoma; colonoscopy; colon; colonic polyps; prospective studies.

## INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer-related death worldwide[1,2]. Colonoscopy is the gold standard for diagnosing and treating adenomas, leading to decreased colorectal cancer incidence and mortality[3]. Adenoma resection can lead to a 53–70% reduction in CRC mortality rate during the first ten years after colonoscopy[4]. However, we also know that lesions can go unnoticed during routine colonoscopy[5].

The primary indicator of colonoscopy quality is the adenoma detection rate (ADR), calculated by the number of patients in which at least one adenoma was found, divided by the total number of patients over 50 years who underwent screening colonoscopy. Thus, physician's efforts and endoscope manufacturers have been focusing to increase ADR. According to Zimmermann-Fraedrich et al.[6], the only way to assess whether these measures are more successful in increasing ADR than conventional state-of-the-art colonoscopies, is to perform randomized controlled trial studies. There are two types of methodologies: one is to perform simple parallel randomized trials with two groups with or without the intervention and ADR as the primary outcome. The other study type is the so-called "tandem study", which includes two colonoscopies with the new and conventional scope in random order. The second pass will reveal adenomas missed by the first one, the so-called adenoma miss rates (AMR). Several reports indicate that the AMR by observing standard high-definition white-light (HDWL) images ranges from 6% to 60%[7]. There are some risk factors for increasing AMR, such as inadequate preparation, flat polyp morphology, smaller size, and patients with multiple polyps detected within first colonoscopy. There are reports of cancer cases diagnosed between screening colonoscopies, some of which are advanced stages. Thus, detecting adenomas during screening colonoscopy remains the main objective in performing a high-quality exam, and the results suggest need for improvements in colonoscopic technology[2,8,9].

In addition to standard HDWL colonoscopy, enhancing imaging technologies, including chromoendoscopy and magnification, have been increasingly adopted to assist in the accurate diagnosis of colorectal neoplasms[10]. Among chromoendoscopy techonologies, narrow band image – NBI (Olympus: Tokyo, Japan), I-scan (Pentax: Tokyo, Japan), blue-laser imaging – BLI-bright, and Linked-color imaging – LCI (Fujifilm: Tokyo, Japan) are currently available[10-11-12].

Declared conflict of interest of all authors: none.
Disclosure of funding: no funding received.
Hospital Beneficência Portuguesa de São Paulo, Departamento de Endoscopia, São Paulo, SP, Brasil.
Corresponding author: Bruna Suelen Raymundo Luz. E-mail: brunaluz04@gmail.com

According to Fujishiro and Kodashima (2010)[13], the image processing of I-scan provides enhanced view by a digital image, a software-oriented technology that allows pixel modifications of sharpness, tone, and contrast. This consists of three types of algorithms: Surface enhancement (SE) = I-scan 1, contrast enhancement (CE) = I-scan 2, and tone enhancement (TE) = I-scan 3.

Few studies aimed to compare the use of digital chromoendoscopy methods versus standard HDWL colonoscopy imaging in the detection of adenomas. Recently, in Brazil, a study determined the positive impact of LCI (Fujifilm) on detecting colorectal adenomas compared with HDWL and BLI-bright (Fujifilm)[14]. In a prospective randomized trial, to compare the detection of adenomas by I-scan vs standard HDWL colonoscopy, higher proportions of patients with adenomas were identified in a group that underwent colonoscopy I-scan than in a group evaluated by HDWL colonoscopy[15]. In another prospective randomized trial, patients underwent a tandem colonoscopy and concluded that with I-scan technology more adenomas were detected compared with HDWL colonoscopy[7].

Our aim was to evaluate whether processing technology and chromoendoscopy I-scan with algorithm surface enhancement – SE (I-scan 1) can impact the AMR, during screening colonoscopy.

## METHODS

In this randomized, controlled trial we enrolled patients aged from 50 to 80 years, underwent screening, tandem colonoscopies at the endoscopy unit of a tertiary hospital in São Paulo-SP (Brazil). The trial was approved by the local an institutional review board (CAAE 08114319.0.0000.5483).

The exclusion criteria were: patients with ASA III or IV[16]; with history of inflammatory bowel disease (IBD); with history of colorectal resection; using anticoagulants; and those with inadequate colon preparation (Boston Bowel Preparation Score ≤6)[17].

We recorded patients' age, sex, indication for the colonoscopy (symptoms investigation, surveillance, or screening), whether there was a family history of colorectal cancer. Antegrade colon preparation with a 10% mannitol solution was offered to all patients. Anesthesiologists provided deep sedation with propofol and fentanyl. The equipment used was the video colonoscopy (MagniView EC-3890LZi; Pentax Corporation, Tokyo, Japan), high-resolution video processor (Optivista EPK-i7010; Pentax Corporation, Tokyo, Japan), and high-resolution monitor (NDSsi 26 Radiance G2 HB; NDS Surgical Imaging, San Jose, United States of America). The chromendoscopy with surface enhancement (SE) was: I-scan mode 1.

Patients were allocated in two groups, using the electronic randomization method. Group 1: first inspection with standard HDWL followed by a second inspection with I-scan 1; Group 2: the first inspection with I-scan 1 followed by a second inspection with HDWL.

Three physicians performed the colonoscopies, who have routinely used magnification and chromoendoscopy for more than ten years, performing more than 1000 colonoscopies per year, with an overall ADR target above 25%. A minimum withdrawal time of 6 minutes at each step of the two inspections was the target[18].

All detected lesions were resected, either at the first or the second inspection. Only the adenomatous lesions were considered for analysis. The adenomas were evaluated according to size, morphology, location, and histology. The location was distributed over the proximal colon (cecum, ascending colon, hepatic angle, and transverse colon) and distal colon (splenic, descending, sigmoid colon, and rectum). The polyps were morphologically classified according to Paris classification[19] and size (≤5 mm, 6–9 mm, and ≥10 mm)[20].

The anatomopathological variables found were classified according to World Health Organization (WHO)[21]. Adenomas were separated into non-advanced and advanced[22,23] (TABLE 1).

**TABLE 1.** Classification of polyps according to the risk of malignancy.

| |
|---|
| **ADVANCED ADENOMA** |
| Size ≥10 mm |
| hight-grade dysplasia |
| Villous component |
| **NON-ADVANCED ADENOMA** |
| Tubular adenoma low-grade dysplasia < 10mm |

By: Modified de Baron et al.[16].

The primary outcome was AMR from first inspection of examination. We calculated this rate by dividing the number of adenomas, detected during the second inspection of examination by the total number of adenomas.

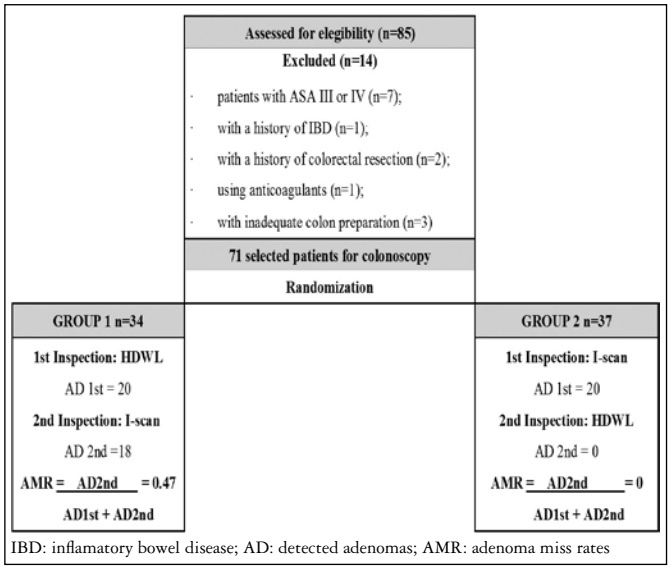
The failure rate in detecting adenoma using HDLW was 30%, regardless of gender. Thus, 50% of patients would have at least one adenoma, and participants with adenomas have an average of two adenomas. For this study, the sample size was calculated to compare proportion between two groups (considering that the number of detection with I-scan 1 is greater than the number with HDWL), with the significance of 0.05, varying the power test (0.8) to detect a 3-fold reduction in adenoma rates, the study required 69 adenomas per group. Thus, the researchers consider that a sample of 138 patients, which results in an 80% test power and a clinically relevant difference of 25%, is adequate for the experiment in question – considering a 10% loss rate. Sample size calculations were based on the simplified assumption of statistical independence among polyps from the same patient and the use of the $X^2$ test.

The mean, standard deviation, minimum, maximum, and quartiles were considered for quantitative variables and frequency tables for qualitative variables. To verify homogeneity between groups 1 and 2, the chi-square test was used for qualitative variables and Student's t-test for quantitative variables. To check the method's effectiveness, numbers of lesions were adjusted using linear model with mixed effects, with groups (1 and 2), chromoendoscopy, and interaction between group and chromoendoscopy being considered fixed effects and patient as effect random. In every study, significance of $P \leq 0.05$ was considered. REDCap platform was used to tabulate data and SPSS v25 software for analysis.

## RESULTS

From June to September 2019, we found 85 patients (38 Male) eligible for the study. Of these, 14 (16.5%) were excluded (FIGURE 1).

Thus, this study included 71 patients who underwent colonoscopy. Of the 71 patients, 32 (45%) were men, ranging from 50 to 79 years, with an average of 64 years. According to age groups, 28% were from 50 to 60 years old, 53% from 61 to 70, and 19%, from 71 and 80.



IBD: inflamatory bowel disease; AD: detected adenomas; AMR: adenoma miss rates

**FIGURE 1.** Flow diagram of study randomization.

As indicated by the exam, 7 (9.8%) were for symptoms investigation, 24 (33.8%) for surveillance, and 40 (56.3%) for screening. Ten (14%) patients had a family history of colorectal cancer. About 16 (23%) of the studied had never undergone a colonoscopy exam (TABLE 2).

The random division of the groups was 34 (48%) to Group 1: first inspection with HDWL, and the second with I-scan 1, and 37 (52%) to Group 2: first inspection with I-scan 1, and the second with HDWL (FIGURE 1).

The cecum was reached in all colonoscopies.

One hundred one lesions were resected and sent for histopathological analysis. Histologically, 41 (40.6%) non-neoplastic polyps, 58 (57.4%) adenomas, and 2 (2%) moderately differentiated adenocarcinomas were detected.

In 31 patients, at least one adenoma was diagnosed, with an average of 1.5 adenomas per patient. Of these, 18 (58%) were women.

According to the adenomas' endoscopic aspects, most were in the proximal colon (69%), 93% tubular adenoma with low-grade dysplasia, 86% sessile, 81% ≤5 mm, and 88% non-advanced adenomas (TABLE 3).

In group 1, 38 adenomas were detected, with 20 adenomas in the first observation (with HDWL) and 18 adenomas in the second (with I-scan 1), representing an overall AMR of 47.4%. In Group 2, 20 adenomas detected in the first observation, and there was no adenoma in the second, with a null missed adenoma rate. The AMR was significantly higher for the group 1 compared to the group 2 ($P$=0.0002) TABLE 4.

Regarding the adenomas detected in the second inspection, all under the I-scan 1, there were only 2 (11%) advanced adenomas, one measuring 10 mm with tubular histology and one with 5 mm and a villous component, both located in the distal colon. There was no difference between location (50% in the distal colon and 50% in the

**TABLE 2.** Group comparison (71 patients who underwent colonoscopy).

| Characteristics | | N | % | G.1 n | G.1 % | G.2 n | G.2 % | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | 71 | 100% | 34 | 48% | 37 | 52% | |
| Sex | | | | | | | | >0.7 |
| | Female | 39 | 55% | 18 | 52.9% | 21 | 56.8% | |
| | Male | 32 | 45% | 16 | 47.1% | 16 | 43.2% | |
| Indicated by the exam | | | | | | | | >0.7 |
| | Diagnosis | 7 | 10% | 3 | 8.8% | 4 | 10.8% | |
| | Surveillance | 24 | 34% | 13 | 38.2% | 11 | 29.7% | |
| | Screening | 40 | 56% | 18 | 52.9% | 22 | 59.5% | |
| Family history CCR | | | | | | | | 0.5 |
| | No | 61 | 86% | 28 | 82.4% | 33 | 89.2% | |
| | Yes | 10 | 14% | 6 | 17.6% | 4 | 10.8% | |
| First colonoscopy | | | | | | | | >0.8 |
| | No | 55 | 77% | 26 | 76.5% | 29 | 78.4% | |
| | Yes | 16 | 23% | 8 | 23.5% | 8 | 21.6% | |
| Age | | | | | | | | >0.8 |
| | 50-70 years | 57 | 80% | 28 | 80.0% | 29 | 78% | |
| | 71-80 years | 14 | 20% | 6 | 20.0% | 8 | 22% | |

CCR: cancer colon retal.

**TABLE 3.** Characteristics of the 58 resected adenomas.

| ENDOSCOPIC ASPECTS | TOTAL | | | G.2 | | G.1 | | G1 | G1 |
|---|---|---|---|---|---|---|---|---|---|
| | N | % | 1nd | 1nd | 2st | 1nd | 2st | total | AMR |
| | 58 | 100 | 40 | 20 | 0 | 20 | 18 | 38 | 47% |
| Location | | | | | | | | | |
| Proximal and transverse colon | 40 | 69% | 32 | 17 | 0 | 15 | 8 | 23 | 21% |
| Distal and rectum colon | 18 | 31% | 8 | 3 | 0 | 5 | 10 | 15 | 26% |
| Anatomopathological | | | | | | | | | |
| Tubular adenoma low-grade dysplasia | 54 | 93% | 37 | 19 | 0 | 18 | 17 | 35 | 45% |
| Tubular adenoma hight-grade dysplasia | 1 | 2% | 1 | 1 | 0 | 0 | 0 | 0 | 0% |
| Adenoma with Villous component | 3 | 5% | 2 | 0 | 0 | 2 | 1 | 3 | 3% |
| The Paris Classification | | | | | | | | | |
| pedunculated (0-Ip) | 4 | 7% | 3 | 1 | 0 | 2 | 1 | 3 | 3% |
| sessile (0-Is) | 50 | 86% | 33 | 15 | 0 | 18 | 17 | 35 | 45% |
| no polypoid (0-II) | 4 | 7% | 4 | 4 | 0 | 0 | 0 | 0 | 0% |
| Size | | | | | | | | | |
| ≤5mm | 47 | 81% | 32 | 15 | 0 | 17 | 15 | 32 | 39% |
| 6-9mm | 5 | 9% | 3 | 3 | 0 | 0 | 2 | 2 | 5% |
| ≥10mm | 6 | 10% | 5 | 2 | 0 | 3 | 1 | 4 | 3% |
| Adenoma | | | | | | | | | |
| Non-advanced adenomas | 51 | 88% | 35 | 17 | 0 | 18 | 16 | 34 | 42% |
| Advanced adenomas | 7 | 12% | 5 | 3 | 0 | 2 | 2 | 4 | 5% |

AMR: adenoma miss rates.

**TABLE 4.** Representing an overall adenoma miss rates - AMR (*P*=0.0002).

| GROUPS | 1st | 2nd | N | AMR | *P* value |
|---|---|---|---|---|---|
| Total | 40 | 18 | 58 | | 0.0002 |
| 1 | 20 | 18 | 38 | 47% | |
| 2 | 20 | 0 | 20 | 0% | |

proximal), and almost all were sessile, <5 mm (94%). In group 1, AMR was also stratified by size: the rate was 3% for adenomas greater than 10 mm, 5% for those measuring 6–10 mm, and 39% for those measuring 1–5 mm; by location: 21% proximal colon, 26% distal colon and by anatomopathological: 45% tubular adenoma with low grade dysplasia, 3% villous component (TABLE 3).

## DISCUSSION

Impact of chromoendoscopy and image magnification on increase ADR or reduce AMR is still a matter of debate because controversial results have been reported[24]. Few tandem or parallel studies are using either ADS or AMR as primary outcome to compare digital chromoendoscopy methods versus standard HDWL colonoscopy for adenoma detection. Evidence about the impact of using I-scan (Pentax, Tokyo, Japan) on detection rate of colorectal adenoma is scarce in the literature[10].

In this study, as we used the "tandem" methodology, all patients were subjected to two inspections in a row to define the AMR. Second inspection revealed the AMR, the adenomas lost in the first colonoscopy inspection. The AMR was significantly higher for the group that observed first with HDWL than the group that first observed with I-scan1 (47% vs 0%, *P*=0.0002). Our results were similar to Hoffman et al. (2014)[7], which showed a higher miss rate for adenomas of 62.5% of white light imaging versus 30% of I-scan 2 – contrast enhancement, (*P*<0.05, chi-square test) in tandem screening colonoscopies. In our study, we employed the surface enhancement (SE) algorithm or I-scan 1. We believe that I-scan 1 should be preferred method to reduce AMR, following the reasoning that I-scan 1 is recommended for polyp detection while I-scan 2 (contrast enhancement), and I-scan 3 (tone enhancement) should be used for polyp characterization[13] (FIGURE 2).

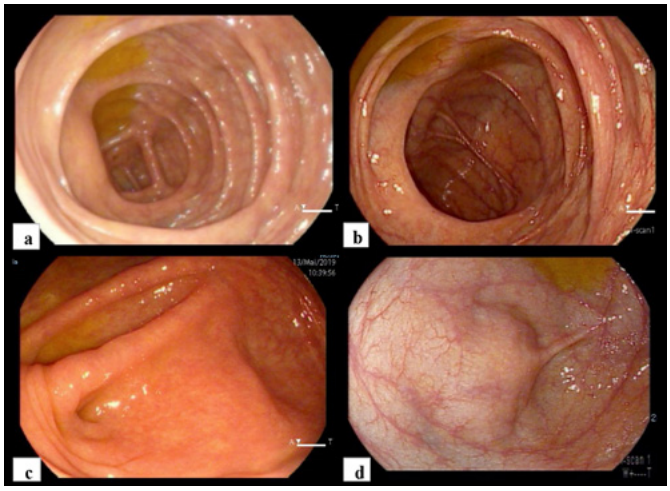



**FIGURE 2.** Endoscopic aspect of normal mucosa of the right colon. A) ascending colon with HDWL; B) ascending colon with I-scan1; C) cecum with HDLW; D) cecum with I-scan1.

Not surprisingly, most adenomas missed under HDWL but detected with I-scan 1 were small (<5 mm), low grade adenomas. In fact, this finding is not different from what is observed when other strategies to increase ADR were adopted: cap[25], retroflexion view of the right colon[26,27], double examination of the right colon[26].

Recently, in Brazil, Santos et al.[14] studied consecutive patients undergoing colonoscopy were randomized (1:1:1) into examination by HDWL, blue-laser imaging (BLI)-bright, or linked-color imaging – LCI (Fujinon) during withdrawal of the colonoscope, and concluded the LCI increases the detection of colorectal adenomas during colonoscopy compared with HDLW. In contrast to our result, using another type of device, Nagorni et al.[28] compared the use of narrow band image – NBI (Olympus) in colonoscopy versus HDWL to detect colorectal adenomas and found no significant difference between the groups. The use of different chromoendoscopy technologies and parallel versus tandem methodology probably accounts for the variation in the results across the studies.

This study has several limitations. We could not reach the calculated sample size of 138 patients. The tandem method has its limitations because it is performed in two moments on the same patient, which can generate anxiety in some patients who refuse to participate in the study. As the study was conducted in a hospital specializing in high complexity, we have many patients with a medical classification ASAIII and IV. Furthermore, we had a limited number of physicians, who were chosen because they have a high ADR. Thus, given the difficulty in reaching the desired sample, we expanded our inclusion criteria, not only limiting the indication to screening colonoscopy. According to a Zimmermann-Fraedrich (2020)[6] in contrast to the homogeneous performance of parallel studies, tandem trials methodology is more variable with more methodological limitations. However, a review concluded that tandem studies would be associated with significant advantages such as lower sample size[29].

In conclusion, the use of I-scan 1 (surface enhancement) – electronic chromoendoscopy, reduced the AMR in screening, surveillance, and diagnostic colonoscopies.

## Authors' contribution

Luz BSR: writing of the text, preparation of the article. Canteras JCCD: study design, data collection, research execution. Gon KC, Ahn TJ and Batista MLD: data collection. Maluf-Filho F: statistical analysis, preparation of the article.

## Orcid

Bruna Suelen Raymundo Luz: 0000-0002-9600-6874.
Juliana Carneiro C Dourado Canteras: 0000-0001-8472-891X.
Karen de Carvalho Gon: 0000-0003-1866-4262.
Maria Luisa de Deus Batista: 0000-0003-1508-4430.
Thomy Jun Ahn: 0000-0001-5669-4732.
Fauze Maluf-Filho: 0000-0001-8875-420X.

Luz BSR, Canteras JCCD, Gon KC, Batista MLD, Ahn TJ, Maluf-Filho F. O impacto da romoendoscopia com aprimoramento da imagem na taxa de perda de adenoma na colonoscopia de rastreio. Arq Gastroenterol. 2021;58(4):450-5.

**RESUMO** – **Contexto** – O câncer colorretal é uma das principais causas de morte relacionada ao câncer em todo o mundo. A colonoscopia é o padrão ouro para diagnosticar e tratar lesões precoces, levando à diminuição da incidência e mortalidade do câncer colorretal. Entretanto, é importante o reconhecimento de que alguns adenomas podem não ser detectados (adenomas perdidos) durante o exame, e todos os esforços vêm sendo destinado a reduzir esta taxa. O aprimoramento das tecnologias de imagem, incluindo cromoendoscopia eletrônica e ampliação, tem sido cada vez mais adotado para melhorar a taxa de detecção de adenomas colorretais. Como exemplo, as imagens obtidas com o I-scan® (Pentax, Tóquio, Japão), que fornecem cromoendoscopia virtual em tempo real durante o exame para visão do padrão de superfície, destacando a microvasculatura da lesão detectada. As evidências sobre o impacto do uso do I-scan® na taxa de detecção de adenoma colorretal são escassas. **Objetivo** – Avaliar se o uso de imagens I-scan® (Pentax, Tóquio, Japão) tem impacto na taxa de perda de adenoma nas colonoscopias de triagem. **Métodos** – Estudo observacional prospectivo de colonoscopias comparando cromoscopia com o aprimoramento de superfície e luz-branca. Pacientes acima de 50 anos submetidos à colonoscopia foram alocados aleatoriamente em dois grupos usando randomização eletrônica – Grupo 1: primeira inspeção com luz branca de alta definição seguida por uma segunda inspeção com o aprimoramento de superfície pelo I-scan 1®; Grupo 2: primeira inspeção com o aprimoramento de superfície I-scan 1® seguida de uma segunda inspeção com luz branca de alta definição. O desfecho primário foi a taxa de perda de adenomas do primeiro exame, calculado com o número de adenomas detectados na segunda inspeção do exame dividido pelo número total de adenomas detectados em ambas inspeções. **Resultados** – Participaram do estudo 85 pacientes, sendo excluídos 14, com amostra final de 71 pacientes. 34 foram alocados para o Grupo 1 e 37 no Grupo 2. Um total de 58 adenomas foram detectados, 40 na primeira inspeção (20 em cada grupo) e 18 na segunda inspeção, somente no Grupo 1. A taxa de perda de adenoma foi maior para o Grupo 1 do que para o Grupo 2 (47,4% vs 0% *P*=0,0002). **Conclusão** – A utilização de aprimoramento de superfície I-scan 1 reduz a taxa de perda de adenomas em exames colonoscópios.

**Palavras-chave** – Adenoma; colonoscopia; cólon; pólipo cólico; estudo prospectivo.

## REFERENCES

1. Siegel R L, Miller K D, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7-30.
2. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med. 2012;366:687-96. doi: 10.1056/NEJMoa1100370.
3. Fujimoto D, Muguruma N, Okamoto K, Fujino Y, Kagemoto K, Okada Y, et al. Linked color imaging enhances endoscopic detection of sessile serrated adenoma/polyps. Endosc Int Open. 2018;6:E322-34. doi: 10.1055/s-0043-124469
4. Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). Estimativa. 2016.[Internet] Availble from: https://santacasadermatoazulay.com.br/wp-content/uploads/2017/06/estimativa-2016-v11.pdf
5. Kaminski MF, Regula J, Kraszewska E, Polkowski M, Wojciechowska U, Didkowska J, et al. Quality indicators for colonoscopy and the risk of interval cancer. N Engl J Med. 2010;362:1795-803. doi: 10.1056/NEJMoa0907667.
6. Zimmermann-Fraedrich K, Pohl H, Rösch T, Rex DK, Hassan C, Dekker E, et al. Designs of colonoscopic adenoma detection trials: more positive results with tandem than with parallel studies - an analysis of studies on imaging techniques and mechanical devices. Gut. 2021;70:268-275. doi: 10.1136/gutjnl-2020-320984.
7. Kamiński MF, Hassan C, Bisschops R, Pohl J, Pellisé M, Dekker E, Ignjatovic-Wilson A, Hoffman A, Longcroft-Wheaton G, Heresbach D, Dumonceau JM, East JE. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2014;46:435-49. doi: 10.1055/s-0034-1365348.
8. Rex DK, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, Lehman GA, Mark DG. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. Gastroenterology. 1997;112:24-8. doi: 10.1016/s0016-5085(97)70214-2.
9. Heresbach D, Barrioz T, Lapalus MG, Coumaros D, Bauret P, Potier P, et al. Miss rate for colorectal neoplastic polyps: a prospective multicenter study of back-to-back vídeo colonoscopies. Endoscopy. 2008;40:284-90. doi: 10.1055/s-2007-995618.
10. Bond A, Burkitt MD, Cox T, Smart HL, Probert C, Haslam N, Sarkar S. Dual-focus Magnification, High-Definition Endoscopy Improves Pathology Detection in Direct-to-Test Diagnostic Upper Gastrointestinal Endoscopy. J Gastrointestin Liver Dis. 2017;26:19-24. doi: 10.15403/jgld.2014.1121.261.gen.
11. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, et al. Serrated lesions of the colorectum: review and recommendations from an expert panel. Am J Gastroenterol. 2012;107:1315-29. doi: 10.1038/ajg.2012.161.
12. Erichsen R, Baron JA, Hamilton-Dutoit SJ, Snover DC, Torlakovic EE, Pedersen L, et al. Increased risk of colorectal cancer development among patients with serrated polyps. Gastroenterology. 2016;150:895-902.e5. doi: 10.1053/j.gastro.2015.11.046.
13. Yamada M, Saito Y, Takamaru H, Sasaki H, Yokota T, Matsuyama Y, et al. Long-term clinical outcomes of endoscopic submucosal dissection for colorectal neoplasms in 423 cases: a retrospective study. Endoscopy. 2017;49:233-242. doi: 10.1055/s-0042-124366.
14. Oliveira Dos Santos CE, Malaman D, Pereira-Lima JC, de Quadros Onófrio F, Ribas Filho JM. Impact of linked-color imaging on colorectal adenoma detection. Gastrointest Endosc. 2019;90:826-34. doi: 10.1016/j.gie.2019.06.045
15. Kidambi TD, Bagatelos KC, Ostroff JW. The Answer Is in the Ampulla. Gastroenterology. 2018;155:e13-e14. doi: 10.1053/j.gastro.2018.04.006.
16. American Society of Anesthesiologists Classification. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Dez. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441940/
17. Lai EJ, Calderwood AH, Doros GD, Fix OK, Jacobson BC. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. Gastrointest Endosc. 2009;69:620-5. doi: 10.1016/j.gie.2008.05.057.
18. Rex DK, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty MB, et al. Quality indicators for colonoscopy. Gastrointest Endosc. 2015;81:31-53. doi: 10.1016/j.gie.2014.07.058.
19. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: november 30 to december 1, 2002. Gastrointest Endosc. 2003;58(6 Suppl): S3-43. doi: 10.1016/s0016-5107(03)02159-x.
20. Ferlitsch M, Moss A, Hassan C, Bhandari P, Dumonceau JM, Paspatis G, et al. Colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. Endoscopy. 2017;49:270-97.
21. Hamilton SR, Aaltonen LA. (Eds.). World Health Organization classification of tumours. pathology and genetics of tumours of the digestive system. Lyon: IARC Press, 2000.
22. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. Gastroenterology. 2012;143:844-57.
23. Baron TH, Smyrk TC, Rex DK. Recommended intervals between screening and surveillance colonoscopies. Mayo Clin Proc. 2013;88:854-8.
24. Hussain ZH, Pohl H. Ancillary imaging techniques and adenoma detection. Gastroenterol. Clin. North Am. 2013;42:547-65.
25. Kondo S, Yamaji Y, Watabe H, Yamada A, Sugimoto T, Ohta M, et al. A randomized controlled trial evaluating the usefulness of a transparent hood attached to the tip of the colonoscope. Am J Gastroenterol. 2007;102:75-81. doi: 10.1111/j.1572-0241.2006.00897.x.
26. Kushnir VA, Barad DH, Gleicher N. Fresh vs Cryopreserved Donor Oocytes--Reply. JAMA. 2015;314:2570. doi: 10.1001/jama.2015.13450.
27. Hewett DG, Rex DK. Miss rate of right-sided colon examination during colonoscopy defined by retroflexion: an observational study. Gastrointest Endosc. 2011;74:246-52. doi: 10.1016/j.gie.2011.04.005.
28. Nagorni A, Bjelakovic G, Petrovic B. Narrow band imaging versus conventional white light colonoscopy for the detection of colorectal polyps. Cochrane Database Syst Rev. 2012;1:CD008361. doi: 10.1002/14651858.CD008361.
29. Van Broek I, Niessen WM, van Dongen WD. Bioanalytical LC-MS/MS of protein-based biopharmaceuticals. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;929:161-79. doi: 10.1016/j.jchromb.2013.04.030.

ORIGINAL ARTICLE

AG-2021-53
doi.org/10.1590/S0004-2803.202100000-83

# Non-adherence to hepatitis C treatment: a Brazilian report

Claudia Alexandra Pontes **IVANTES**[1], Bernardo Carvalho da **SILVA**[2], Gabriel Gonçalves **ACOSTA**[2], Fabiane Beatriz Neves El **TAWIL**[1] and Renato **NISIHARA**[2]

Received: 9 March 2021
Accepted: 3 May 2021

**ABSTRACT** – **Background** – In Brazil, since 2015, the treatment of hepatitis C is provided by SUS (Public Health System) with direct-acting antiviral (DAA). **Objective** – To describe the rate of non-adherence patients to hepatitis C treatment by DAA, investigating the epidemiological data in a large database from Curitiba, Brazil. **Methods** – Retrospective study with patients treated between January 2015 and June 2019. Patients were considered adherent when received all medication doses during their treatment. The following data were evaluated: gender, age, type of treatment, period of treatment, presence of diabetes or HIV, previous therapy, originated from SUS or private medicine, fibrosis grade and HCV genotype. **Results** – 1248 patients (56.8% males) were studied and 102/1248 (8.2%) were non-adherent to treatment. Age or gender not influenced significantly; 10.2% patients from SUS and 3.7% individuals from private medicine were non-adherent ($P$<0.0001; OR=2.9; CI95%=1.6–9.1); 13.1% patients were co-infected with HIV and among them, 15.9% abandoned treatment. Individuals without co-infection presented 7.0% of non-adherence ($P$<0.0001; OR=2.5; CI=1.5–4.1). All the other variables showed no differences in the adhesion rate. **Conclusion** – Our study showed that 8.2% of patients were non-adherent to HCV treatment, and that patients from the Public Health System and co-infected with HIV were significantly less adherent.

**Keywords** – Hepatitis C; antiviral agents; patient compliance.

## INTRODUCTION

Hepatitis C is an infection caused by the hepatitis C virus (HCV) that is transmitted through contact with contaminated blood. Is estimated that around 399,000 people/year will die from complications from Hepatitis C in the world[1]. It is a high prevalence disease worldwide, being present in all continents and with an estimate of approximately 71 million individuals affected in chronic phase[1]. From 1999 to 2018, 359.673 cases of hepatitis C were reported in Brazil. In the year of 2018, the highest virus detection rate, for both genders, was present in the age group between 55 to 59 years old[2]. The deaths associated with hepatitis C are the biggest among all other hepatitis in Brazil, and it has been reaching higher rates year after year. From 2000 to 2017, a total of 53,715 deaths were associated with hepatitis C[2]. In 2017, the mortality coefficient was 1.0 per 100,000 in the male population, while among females the coefficient corresponded to 0.7 per 100,000[2].

In Curitiba, South Region of Brazil, the agency responsible for distributing medicines and counseling patients is the Center of Guidance and Advisor (COA), which works in prevention, diagnosis, and treatment of hepatitis C, all free of charge, provided by SUS (the Brazilian public health system), regardless if the patient had a private health care insurance or is a user of the public health system.

For a long time, hepatitis C treatment was carried out with the combination of 2a or 2b alpha-pegylated interferon and ribavirin (RBV)[3]. This treatment reported a cure rate of about 50% (20% in cirrhotic patients). However, patients showed many collateral effects and discomfort due to weekly injections for months, leading to low adherence to treatment. Since 2013, the Brazilian public health system incorporated three new direct-acting antivirals (sofosbuvir, simeprevir and daclatasvir) that combined and associated or not with RBV were used to treat individuals with hepatitis C. This treatment managed to reach levels close to 93% efficacy in sustained virological response (SVR), also largely reducing the adverse effects[3]. Patients who use this medication generally have few adverse effects, but there are still some individuals that are non-adherent and there are little data about the reasons that support this non-adherence. Knowing these reasons could improve the results of treatment and in the near future, eliminate hepatitis C in Brazil.

This study aimed to describe the rate of non-adherence patients to hepatitis C treatment by direct-acting agents, investigating the profile and epidemiological data registered in a large database from Curitiba, in the period from January 2015 to June 2019.

## METHODS

This study was approved by the Ethics and Research Committee of the Prefeitura Municipal de Curitiba, under number CAAE 65272117.3.1001.0101.

This is a retrospective study, conducted in the database of hepatitis C, attended and registered in the database of COA – Municipal Health Department of Curitiba, Brazil. In this study were included patients diagnosed with hepatitis C who received medication and follow-up by COA from January 2015 to June 2019. Patients whose data are not complete in the database have been excluded.

The following patient data were collected and analyzed: gender,

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Centro de Orientação e Aconselhamento (COA), Curitiba, PR, Brasil. [2] Universidade Positivo, Departamento de Medicina, Curitiba, PR, Brasil.
Corresponding author: Renato Nisihara. E-mail: renatonisihara@up.edu.br

age, type of treatment, date of beginning and end of treatment, period of treatment, presence of diabetes or HIV, previous therapy, originated from Public Health System (SUS) or private medicine, liver fibrosis grade, HCV genotype, and need of liver transplant. The treatment effectiveness was assessed by SVR 12 weeks after the end of treatment. The patients return each month, usually 4 days before finishing their medication, to receive a new prescription and withdraw your medicine free.

Patients were considered adherent when received all medication doses in COA during their treatment, which lasted for 12 or 24 weeks, depending on the treatment chosen by their physicians. Therefore, were considered non-adherent individuals that, for some reason, did not show up to get all the medicine doses in COA, or reported treatment interruption. Patients who died before treatment were not counted as adherent or non-adherent. Patients who died during treatment were considered non-adherent. Patients who died after the end of treatment, but sought all medications within the established deadlines were considered adherent. Patients who needed hospitalization for any reason and did not seek their medication during the established period were considered non-adherent.

The choice of 12 or 24 weeks of treatment is based on type of medication and patient's health condition according to the Brazilian Clinical Protocol and Therapeutic Guidelines[2].

### Statistical analysis

Distribution of numerical data was analyzed by the Shapiro Wilk test and central tendency was expressed in median and interquartile range (IQR). Nominal and categorical data were expressed in percentage (%). Chi-squared and Fisher tests were used to compare male and female nominal data and the Mann Whitney test was used for numeric data. The adopted significance was 5%.

## RESULTS

From January 2015 to June 2019, 1248 patients (56.8% males) received treatment at COA. TABLE 1 shows the demographic and clinical data on studied patients. In general, the mean age was 53.6±11.55 years, being men significantly younger than women (51.2±10.74 and 56.4±11.97 respectively, $P<0.0001$). In addition, male patients were more affected by hepatitis C than female patients (OR=1.7; CI95%=1.4–2.0).

A total of 102/1248 (8.2%) of patients attended were considered non-adherent to treatment. Age or gender not influenced significantly ($P=0.41$ and $P=0.16$, respectively) on adhesion to treatment. Data about non-adherent patients were in TABLE 1.

Comparing the adhesion rate between SUS or private medicine, 87/859 (10.2%) patients from SUS were non-adherent and 15/406 (3.7%) individuals from private medicine were considered non-adherent ($P<0.0001$; OR=2.9; CI95%=1.6–9.1).

Regarding co-infection, 163/1248 (13.1%) patients were co-infected with HIV and among them, 26/163 (15.9%) abandoned treatment. Individuals without co-infection presented 76/1085 (7.0%) of non-adherence. Thus, the rate of non-adherence was significantly higher in patients infected with HIV ($P<0.0001$; OR=2.5; CI=1.5–4.1). Among co-infected with HIV patients 75.1% were men and the gender not influenced in the adherence rate ($P=0.80$).

Among the adherent patients, 12/1112 (1.1%) deaths were observed and in non-adherent patients 8/102 (7.8%; $P<0.0001$; OR=8.4; CI95%=3.3–21.1). There is no difference in age or gender between patients who died in both groups.

In this sample, all liver transplant patients underwent surgery before starting treatment; therefore, this factor did not interfere on patients' adherence. In addition, previous treatment, presence of diabetes, grade of fibrosis, genotype of virus and type of treatment not influenced significantly the adherence rate.

## DISCUSSION

Hepatitis C elimination requires widespread access to treatment and responses at health-service. Having knowledge about the profile of patients who abandoned treatment for hepatitis C is crucial for disease control strategies. This study, from a large database of patients attended in the capital of the state of South Brazil, showed that a rate of 8.2% of patients who started the treatment did not complete this purpose. In addition, co-infected patients and patients from the SUS were more susceptible to non-adherence to HCV treatment, and these factors increased the death risk significantly.

Epidemiological data about infected patients did not differ from other studies performed in Brazil[4,5]. Men were significantly more affected and younger than women. Predominantly were attended patients from SUS, being diabetes and HIV co-infection the main comorbidities. Data about liver fibrosis grade, genotype of virus, treatment and previous treatment is also similar to other reports [4,5].

A major predictor of response to DAAs is adherence rate[6]. Therefore, the focus of this study is about non-adherence rate and to know the factors that influences this finding. The rate of 8.2% of non-adherence to HCV treatment can be considered high and worrisome. The objective is to eliminate hepatitis C in the world by 2030[6]. Although it tried, Brazil should not be able to achieve this goal and it is failing to test enough people mainly due to the SARS-COV 2 pandemic.

About treatment, nowadays the frequency of side effects and duration (12 or 24 weeks) of treatment are smaller than other previous therapy[7]. Our data showed that duration or type of drugs used as treatment did not influence significantly in the adherence rate, suggesting that both were well tolerated by the patients and did not contribute to improving the non-adherence rate[8,9]. In Brazil, since 2015, all the current drugs used as treatment to HCV are free of charge and available for the patients for both the public and private health systems. Monthly, they took the medication from a public pharmacy and were asked for side effects. However, the rate of non-adherence among patients from SUS is three times greater than private medicine. This finding suggests that socioeconomic status influence in the adherence to HCV treatment, as described by other authors[10] and highlighted to a target population to follow-up the treatment. Gender and age did not influence the treatment's adherence.

In this study, among the patients co-infected with HIV, the rate of non-adherence was 2.5 greater than non co-infected. Lakshmi et al., in the United States, described that 11.9% of patients co-infected (HCV + HIV) were non-adherent to treatment and that HCV cure rates among co-infected patients seeking care in real world settings are lower than those reported in clinical trials[11]. Other authors described that the rate of HCV cure is similar in coinfected patients[12]. Some authors described reasons for lower adherence of these individuals to treatment such as type of treatment, frequency of clinic visits, depression, sociodemographic factors and others[11,13]. In addition, other authors reported that with the accessibility of HCV direct-acting antivirals, efficacy and adverse event rates among those with HIV/HCV coinfection are

**TABLE 1.** Clinical and demographical data of patients adherent and non-adherent to hepatitis C treatment with direct-acting antivirals.

| | Adherent patients (n=1146) | | Non – Adherent patients (n=102) | | *P* value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Gender | | | | | 0.16 |
| Male | 644 | 56.1 | 65 | 63.7 | |
| Female | 502 | 43.9 | 37 | 36.3 | |
| Health System | | | | | <0.0001 |
| Public | 761 | 66.4 | 87 | 85.3 | |
| Private | 385 | 33.6 | 15 | 14.7 | |
| Comorbidities | | | | | |
| Diabetes type 1 or 2 | 167 | 14.5 | 10 | 9.8 | 0.12 |
| HIV+ | 137 | 11.9 | 26 | 25.5 | <0.0001 |
| Liver transplantation | 47 | 4.1 | 3 | 2.9 | 0.78 |
| Liver Fibrosis grade | | | | | 0.65 |
| F0 | 20 | 1.7 | 0 | 0 | |
| F1 | 29 | 2.5 | 0 | 0 | |
| F2 | 170 | 14.8 | 6 | 5.9 | |
| F3 | 173 | 15.0 | 10 | 9.8 | |
| F4 | 200 | 17.4 | 20 | 19.6 | |
| Not evaluate | 554 | 48.3 | 66 | 64.7 | |
| Genotype of virus C | | | | | 0.52 |
| 1 | 49 | 4.2 | 4 | 3.9 | |
| 1A | 397 | 34.6 | 46 | 45.1 | |
| 1B | 319 | 27.8 | 14 | 13.7 | |
| 2 | 27 | 2.3 | 3 | 2.9 | |
| 3 | 334 | 29.1 | 33 | 32.4 | |
| 4 | 16 | 1.3 | 1 | 1 | |
| Not evaluated | 4 | 0.3 | 1 | 1 | |
| Treatment | | | | | 0.34 |
| Sofosbuvir + daclatasvir | 405 | 35.3 | 37 | 36.3 | |
| Sofosbuvir + daclatasvir + ribavirin | 364 | 31.7 | 43 | 42.2 | |
| Sofosbuvir + simeprevir | 173 | 15.0 | 7 | 6.9 | |
| Sofosbuvir + simeprevir + ribavirin | 20 | 1.7 | 5 | 4.9 | |
| 3D | 117 | 10.2 | 6 | 5.9 | |
| 3D + ribavirin | 51 | 4.4 | 4 | 3.8 | |
| Others | 16 | 1.3 | 0 | 0 | |
| Duration of treatment | | | | | 0.24 |
| 12 weeks | 917 | 80.0 | 81 | 79.4 | |
| 24 weeks | 161 | 14.0 | 20 | 19.6 | |
| Other | 68 | 5.9 | 1 | 1 | |
| Previous treatment | 317 | 27.6 | 27 | 26.5 | 0.88 |
| Relapsed | 166 | 14.4 | 11 | 10.8 | |
| Null response | 120 | 10.4 | 11 | 10.8 | |
| Partial response | 26 | 2.2 | 4 | 3.9 | |
| No data | 15 | 1.3 | 1 | 1 | |

F0: absence of fibrosis; F1: portal fibrosis without septa; F2: portal fibrosis with few septa; F3: numerous septa without cirrhosis; F4: liver cirrhosis 3D: ombitasvir + veruprevir + ritonavir + dasabuvir.

similar to those observed with HCV monoinfection[14]. In our study, we did not find significant differences between types of treatment and, unfortunately, we do not have data about depression and socioeconomic status, therefore we believe that these factors probably influence adhesion rates. On the other hand, a prospective study with 74 marginalized patients from Canada[15], showed that in the context of social marginalization and high rates of substance use, a community-based, supportive model of HCV treatment can promote high levels of adherence and achieve treatment outcomes that are comparable to registered clinical trials[15].

Our data showed that the number of deaths among non-adherent patients was eight times higher than those adherent to treatment. Although it is expected, this data should be analyzed with caution, because many factors that were not possible to be evaluated could influence this finding.

Limitations of our study must be recognized, mainly due to retrospective design. We had no information on the socioeconomic status of subjects in our sample. Because lower socioeconomic status is associated with lower adherence to treatment, its omission may have biased to an unknown extent. It is possible that missing data does not actually reflect non-adherence and thus non-adherence may be overestimated in some cases. In addition, in this study the adherence rate was based on evidence of the drug being dispensed but not ingested.

Concluding, our study showed that 8.2% of patients were non-adherent to HCV infection treatment, and that the factors that significantly influenced this finding were patients in the Public Health System and co-infection with HIV.

## Authors' contribution

Ivantes CAP, Silva BC, Acosta GG and Nisihara R conceived and carried out the study; Ivantes CAP, Silva BC, Tawil FBNE and Nisihara R organized and analyzed data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

## Orcid

Claudia Alexandra Pontes Ivantes: 0000-0001-5422-557X.
Bernardo Carvalho da Silva: 0000-0002-6797-1341.
Gabriel Gonçalves Acosta: 0000-0003-4267-1331.
Fabiane Beatriz Neves El Tawil: 0000-0002-5900-9056.
Renato Nisihara: 0000-0002-1234-8093.

Ivantes CAP, Silva BC, Acosta GG, Tawil FBNE, Nisihara R. Não aderência ao tratamento para hepatite C: um estudo brasileiro. Arq Gastroenterol. 2021;58(4):456-60.

**RESUMO** – **Contexto** – No Brasil, desde 2015, o tratamento da hepatite C é prestado pelo Sistema Público de Saúde (SUS) com antivirais de ação direta. **Objetivo** – Avaliar a taxa de não adesão de pacientes ao tratamento da hepatite C pelo antiviral de ação direta investigando os dados epidemiológicos em um banco de dados de Curitiba, Brasil. **Métodos** – Estudo retrospectivo com pacientes atendidos entre janeiro de 2015 e junho de 2019. Os pacientes foram considerados aderentes quando receberam todas as doses da medicação durante o tratamento. Foram avaliados os seguintes dados: sexo, idade, tipo de tratamento, tempo de tratamento, presença de diabetes ou HIV, terapia anterior, proveniente do SUS ou medicina privada, grau de fibrose e genótipo da hepatite C. **Resultados** – Um total de 1.248 pacientes (56,8% homens) foram estudados e desses, 102/1248 (8,2%) não aderiram ao tratamento. Idade ou sexo não influenciou significativamente; 10,2% pacientes do SUS e 3,7% da medicina privada eram não aderentes ($P$<0,0001; OR=2,9; IC95%=1,6–9,1); 13,1% dos pacientes foram coinfectados pelo HIV e, entre eles, 15,9% abandonaram o tratamento. Indivíduos sem coinfecção apresentaram 7,0% de não adesão ($P$<0,0001; OR=2,5; IC=1,5–4,1). Todas as outras variáveis não mostraram diferenças na taxa de adesão. **Conclusão** – Nosso estudo mostrou que 8,2% dos pacientes não aderiram ao tratamento para hepatite C e que os pacientes do SUS e coinfectados pelo HIV eram significativamente menos aderentes.

**Palavras-chave** – Hepatite C; agentes antivirais; cooperação do paciente.

## REFERENCES

1. WHO – World Health Organization; Hepatitis C, 2020 – Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.
2. Brasil. Ministério da saúde; Boletim Epidemiológico de Hepatites Virais 2019 – Secretaria de Vigilância em Saúde - Ministério da saúde. Available from: http://pncq.org.br/uploads/boletim_hepatites_2019_c_.pdf. Last visited: 15/12/2020
3. Ghany MG, Morgan TR, AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. Hepatology. 2020;71:686-721. doi: 10.1002/hep.31060.
4. Portari-Filho LH, Álvares-da-Silva MR, Gonzalez A, Ferreira AP, Villela-Nogueira CA, Mendes-Correa MC, et al. How are HCV-infected patients being identified in Brazil: a multicenter study. Braz J Infect Dis. 2019;23:34-9. doi:10.1016/j.bjid.2019.01.006.
5. Lobato CMO, Codes L, Silva GF, Souza AFM, Coelho HSM, Pedroso MLA, et al. Direct antiviral therapy for treatment of hepatitis C: A real-world study from Brazil. Ann Hepatol. 2019;18:849-54. doi:10.1016/j.aohep.2019.08.001.
6. WHO – World Health Organization. Towards the elimination of hepatitis B and C by 2030. Draft WHO Global Hepatitis Strategy, 2016-2021. Available from: https://www.who.int/hepatitis
7. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; Clinical Practice Guidelines Panel: Chair: EASL Governing Board representative; Panel members. EASL recommendations on treatment of hepatitis C: Final update of the series. J Hepatol. 2020;73:1170-1218. doi: 10.1016/j.jhep.2020.08.018.
8. Falade-Nwulia O, Sutcliffe C, Moon J, Chander G, Wansom T, Keruly J, et al. High hepatitis C cure rates among black and nonblack human immunodeficiency virus-infected adults in an urban center. Hepatology. 2017;66:1402-12. doi:10.1002/hep.29308.

9. Pecoraro V, Banzi R, Cariani E, Chester J, Villa E, D'Amico R, et al. New Direct-Acting Antivirals for the Treatment of Patients With Hepatitis C Virus Infection: A Systematic Review of Randomized Controlled Trials. J Clin Exp Hepatol. 2019;9:522-38. doi: 10.1016/j.jceh.2018.07.004.
10. Luma HN, Eloumou SAFB, Noah DN, Eyenga BA, Nko'Ayissi G, Taku TS, et al. Hepatitis C Continuum of Care in a Treatment Center in Sub-Saharan Africa. J Clin Exp Hepatol. 2018;8:335-41. doi:10.1016/j.jceh.2018.01.001.
11. Lakshmi S, Alcaide M, Palacio AM, Shaikhomer M, Alexander AL, Gill-Wiehl G, et al. Improving HCV cure rates in HIV-coinfected patients - a real-world perspective. Am J Manag Care. 2016;22(6 Spec No.):SP198-SP204.
12. Milazzo L, Lai A, Calvi E, Ronzi P, Micheli V, Binda F, et al. Direct-acting antivirals in hepatitis C virus (HCV)-infected and HCV/HIV-coinfected patients: real-life safety and efficacy. HIV Med. 2017;18:284-91. doi:10.1111/hiv.12429.
13. DiMatteo MR, Lepper HS, Croghan TW. Depression is a risk factor for noncompliance with medical treatment: meta-analysis of the effects of anxiety and depression on patient adherence. Arch Intern Med. 2000;160:2101-7. doi: 10.1001/archinte.160.14.2101.
14. Rockstroh JK, Lacombe K, Viani RM, Orkin C, Wyles D, Luetkemeyer AF, et al. Efficacy and Safety of Glecaprevir/Pibrentasvir in Patients Coinfected With Hepatitis C Virus and Human Immunodeficiency Virus Type 1: The EXPEDITION-2 Study. Clin Infect Dis. 2018;67:1010-7. doi: 10.1093/cid/ciy220.
15. Mason K, Dodd Z, Guyton M, Tookey P, Lettner B, Matelski J, et al. Understanding real-world adherence in the directly acting antiviral era: A prospective evaluation of adherence among people with a history of drug use at a community-based program in Toronto, Canada. Int J Drug Policy. 2017;47:202-8. doi: 10.1016/j.drugpo.2017.05.025.

ORIGINAL ARTICLE

AG-2021-58
doi.org/10.1590/S0004-2803.202100000-84

# Severity of irritable bowel syndrome symptoms and FODMAPs intake in University students

Mariana Cerne **AUFIERI**, Juliana Masami **MORIMOTO** and Renata Furlan **VIEBIG**

Received: 12 March 2021
Accepted: 23 June 2021

**ABSTRACT** – **Background** – Irritable bowel syndrome (IBS) symptoms such as diarrhea, bloating and abdominal pain can reduce University student's productivity and learning ability. One of the possible treatments for IBS is the temporarily exclusion of foods that have a high content of short-chain fermentable carbohydrates, the fermentable, oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). **Objective** – This study aimed to assess University student's intake of foods that are rich in FODMAPs, looking for possible associations with the severity of IBS symptoms. **Methods** – A cross-sectional study was carried out, with undergraduate students from a private University in the city of São Paulo, Brazil, aged between 19 and 46 years old and that were enrolled in different courses and stages. Students were invited to participate and those who gave their formal consent were included in this research. A sociodemographic and lifestyle questionnaire was applied, in addition to the Gastrointestinal Symptom Rating Scale – GSRS. Students also responded a short Food Frequency Questionnaire, developed to investigate habitual FODMAPs intake of Brazilian adult population. Spearman's correlation analysis between the student's GSRS scores and the frequency of foods rich in FODMAPs intake were performed in SPSS v.21. **Results** – Fifty-six students were interviewed, with mean age of 21.4 years old (SD=4.41), with a predominance of women (76.8%). The GSRS results showed that 58.9% of students felt minimal to moderate abdominal discomfort and 14.3% had moderately severe to very severe abdominal pain during the prior week to the interview. Besides abdominal pain, the gastrointestinal symptoms that were most reported by students were flatulence (98.2%), stomach rumbling (89.3%) and eructations (85.7%). Greater symptom severity was observed in women ($P$=0.004) and sedentary students ($P$=0.003). Regarding FODMAPs consumption, honey ($P$=0.04), chocolate ($P$=0.03) and milk table cream ($P$=0.001) intakes were positively correlated with the greater severity of symptoms. **Conclusion** – Although clinical diagnosis is necessary to establish IBS, 73.2% of the students presented minimal to very severe abdominal pain during the prior week. Female had sedentary students had greater severity of gastrointestinal symptoms. A low FODMAP diet, well oriented, could bring some symptoms relief to these University students.

**Keywords** – FODMAPs; irritable bowel syndrome; gastrointestinal symptoms.

## INTRODUCTION

Young University students present important changes in their lifestyles and eating habits throughout their undergraduate course, as a result of their study routines and curricular internships. These students go through constant stress, being a vulnerable group to trigger metabolic and physiological changes that can result in a symptom picture related to irritable bowel syndrome (IBS), including diarrhea, constipation, abdominal pain, in addition to flatulence and abdominal distention[1,2]. Undergraduate students suffering from these symptoms state that is difficult to deal with social life and University appointments and tasks because they cannot stand being away from home for many hours, having difficulty concentrating on academic activities and exams, and they often miss classes[3].

IBS is a chronic, often debilitating, and highly prevalent disorder of gut-brain interaction (previously called functional gastrointestinal [GI] disorders)[1,2]. Although the etiology of IBS is still unclear, several factors contribute to its appearance, such as eating habits, stress, psychosocial factors, the presence of comorbidities, intestinal dysbiosis, among others[4]. Research carried out, especially in the last decade, has linked the increase in IBS symptoms not only to stress, but to the consumption of short-chain carbohydrates, which are poorly absorbed and fermented by the intestinal flora, called fermentable, oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs)[4-8].

Australian researchers were the first to define what these carbohydrates were and designated them by the acronym FODMAPs, representing: fermentable, oligosaccharides, disaccharides, monosaccharides and polyols[4,9]. The exclusion of foods that have a high content of these carbohydrates in patient's diet is currently one of the main non-pharmacological therapy for IBS[1,7], following gradual reintroduction between the eighth to the twelfth week after the disappearance of symptoms[6].

The genesis of the clinical presentation of IBS after consumption of rich foods in FODMAPs, it is attributed to several factors, with emphasis on the intestinal malabsorption resulting from the absence of enzymes capable of hydrolyzing the luminous glycosidic bonds of carbohydrates, the low activity of brush edge enzymes (for example, lactase) or even the reduced capacity of epithelial transporters[10]. Furthermore, the FODMAPs can increase GI water secretion and fermentation in the colon, leading to bloating and triggering IBS common symptoms[1].

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
Research performed at: Mackenzie Presbyterian University, Biological Sciences and Health Center, São Paulo, SP, Brazil.
Universidade Presbiteriana Mackenzie, Centro de Ciências Biológicas e Saúde, Curso de Graduação em Nutrição, São Paulo, SP, Brasil.
Correspondence author: Mariana Cerne Aufieri. E-mail: marianaaufieri@gmail.com

Therefore, knowledge of the importance of the low FODMAPs diet and its adoption could bring relief from gastrointestinal symptoms presented by undergraduate students. On the other hand, although it may play a positive role in improving symptoms, the application of this diet should be well indicated, as it is necessary to exclude a significant list of foods that are present in the dietary patterns of the Brazilian population[10], which makes it difficult its application and justifies that this intervention should always be carried out with the guidance and monitoring of dieticians[1] and gastroenterologists.

In this panorama, the present study aimed to evaluate the consumption of foods rich in FODMAPs from University students seeking associations with the symptoms of IBS.

## METHODS

It was a cross-sectional study, carried out with a group of individuals, voluntary participants, men and women, aged between 19 and 46 years old, students from different Undergraduate courses and stages from a private University in São Paulo, SP, Brazil. The study was conducted between January and March of 2020.

After presenting the study's objectives and procedures to potential participants, an individual invitation was made to students to integrate the research sample. Institution and individuals consented to their participation by formally signing the Free and Informed Consent Form (ICF). It was explained to the students that there would be no harm in giving up their collaboration with the research and, in addition, the confidentiality and anonymity of the participants in all stages of the study was guaranteed.

Initially, a standardized questionnaire was applied to students, in person and individually, at University's classrooms, containing questions about age, sex, education, occupation, residence (local and if live alone), lifestyle (smoking, alcohol consumption and physical activity) and self-perceived health.

Then, the participants answered a questionnaire that assessed gastrointestinal symptoms, called Gastrointestinal Symptom Rating Scale (GSRS), which was translated into Portuguese in a study by Souza et al.[11]. The design of this instrument was carried out by health professionals and the translation was done by translators fluent in the English language, with the questionnaire's efficiency tested by volunteers at the end of the study. It refers to a self-administered questionnaire that has 15 questions and includes five symptoms in the week prior to the application: diarrhea, constipation, abdominal pain, reflux and indigestion. Their responses are organized according to the 7-point Likert scale, in which one indicates absence of the symptom and seven, greater severity. The score is obtained by the average of the items on the scale of each component, being and indicator of greater severity of symptoms (moderate to severe), a score greater than 2.4[12].

The analysis of the student's FODMAPs was assessed using the self-filling of the Short Food Frequency Questionnaire (FFQ), designed and proposed by Brazilian researchers, to evaluate Brazilian adult population[13]. The FFQ use for the present study was formally authorized by the authors. The daily consumption of each food, by each participant, was estimated by dividing the frequency of consumption reported by them by the referred time unit (times a week; times a month).

Data were analyzed using the statistical package SPSS v. 21, considering a significance level of 5%. Results were evaluated according to central tendency and frequency measures. Student's *t*-test was used to assess participants according to differences between average ages and scores on the questionnaires. Spearman's correlation analysis was used to assess the correlation between the final score on the GSRS questionnaire and the daily consumption of high FODMAPs foods.

This research was submitted to the local Research Ethics Committee and received approval under number CAAE 24296819.2.0000.0084.

## RESULTS

Fifty-six Undergraduate students were interviewed, being 76.8% (n=43) women. The mean age of the participants was 21.4 years (SD=4.41) and only 10.7% (n=6) students were 25 years old or more (TABLE 1).

There was a higher prevalence of students of the Biological Sciences and Health courses, being the most cited: Psychology (35.7%) and Nutrition (26.8%). Nine (16.1%) students reported working in addition to studying and only 12.5% of the participants were living by themselves (TABLE 1).

Regarding to self-perceived health, 23.3% of female and 7.7% of male students considered themselves "healthy". Sixty-six percent of the students (n=37) reported consuming alcoholic beverages weekly and 6 (10.7%) individuals never consumed alcohol before. In contrast, the majority of students (n=35; 62.5%) said they had never smoked cigarettes, while 12 (21.4%) individuals had a frequent smoking habit (TABLE 1).

Almost half of the students (46.4%) declared themselves physically active, practicing exercises for 4 days a week, in average, with a mean session duration of 1 hour and 35 minutes. On the other hand, 53.6% of the students declared not to practice any physical activity, being sedentary (TABLE 1).

When asked about the frequency of IBS-related symptoms in the week prior to the application of the GSRS questionnaire, 26.8% of students reported that they did not experience abdominal pain at all, 58.9% felt minimal to moderate discomfort and 14.3% had moderately severe to very severe pain. Regarding the appearance of the stools, 12.5% said they had soft stools at many times and 16.1%, hard stools. On the other hand, almost half of the students stated that they did not present not once soft (46.4%) and hardened (41.4%) stools in the week prior to the interview.

The most frequently gastrointestinal symptoms cited by students were flatulence (98.2%), stomach rumbling (89.3%) and eructation (85.7%). The least prevalent symptoms in the last week, among the participants, were diarrhea (37.5%), acid reflux (37.5%), nausea (39.3%) and urgent need to evacuate (46.4%). TABLE 2 shows the severity and frequency of the symptoms mentioned by the students.

Greater symptom severity was observed in women ($P$=0.004), with 67.4% of them having a total score greater than 2.4 on the GSRS. On the other hand, 23.1% of men had a score that correspond to higher severity of symptoms, according to the GSRS.

No statistically significant associations were found between the severity of gastrointestinal symptoms, given by the GSRS score, and the type of Undergraduate course ($P$=0.19), student occupation ($P$=0.52) or smoking ($P$=0.61). It was observed that the majority of young people who consumed alcoholic beverages had greater severity and frequency of symptoms, however, the result was also not statistically significant ($P$=0.11). In addition, sedentary students had a higher severity of IBS symptoms ($P$=0.03).

**TABLE 1.** Distribution of the University students according to general and lifestyle characteristics.

| Variables | Men | | Women | | Total | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Age | | | | | | |
| 19–24 | 11 | 84.6 | 39 | 90.6 | 50 | 89.3 |
| 25–29 | 2 | 15.4 | 2 | 4.7 | 4 | 7.1 |
| 30+ | – | – | 2 | 4.7 | 2 | 3.6 |
| Undergraduate course | | | | | | |
| Biology | 2 | 15.4 | – | – | 2 | 3.6 |
| Pharmacy | 1 | 7.7 | 11 | 25.5 | 12 | 21.4 |
| Physiotherapy | 3 | 23.0 | 2 | 4.7 | 5 | 8.9 |
| Nutrition | 2 | 15.4 | 13 | 30.2 | 15 | 26.8 |
| Psychology | 5 | 38.5 | 15 | 34.9 | 20 | 35.7 |
| Others | – | – | 2 | 4.7 | 2 | 3.6 |
| Occupation | | | | | | |
| Student | 9 | 69.2 | 38 | 88.4 | 47 | 83.9 |
| Student and worker | 4 | 30.8 | 5 | 11.6 | 9 | 16.1 |
| Living alone | | | | | | |
| Yes | 1 | 7.7 | 6 | 14.0 | 7 | 12.5 |
| No | 12 | 92.3 | 37 | 86.0 | 49 | 87.5 |
| Self-perceived health | | | | | | |
| Healthy | 1 | 7.7 | 10 | 23.3 | 11 | 19.6 |
| Relatively healthy | 10 | 76.9 | 27 | 62.7 | 37 | 66.1 |
| Not healthy | 2 | 15.4 | 6 | 14.0 | 8 | 14.3 |
| Smoking | | | | | | |
| Frequently | – | – | 1 | 2.3 | 1 | 1.8 |
| Sometimes | 6 | 46.1 | 6 | 14.0 | 12 | 21.4 |
| Rarely | 2 | 15.4 | 5 | 11.6 | 7 | 12.5 |
| Never | 4 | 30.8 | 31 | 72.1 | 35 | 62.5 |
| Ex-smoker | 1 | 7.7 | – | – | 1 | 1.8 |
| Alcohol consumption | | | | | | |
| Frequently | 3 | 23.1 | 6 | 14.0 | 9 | 16.1 |
| Sometimes | 5 | 38.4 | 23 | 53.5 | 28 | 50.0 |
| Rarely | 3 | 23.1 | 10 | 23.3 | 13 | 23.2 |
| Never | 2 | 15.4 | 4 | 9.2 | 6 | 10.7 |
| Physical activity | | | | | | |
| Yes | 9 | 69.2 | 17 | 39.5 | 26 | 46.4 |
| No | 4 | 30.8 | 26 | 60.5 | 30 | 53.6 |
| Total | 13 | 100 | 43 | 100 | 56 | 100 |

**TABLE 2.** Severity and frequency of gastrointestinal symptoms presented by the University students, according to GSRS (Gastrointestinal Symptom Rating Scale).

| Gastrointestinal symptoms | No discomfort/ not once | | Minor to moderate discomfort/rare to few times | | Severe to a very severe discomfort/ some to many times | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Abdominal pain | 15 | 26.8 | 33 | 59.0 | 8 | 14.3 |
| Heartburn | 28 | 50.0 | 21 | 37.5 | 7 | 12.5 |
| Acid reflux | 35 | 62.5 | 19 | 33.9 | 2 | 3.6 |
| Hungry feeling | 12 | 21.4 | 30 | 53.5 | 14 | 25.1 |
| Nausea | 34 | 60.7 | 19 | 33.9 | 3 | 5.4 |
| Stomach rumbling | 6 | 10.7 | 37 | 66.1 | 13 | 23.2 |
| Abdominal distention | 27 | 48.2 | 16 | 28.5 | 13 | 23.3 |
| Eructation | 8 | 14.3 | 30 | 53.5 | 18 | 32.1 |
| Flatulence | 1 | 1.8 | 30 | 53.6 | 25 | 44.6 |
| Constipation | 27 | 48.2 | 21 | 37.5 | 8 | 14.2 |
| Diarrhea | 35 | 62.5 | 17 | 30.4 | 4 | 7.2 |
| Soft stools | 26 | 46.4 | 23 | 41.0 | 7 | 12.5 |
| Hard stools | 23 | 41.1 | 24 | 42.8 | 9 | 16.1 |
| Urgent need to evacuate | 30 | 53.6 | 20 | 35.8 | 6 | 10.7 |
| Feeling of incomplete bowel emptying | 14 | 25.0 | 24 | 42.9 | 18 | 32.1 |

Honey ($P$=0.04), chocolate ($P$=0.03) and milk table cream ($P$=0.001) intakes were positively associated with the greater severity of symptoms presented by the students, although the correlations found are considered weak to moderate. On the other hand, a statistically significant and inverse association was observed between the consumption of sweet potatoes, soy protein, cassava and oilseeds and the score of the symptom questionnaire. Again, the correlations were shown to be weak to moderate with the exception of soy protein, which appears to be strongly correlated with minor gastrointestinal symptoms (TABLE 3).

**TABLE 3.** Correlation coefficients between gastrointestinal symptoms and the intake of foods rich in FODMAPs of University students.

| Food rich in FODMAPs | Student's intake | | r* | *P* |
|---|---|---|---|---|
| | n | % | | |
| Honey | 30 | 53.6 | 0.37 | 0.04 |
| Oilseeds | 38 | 67.9 | -0.31 | 0.05 |
| Soy protein | 6 | 10.7 | -0.81 | 0.05 |
| Cassava | 42 | 75.0 | -0.37 | 0.01 |
| Chocolate | 53 | 94.6 | 0.30 | 0.03 |
| Milk table cream | 45 | 80.4 | 0.46 | 0.001 |

*Spearman's correlation coefficient.

## DISCUSSION

The University students of this research were mostly women, of Biological Sciences and Health courses, non-smokers, insufficiently physically active and with regular intake of alcoholic beverages. In a review study carried out by Fernandes et al.[14], 29 articles were analyzed that investigated the use of psychoactive substances in Brazilian University students and, among students from the Southeast region, there was a predominance of women and an elevated consumption of alcohol was observed, as well as in the present study. Another study conducted by Guimarães et al.[15] analyzed the lifestyle of 550 Brazilian University students from a public Institution of Higher Education in Piauí, Brazil, showing an even more elevated prevalence of sedentarism than in the present study (71.6%).

The authors of the present study did not aim to diagnose IBS, which is a very difficult task to clinicians, due to the fact that, at this moment, there is no efficient biological marker that indicates that the person has IBS and also that their symptoms can be shared with other diseases such as, for example, inflammatory bowel disease and celiac disease, which can lead to misdiagnosis[2,7,16].

However, the presence of IBS-related symptoms in the last week was identified among the University students, and approximately a quarter of the students had at least one symptom causing severe to very severe discomfort, and some of them presented these symptoms many times in their daily lives. In addition, female students were more affected by the gastrointestinal symptoms, with a higher severity. Brazilian and international studies show that the

prevalence of IBS is higher between women[1,10], but some of them also refer an increased incidence of this syndrome among men[10].

There are few studies that assessed the prevalence of IBS or the IBS-related symptoms in Brazilian University students. Pedreira et al.[17] analyzed 18 medical students who met the IBS criteria, however, 77.8% had not received a previous medical diagnosis of the disease. Stools alterations (66.7%) and feeling of incomplete emptying (50%) were mentioned by these undiagnosed students. In the present study, there was a lower prevalence of changes in stools (58.9%), but a similar frequency of feeling of incomplete bowel emptying (51.8%).

Real[12] carried out a study with 80 young Portuguese physical activity practitioners, with an average age of 30.6 years, using the GSRS Questionnaire and observed that reflux and abdominal pain were referred to as "moderate to severe" symptoms in 13.8% and 6.3% of the sample, respectively. In the present study, the same symptoms were mentioned by 19.2% and 73.1% of University students who practiced physical activity, respectively, which may suggest the protective role of physical activity in the frequency of the onset of IBS symptoms.

A diet rich in high FODMAPs may increase gastrointestinal symptoms even in healthy individuals. Ong et al.[18] studied 30 individuals (15 healthy and 15 diagnosed with IBS) and observed that a higher rate of hydrogen gas was produced by both groups throughout the day they adhered to a diet rich in FODMAPs ($P$<0.001), representing that a certain intolerance to the diet was observed. The group of undiagnosed volunteers showed intensification in flatulence.

Among the foods cited sources of FODMAPs surveyed, the consumption of milk table cream was positively associated with greater severity of symptoms among the University students studied. Like any other cow's milk derived food, milk table cream can cause discomfort due to lactose malabsorption. On the other hand, it is a food richer in fats than milk itself, because in its production the cream is removed from the milk in order to obtain a fatty cream[19] and the ingestion of fatty foods can favor the appearance of gastrointestinal symptoms[20].

The consumption of chocolate also showed a positive association with the greater severity of symptoms presented by the students of this research, which may be related to the fact that, depending on its type, in addition to containing carbohydrates, this can be a fatty food that contributes to the symptoms. Half of the sample studied stated that they had some discomfort with heartburn in the last week and, according to the World Gastroenterology Organization (WGO)[21], individuals with heartburn should avoid consuming chocolate because it is a food that triggers this symptom. An alternative source of low FODMAPs content than it is in normal chocolate would be dark chocolate[22].

Honey, as well as milk table cream and chocolate, was positively associated with greater severity of symptoms among the University students, being a food highly rich in fructose[23], a monosaccharide not completely absorbed by the intestine and responsible for the production of gases and abdominal distention[6]. In the study conducted by Murray et al.[6], with 16 volunteers without a diagnosis of IBS, liquids containing glucose, fructose or inulin or a mixture of glucose and fructose were offered. After drinking the drink with fructose, there was an immediate increase in expired hydrogen, as well as the appearance of both symptoms. In the present study, flatulence and abdominal distension caused discomfort in approximately 98% and 52% of students, respectively.

The relation found between soy protein and a lower severity of gastrointestinal symptoms could be explained by the fact that this food is a marker of a healthier or plant-based diet, with few industrialized and dairy products. However, this fact still needs to be further investigated and no studies have been found in this regard.

Studies about the FODMAPs intake are still scarce in Brazil, especially when it comes to University students. In a study conducted at FMUSP Clinical Hospital, in São Paulo, it was observed that beans, coffee and pizza were considered the most aggressive and symptom-causing foods in patients with IBS (n=140)[24], which were consumed by 41.4%, 40.7% and 36.6% of participants in the current survey, respectively.

In another study carried out with patients from the school clinic of University of Piauí, wheat, beans and milk and their derivatives were the foods with the lowest consumption by patients with IBS, and their relation with intestinal symptoms increase was observed[25]. Foods rich in wheat, present in the FFQ used in the present research, such as loaf of bread, bread roll, pasta and pizza were consumed by most students (85%, 87.5%, 100% and 98.2%, respectively), but it was not found a significant correlation between these and gastrointestinal symptoms, as well as beans.

Roest et al.[26] studied a sample of 90 patients diagnosed with IBS who started to follow a diet low in FODMAPs and, at the end of the study, there was a significant reduction in the following symptoms: abdominal pain, bloating, flatulence and diarrhea ($P$<0.001). In the current study, even though a medical diagnosis of IBS was not performed prior to the sample composition, some of the gastrointestinal symptoms of the students could be part of the IBS spectrum of symptoms, being flatulence the most prevalent (98.2%). Therefore, a restricted diet of these foods could reduce the symptoms of these individuals, and certainly, the precise definitive clinical diagnosis performed by a gastroenterologist should be made.

The relationship between certain foods and the triggering or worsening of gastrointestinal symptoms is not yet fully explained, which leads to the need for further investigation on the role of food intolerance as a contributor to IBS[10]. Furthermore, the rich FODMAPs foods that are part of the Brazilian diets are different than other world populations, or even between Brazilians of different regions and age groups. In this context, even though several observational studies have exposed the benefits of FODMAPs-restricted diet as an innovative approach to symptom relief, more research is needed to explain pathophysiological mechanisms involved.

The present study has limitations regarding the sample size and its composition by students who did not necessarily have a diagnosis of IBS. In addition, the questionnaires used depended on the volunteers' memory and the chosen Food Frequency Questionnaire, specific for assessing Brazilians FODMAPs intake, was recently published and has not yet been used on a large scale, so that more effective comparisons could be made.

Another limitation of the current research is that the effects of food sources of FODMAPs are mainly related to intestinal symptoms and GSRS also takes into account gastric symptoms, which could somehow confuse the observed correlations.

Furthermore, the GSRS questionnaire assessed the recent gastrointestinal symptoms of the participants, related to the previous week of data collection, that is, the symptoms presented may not be chronic and mean intolerance, but rather, acute symptoms of that specific week.

## CONCLUSION

It was observed that several gastrointestinal symptoms, IBS-related, were part of the of the University students' life, mostly female students, and that some rich FODMAPs foods could worsen the severity of these symptoms. There was a greater significant severity of gastrointestinal symptoms among women and students who did not practice physical activity.

In addition, the correlations between a more severe gastrointestinal symptomatology and the consumption of chocolate, milk table cream and honey were significant, and the intake of these foods should be avoided or reduced by symptomatic students.

We reinforce that IBS diagnosis, carried out by a trained medical professional, could bring symptoms relief and a better quality of life for students, as well as the subsequent guidance of an expert dietician.

Further studies should be carried out, using similar instruments to assess FODMAPs intake among Brazilian populations to find the benefits of the low FODMAP and improve gastrointestinal symptoms control and quality of life.

## ACKNOWLEDGEMENTS

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for the scholarship provided to Mariana Cerne Aufieri.

### Authors' contribution

Aufieri MC: study conception and design; analysis and interpretation of the data; data acquisition; drafting of the article. Morimoto JM: analysis and interpretation of the data. Viebig RF: scientific initiation program orientation; study conception and design; analysis and interpretation of the data; drafting of the article; critical revision of the article; final approval of the article.

### Orcid

Mariana Cerne Aufieri: 0000-0002-4393-8424.
Juliana Masami Morimoto: 0000-0003-3394-1723.
Renata Furlan Viebig: 0000-0001-8110-6277.

Aufieri MC, Morimoto JM, Viebig RF. Severidade dos sintomas da síndrome do intestino irritável e consumo de FODMAPs em estudantes universitários. Arq Gastroenterol. 2021;58(4):461-7.

**RESUMO** – **Contexto** – Os sintomas da síndrome do intestino irritável (SII), como diarreia, "inchaço" e dor abdominal, podem reduzir a produtividade e a capacidade de aprendizagem do estudante universitário. Um dos possíveis tratamentos para a SII é a exclusão temporária de alimentos que possuem alto teor de carboidratos fermentáveis de cadeia curta, os fermentáveis, oligossacarídeos, dissacarídeos, monossacarídeos e polióis (FODMAPs). **Objetivo** – Este estudo teve como objetivo avaliar a ingestão de alimentos ricos em FODMAPs por estudantes universitários, buscando possíveis associações com a gravidade dos sintomas da SII. **Métodos** – Foi realizado um estudo transversal, com alunos de graduação de uma Universidade privada da cidade de São Paulo, Brasil, com idades entre 19 e 46 anos, matriculados em diferentes cursos e estágios. Os alunos foram convidados a participar e aqueles que deram seu consentimento formal foram incluídos nesta pesquisa. Foi aplicado um questionário sociodemográfico e de estilo de vida, além da *Gastrointestinal Symptom Rating Scale* – GSRS. Os alunos também responderam a um pequeno Questionário de Frequência Alimentar, desenvolvido para investigar o consumo habitual de FODMAPs na população adulta brasileira. A análise de correlação de Spearman entre os escores GSRS do aluno e a frequência do consumo de alimentos ricos em FODMAPS foi realizada no SPSS v.21. **Resultados** – Foram entrevistados 56 alunos, com média de idade de 21,4 anos (DP=4,41) e predomínio do sexo feminino (76,8%). Os resultados do GSRS mostraram que 58,9% dos alunos sentiram desconforto abdominal mínimo a moderado e 14,3% tiveram dor abdominal moderadamente intensa a muito intensa durante a semana anterior à entrevista. Além das dores abdominais, os sintomas gastrointestinais mais referidos pelos estudantes foram flatulência (98,2%), roncos estomacais (89,3%) e eructações (85,7%). Maior gravidade dos sintomas foi observada em mulheres ($P$=0,004) e estudantes sedentários ($P$=0,003). Em relação ao consumo de FODMAPs, a ingestão de mel ($P$=0,04), chocolate ($P$=0,03) e creme de leite ($P$=0,001) se correlacionou positivamente com a maior gravidade dos sintomas. **Conclusão** – Embora o diagnóstico clínico seja necessário para o estabelecimento da presença da SII, 73,2% dos alunos apresentaram dor abdominal mínima a muito intensa na semana anterior ao estudo. Estudantes do sexo feminino e sedentários apresentaram maior gravidade dos sintomas gastrointestinais. Uma dieta pobre em FODMAP, bem orientada, poderia trazer algum alívio destes sintomas a estes universitários.

**Palavras-chave** – FODMAPs; síndrome do intestino irritável; sintomas gastrointestinais.

## REFERENCES

1. Lacy BE, Pimentel M, Brenner DM, Chey WD, Keefer LA, Long MD, et al. ACG clinical guideline: management of irritable bowel syndrome. Am J Gastroenterol. 2021;116:17-44.
2. Camilleri M. Diagnosis and treatment of irritable bowel syndrome: a review. JAMA. 2021;325:865-77.
3. International Foundation for Gastrointestinal Disorders [Internet]. [place unknown]: IFFGD; c2015 [cited 2019 Feb 11]. Personal Stories. Available from: https://aboutibs.org/living-with-ibs-main/personal-stories.html
4. Bastos TF. Síndrome do Intestino Irritável e dieta com restrição de FODMAPs [dissertation]. Lisboa: Faculdade de Medicina, Universidade de Lisboa; 2016. 31p.
5. Gibson PR. Food intolerance in functional bowel disorders. J Gastroenterol Hepatol. 2011;26:128-31.
6. Murray K, Wilkinson-Smith V, Hoad C, Costigan C, Cox E, Lam C, et al. Differential effects of FODMAPs (Fermentable Oligo-, Di-, Mono-saccharides and Polyols) on small and large intestinal contents in healthy subjects shown by MRI. Am J Gastroenterol. 2014;109:110-9.
7. Barrett JS. How to institute the low-FODMAP diet. J Gastroenterol Hepatol. 2017;32:8-10.
8. Zanetti AJ, Rogero MM, Von Atzingen MC. Low-FODMAP diet in the management of irritable bowel syndrome. Nutrire (Online). 2018;43:1-5.
9. Gibson PR. History of the low FODMAP diet. J Gastroenterol Hepatol. 2017;32:5-7.
10. Andrade VL, Fonseca TN, Gouveia CA, Kobayashi TG, Leite RG, Mattar RA, et al. FODMAPs restricted diet as a treatment option in irritable bowel syndrome: systematic review. Gastroenterol Endosc Dig. 2014;34:34-41.

11. Souza GA, Sardá FA, Giuntini EB, Gumbrevicius I, Morais MB, Menezes EW. Translation and validation of the brazilian portuguese version of the Gastrointestinal Symptom Rating Scale (GSRS) Questionnaire. Arq Gastroenterol. 2016;53:146-51.
12. Real II. Ingestão alimentar e sintomas gastro intestinais em praticantes de exercício físico [dissertation]. Lisboa: Faculdade de Medicina, Universidade de Lisboa; 2014. 54p.
13. Solar I, Santos LA, Yamashita LM, Barret JS, Nagasako CK, Montes CG, et al. Irritable bowel syndrome: associations between FODMAPs intake, problematic foods, adiposity, and gastrointestinal symptoms. Eur J Clin Nutr. 2019;73: 637-41.
14. Fernandes TF, Monteiro BM, Silva JB, De Oliveira KM, Viana NA, Da Gama CA, et al. Use of psychoactives substances among college students: epidemiological profile, settings and methodological limitations. Cad Saúde Colet. 2017;25:498-507.
15. Guimarães MR, Batista AM, Santos IM, Vale MP, De Moura IH, Da Silva AR. Estilo de vida e fatores associados entre estudantes universitários. Rev Enferm UFPE on line. 2017;1:3228-35. Portuguese.
16. Lacy BE, Patel NK. Rome criteria and a diagnostic approach to irritable bowel syndrome. J Clin Med. 2017;6:1-8.
17. Pedreira M, Carneiro A, Dunningham W, De Pinho SL, De Aguiar WM. Prevalência de Síndrome do Intestino Irritável em estudantes de Medicina. Rev Bras Neurol Psiquiatr. 2013;7:54-64. Portuguese.
18. Ong DK, Mitchell SB, Barrett JS, Sheperd SJ, Irving PM, Biesiekierski JR, et al. Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. J Gastroenterol Hepatol. 2010;25:1366-73.
19. Rossi GA, Vidal AM, Netto AS, Aguilar CE. Fluxograma de produção de leite e derivados. In: Vidal AM, Netto AS, editors. Obtenção e processamento do leite e derivados. Pirassununga: Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo; 2018. p. 213-15.
20. Villela NB, Rocha R. Manual básico para atendimento ambulatorial em nutrição. [Article in Portuguese]. [online]. 2. Ed. Salvador: EDUFBA, 2008. 120 p. Available from: http://books.scielo.org.
21. World Gastroenterology Organisation. Coping with common GI symptoms in the community: a global perspective on heartburn, constipation, bloating, and abdominal pain/discomfort [Internet]. WGO Global Guidelines; 2013 [cited 2019 Feb 11]. 37 p. Available from: https://www.worldgastroenterology.org/UserFiles/file/guidelines/common-gi-symptoms-english-2013.pdf
22. Oliveira PD, Reis JE, Reis MA, Ferreira SM, Candelária Al. A dieta com restrição de FODMAP reduz os sintomas no síndroma do intestino irritável? Uma revisão baseada na evidência. [Article in Portuguese]. Rev Port Med Geral Fam. 2020;36:126-34.
23. Da Silva RA, Maia GA, De Souza PH, Da Costa JM. Composição e propriedades terapêuticas do mel de abelha. [Article in Portuguese]. Aliment Nutr. 2006; 17:113-20.
24. Amarante D. Aspectos nutricionais na população de pacientes com Síndrome do Intestino Irritável atendidos nos Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo [dissertation]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2013. 93p.
25. Teles KB, De Souza SM, Landim LA. Ingestão dietética de alimentos ricos em FODMAPs em portadores da síndrome do intestino irritável. [Article in Portuguese]. REAC. 2020;9:1-8.
26. Roest RD, Dobbs BR, Chapman BA, Batman B. The low FODMAP diet improves gastrointestinal symptoms in patients with irritable bowel syndrome: a prospective study. Int J Clin Pract. 2013;67:895-903.

ORIGINAL ARTICLE

AG-2021-60
doi.org/10.1590/S0004-2803.202100000-85

# *Helicobacter pylori cagA* virulence gene and severe esogastroduodenal diseases: is there an association?

Ana Karoline Silva **OLIVEIRA**[1], Lucas Luiz de Lima **SILVA**[1], Marina Pacheco **MIGUEL**[2], Angel José Vieira **BLANCO**[3], Lilian Carla **CARNEIRO**[2] and Mônica Santiago **BARBOSA**[1,2]

Received: 15 March 2021
Accepted: 5 July 2021

**ABSTRACT** – **Background –** *Helicobacter pylori* colonizes approximately half of the world's human population. Its presence in the gastric mucosa is associated with an increased risk of gastric adenocarcinoma, gastric lymphoma, and peptic ulcer disease. In Brazil, the high prevalence of *H. pylori* infection is a serious health problem. *H. pylori* virulence factors are associated with an increased risk of serious gastrointestinal disorders. The *cagA* gene encodes a cytotoxin-A-associated antigen (CagA) that is involved in bacterial pathogenicity. *H. pylori* strains carrying the cag pathogenicity island (cag-PAI) are significantly associated with severe clinical outcomes and histopathological changes. **Objective –** The present study aims to investigate the prevalence of the *cagA* gene among *H. pylori* isolates from patients with different gastric pathologies. Further, the study hopes to verify its association with clinical outcomes. In addition, phylogenetic analysis was performed on *cagA*-positive *H. pylori* strains from patients with severe and non-severe diseases. **Methods –** Gastric specimens were collected through a biopsy from 117 patients with different esogastroduodenal diseases. DNA was extracted from these gastric specimens and the polymerase chain reaction was performed to amplify the gene fragments corresponding to the 16S ribosomal RNA and *cagA* genes using specific primers. The polymerase chain reaction products of selected samples positive for *cagA* were sequenced. The sequences were aligned with reference sequences from the National Center for Biotechnology Information (NCBI) (Bethesda/USA), and a phylogenetic tree was constructed. **Results –** *H. pylori* was detected in 65.9% (77/117) of Brazilian patients with different gastroduodenal disorders. Overall, 80.5% (62/77) of the strains were *cagA*-positive. The ages of patients with *cagA*-positive strains (15 males and 47 females) ranged from 18 to 74 years. The lesions were categorized as non-severe and severe according to the endoscopic and histopathological reports the most prevalent non-severe esogastroduodenal lesion was gastritis 54/77 (70.12%), followed by esophagitis 12/77 (15.58%) and duodenitis 12/77 (15.58%). In contrast, the most prevalent severe lesions were atrophy 7/77 (9.09%), followed by metaplasia 3/77 (3.86%) and gastric adenocarcinoma 2/77 (2.59%). Phylogenetic analyses performed with the partial sequences of the *cagA* gene obtained from local strains were grouped in the same clade. No differences in phylogenetic distribution was detected between severe and non-severe diseases. **Conclusion** – The *cagA* gene is highly prevalent among *H. pylori* isolates from gastric lesions in Brazilian patients. The presence of the *cagA* gene was not considered a marker of the severity of esogastroduodenal lesions in the present study. This is the first study to investigate the phylogenetic population structure of *H. pylori* strains in a Brazilian capital, which may improve our understanding of the clinical outcome of *H. pylori* infection.

**Keywords –** Molecular epidemiology; phylogeny; virulence factors; gene bacterial.

## INTRODUCTION

*Helicobacter pylori* is a gram-negative bacterium that colonizes the human gastric mucosa[1]. It is estimated that approximately half of the world's population is colonized by *H. pylori*[2]. Transmission routes of *H. pylori* are associated with precarious socioeconomic conditions. Therefore, the prevalence of *H. pylori* infection is significantly higher in developing countries[3]. In Brazil, the prevalence of infection in some regions is comparable to that of infection rates in Africa, where rates can reach 90%[4,5].

*H. pylori* infection favors the development of gastrointestinal diseases, such as gastritis, ulcers, atrophy, lymphoma of the lymphoid tissue of the mucosa (MALT), and gastric adenocarcinoma (GA)[6]. Due to its well-established role in carcinogenesis, *H. pylori* was classified as a Group I carcinogen by the World Health Organization (WHO) in 1994[7]. In addition, *H. pylori* infection is associated with gastroesophageal reflux (GERD). The mechanism underlying *H. pylori* infection-associated GERD is not yet fully understood. Some studies have shown that *H. pylori*-positive patients with arthritis have hypergastrinemia with a consequent decrease in gastric pH. This leads to the worsening of GERD symptoms. However, other studies have reported that the use of proton pump inhibitors in the treatment of *H. pylori* infection can lead to an increase in gastric pH levels, reducing the symptoms of GERD[8-10].

The development of esogastroduodenal lesions depends on the complex and dynamic relationship between the parasite and the

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade Federal de Goiás, Núcleo de Estudos da *Helicobacter pylori*, Goiânia, GO, Brasil. [2] Universidade Federal de Goiás, Instituto de Patologia Tropical e Saúde Pública,Goiânia, GO, Brasil. [3] Instituto Federal de Educação, Ciência e Tecnologia de Goiás – Campus Inhumas, GO, Brasil.
Corresponding author: Mônica Santiago Barbosa. E-mail: santiagosant@gmail.com

host. This, in turn, is determined by several factors. These include genetic susceptibility of the host, environmental factors, and bacterial virulence[11]. The bacterial strains have various virulence genes that influence the pathogenicity of the infection; these include *ureA*, *ureB*, *vacA*, *cagE*, *sabA*, *ice*, and the gene associated with cytotoxin A (*cagA*)[12-14].

The *cagA* oncogene, located on the pathogenicity island (*cag*-PAI), encodes the cytotoxin associated with gene A (CagA)[15,16]. The interaction of CagA, with molecules in the gastric epithelium of the host, can increase the severity of esogastroduodenal diseases[17]. The bacterium uses the type IV secretion system (T4SS) to inject bacterial factors into the host's intracellular environment[18,19]. Once in the intracellular medium, CagA can follow two pathways: independent phosphorylative and dependent phosphorylative.

In the independent phosphorylative pathway, the main cellular changes are interruption of mitogenic signals, changes in cell-cell junctions, and the exacerbation of the activity of inflammatory pathways[15]. The dependent phosphorylative pathway occurs when CagA is initially phosphorylated in the EPYIA region (Glu-Pro-Ile-Try-Ala) by the kinases of the SCR and Abl family, followed by binding to the SH2 domain of SHP-2 phosphatase[16,20]. The formation of the CagA-SHP-2 complex causes abnormal cell events. These include the dysregulation of cell growth, changes in the cytoskeleton, increased proliferation and mobility, changes in cell junctions, and the expression of pro-inflammatory, pro-mitogenic, and pro-apoptotic proteins[12].

*cagA* is one of the most characterized virulence genes as severe esogastroduodenal lesions, such as GA, are frequently associated with *cagA*-positive *H. pylori* strains[21,22]. Although some studies have demonstrated the association of this gene with severe esogastroduodenal diseases, there is still a lack of information regarding the Brazilian population. The aim of this study was to investigate the prevalence of *cagA* in dyspeptic patients, as well as the association of the oncogene with the severity of different esogastroduodenal lesions. In addition, the present study evaluated the phylogenetic relationship of *cagA*-positive *H. pylori* strains isolated from patients with severe and non-severe diseases.

## METHODS

### Ethical considerations

The study protocol was reviewed and approved by the Research Ethics Committee of the Federal University of Goias (CEP/UFG). The study was conducted in accordance with the Ethical Standards of the Brazilian National Committee of Ethics in Human Research, which follows the principles of the Declaration of Helsinki. The approval number is: 2.519.032 (CAAE: 83422017.7.0000.5078). Informed consent was obtained from all the participants.

### Study participants

The study was conducted in Goiânia, State of Goiás, Central West Brazil. Both male and female participants and those aged 18 years and older who agreed to participate in the study were recruited from a referral center for gastric diseases. Participants were excluded from the study if they used antibiotics and immunosuppressants eight weeks prior to sample collection, the use of proton pump inhibitors two weeks prior to sample collection, gestation, lactation, active gastrointestinal bleeding, and a history of gastrectomy. The participants were recruited from January to December 2018. A total of 117 participants were included in the study.

### Samples

After clinical evaluation, the participants underwent endoscopy, which was performed by a trained endoscopist. Macroscopic data included topography, localization, and type of injury. During the procedure, gastric biopsies were carried out (two in the antrum, two in the body) in accordance with the recommendations of the IV Consensus on *Helicobacter pylori* infection[23]. The samples were sent to the clinical pathology laboratory of the University hospital for histopathological analysis and to the Nucleus for the Study of *Helicobacter pylori* at the Federal University of Goiás (NEHP/UFG) for molecular analysis.

### Histopathological analysis

Histopathological examination was performed at the pathology laboratory of the reference hospital. All clinical specimens were fixed in 10% formaldehyde and stained with hematoxylin-eosin and Giemsa stain[24]. The gastric mucosa was assessed according to the Sydney system[25].

### Esogastroduodenal injuries and severity criteria

Endoscopic and histopathological reports were used to segregate patients with severe and non-severe esophagogastroduodenal lesions. According to the recommendations of Paredes-Osses et al., 2017[26] and Bellolio et al., 2019[27], injuries categorized as severe were GA, gastric atrophy, intestinal and non-severe metaplasia, esophagitis, duodenitis, gastritis, and ulcers.

### DNA extraction and genotyping of *H. pylori*

Molecular analysis was carried out at the Nucleus for the Study of *Helicobacter pylori* at the Federal University of Goiás (NEHP/UFG). The clinical specimens were subjected to DNA extraction using the commercial kit KitQIamp DNA Minikit® (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. The DNA concentration was determined using NanoDrop® (ND-1000 UV-Vis).

*H. pylori* was detected by polymerase chain reaction (PCR) using the ribosomal 16S rRNA gene as described previously by Luscenti and Gatti, 2008[28]. Positive samples for the 16S rRNA gene were subjected to amplification of the *cagA* virulence gene by PCR as described by Dadashzadeh et al., 2017[29]. The primer sequences, reaction conditions, and sizes of the amplified fragments are listed in TABLE 1.

The PCR products were stained with Blue Green Loading Dye I (LGC Biotecnologia, São Paulo, Brazil) and then subjected to electrophoresis on 2% agarose gel. The product was visualized under ultraviolet light and the images were documented.

### Sequencing and phylogenetic analysis of the *cagA* gene

Sequencing was performed according to the method proposed by Sanger et al., 1978[30], using the DYEnamicTM ET Terminator Cycle Sequencing Kit (GE Healthcare, USA) and ABI Prism 3100 (Applied Biosystems). Phylogenetic analyses were performed using the Molecular Evolutionary Genetics Analysis Software (MEGA), version 10.1[31]. The phylogenetic tree was constructed using the maximum parsimony method with 1000 bootstraps considering the gaps generated in the alignment as a fifth base.

### Data analysis

Statistical analyses was performed using the GraphPad Prism version 7.0, and SAS version 9.1 packages. The probability of se-

**TABLE 1**. Sequence of primers, reaction conditions and sizes of amplified fragments of the 16S rRNA and *cagA* genes.

| Gene | Primer | Primer sequence | Amplification Conditions | bp | Reference |
|---|---|---|---|---|---|
| 16S rRNA | *hpx1*<br>*hpx 2* | CTGGAGARACTAAGYCCTCC<br>GAGGAATACTCATTGCGAAGGCGA | 94°C 5', 40 cycles 94°C 1'<br>59°C 1' / 72°C 1' and 72°C 7'. | 150 | Lusceti and Gatti, 2008 |
| *cagA* | *cag1*<br>*cag2* | ATGACTAACGAAACTATT<br>CAGGATTTTTGATCGCTTTATT | 94°C 5', 25 cycles 94°C 1'<br>53°C 1' / 72°C 1' and 71°C 7'. | 232 | Dadashzadeh et al., 2017 |

verity, as well as its association with the *cagA* gene, was estimated using odds ratios (ORs). Fisher's exact test was used to analyze the sex and severity. The relationship between age and severity was evaluated using two-way ANOVA with Tukey's post-hoc test. Data will be presented as mean and standard deviation (mean ± standard deviation). The results were considered statistically significant in case of $P<0.05$, with a 95% confidence interval (CI).

## RESULTS

DNA extracted from gastric biopsies was used for molecular screening of the bacteria by amplifying the 16S rRNA gene. Samples that amplified a 150 bp fragment were considered positive (FIGURE 1A). *H. pylori*-positive samples were used to amplify a 232 bp fragment of the *cagA* virulence gene (FIGURE 1B).

*H. pylori* was detected in 65.9% (77/117) of samples from gastric specimens. Among *H. pylori*-positive samples, 80.5% (62/77) were *cagA*-positive and 19.5% (15/77) were *cagA*-negative. *H. pylori*-positive patients were segregated into two groups based on the type of lesion (non-severe and severe).

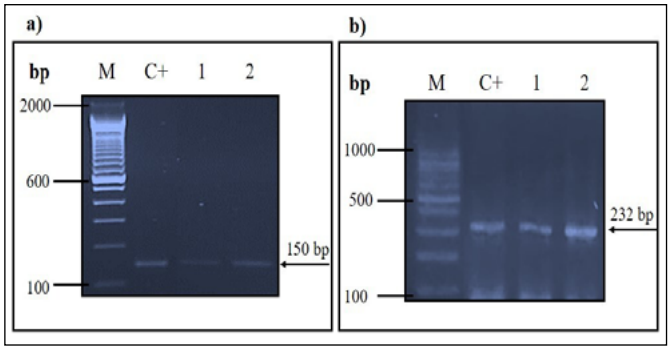



**FIGURE 1.** Amplification products of the *H. pylori* 16S rRNA and *cagA* genes. M: molecular weight labeled (1a. 2000 bp / 1b. 1000 bp); C +: positive control; **(a)** lanes 1 and 2: 16S rRNA gene amplification product (150 bp amplicon); **(b)** lanes 1 and 2: *cagA* gene amplification product (232 bp amplicon).

A total of 90 clinical outcomes were detected, 78 of which were non-severe and 12 were severe. Since the same patient could have more than one esogastroduodenal lesion, the number of clinical outcomes was higher than the number of patients in the study. The most prevalent non-severe esogastroduodenal lesion was gastritis 54/77 (70.12%), followed by esophagitis 12/77 (15.58%) and duodenitis 12/77 (15.58%). In contrast, the most prevalent severe lesions were atrophy 7/77 (9.09%), followed by metaplasia 3/77 (3.86%) and GA 2/77 (2.59%).

Gastric biopsy samples from *cagA*-positive *H. pylori* patients with severe and non-severe lesions were used for photomicrography, as shown in FIGURE 2. Photomicrographs 2a and 2b show severe lesions in the gastric tissues of patients with atrophy and GA, respectively. FIGURES 2c and 2d show gastritis and duodenitis, respectively, considered non-severe lesions.

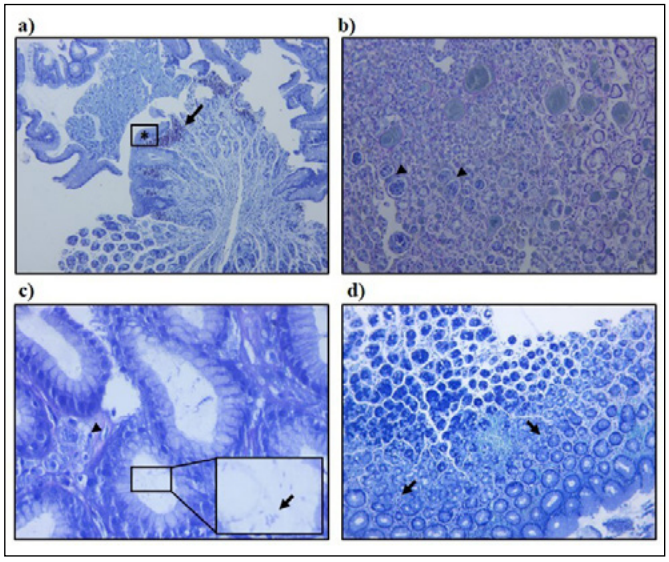



**FIGURE 2.** Photomicrograph of gastric tissue lesions in patients infected with positive *H. pylori cagA* strains. **(a)** Atrophy in the gastric mucosa (*) and multifocal inflammatory infiltrate in the submucosa (arrow). 10x. Giemsa. **(b)** GA – it is possible to observe proliferation of tubular structures containing cells with atypical cells and evidence of malignancy (arrowhead). 10x. Giemsa. **(c)** Gastritis, discrete inflammatory aggregates (arrowhead), and the presence of *H. pylori* (arrow) are observed. 10x. Giemsa **(d)** Duodenitis – it is possible to observe multifocal inflammatory infiltrate (arrow). 10x. Giemsa.

The analysis of the association between the 90 clinical outcomes found 78 non-severe cases and 12 severe cases. In addition, it found the presence of *cagA* was performed to assess the role of this gene in the severity of esogastroduodenal diseases. The results showed that there was no statistically significant association between *cagA* and lesion severity (OR=0.90, CI: 0.2200–3.6821, $P$=0.8835) (TABLE 2).

Clinical outcomes were also analyzed individually to assess whether the *cagA* gene was specifically associated with the severity of esogastroduodenal lesions. The odds ratios, confidence intervals, and *P*-values, are listed in TABLE 3. Additionally, we evaluated whether the *cagA* gene acted as a risk or protection factor in the development of specific gastropathy. Statistical analyses did not show an association between *cagA* and the various isolated clinical outcomes (TABLE 3).

**TABLE 2**. Relationship between the presence of *cagA* and the severity of esogastroduodenal injuries.

| | Non-severe diseases (n=78) | Severe diseases (n=12) | OR | CI 95% | *P* |
|---|---|---|---|---|---|
| *cagA*+ (n=62) | 60 (76.92%) | 9 (75.00%) | 0.90 | 0.2200–3.6821 | 0.8835 |
| *cagA*- (n=15) | 18 (23.08%) | 3 (25.00%) | | | |

OR: odds ratio, was used for statistical analysis.

**TABLE 3**. Relationship between clinical outcomes* and the *H. pylori cagA* virulence gene.

| Non-severe diseases | | | | | |
|---|---|---|---|---|---|
| **cagA** | **Disease** | | **OR** | **CI 95%** | ***P*** |
| | Gastritis | | | | |
| | No (n=23) | Yes (n=54) | | | |
| cagA+ | 20 (86.96%) | 42 (77.78%) | 0.5250 | 0.1331–2.0716 | 0.3575 |
| cagA- | 3 (13.04%) | 12 (22.22%) | | | |
| | Esophagitis | | | | |
| | No (n=65) | Yes (n=12) | | | |
| cagA+ | 53 (81.54%) | 9 (75.00%) | 0.6792 | 0.1595–2.8932 | 0.6009 |
| cagA- | 12 (18.46%) | 3 (25.00%) | | | |
| | Duodenitis | | | | |
| | No (n=65) | Yes (n=12) | | | |
| cagA+ | 53 (81.54%) | 9 (75.00%) | 0.6792 | 0.1595–2.8932 | 0.6009 |
| cagA- | 12 (18.46%) | 3 (25.00%) | | | |
| Severe diseases | | | | | |
| cagA | **Disease** | | **OR** | **CI 95%** | ***P*** |
| | Atrophy | | | | |
| | No (n=70) | Yes (n=7) | | | |
| cagA+ | 57 (81.43%) | 5 (71.43%) | 0.5702 | 0.0994–3.2713 | 0.5285 |
| cagA- | 13 (18.57%) | 2 (28.7%) | | | |
| | Metaplasia | | | | |
| | No (n=74) | Yes (n=3) | | | |
| cagA+ | 60 (81.08%) | 2 (66.67%) | 0.4667 | 0.0395–5.5171 | 0.5453 |
| cagA- | 14 (18.92%) | 1 (33.33%) | | | |
| | Gastric adenocarcinoma | | | | |
| | No (n=75) | Yes (n=2) | | | |
| cagA+ | 60 (80.00%) | 2 (100.00%) | 1.281 | 0.0584–28.0755 | 0.8751 |
| cagA- | 15 (20.00%) | 0 (0.00%) | | | |

OR: odds ratio, was used for statistical analysis. *Clinical outcomes were detected by endoscopic and histopathological reports.

The relationship between severity and sex showed that in severe esogastroduodenal lesions, 18.8% of female patients were infected with *cagA*-negative *H. pylori* strains and 10.6% were infected with *cagA*-positive *H. pylori* strains (18.18±11.62 vs 10.63±4.49). In male patients with severe diseases, 25% were infected with *cagA*-negative *H. pylori* strains and 20% were infected with *cagA*-positive *H. pylori* strains (25.0±21.65 vs 20.0±10.32). No statistically significant differences were observed between the groups (FIGURE 3).

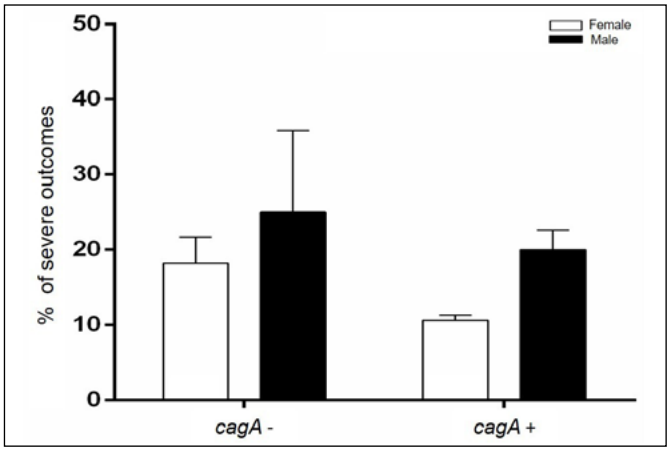



**FIGURE 3.** Role of the *cagA* gene in the severity of disease outcome according to sex. Black bars, male; white bars, female. Test used: Fisher's exact test.

The age of the evaluated patients ranged between 18 and 71 years; the average age was 44.4 years. The analysis of the age groups of patients with non-severe esogastroduodenal injuries with *cagA*-positive and *cagA*-negative gastric specimens showed that there was no statistically significant association in the non-severe injury group (mean ± SD; 14.28±13.43). In patients with severe injuries, no statistically significant association was found between the oncogene and the age group (mean ± SD; 15.08±18.53) (FIGURE 4).

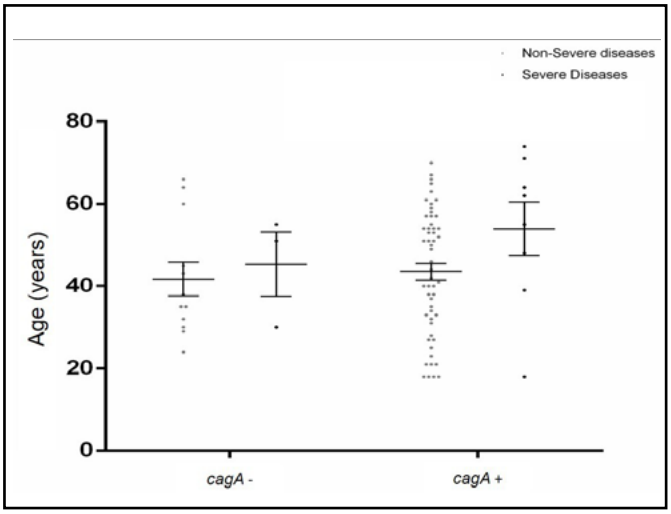



**FIGURE 4.** Evaluation of possible association between the *cagA* oncogene and the severity of esogastroduodenal lesions according to the age group of the patients. Circles filled in black: severe disease; circles filled in gray: non-severe disease. The two-way ANOVA test with Tukey's post-hoc test was used for statistical analysis.

The phylogenetic tree was constructed from the alignment of 16 *cagA* sequences from local strains in the present study; 20 reference sequences obtained from the NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). The sequences used in the construction of the phylogenetic tree were obtained from *H. pylori* strains isolated from patients with severe and non-severe esogastroduodenal lesions.

Phylogenetic analysis showed that local strains were similar. In addition, it was observed that sequences obtained from patients five and six showed a strong genetic association between them and most of the reference sequences. *H. pylori* strains from patients with severe and non-severe pathologies were scattered in the phylogenetic tree. Moreover, our study did not detect any difference in the phylogenetic distribution between severe and non-severe diseases (FIGURE 5).

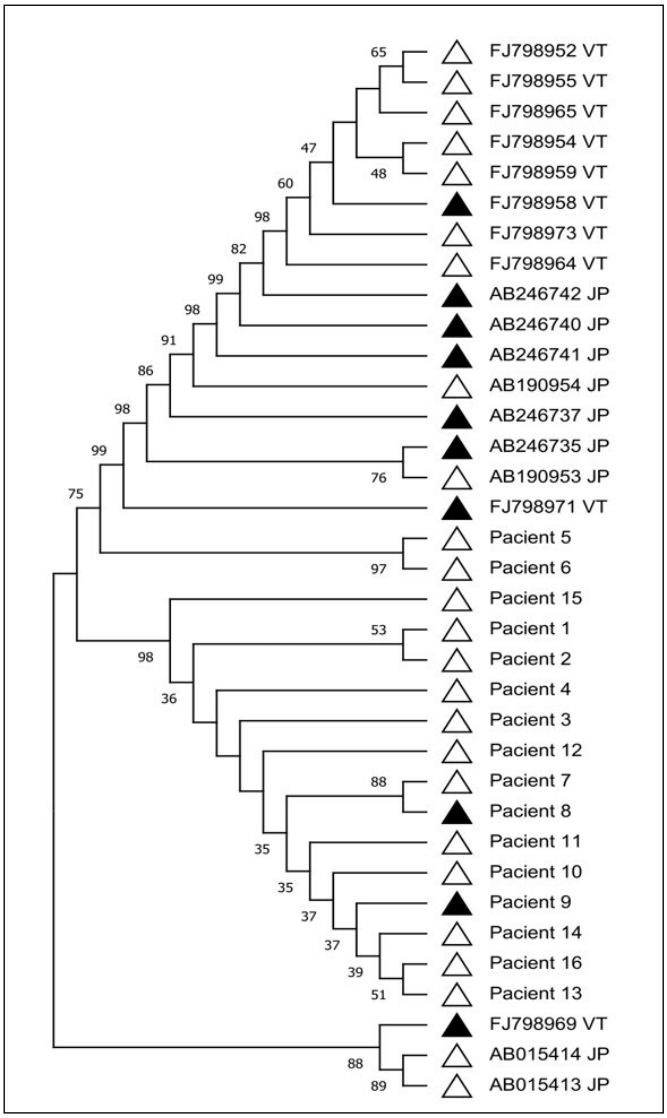



**FIGURE 5.** Phylogenetic tree constructed with sequences of the *H. pylori cagA* gene. Triangles filled in white represent non-severe strains; black triangles represent severe strains. Neighbor joining was built on MEGA 5.0, using maximum parsimony and 1000 repetition bootstraps, based on the 16 local *cagA* sequences and another 10 reference sequences obtained from the GenBank database.

## DISCUSSION

*H. pylori* is an oncobacterium widely recognized globally and it is estimated that approximately half of the world's population is infected by it[2]. Different genotypes of *H. pylori* produce different virulence factors. Our study focused on the characterization of the *H. pylori cagA* virulence gene from gastric biopsy samples which were obtained from patients with diseases of the upper gastrointestinal tract and its relationship with clinical status.

The presence of the *cagA* oncogene is extremely important for the establishment of severe and non-severe esogastroduodenal lesions. In the present study, the *cagA* gene was detected in 80.5% (62/77) of the patients infected with *H. pylori*. This infection rate is considered high compared to other regions of the world. Our results are similar to those of studies conducted in Iran, Greece, and Bulgaria which showed detection rates of 70.0%, 73.0%, and 83.0% respectively[32]. On the other hand, some countries showed lower rates of *cagA* detection. These included Chile (15.2%) and Malaysia (43.0%)[24,33]. The different rate of detection of the *cagA* gene can possibly be attributed to the genetic polymorphism, characteristics of different *H. pylori* strains, as well as the different rates of prevalence of infection in these countries[34].

Several severe and non-severe clinical outcomes were associated with the presence of *cagA*-positive *H. pylori* strains. The association between severity and the oncogene may be due to factors such as the genetic profile of the strains circulating in the region, the sample size of the study, and variations in socioeconomic conditions[24,35]. In the present study, a comparison was made between the different clinical outcomes (severe and non-severe) in patients infected with *H. pylori cagA*-positive strains. There was no statistical difference between the presence of the gene and the severity of esogastroduodenal lesions in the study population. Similar results were found in Chile where research showed that there was no association between the gene and the severity of esogastroduodenal lesions[24]. In contrast, studies conducted in the Brazilian population by Ramis et al., 2010[36] demonstrated a relationship between *cagA* and severe diseases.

In the present study, the most prevalent severe and non-severe injuries were atrophy and gastritis, respectively. Similar results were found in a study by Cavalcante et al. 2012[37] where gastritis was the most prevalent non-severe injury. In contrast, in the study by Oliveira et al. 2003[38] – carried out in South-West Brazil – showed that one of the most prevalent severe injury was gastric cancer. The individual evaluation of several clinical outcomes associated with the *cagA* gene did not demonstrate statistical significance.

To assess the association of the *cagA* gene with the severity of gastric diseases in different countries, a bibliographic survey of global gastric cancer incidence rates was carried out in both sexes and all ages which was standardized by age estimated in 2018 (age-standardised incidence rates, ASIR). The levels of gastric cancer risk were segregated according to the geographical location, in low, medium, and high categories, as per the International Agency for Research on Cancer (IARC) recommendations[39]. As shown in TABLE 4, we observed that in several regions (with a high incidence of gastric cancer), the *cagA* gene was not associated with severity corroborating our data.

The photomicrographs from the present study showed histopathological patterns of severe (atrophy and GA) and non-severe (gastritis and duodenitis) lesions. The mechanisms involved in the genesis of esogastroduodenal diseases associated with *H. pylori* infection have not yet been fully elucidated. However, it is well understood

**TABLE 4.** Association of the *cagA* gene with the risk of severe diseases in areas of low, moderate, or high risk of gastric cancer.

| Continents | Countries | ASIR in both sexes (2018) | *cagA* severity-related | References |
|---|---|---|---|---|
| Africa | South Africa | 4.0 | No | Tanih NF, et al. 2010 |
| | Egypt | 2.8 | No | El-Khlousy M, et al. 2016 |
| | Gambia | 1.4 | Yes | Secka O, et al. 2011 |
| | Ghana | 5.7 | Yes | Archampong TN, et al. 2017 |
| | Morocco | 4.7 | No | Boukhris AS, et al. 2012 |
| | Senegal | 6.8 | Yes | Breurec S, et al. 2012 |
| Asia | Iran | 15.8 | Yes | Bakhti SZ, et al. 2020 |
| | Bangladesh | 5.2 | No | Aftab H, et al. 2017 |
| | China | 20.7 | No | Pinto-Ribeiro I, et al. 2016 |
| | South Korea | 39.6 | Yes | Boonyanugomol W, et al. 2020 |
| | India | 4.5 | No | Jeyamani L, et al. 2018 |
| | Japan | 27.5 | Yes | Matsunari O, et al. 2011 |
| | Malaysia | 5.2 | No | Osman HA, et al. 2015 |
| | Thailand | 3.6 | Yes | Boonyanugomol W, et al. 2020 |
| Latin America | Brazil | 7.9 | Yes | Cavalcante MQF, et al. 2012 |
| | Brazil | 7.9 | Yes | Vinagre RMDF, et al. 2013 |
| | Brazil | 7.9 | No | Gatti LL, et al. 2005 |
| | Chile | 17.8 | No | Paredes-Osses E, et al. 2017 |
| | Colombia | 12.8 | Yes | Watada M, et al. 2011 |
| | Costa Rica | 13.4 | Yes | Molina-Castro S, et al. 2019 |
| | Cuba | 5.7 | No | Feliciano O, et al. 2015 |
| North America | United States | 4.1 | No | Homan M, et al. 2014 |
| | United States | 4.1 | No | Niknam R, et al. 2014 |
| | Dominican Republic | 6.6 | No | Shiota S, et al. 2014 |
| Europe | Spain | 6.6 | Yes | González CA, et al. 2011 |
| | Estonia | 11.4 | No | Andreson H, et al. 2002 |
| | Italy | 7.2 | Yes | Chiarini A, et al. 2009 |
| | Poland | 8.3 | No | Biernat MM, et al. 2014 |
| | Portugal | 11.0 | Yes | Almeida N, et al. 2014 |
| | Russia | 13.3 | No | Momynaliev K, et al. 2003 |

Age-standardised incidence rates were obtained from GLOBOCAN 2018 (http://gco.iarc.fr/today).

that the presence of the bacterium in the gastric mucosa can cause changes in gastric homeostasis[38]. In addition, from phosphorylative and non-phosphorylative pathways, the CagA oncoprotein can induce exacerbated inflammation and GA induction[40].

The prevalence of infection in patients with *cagA*-positive *H. pylori* strains may vary according to the sex of the individual. Studies have shown that these variations may be due to differences in lifestyle between men and women[4]. In the present study, the relationship between the sex of patients and the severity of esogastroduodenal diseases was evaluated. No statistical significance was observed in patients of both sexes. The proportion of female patients in the present study was almost three times higher than that of males. This can be explained by the greater demand for women by basic health services facilitating early detection and effective treatment[41-44].

Through phylogenetic evaluation, it was not possible to segregate the *cagA* sequences isolated from patients with severe and non-severe esogastroduodenal diseases. In part, this can be explained by partial sequencing of the virulence gene, which was used in the analysis.

The similarity between the sequences of patients five and six suggests that at some point, these patients may have been infected by the same strain from a different region. In addition, it is possible that they may have resided in the same house or city so that the same strain infected both of them through different routes of transmission. It is also possible that, in addition to biogeographic similarities, both share the same non-severe clinical outcomes. The different mechanisms underlying the pathogenesis of these strains can be explained by the genetic variations between them.

The screening of a large number of samples would provide a better understanding of the possible variations in the *cagA* gene. Hence, a more comprehensive phylogeographic analysis is needed to elucidate the impact of genetic heterogeneity of *H. pylori* on dyspeptic patients in Brazil. Although this study involved a small sample number and partial gene sequences, this is the first Brazilian study that used partial *cagA* sequences from *H. pylori* to build a phylogenetic tree.

The present study has high scientific relevance, since information about the mechanisms involved in the parasite-host relationship is scarce in the researched population. Some limitations were observed in the study, such as the low sample size of patients with severe esogastroduodenal lesions and the partial sequencing of the *cagA* gene. Despite the limitations, the study opens perspectives for the development of personalized medicine in the Brazilian territory.

## CONCLUSION

*H. pylori* infection is highly prevalent in patients with dyspepsia in central Brazil. The *cagA* oncogene was not considered to be a molecular marker of the severity of esogastroduodenal lesions. Through phylogenetic analyses, it was not possible to segregate strains from patients with severe and non-severe diseases. This study provides additional insight into the *cagA* profiles of the different strains of *H. pylori* and opens perspectives for studies with a larger sample size of esogastroduodenal disease patients as well as research that aims to assess the association between genetic variability and clinicopathological outcomes.

## ACKNOWLEDGMENTS

We would like to thank the Genetics and Biodiversity Laboratory at the Federal University of Goiás. We also like to thank Editage for English language editing.

### Authors' contribution

Oliveira AKS: responsible for the study, performed data collection, execution of molecular techniques, text writing and statistical analysis. Silva LLL: study collaborator, responsible for intellectual contribution, data analysis and execution of experiments. Miguel MP: responsible for assisting in histopathological analysis and assessments. Blanco AJV: collaboration in phylogenetic analyzes. Carneiro LC: intellectual contribution to the study. Barbosa MS: responsible for guidance in the execution of molecular techniques, manuscript correction, data analysis and intellectual contribution.

### Orcid

Ana Karoline Silva Oliveira: 0000-0001-7576-5735.
Lucas Luiz de Lima Silva: 0000-0001-6510-4175.
Marina Pacheco Miguel: 0000-0002-6639-2452.
Angel José Vieira Blanco: 0000-0003-2712-7952.
Lilian Carla Carneiro: 0000-0003-4067-1506.
Mônica Santiago Barbosa: 0000-0001-6964-5219.

Oliveira AKS, Silva LLL, Miguel MP, Blanco AJV, Carneiro LC, Barbosa MS. Gene de virulência *cagA* de *Helicobacter pylori* e doenças esogastroduodenais severas: existe uma associação? Arq Gastroenterol. 2021;58(4):468-75.

**RESUMO – Contexto –** *Helicobacter pylori* coloniza aproximadamente metade da população humana mundial. A presença do microrganismo na mucosa gástrica está associada a um risco aumentado de adenocarcinoma gástrico, linfoma gástrico e úlcera péptica. No Brasil, a alta prevalência de infecção por *H. pylori* é um grave problema de saúde. Os fatores de virulência de *H. pylori* estão associados a risco aumentado de distúrbios gastrointestinais severos. O gene *cagA* codifica um antígeno associado à citotoxina A (CagA) que está envolvido na patogenicidade bacteriana. As cepas de *H. pylori* portadoras da ilha de patogenicidade *cag* (*cag*-PAI) estão significativamente associadas a desfechos clínicos severos e alterações histopatológicas. **Objetivo –** O presente estudo tem como objetivo investigar a prevalência do gene *cagA* entre isolados de *H. pylori* de pacientes com diferentes desordens gástricas, bem como verificar sua associação com desfechos clínicos. Além disso, a análise filogenética foi realizada em cepas de *H. pylori cagA*-positivas de pacientes com doenças severas e não severas. **Métodos –** Amostras gástricas foram coletadas por meio de biópsia gástrica de 117 pacientes com diferentes doenças esogastroduodenais. O DNA foi extraído das amostras e utilizado para amplificar os fragmentos gênicos correspondentes aos genes RNA ribossomal 16S e *cagA,* através da reação em cadeia da polimerase. Os produtos da reação em cadeia da polimerase de amostras selecionadas positivas para *cagA* foram sequenciados e as sequências foram alinhadas com sequências de referência do *National Center for Biotechnology Information* (NCBI) (Bethesda/EUA). As análises filogenéticas foram realizadas a partir do sequenciamento e construção da árvore filogenética. **Resultados –** *H. pylori* foi detectado em 65,9% (77/117) dos pacientes brasileiros com diferentes distúrbios gastroduodenais. No total, 80,5% (62/77) das cepas foram *cagA*-positivas. As idades dos pacientes com cepas *cagA*-positivas (15 homens e 47 mulheres) variaram de 18 a 74 anos. As lesões foram categorizadas como não severas e severas de acordo com o laudo endoscópico e histopatológico. A lesão esogastroduodenal não severa mais prevalente foi gastrite 54/77 (70,12%), seguida de esofagite 12/77 (15,58%) e duodenite 12/77 (15,58%). Em contraste, as lesões severas mais prevalentes foram atrofia 7/77 (9,09%), seguida de metaplasia 3/77 (3,86%) e adenocarcinoma gástrico 2/77 (2,59%). As análises filogenéticas realizadas com as sequências parciais do gene *cagA* obtidas de cepas locais foram agrupadas no mesmo clado. Nenhuma diferença na distribuição filogenética foi detectada entre doenças severas e não severas. **Conclusão –** O gene *cagA* é altamente prevalente entre isolados de *H. pylori* de lesões gástricas em pacientes brasileiros. A presença do gene *cagA* não foi considerada um marcador de severidade das lesões esogastroduodenais no presente estudo. Este é o primeiro estudo a investigar a estrutura filogenética da população de cepas de *H. pylori* em uma capital brasileira. Esses resultados irão contribuir para o entendimento sobre o desfecho clínico da infecção por *H. pylori*.

**Palavras-chave –** Epidemiologia molecular; filogenia; fatores de virulência; gene bacteriano.

## REFERENCES


1. Warren JR, Marshall B. Unidentified Curved Bacilli on Gastric Epithelium in Active Chronic Gastritis. Lancet. 1983;321:1273-5.
2. Zamani M, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, et al. Systematic review with meta-analysis: the worldwide prevalence of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2018;47:868-76.
3. Zamani M, Vahedi A, Maghdouri Z, Shokri-Shirvani J. Role of food in environmental transmission of *Helicobacter pylori*. Casp J Intern Med. 2017;8:146-52.
4. Basílio ILD, Catão MDFC, Carvalho JDDS, Freire-Neto FP, Ferreira LC, Jerônimo SMB. Risk factors of *Helicobacter pylori* infection in an urban community in Northeast Brazil and the relationship between the infection and gastric diseases. Rev Soc Bras Med Trop. 2018;51:183-9.
5. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153:420-9.
6. Kamboj AK, Cotter TG, Oxentenko, Amy S. Oxentenko M. *Helicobacter pylori*: The Past, Present, and Future in Management. Mayo Clin Proc. 2017;92:599-604.
7. Cortes MCC, Yamakawa A, Casingal CR, Fajardo LSN, Juan MLG, De Guzman BB, et al. Diversity of the *cagA* gene of *Helicobacter pylori* strains from patients with gastroduodenal diseases in the Philippines. FEMS Immunol Med Microbiol. 2010;60:90-7.
8. Junior LR, Miler C, Geocze S, Chehter L. *Helicobacter pylori* eradication does not influence gastroesophageal reflux disease: a prospective, parallel, randomized, open-label, controlled trial. Arq Gastroenterol. 2012;49:56-63.
9. Scida S, Russo M, Miraglia C, Leandro G, Franzoni L, Meschi T, et al. Relationship between *Helicobacter pylori* infection and GERD. Acta Biomed. 2018;89:40-3.
10. Liu L, Gao H, Wang H, Zhu K, Yu W, Zhang Y, et al. Comparison of esophageal function tests to investigate the effect of *Helicobacter pylori* infection on gastroesophageal reflux disease (GERD). Med Sci Monit. 2018;24:4791-7.
11. Araújo-Filho I, Brandão-neto J, Araújo L, Pinheiro M, Medeiros AC. Prevalence of *Helicobacter pylori* infection in advanced carcinoma. Arq Gastroenterol. 2006;43:288-92.
12. Zhang RG, Duan GC, Fan QT, Chen SY. Role of *Helicobacter pylori* infection in pathogenesis of gastric carcinoma. World J Gastrointest Pathophysiol. 2016;7:97.
13. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. 1997;388:539-47.
14. Pandya HB, Agravat HH, Patel JS. Prevalence of specific *Helicobacter pylori* CagA, VacA, IceA, UreC genotypes and its clinical relevance in the patients with acid-peptic diseases. J Clin Diagnostic Res. 2017;11:23-6.
15. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nat Rev Cancer. 2004;4:688-94.
16. Braga LLBC, Oliveira MAA, Gonçalves MHRB, Chaves FK, Benigno TGS, Gomes AD, et al. CagA phosphorylation EPIYA-C motifs and the vacA i genotype in *Helicobacter pylori* strains of asymptomatic children from a high-risk gastric cancer area in northeastern Brazil. Mem Inst Oswaldo Cruz. 2014;109:1045-9.
17. Tegtmeyer N, Wessler S, Necchi V, Rohde M, Harrer A, Rau TT, et al. *Helicobacter pylori* employs a unique basolateral type IV secretion mechanism for CagA delivery. Cell Host Microbe. 2017;22:552-60.
18. Nishizawa T, Suzuki H. Gastric carcinogenesis and underlying molecular mechanisms: *Helicobacter pylori* and novel targeted therapy. Biomed Res Int. 2014;2015.
19. Miftahussurur M, Yamaoka Y. *Helicobacter pylori* virulence genes and host genetic polymorphisms as risk factors for peptic ulcer disease. Expert Rev Gastroenterol Hepatol. 2015;9:1535-47.
20. Lind J, Backert S, Pfleiderer K, Berg DE, Yamaoka Y, Sticht H, et al. Systematic analysis of phosphotyrosine antibodies recognizing single phosphorylated EPIYA-motifs in CagA of western-type *Helicobacter pylori* strains. PLoS One. 2014;9:14-8.
21. Rugge M. Gastric cancer risk in patients with *Helicobacter pylori* infection and following its eradication. Gastroenterol Clin North Am. 2015;44:609-24.
22. Matos JI, Sousa HAC, Marcos-Pinto R, Dinis-Ribeiro M. *Helicobacter pylori* CagA and VacA genotypes and gastric phenotype: A meta-analysis. Eur J Gastroenterol Hepatol. 2013;25:1431-41.
23. Coelho LGV, Marinho JR, Genta R, Ribeiro LT, Passos M do CF, Zaterka S, et al. IVth Brazilian consensus conference on *Helicobacter pylori* infection. Arq Gastroenterol. 2018;55:97-121.
24. Giemsa G. A simplification and perfection of my methylene azure-methylene blue-eosin staining method for the political purpose of Romanowsky-Nochtenschen chromatin staining. Zentralbl Bakteriol. 1904;308-11.
25. Stolte M, Meining A. The Updated Sydney System: Classification and grading of gastritis as the basis of diagnosis and treatment. Can J Gastroenterol. 2001; 15:591-8.
26. Paredes-Osses E, Sáez K, Sanhueza E, Hebel S, González C, Briceño C, et al. Association between *cagA*, vacAi, and dupA genes of *Helicobacter pylori* and gastroduodenal pathologies in Chilean patients. Folia Microbiol (Praha). 2017;62:437-44.
27. Bellolio E, Riquelme I, Riffo-Campos AL, Rueda C, Ferreccio C, Villaseca M, et al. Assessment of gastritis and gastric cancer risk in the Chilean population using the OLGA system. Pathol Oncol Res. 2019;25:1135-42.
28. Luscenti RS, Gatti LL. Molecular diagnosis of *Helicobacter pylori* infection in the gastric mucosal. Rev Para Med. 2008;22:21-6.
29. Dadashzadeh K, Peppelenbosch MP, Adamu AI. *Helicobacter pylori* pathogenicity factors related to gastric cancer. Can J Gastroenterol Hepatol. 2017;2017:1-6.
30. Sanger F, Coulson AR, Friedmann T, Air GM, Barrell BG, Brown NL, et al. The nucleotide sequence of bacteriophage ϕX174. J Mol Biol. 1978;125:225-46.
31. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018; 35:1547-9.
32. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2014;19(S1):1-5.
33. Leja M, Grinberga-Derica I, Bilgilier C, Steininger C. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2019;24(S1):2-6.
34. Román-Román A, Martínez-Santos VI, Castañón-Sánchez CA, Albañil-Muñoz AJ, González-Mendoza P, Soto-Flores DG, et al. CagL polymorphisms D58/K59 are predominant in *Helicobacter pylori* strains isolated from mexican patients with chronic gastritis. Gut Pathog. 2019;11:1-11.
35. Abu-Taleb AMF, Abdelattef RS, Abdel-Hady AA, Omran FH, El-Korashi LA, Abdel-Aziz El-Hady H, et al. Prevalence of *Helicobacter pylori cagA* and *iceA* genes and their association with gastrointestinal diseases. Int J Microbiol. 2018;2018.
36. Ramis IB, Fonseca TL, de Moraes EP, Fernandes MS, Mendoza-Sassi R, Rodrigues O, et al. Molecular Basis of pathogenicity in *Helicobacter* pylori clinical isolates. J Clin Microbiol. 2010;48:3776-8.
37. Cavalcante MQ, Silva CI, Braga-Neto MB, Fialho AB, Nunes Fialho A, Barbosa AM, et al. *Helicobacter pylori vacA* and *cagA* genotypes in patients from northeastern Brazil with upper gastrointestinal diseases. Mem Inst Oswaldo Cruz. 2012;107:561-3.
38. Oliveira AG, Santos A, Guerra JB, Rocha GA, Rocha AM, Oliveira CA, et al. babA2- and *cagA*-positive *Helicobacter pylori* strains are associated with duodenal ulcer and gastric carcinoma in Brazil. J Clin Microbiol. 2003;41:3964-6.
39. Estimated age-standardized incidence rates (World) in 2018, all cancers, both sexes, all ages [Internet]. Lyon, France: International Agency for Research on Cancer; 2018 [cited 2020 Mar 1]. Available from: http://gco.iarc.fr/today.
40. Gomes LI, Rocha GA, Rocha AMC, Soares TF, Oliveira CA, Bittencourt PFS, et al. Lack of association between *Helicobacter pylori* infection with dupA-positive strains and gastroduodenal diseases in Brazilian patients. Int J Med Microbiol. 2008;298:223-30.
41. Backert S, Tegtmeyer N. Type iv secretion and signal transduction of *Helicobacter pylori cagA* through interactions with host cell receptors. Toxins (Basel). 2017;9:115.
42. Ferro A, Morais S, Pelucchi C, Dierssen-Sotos T, Martín V, López-Carrillo L, et al. Sex differences in the prevalence of *Helicobacter pylori* infection: An individual participant data pooled analysis (StoP Project). Eur J Gastroenterol Hepatol. 2019;31:593-8.
43. Levorato CD, de Mello LM, da Silva AS, Nunes AA. Factors associated with the demand for health services from a gender-relational perspective. Cienc e Saude Coletiva. 2014;19:1263-74.
44. Ibrahim A, Morais S, Ferro A, Lunet N, Peleteiro B. Sex-differences in the prevalence of *Helicobacter pylori* infection in pediatric and adult populations: Systematic review and meta-analysis of 244 studies. Dig Liver Dis. 2017; 49:742-9.

ORIGINAL ARTICLE

AG-2021-76
doi.org/10.1590/S0004-2803.202100000-86

# Major depressive disorder is associated with type 2 diabetes in patients with chronic hepatitis C infection

Luciana Rodrigues da **CUNHA**[1,2], Maria Carolina Magalhães de **CASTRO**[1], Gabriela Silva **DUARTE**[1], Graziela Cançado e **NASCIMENTO**[1], Gifone Aguiar **ROCHA**[3] and Luciana Diniz **SILVA**[1,4]

Received: 9 April 2021
Accepted: 11 June 2021

**ABSTRACT** – **Background** – Major depressive disorder (MDD) is commonly reported in patients with chronic hepatitis C (CHC); however, the factors behind the co-occurrence of these conditions have not been completely clarified yet. **Objective** – We aimed to evaluate the frequency of mental disorders in CHC patients and to investigate variables associated with MDD. **Methods** – CHC patients (n=151) attending a referral Centre for hepatitis were evaluated using the Mini-International Neuropsychiatry Interview and the Cut-Annoyed-Guilty-Eye (CAGE) Questionnaire. Multivariate analysis was used to evaluate independent covariates associated with current MDD. **Results** – Seventy-six (50.3%) patients had, at least, one current psychiatric diagnosis with MDD (33.1%) being the most common. Current MDD was independently associated with age (≤50 yr.) (OR=2.57; 95%CI=1.25–5.29; *P*=0.01) and type 2 diabetes mellitus (OR=2.80, 95%CI=1.17–6.70; *P*=0.02). Cirrhosis was associated with type 2 diabetes mellitus (OR=5.09; 95%CI=1.73–15.04; *P*=0.03) and current alcohol abuse/dependence (OR=2.54; 95%CI=1.04–6.22; *P*=0.04). **Discussion** – MDD is associated with type 2 diabetes in CHC patients. Even in the direct-acting antivirals (DAAs) era, characterized by great perspectives for the first ample cure of a chronic viral infection, we should ensure that the screening for psychiatric disorders takes place in the course of routine clinical care of patients chronically infected with hepatitis C virus.

**Keywords** – Hepatitis C; chronic psychiatric disorders; major depressive disorder; anxiety disorder; type 2 diabetes mellitus; alcohol abuse/dependence; non-alcohol drug abuse/dependence; cirrhosis.

## INTRODUCTION

Hepatitis C virus (HCV) blood screening and the revolutionary development of direct-acting antivirals (DAAs) paved the way for saving millions of lives around the world[1,2]. The 2020 Nobel Prize in Physiology or Medicine honours the hepatitis C discoverers[1,2]. It should be highlighted that DAAs have been offering great perspectives for the first ample cure of a chronic infection in human, i.e., a sustained viral response rates that surpass 95.0%[3,4].

Although outstanding changes in the management of patients with chronic hepatitis C (CHC), the frequency of advanced liver disease and hepatic related deaths are expected to rise around the world over the next 15 years[5]. Furthermore, most countries have been focusing on treating patients in whom the HCV infection was already diagnosed without robust strategies to screen new hepatitis C cases[6].

Approximately 80.0% of acutely infected patients develop CHC, of whom, 20.0% will progress to liver cirrhosis and/or carcinoma hepatocellular after 2/3 decades of viral infection[7]. Almost one quarter of all cases of hepatic cirrhosis and hepatocellular carcinoma worldwide were identified in individuals with CHC, which accounted for 400,000 deaths per year in 2015[8].

In addition, several HCV-associated comorbidities have significantly contributed the health burden related to HCV. Among them, type 2 diabetes, insulin resistance, atherosclerosis, mixed cryoglobulinemia vasculitis, lymphoproliferative disorders, renal disease, sicca syndrome, rheumatoid arthritis-like polyarthritis, and autoantibody production[9]. An augmented prevalence of psychiatric disorders and symptoms, particularly fatigue, depression, anxiety, bipolar disorder and schizophrenia, has also been verified in individuals with CHC in comparison with general population[10]. Otherwise, increased risk for acquiring HCV has been identified in psychiatric populations. The prevalence of HCV infection among patients with chronic mental illnesses ranges from 0.4% to 38.0%[11].

Although the development of DAAs has been causing immense modifications in the treatment of CHC[3,4], these innovations have a high cost[3,4] and represent an obstacle for many health systems around the world. Recently, Hengstet et al. (2016) demonstrated that DAA-induced viral clearance does not completely restore the altered cytokine and chemokine milieu in CHC patients[12]. Furthermore, in previous studies, the authors observed the persistence of neuropsychiatric impairment in patients with CHC despite the clearance of the virus[13,14].

Therefore, the aim of the present study was to identify the frequency of mental disorders in outpatients with CHC. We further investigated the association between demographic, clinical, lifestyle, biochemical, and virological variables and current major depressive disorder (MDD).

Declared conflict of interest of all authors: none
Disclosure of funding: supported by *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG) and *Pró-Reitoria de Pesquisa da Universidade Federal de Minas Gerais*, Belo Horizonte, MG, Brasil.
[1] Universidade Federal de Minas Gerais, Faculdade de Medicina, Ambulatório de Hepatites Virais, Instituto Alfa de Gastroenterologia, Belo Horizonte, MG, Brasil. [2] Universidade Federal de Minas Gerais, Programa de Pós-Graduação em Neurociências, Instituto de Ciências Biológicas, Belo Horizonte, MG, Brasil. [3] Universidade Federal de Minas Gerais, Faculdade de Medicina, Laboratório de Pesquisa em Bacteriologia, Belo Horizonte, MG, Brasil. [4] Universidade Federal de Minas Gerais, Faculdade de Medicina, Departamento de Clínica Médica, Belo Horizonte, MG, Brasil. Corresponding author: Luciana Diniz Silva. E-mail: lucianadinizsilva@ufmg.br

## METHODS

### Study population

From 2013 to 2018, we, prospectively, invited 178 consecutive outpatients with CHC attending the viral hepatitis outpatient Clinic of the University Hospital of *Universidade Federal de Minas Gerais* (UFMG), Belo Horizonte, Brazil. The Viral Hepatitis Outpatient Clinic is an outpatient care ambulatory of a metropolitan tertiary teaching hospital that admits patients for the treatment of chronic viral hepatitis B or C.

### Patients

The study was approved by the Ethics Committee of Federal University of Minas Gerais, UFMG (ETIC 0631.0.203.000-09).

The patients with CHC were screened for other hepatic diseases. The exclusion criteria were pregnancy, hepatic encephalopathy, hepatitis B virus (HBV)/HCV or HCV/human immunodeficiency virus (HIV) co-infection, recurrent MDD, current antiviral or antidepressant treatment, use of non-steroidal anti-inflammatory drugs or corticosteroids, and the presence of advanced disease such as chronic kidney disease, heart failure, chronic pulmonary disease, and neoplasia, including hepatocellular carcinoma.

The diagnosis of cirrhosis was based on standard clinical, biochemical, radiological, and histological parameters[15]. Compensated cirrhosis was defined as the absence of variceal bleeding, ascites, and oedema on physical examination; jaundice or symptomatic encephalopathy; and decompensated cirrhosis as the presence of any of these complications[16]. The Child-Turcotte-Pugh[17] was scored for all compensated cirrhotic patients.

Twenty-seven patients were not included because one spontaneously cleared the virus, one had hepatic encephalopathy; one had HBV/HCV co-infection and eight had chronic kidney disease; four refused to participate and twelve failed to complete the questionnaire. One hundred and fifty-one patients remained in the study. The patients were from a similar socioeconomic level, as assessed by a previously validated questionnaire[18], which was based on income and educational level.

All included subjects underwent a psychiatric evaluation, by assessing the psychiatric history and mental status. Thereafter, the Brazilian version of the Mini International Neuropsychiatric Interview (M.I.N.I. Plus) was administered[19]. This instrument is a semi structured diagnostic interview comprising the primary Axis I Disorders of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) and the International Classification of Diseases (ICD-10), which was designed for clinical practice and research in psychiatric and primary care settings[20]. The diagnosis of the first episode of MDD and alcohol misuse was performed according to the ICD-10 and the DSM-IV (American Psychiatric Association, 1994)[20]. Regarding alcohol use, the individuals answered a self-administered questionnaire. The first part was the standard Cut-Annoyed-Guilty-Eye (CAGE) Questionnaire that consists of four items asking whether patients have ever tried to cut down on drinking, gotten annoyed with people asking about their drinking, felt guilty about drinking, or had an eye opener. The presence of two or more positive answers to the CAGE Questionnaire suggests an alcohol problem[21]. The second part consisted in responding a detailed questionnaire regarding past and current alcohol consumption in terms of amount, frequency, and duration of use.

The diagnosis of type 2 diabetes mellitus was based on documented use of oral hypoglycaemic medication or insulin; random plasma glucose levels ≥200 mg/dL in the presence of classic symptoms of hyperglycaemia or hyperglycaemic crisis; fasting plasma glucose ≥126 mg/dL on two occasions; 2 hours plasma glucose ≥200 mg/dL during oral glucose tolerance test or haemoglobin A1c ≥6.5% on two occasions[22].

### Laboratory parameters

Venous blood samples were obtained from each patient for HCV diagnosis and biochemical evaluation. Antibody to HCV was investigated by a commercial enzyme-linked immunosorbent assay (ELISA) (AxSYM HCV, version 3.0; Abott GmbH & Co., Wiesbaden, Germany), and HCV status was confirmed by a qualitative polymerase chain reaction for HCV RNA (AMPLICOR 2.0 assay; Roche Diagnostics, Branchburg, NJ) according to the manufacturers' instructions. HCV genotyping and viral load were determined by using a commercial test (Cobas TaqMan HCV test V.2.0; Roche Molecular Systems, Pleasanton, CA) and a line probe assay (VERSANT HCV genotyping assays; Bayer's Diagnostic Corporation, Tarrytown, NY), respectively. The assays were carried out according to the manufacturers' recommendations. Viral load and HCV genotyping are only performed in patients who fulfil the criteria for antiviral therapy adopted in our service, being available in 74.8% of the included patients.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum albumin were evaluated by routine laboratory methods.

### Data analyses

Data were analysed using the Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, IL).

Descriptive statistics were used to provide information regarding the demographic and disease-specific characteristics. The Shapiro-Wilk test was used to evaluate whether the data were normally distributed. For the comparison of the percentages and the median, the asymptotic Pearson's chi-square test and the Mann-Whitney U test were used, respectively.

Logistic regression models were created to quantify independent associations between the psychiatric diagnosis, MDD, and the following variables: sociodemographic, clinical and lifestyle data. All variables with $P \leq 0.25$ in the univariate analysis were included in the full models of logistic regression using the additive and subtractive stepwise approach. Odds ratio (OR) and 95.0% confidence interval (95% CI) were used as an estimate of the risk. The Hosmer-Lemeshow test was used to assess the adequacy of the models. Variables that had more than 10.0% missing data were not selected for the models of multivariate analysis.

Logistic regression models were created to quantify the independent association between the cirrhosis and the following variables: clinical comorbidities, and psychiatric disorders data, adjusted by age and sex.

*P* values ≤0.05 were considered significant.

## RESULTS

### Characteristics of the study population

The sociodemographic, clinical, virological, biochemical and lifestyle data of the CHC patients with (n=76) and without (n=75) current psychiatric disorders are shown in TABLE 1. Patients with psychiatric disorders were younger than those without these disorders ($P$=0.03). The frequency of current smoking was significantly

**TABLE 1**. Sociodemographic, clinical, virological, biochemical and lifestyle data of the chronic hepatitis C patients with (n=76) and without (n=75) current psychiatric disorders.

| Variables | Current psychiatric disorders | | |
|---|---|---|---|
| | Present | Absent | *P* |
| Sociodemographic data n (%) | | | |
| Sex | | | 0.47 |
| Male | 33 (43.4) | 37 (49.3) | |
| Female | 43 (56.6) | 38 (50.7) | |
| Age (years) median (IQR)* | 50.5 (15) | 55.0 (15) | 0.03 |
| Marital status n (%) | | | 0.10 |
| Single | 18 (23.7) | 24 (32.0) | |
| Married | 46 (60.5) | 45 (60.0) | |
| Separated/divorced | 6 (7.9) | 3 (4.0) | |
| Widowed | 6 (7.9) | 3 (4.0) | |
| Educational level n (%) | | | 0.12 |
| ≤9 years | 43 (56.6) | 33 (44.0) | |
| >9 years | 33 (43.4) | 42 (56.0) | |
| Total household income/month n (%)[a] | | | 0.29 |
| ≤1 minimum wage | 17 (22.4) | 12 (16.0) | |
| 1.001 – 3 minimum wages | 35 (46.1) | 32 (42.7) | |
| 3.001 – 5 minimum wages | 13 (17.1) | 20 (26.7) | |
| >5 minimum wages | 11 (14.5) | 11 (14.7) | |
| Clinical Comorbidities n (%) | | | |
| Type 2 diabetes mellitus | 17 (22.4) | 11 (14.7) | 0.23 |
| Hypertension | 25 (32.9) | 30 (40.0) | 0.37 |
| Liver disease stage n (%) | | | 0.30 |
| Chronic hepatitis | 63 (82.9) | 57 (76.0) | |
| Cirrhosis | 13 (17.1) | 18 (24.0) | |
| Cirrhosis stage – Child A/B/C | 9/3/1 | 14/3/1 | 0.83 |
| Virological parameters | | | |
| Viral load[b] | | | |
| HCV-RNA (IU/mL) median (IQR) | 698,000 (2,014,842) | 580,500 (1,209,211) | 0.77 |
| Genotype (1a, 1b, 3a) n (%)[b] | 48 (92.3) | 60 (98.4) | 0.26 |
| Genotype others n (%)[b, c] | 4 (7.7) | 1 (1.6) | |
| Biochemical parameters median (IQR) | | | |
| ALT (U/L) | 61 (50.0) | 60 (50.0) | 0.63 |
| AST (U/L) | 56 (43.5) | 63 (46) | 0.60 |
| Albumin (g/dL) | 4.3 (0.5) | 4.3 (0.8) | 0.51 |
| Lifestyle data n (%) | | | |
| Alcohol use | | | |
| At least once/adult lives | 65 (85.5) | 60 (80.0) | 0.37 |
| Current use | 24 (31.6) | 12 (16.0) | 0.06 |
| Positive CAGE screen[d]* | 21 (28.8) | 12 (15.7) | 0.03 |
| Smoking | | | |
| Previous | 41 (54.7) | 38 (52.1) | 0.75 |
| Current use* | 18 (24.3) | 8 (10.7) | 0.03 |

a: the Brazilian national minimum wage was R$ 724.00 ($ 211.70) (minimum wage law: 8.166/2013) in 2014. b: data from 113 (74.8%) patients; c: 1a + 1b, 2b, 2a + 2b; d: CAGE (cut down on drinking, gotten angry with people asking about their drinking, felt guilty about drinking or had an eye opener). n: number of subjects; IQR, interquartile range; CHC: chronic hepatitis C; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CAGE: Cut-Annoyed-Guilty-Eye. *$P \leq 0.05$.

higher ($P$=0.03) and current alcohol use tended to be significantly more frequent ($P$=0.06) in patients with psychiatric disorders. Positive CAGE screen was associated with psychiatric disorders in patients with CHC ($P$=0.03). At the time of the study, no patient was receiving interferon-based drug therapy or DAAs.

## Psychiatric disorders

Prevalence of current and lifetime psychiatric disorders are summarized in TABLE 2. The psychiatric diagnoses included six categories, anxiety disorders, mood disorders, psychotic disorders, alcohol use disorders, non-alcohol substance use disorders (abuse and/or dependence) and other psychiatric disorders. Fifty of 151 CHC patients (33.1%) had current MDD, i.e., 50 of 76 (65.8%) patients diagnosed with current psychiatric disorder. MDD was the most common psychiatric disorder observed among these patients. Seventeen of the 151 (11.2%) patients had at least one current anxiety disorder (TABLE 2).

**TABLE 2**. Current and lifetime psychiatric disorders diagnosed in patients with chronic hepatitis C (n=151).

| Psychiatric diagnoses | Current n (%) | Lifetime n (%) |
|---|---|---|
| Any psychiatric disorders | 76 (100.0) | 102 (100.0) |
| Anxiety disorders | | |
| Generalized anxiety | 17 (22.4) | 17 (16.7) |
| Panic | 4 (5.3) | 4 (3.9) |
| Obsessive compulsive | 2 (2.6) | 2 (2.0) |
| Post-traumatic stress | 1 (1.3) | 1(1.0) |
| Other anxiety disorder | 15 (19.7) | 15 (14.7) |
| Mood disorders | | |
| Major depressive | 50 (65.8) | 27 (26.5) |
| Bipolar | 1 (1.3) | 1 (1.0) |
| Psychotic disorders | 6 (7.9) | 6 (5.9) |
| Substance use disorders (abuse and/or dependence) | | |
| Alcohol | 17 (22.4) | 46 (45.0) |
| Non-alcohol | 7 (9.2) | 36 (35.3) |
| Other psychiatric disorders | 2 (2.6) | 2 (2.0) |

Lifetime alcohol use disorder was verified in 46/151 (30.5%) of the patients. Twenty-seven patients with drug addiction (27/36; 75.0%; $P$<0.001) meeting criteria for alcohol abuse and/or dependence. Cocaine, crack and cannabis were the most commonly used illicit drugs. Alcohol abuse ($P$<0.001) and non-alcohol substance abuse ($P$=0.005) were more frequently observed in men than in women (58.6% vs 11.1%) and (34.3% vs 14.8%), respectively.

Individuals who received diagnosis of alcohol abuse and/or dependence (49.3±8.3 yr.) were younger ($P$=0.002) than those without this psychiatric disorder (54.6±11.9 yr.).

### Factors associated with current major depressive disorder in patients with chronic hepatitis C

In the univariate analysis, age, educational level, type 2 diabetes mellitus, positive CAGE screen as well as current smoking use were selected. Younger age and type 2 diabetes mellitus remained significantly and independently associated with current major depressive disorder in the multivariate analysis (TABLE 3).

**TABLE 3.** Variables associated with current major depressive disorder in patients with chronic hepatitis C.

| Variables | Univariate analysis | Multivariate analysis | | |
|---|---|---|---|---|
| | *P* | OR | 95%CI | *P* |
| Sociodemographic data | | | | |
| Age (≤50 years) | 0.02 | 2.57 | 1.25–5.29 | 0.01 |
| Marital status | 0.43 | – | – | – |
| Educational level | 0.15 | 1.39 | 0.66–2.91 | 0.38 |
| Clinical comorbidities | | | | |
| Type 2 diabetes mellitus | 0.05 | 2.80 | 1.17–6.70 | 0.02 |
| Lifestyle data | | | | |
| Positive CAGE screen[a] | 0.11 | 1.31 | 0.55–3.11 | 0.55 |
| Current smoking use | 0.13 | 1.77 | 0.72–4.33 | 0.21 |

a: CAGE (cut down on drinking, gotten angry with people asking about their drinking, felt guilty about drinking, or had an eye opener). CAGE: Cut-Annoyed-Guilty-Eye.

### Factors associated with cirrhosis in patients with chronic hepatitis C

In the univariate analysis, type 2 diabetes mellitus, current anxiety disorders and current alcohol abuse and/or dependence were selected. In the multivariate analysis, type 2 diabetes mellitus and current alcohol abuse/dependence remained significantly and independently associated with cirrhosis (TABLE 4).

### HCV viral load, HCV genotype and the psychiatric disorders in patients with hepatitis C

Neither the viral load nor the HCV genotype was associated with the psychiatric disorders in patients with hepatitis C.

## DISCUSSION

To the best of our knowledge, this is the first study to demonstrate that MDD is associated with type 2 diabetes in non-cirrhotic and compensated cirrhotic patients with CHC. Growing body of evidence has shown that HCV infection not only causes hepatic cirrhosis and hepatocarcinoma but also is associated with several extrahepatic manifestations, including neuropsychiatric disorders. Yovtcheva et al. (2001) in a pioneering study, demonstrated a strong association between HCV infection and psychiatric disorders[23]. Remarkably, the 2020 Nobel Prize in Physiology or Medicine highlighted the critical significance of hepatitis C in world health[24]. Even in the face of development of safe, well tolerated, and highly effective direct-acting antiviral agents that promote a cure of the chronic infection in more than 95.0% of patients[3,4,24], several aspects of HCV-related psychiatric illnesses have not been completely clarified.

A strength of our study was that we studied only patients with CHC without decompensated conditions as encephalopathy, ascites, gastrointestinal bleeding, and infections, which might interfere in the brain function. In addition, the depressive disorder was diagnosed by a psychiatrist, using a detailed psychiatric approach in combination with a semi-structured interview validated in Portuguese[19] and widely used by Brazilian researchers[25]. Another strength is that we performed logistic regression models to analyse the associations between current MDD and sociodemographic, clinical, and lifestyle variables. One limitation is that psychiatric evaluation of non-HCV chronically infected patients with type 2 diabetes of the same geographic area was not available, therefore an epiphenomenon should not be completely ignored.

It has been shown that the prevalence of depression was moderately and markedly increased in pre-diabetic and diabetic patients, respectively, when these subjects were compared to healthy individuals[26,27]. Concerning the hepatitis C scenario, depressive disorders[28-30] and type 2 diabetes[31,32] are both associated with HCV infection. Especially, CHC patients with diabetes mellitus

**TABLE 4.** Variables associated with cirrhosis in patients with chronic hepatitis C.

| Variables | Univariate analysis | Multivariate analysis | | |
|---|---|---|---|---|
| | *P* | OR | 95%CI | *P* |
| Sociodemographic data | | | | |
| Age | 0.98 | – | – | – |
| Sex | 0.51 | – | – | – |
| Clinical comorbidities | | | | |
| Hypertension | 0.34 | – | – | – |
| Type 2 diabetes mellitus | 0.008 | 5.09 | 1.73–15.04 | 0.003 |
| Psychiatric disorders | | | | |
| Current major depressive disorder | 0.51 | – | – | – |
| Current anxiety disorders | 0.05 | 0.25 | 0.05–1.23 | 0.09 |
| Current alcohol abuse or dependence | 0.02 | 2.54 | 1.04–6.22 | 0.04 |
| Current non-alcohol abuse or dependence | 0.44 | – | – | – |

are likely to have a more complicated course of hepatopathy such as cirrhosis and carcinoma hepatocellular[31,32]. In the study population, the presence of cirrhosis was independently associated with current alcohol abuse/dependence as well as type 2 diabetes mellitus.

It is important to bear in mind that inflammation and host immune response to HCV may contribute to the pathophysiology and progression of psychiatric disorders. This hypothesis may shed light on the role played by the host's immune response in HCV-related extrahepatic manifestations. Given the potential relevance of cytokines in mediating depressive symptoms[33] and diabetes mellitus clinical course[27], it might be speculated that inflammatory mediators are overexpressed in CHC patients with concurrent type 2 diabetes and depressive disorder. Recently, we demonstrated that the IL-10 low producer ATA haplotype was associated with the first major depressive episode[34] and combined polymorphisms of IL-6-174GG genotype, high-producer IL-6 phenotype, and IL-10 ATA haplotype were associated with a poor quality of life in patients with CHC[31]. More recently, our group also demonstrated that patients with chronic HCV infection carrying IL-6-174GG homozygous genotype were at increased risk of type 2 diabetes[35]. These findings suggest that an imbalance between pro-inflammatory and anti-inflammatory cytokines might induce an immune activation, and consequently, generates extrahepatic disorders as depressive symptoms as well as glucose intolerance.

Besides a high prevalence of psychiatric disorders found in our outpatients with CHC, irrespective of HCV antiviral therapy, notably, psychiatric illness overlap was also a common finding. At least, 35.8% of them suffer from two or more psychiatric disorders. Among the lifetime psychiatric illness, alcohol use disorder (30.5%), non-alcohol drug disorder (23.8%), MDD (17.9%) and generalized anxiety (11.3%) were the most prevalent disorders identified in agreement with previous studies[23,28]. However, the patients evaluated in those studies were veterans followed at the US Department of Veterans Affairs Hospital, where a large number of them were male and had a high prevalence of psychiatric illness and history of substance abuse[23,28] consequently, in CHC setting, health professionals have a great challenge in the management of these patients because two or more psychiatric disorders associated with clinical comorbidities, can be present. Additionally, the alcohol and/or non-alcohol substance were common in the study population. Thus, these findings precluded the possibility to recognize the influence of each specific variable in a particular psychiatric disorder occurrence. On other hand, the overlapping between host-, HCV- and environmental- related factors reflects the complexities of analysing chronic diseases in the real world[36].

Preceding HCV infection significantly increases the risk of developing psychiatric comorbidities. On the other hand, patients with psychiatric illness are at an increased risk of acquiring HCV infection[11]. Thus, this two-way interface, i.e., the relationship linking HCV and neuropsychiatric manifestations, is possibly determined by complex and multifaceted interactions among the hepatitis virus, the environment and the host.

Our current data show that neither different HCV genotypes nor viral load was significantly associated with psychiatric comorbidities. In a systematic review conducted by Perry et al. (2008) neither the viral load nor the HCV genotype was associated with cognitive impairment in patients with CHC[37]. Despite the pathogenesis of HCV-related psychiatric symptoms has not been completely understood, evidences that the virus is able to cross the blood-brain-barrier should not be disregarded[38].

In summary, we have clearly demonstrated that psychiatric disorders are prevalent in patients with CHC. Even in the DAAs era, characterized by great perspectives for the first ample cure of a chronic viral infection in humans[3,4], we should pursue the patient care integration. Therefore, efforts should be made to ensure that the screening for psychiatric disorders takes place in the course of routine clinical care. Considering the hepatitis C as a systemic disease, integrated clinical/psychiatric/psychological care must be enhanced in the management of individuals chronically infected with HCV. Beyond the virus and the liver disease, the psychiatric disorders must be recognized in patients with CHC. This approach will able to prioritize the patients' needs, values and goals[39].

Concerning the association between current MDD and type 2 diabetes our patients with CHC were recruited from a referral centre and, consequently, may not be representative of all chronically HCV-infected patients. Furthermore, the cross-sectional nature of the investigation hindered the likelihood to recognize any cause-effect relationship between current MDD and demographic, clinical, lifestyle, and virological variables in patients with CHC.

In conclusion, MDD is associated with type 2 diabetes in CHC patients. Even in the direct-acting antivirals (DAAs) era, characterized by great perspectives for the first ample cure of a chronic viral infection, we should ensure that the screening for psychiatric disorders takes place in the course of routine clinical care of patients chronically infected with hepatitis C virus (HCV).

## Authors' contribution

Cunha LR: designed research, project conception, development of overall research plan, and study oversight. Castro MCM: conducted research, hands-on conduct of the experiments and data collection. Duarte GS: conducted research, hands-on conduct of the experiments and data collection. Nascimento GC: conducted research, hands-on conduct of the experiments and data collection. Rocha GA: designed research, project conception, development of overall research plan, and study oversight. Silva LD: designed research, project conception, development of overall research plan, and study oversight.

## Orcid

Luciana Rodrigues da Cunha: 0000-0001-5006-6129.
Maria Carolina Magalhães de Castro: 0000-0001-7371-1979.
Gabriela Silva Duarte: 0000-0003-1755-3253.
Graziela Cançado e Nascimento: 0000-0003-2801-0326.
Gifone Aguiar Rocha: 0000-0002-1858-3166.
Luciana Diniz Silva: 0000-0003-0061-7361.

Cunha LR, Castro MCM, Duarte GS, Nascimento GC, Rocha GA, Silva LD. O transtorno depressivo maior está associado ao diabetes mellitus tipo 2 em pacientes com hepatite C crônica. Arq Gastroenterol. 2021;58(4):476-82.

**RESUMO** – **Contexto** – O transtorno depressivo maior (TDM) é comumente detectado em pacientes com hepatite C crônica. Entretanto, fatores potencialmente associados à coocorrência destas condições não são completamente conhecidos. **Objetivo** – Avaliar a frequência de transtornos mentais em pacientes com hepatite C crônica e investigar variáveis associadas ao TDM. **Métodos** – Pacientes com hepatite C crônica (n=151) atendidos em um centro de referência para hepatite foram avaliados usando o *Mini-International Neuropsychiatry Interview* e o questionário *Cut-Annoyed-Guilty-Eye* (CAGE). Análise multivariada foi usada para avaliar as covariáveis independentes associadas ao TDM atual. **Resultados** – Setenta e seis (50,3%) pacientes apresentaram pelo menos um diagnóstico psiquiátrico atual; dentre eles destaca-se o TDM (33,1%). TDM atual foi independentemente associado à idade (≤50 anos) (OR=2,57; IC95% = 1,25–5,29; *P*=0,01) e diabetes mellitus tipo 2 (OR=2,80, IC95% = 1,17–6,70; *P*=0,02). Cirrose foi associada ao diabetes mellitus tipo 2 (OR=5,09; IC95% = 1,73–15,04; *P*=0,03) e abuso/dependência de álcool atual (OR=2,54; IC95% = 1,04–6,22; *P*=0,04). **Discussão** – TDM está associado a diabetes tipo 2 em pacientes com hepatite C crônica. Em vigência da era dos antivirais de ação direta, caracterizada por grandes perspectivas para a primeira cura ampla de uma infecção viral crônica, devemos assegurar que a triagem dos transtornos psiquiátricos ocorra durante o atendimento clínico de rotina de pacientes com infecção crônica pelo vírus da hepatite C.

**Palavras-chave** – Hepatite C; transtornos psiquiátricos crônicos; transtorno depressivo maior; transtorno de ansiedade; diabetes mellitus tipo 2; abuso/dependência de álcool; abuso/dependência de substâncias não alcoólicas; cirrose hepática.

## REFERENCES

1. Burki T. Nobel Prize for hepatitis C virus discoverers. Lancet. 2020;396:1058. doi: 10.1016/S0140-6736(20)32111-5.
2. Gretchen-Vogel. Medicine Nobel honours three scientists for discoveries on hepatitis C virus. Available from: https://www.sciencemag.org/news/2020/10/medicine-nobel-honors-three-scientists-discoveries-hepatitis-c-virus. doi:10.1126/science.abf0538.
3. Baumert TF, Berg T, Lim JK, Nelson DR. Status of Direct-Acting Antiviral Therapy for Hepatitis C Virus Infection and Remaining Challenges. Gastroenterology. 2019;156:431-45. doi: 10.1053/j.gastro.2018.10.024.
4. Daniel KE, Saeian K, Rizvi S. Real-world experiences with direct-acting antiviral agents for chronic hepatitis C treatment. J Viral Hepat. 2020;27:195-204. doi: 10.1111/jvh.13218.
5. Hatzakis A, Chulanov V, Gadano AC, Bergin C, Ben-Ari Z, Mossong J, et al. The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm - volume 2. J Viral Hepat. 2015;22 (Suppl 1):26-45. doi: 10.1111/jvh.12351.
6. Razavi H. Global Epidemiology of Viral Hepatitis. Gastroenterol Clin North Am. 2020;49:179-89. doi: 10.1016/j.gtc.2020.01.001.
7. Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. Semin Liver Dis. 2000;20:17-35. doi: 10.1055/s-2000-9505.
8. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017. Available from: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/
9. Negro F, Forton D, Craxì A, Sulkowski MS, Feld JJ, Manns MP. Extrahepatic morbidity and mortality of chronic hepatitis C. Gastroenterology. 2015;149:1345-60. doi: 10.1053/j.gastro.2015.08.035.
10. Schaefer M, Capuron L, Friebe A, Diez-Quevedo C, Robaeys G, Neri S, et al. Hepatitis C infection, antiviral treatment and mental health: a European expert consensus statement. J Hepatol. 2012;57:1379-90. doi: 10.1016/j.jhep.2012.07.037.
11. Campos LN, Guimarães MD, Carmo RA, Melo AP, Oliveira HN, Elkington K, et al. HIV, syphilis, and hepatitis B and C prevalence among patients with mental illness: a review of the literature. Cad Saude Publica. 2008;24(Suppl 4):s607-20.
12. Hengst J, Falk CS, Schlaphoff V, Deterding K, Manns MP, Cornberg M, et al. Direct-acting antiviral-induced hepatitis C virus clearance does not completely restore the altered cytokine and chemokine milieu in patients with chronic hepatitis C. J Infect Dis. 2016;214:1965-74. doi.org/10.1093/infdis/jiw457.
13. Dirks M, Pflugrad H, Haag K, Tillmann HL, Wedemeyer H, Arvanitis D, et al. Persistent neuropsychiatric impairment in HCV patients despite clearance of the virus?! J Viral Hepat. 2017;24:541-50. doi: 10.1111/jvh.12674.
14. Gallach M, Vergara M, da Costa JP, Miquel M, Casas M, Sanchez-Delgado J, et al. Impact of treatment with direct-acting antivirals on anxiety and depression in chronic hepatitis C. PLoS One. 2018;13:e0208112. doi: 10.1371/journal.pone.0208112.
15. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383:1749-61. doi: 10.1016/S0140-6736(14)60121-5.
16. D'Amico G, Pasta L, Morabito A, D'Amico M, Caltagirone M, Malizia G, et al. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients. Aliment Pharmacol Ther. 2014;39:1180-93, doi.org/10.1111/apt.12721.
17. Child CG, Turcotte JG. Surgery and portal hypertension. Major Probl Clin Surg. 1964;1:1-85.
18. Silva LD, Cunha CC, Cunha LR, Araújo RF, Barcelos VM, Menta PL, et al. Depression rather than liver impair-ment reduces quality of life in patients with hepati-tis C. Rev Bras Psiquiatr. 2015;37:21-30. doi.org/10.1590/1516-4446-2014-1446.
19. Amorim P. Mini International Neuropsychiatric Interview (MINI): validation of a short structured diagnostic psychiatric interview. Rev Bras Psiquiatr. 2000;22:106-15. doi.org/10.1590/S1516-44462000000300003.
20. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Inter-view (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(Suppl 20):22-33;quis 34-57.
21. Dhalla S, Kopec JA. The CAGE questionnaire for alcohol misuse: a review of reliability and validity studies. Clin Invest Med. 2007;30:33-41. doi: 10.25011/cim.v30i1.447.
22. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S13-S27. doi: 10.2337/dc18-S002.
23. Yovtcheva SP, Rifai MA, Moles JK, Van der Linden BJ. Psychiatric comorbidity among hepatitis C-positive patients. Psychosomatics. 2001;42:411-5. doi: 10.1176/appi.psy.42.5.411.
24. Hoofnagle JH, Feinstone SM. The Discovery of Hepatitis C - The 2020 Nobel Prize in Physiology or Medicine. N Engl J Med. 2020;383:2297-9. doi: 10.1056/NEJMp2031110.
25. Batista-Neves SC, Quarantini LC, de Almeida AG, Bressan RA, Lacerda AL, de-Oliveira IR, et al. High frequency of unrecognized mental disorders in HCV-infected patients. Gen Hosp Psychiatry. 2008;30:80-2. doi: 10.1016/j.genhosppsych.2007.08.014.
26. Chen S, Zhang Q, Dai G, Hu J, Zhu C, Su L, Wu X. Association of depression with pre-diabetes, undiagnosed diabetes, and previously diagnosed diabetes: a meta-analysis. Endocrine. 2016;53:35-46. doi: 10.1007/s12020-016-0869-x.
27. Moulton CD, Pickup JC, Ismail K. The link between depression and diabetes: the search for shared mechanisms. Lancet Diabetes Endocrinol. 2015;3:461-71. doi: 10.1016/S2213-8587(15)00134-5.
28. El-Serag HB, Kunik M, Richardson P, Rabeneck L. Psychiatric disorders among veterans with hepatitis C infection. Gastroenterology. 2002;123:476-82. doi: 10.1053/gast.2002.34750.

29. Lee K, Otgonsuren M, Younoszai Z, Mir HM, Younossi ZM. Association of chronic liver disease with depression: a population-based study. Psychosomatics. 2013;54:52-9. doi.org/10.1016/j.psym.2012.09.005.
30. Vieira DA, da Cunha LR, da Silva CB, Almeida MTB, Gomes AD, de Faria CLL Jr, et al. The combined polymorphisms of interleukin-6-174GG genotype and interleukin-10 ATA haplotype are associated with a poor quality of life in patients with chronic hepatitis C. Qual Life Res. 2019;28:1531-42. doi: 10.1007/s11136-019-02129-5.
31. Negro F, Alaei M. Hepatitis C virus and type 2 diabetes. World J Gastroenterol. 2009;15:1537-47. doi: 10.3748/wjg.15.1537.
32. Li J, Gordon SC, Rupp LB, Zhang T, Trudeau S, Holmberg SD, et al. CHeCS Investigators. Sustained virological response to hepatitis C treatment decreases the incidence of complications associated with type 2 diabetes. Aliment Pharmacol Ther. 2019;49:599-608. doi: 10.1111/apt.15102.
33. Loftis JM, Huckans M, Ruimy S, Hinrichs DJ, Hauser P. Depressive symptoms in patients with chronic hepatitis C are correlated with elevated plasma levels of interleukin-1beta and tumour necrosis factor-alpha. Neurosci Lett. 2008;430:264-8. doi: 10.1016/j.neulet.2007.11.001.
34. Cunha LRD, Vieira DA, Giampietro YG, Gomes AD, Lopes de Faria CL Jr, Freire de Melo F, et al. Interleukin-10 promoter gene polymorphisms are associated with the first major depressive episode in chronic hepatitis C patients. Clin Res Hepatol Gastroenterol. 2019;43:417-26. doi: 10.1016/j.clinre.2018.11.015.
35. da Silva CB, Vieira DA, de Melo LF, Chagas ALS, Gomes AD, de Faria CLL Jr, et al. Interleukin-6-174G/C polymorphism is associated with a decreased risk of type 2 diabetes in patients with chronic hepatitis C virus. World J Hepatol. 2020;12:137-48. doi: 10.4254/wjh.v12.i4.137.
36. Helbling B, Overbeck K, Gonvers JJ, Malinverni R, Dufour JF, Borovicka J, et al. Swiss Hepatitis C Cohort Study. Host- rather than virus-related factors reduce health-related quality of life in hepatitis C virus infection. Gut. 2008;57:1597-603. doi: 10.1136/gut.2007.142844.
37. Perry W, Hilsabeck RC, Hassanein TI. Cognitive dysfunction in chronic hepatitis C: a review. Dig Dis Sci. 2008;53:307-21. doi: 10.1007/s10620-007-9896-z.
38. Fletcher NF, Wilson GK, Murray J, Hu K, Lewis A, Reynolds GM, et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. Gastroenterology. 2012;142:634-43. doi.org/10.1053/j.gastro.2011.11.028.
39. Verma M, Navarro V. Patient-centred care: A new paradigm for chronic liver disease. Hepatology. 2015;62:988-90. doi: 10.1002/hep.28022.

ORIGINAL ARTICLE

AG-2021-81
doi.org/10.1590/S0004-2803.202100000-87

# Epidemiological profile and clinical characteristics of inflammatory bowel diseases in a Brazilian referral center

Luiza Maria Pilau **FUCILINI**, Lívia Moreira **GENARO**, Daniela Cunha e **SOUSA**, Cláudio Saddy Rodrigues **COY**, Raquel Franco **LEAL** and Maria de Lourdes Setsuko **AYRIZONO**

Received: 18 April 2021
Accepted: 13 July 2021

**ABSTRACT – Background** – The increase in the incidence and prevalence rates of inflammatory bowel disease (IBD) is evident in many newly industrialized countries in Asia, Africa, Eastern Europe, and the American continent. In Brazil, records are still scarce, and further studies on this topic are needed. **Objective** – To evaluate the epidemiological profile and clinical characteristics of patients with IBD who were followed up at a reference service in the state of São Paulo. **Methods** – We retrospectively analyzed the medical records of patients with IBD who were followed up in a Brazilian Referral Center. **Results** – A total of 625 patients was evaluated, 416 with Crohn's disease (CD), 190 with ulcerative colitis (UC), and 19 with indeterminate colitis. The average age of the patients was 31.6 years, with a homogeneous distribution between males and females patients. In patients with CD, the most predominant Montreal classification was A2, L3, and B1, with 44.8% of patients presenting with perianal disease; in UC, it was E2, and S0. The main extraintestinal manifestation was rheumatologic, followed by cutaneous and ophthalmic lesions. The majority of patients (85.4%) used some type of medication, the most frequent being aminosalicylates in patients with UC and biological therapy in patients with CD. Regarding surgeries, in CD, a significant percentage of patients underwent some type of surgical procedure, unlike the UC patients, including fistulotomies and placement of seton, derivative ostomies, enterectomy, ileocecectomy/right colectomy, total or partial colectomy, and strictureplasty. Only 195 (31.2%) patients lived in the city of Campinas, while 443 (70.9%) were from the 7th Regional Health Department (RHD), which corresponds to the macro-region of Campinas. **Conclusion** – In this study, most patients came from the 7th RHD of Campinas; the patients were young, with no predominance of either sex; there was a higher frequency of patients with CD (66.6%). Most of them (85.4%) were undergoing pharmacological treatment, and a significant percentage of CD patients had undergone surgery.

**Keywords** – Crohn's disease; ulcerative colitis; epidemiology; gastrointestinal tract.

## INTRODUCTION

Inflammatory bowel disease (IBD), most prominently represented by Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic diseases of the gastrointestinal tract (GIT). When the distinction between CD and UC is not possible, colitis is classified as indeterminate colitis (IC)[1]. All of these diseases are characterized by periods of activity and remission, and they affect mostly young individuals, with no predominance of sex[2,3]. UC affects the mucosal and submucosal layers of the colon and rectum, whereas CD can involve the entire GIT transmurally[4]. The phenotypes that characterize CD can be described as inflammatory (no stricturing no penetrating), stricturing, or penetrating, which are the three behaviors of the disease's presentation[5]. IBD corresponds to a group of immune-mediated diseases with a multifactorial etiology, and probably results from an interaction between genetic and environmental factors, which favors an uncontrolled immune response to the intestinal microbiota, causing tissue damage[6].

The highest incidence and prevalence rates of IBD are mainly found in developed countries. In recent years, especially in the first two decades of the 21st century, an increase in IBD cases has been observed in newly industrialized and developing countries. This epidemiological change in newly industrialized countries is comparable to the patterns observed in Western developed countries more than 50 years ago[7]. Some factors may be responsible for this increased frequency of IBD in these countries, including a higher rate of urbanization that resulted in a transformation of lifestyle behaviors, higher rates of smoking, sedentary occupations, and lower rates of breastfeeding[8].

In Brazil, where IBD is considered an emerging disease, there are only a few population-based studies. A justification for this is the lack of medical surveillance, reliable and unified records, and databases[9,10]. Another factor that could explain this deficit is the disorganization of the health system in a country of continental size and its associated economic problems, which makes it difficult to maintain adequate records[10]. In addition, published epidemiological data can often be unreliable when measuring the real dimension of the problem, owing to the great heterogeneity of the characteristics presented in IBD, lack of access and availability of diagnostic methods, experience of the professionals involved, and information and notification systems in different regions of the country[11].

Declared conflict of interest of all authors: none
Disclosure of funding: none
Universidade de Campinas, Departamento de Cirurgia, Campinas, SP, Brasil.
Corresponding author: Maria de Lourdes Setsuko Ayrizono. E-mail: luayrizono@terra.com.br

A few studies have demonstrated an increase in incidence and prevalence rates of IBD in Brazil, confirming that IBD is a public health problem because of its chronicity, associated with high morbidity and treatment costs[12]. Therefore, this study aimed to assess the epidemiological profile and clinical characteristics of patients with IBD followed up at a Brazilian Referral Center by analyzing their sociodemographic characteristics, their stratification according to the Montreal classification, and by identifying the medical approach chosen for these patients.

## METHODS

The study enrolled patients with CD, UC, and IC diagnosed at the IBD outpatient clinic, Prof. Dr. Juvenal Ricardo Navarro Góes, of the Center for Diagnosis of Diseases of the Digestive System (Gastrocenter), University of Campinas (Unicamp), and those who were treated between January 1991 and December 2019. The exclusion criteria were as follows: patients with other types of colitis, those under 18 years of age, those who missed follow-up, and deaths. Patient information was collected from the database of the outpatient clinic (attendance files and electronic registration). It was complemented, if necessary, with the medical records from the Unicamp Clinical Hospital, and more recently, with the hospital's electronic record system (AGHuse).

The variables collected and analyzed were sociodemographic characteristics (age, sex, city of origin, and the Regional Health Department [RHD]), clinical aspects (time of symptom onset until diagnosis, presence of extraintestinal manifestations (EIMs), the Montreal classification, personal history of smoking, and family history of IBD), and the performed treatment (medication and surgery). The Montreal classification was used to classify CD and UC, and the parameters for CD were as follows: the age of the first clinical presentation of the disease (A = age), the location of the involvement in the GIT (L = location), and the clinical behavior of the disease (B = behavior). For UC, it was based on the endoscopic appearance of the colon (remission, mild, moderate, or severe disease), and the maximum extent of intestinal involvement (distal, left colitis, or pancolitis) observed during the colonoscopy exam[13].

The study was approved by the Research Ethics Committee of the Faculty of the University of Campinas (Unicamp) under the registered approval number 3,265,851 (CAAE: 02165318.2.0000.5404), and was conducted in accordance with the declaration of Helsinki. The data were plotted in an Excel spreadsheet and statistical analysis was performed using the Statistical Package for Social Sciences program version 25.0 (SPSS Inc., Chicago, IL, USA, 2018). The results are presented using descriptive statistics, using absolute and relative distributions (%), and measures of central tendency (mean and median), and variability (standard deviation, amplitude, and interquartile amplitude), with the study of the symmetry of distributions using the Kolmogorov-Smirnov test.

## RESULTS

During the study period, 1,231 patients were treated at the IBD outpatient clinic. Of these, 574 missed the follow-up appointments over the years. There were 28 deaths, 14 of which were due to disease-related causes; four were excluded from the study because they were underage. Thus, the study population comprised 625 patients, of which 416 (66.6%) had CD, 190 (30.4%) had UC, and 19 (3.0%) had IC (FIGURE 1).

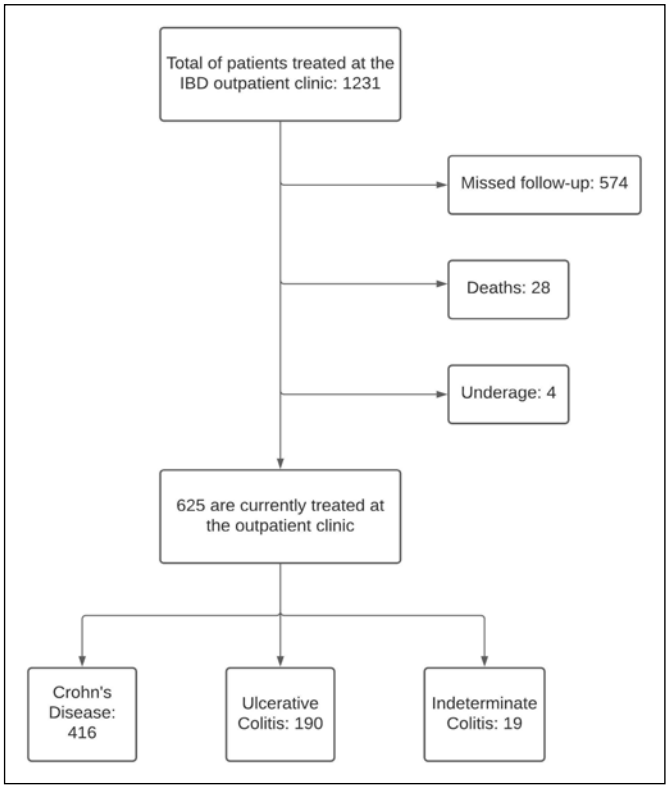



**FIGURE 1.** Population included in the study.
IBD: inflammatory bowel disease.

The outpatient clinic of GASTROCENTER-Unicamp is a reference center for IBD treatment for the 7th RHD, which covers 43 municipalities around Campinas, in São Paulo state, Brazil (TABLE 1).

The population of this region is 4,008,065 inhabitants, According to the latest census of 2010 (Brazilian Institute of Geography and Statistics, 2020). The prevalence of IBD in the 7th RHD was 15.59/100,000 inhabitants, of which 10.38/100,000 inhabitants had CD and 4.74/100,000 inhabitants had UC. Although the majority of patients come from this RHD, some hail from other distant regions, and even from other states, mainly cities in the South of Minas Gerais (TABLE 2).

Regarding the patients' current age, the mean and median for CD, UC, and IC were 44.98 (SD=14.3) and 44 years; 50.35 (SD=15.5) and 52 years; and 45.16 (SD=17.0) and 39 years, respectively. Of the 362 patients with CD in whom information on family history of IBD was obtained, 38 patients had a family history of IBD. Among the 161 UC patients, 18 presented the same history. Furthermore, 52 of 203 patients with CD; and 16 of the 79 patients with UC had a history of smoking (TABLE 3).

The age at the time of IBD diagnosis ranged from 7 to 78 years, with a mean of 31.62 years (SD=13.2). The mean age for CD was 29.61 years (SD=12.0), ranging from 7 to 73 years, and for UC, 35.79 years (SD=14.3), ranging from 10 to 78 years.

According to the Montreal classification for CD, there was a higher frequency of the A2 age Group (72.8%), with a similar distribution between males and females. The L3 location (34.4%) and B1 behavior (46.9%) were observed. Approximately half of the patients (44.5%) had an association with perianal disease (TABLE 4).

**TABLE 1.** Distribution of patients according to the city of origin (7th Regional Health Department) and its population.

| City | Number of IBD patients | Number of UC patients | Number of CD patients | Population defined by BIGS |
|---|---|---|---|---|
| Águas de Lindóia | 4 | 0 | 2 | 17.266 |
| Americana | 11 | 6 | 5 | 210.638 |
| Amparo | 2 | 1 | 1 | 65.829 |
| Artur Nogueira | 5 | 1 | 4 | 44.177 |
| Atibaia | 1 | 0 | 1 | 126.603 |
| Bom Jesus dos Perdões | 0 | 0 | 0 | 19.708 |
| Bragança Paulista | 6 | 1 | 5 | 146.744 |
| Cabreúva | 0 | 0 | 0 | 41.604 |
| Campinas | 195 | 65 | 122 | 1.080.113 |
| Campo Limpo Paulista | 3 | 1 | 2 | 74.074 |
| Cosmópolis | 9 | 4 | 3 | 58.827 |
| Holambra | 2 | 1 | 1 | 11.299 |
| Hortolândia | 32 | 11 | 20 | 192.692 |
| Indaiatuba | 21 | 6 | 15 | 201.619 |
| Itatiba | 6 | 0 | 5 | 101.471 |
| Itupeva | 3 | 0 | 1 | 44.859 |
| Jaguariúna | 9 | 5 | 4 | 44.313 |
| Jarinu | 0 | 0 | 0 | 23 847 |
| Joanópolis | 1 | 0 | 1 | 11.768 |
| Jundiaí | 5 | 1 | 4 | 370.126 |
| Lindóia | 0 | 0 | 0 | 6.712 |
| Louveira | 3 | 0 | 3 | 37.125 |
| Monte Alegre do Sul | 0 | 0 | 0 | 7.152 |
| Monte Mor | 6 | 2 | 4 | 48.949 |
| Morungaba | 3 | 0 | 2 | 11.769 |
| Nazaré Paulista | 1 | 0 | 1 | 16.414 |
| Nova Odessa | 4 | 2 | 2 | 51.242 |
| Paulínia | 8 | 3 | 5 | 82.146 |
| Pedra Bela | 0 | 0 | 0 | 5.780 |
| Pedreira | 3 | 1 | 2 | 41.558 |
| Pinhalzinho | 0 | 0 | 0 | 13.105 |
| Piracaia | 0 | 0 | 0 | 25.116 |
| Santa Bárbara D'oeste | 19 | 2 | 17 | 180.009 |
| Santo Antônio da Posse | 2 | 0 | 2 | 20.650 |
| Serra Negra | 3 | 1 | 2 | 26.387 |
| Socorro | 1 | 0 | 1 | 36.686 |
| Sumaré | 52 | 18 | 34 | 241.311 |
| Tuiuti | 0 | 0 | 0 | 5.930 |
| Valinhos | 7 | 2 | 4 | 106.793 |
| Vargem | 0 | 0 | 0 | 8.801 |
| Várzea Paulista | 2 | 0 | 2 | 107.089 |
| Vinhedo | 8 | 5 | 3 | 63.611 |

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease. BIGS: Brazilian Institute of Geography and Statistics.

**TABLE 2.** Distribution of patients according to RHDs in São Paulo State and other states.

| RHD's number / Other States | RHD's names / Other States | Number of patients |
|---|---|---|
| 1 | Grande São Paulo | 6 |
| 2 | Araçatuba | 2 |
| 3 | Araraquara | 2 |
| 6 | Bauru | 2 |
| 7 | Campinas | 443 |
| 8 | Franca | 1 |
| 9 | Marília | 2 |
| 10 | Piracicaba | 56 |
| 14 | São José da Boa Vista | 45 |
| 15 | São José do Rio Preto | 1 |
| 16 | Sorocaba | 26 |
| 17 | Taubaté | 3 |
| DF | Distrito Federal | 1 |
| MA | Maranhão | 1 |
| MG | Minas Gerais | 31 |
| PR | Paraná | 1 |

RHD: Regional Health Department.

**TABLE 3.** Demographic characteristics.

| Patients' characteristics | Disease type: n (%) | | |
|---|---|---|---|
| | CD: n=416 (66.6%) | UC: n=190 (30.4%) | IC: n=19 (3.0%) |
| Sex (%) | | | |
| Female | 196 (47.1) | 100 (52.6) | 13 (68.4) |
| Male | 220 (52.9) | 90 (47.4) | 06 (31.6) |
| Age (years) | | | |
| Mean ± DP (amplitude) | 44.9 ±14.3 (18.0–80.0) | 50.3±15.5 (20.0–88.0) | 45.2±17.1 (23.0–74.0) |
| Median (1º–3º quartile) | 44.0 (34.0–56.0) | 52.0 (37.0–62.0) | 39.0 (31.0–57.0) |
| Family history (%) | | | |
| No | 324 (77.9) | 144 (75.8) | 13 (68.4) |
| Yes | 38 (9.1) | 17 (8.9) | 02 (10.5) |
| Uninformed | 54 (13.0) | 29 (15.3) | 04 (21.1) |
| Smoking habit (%) | | | |
| No | 151 (36.3) | 63 (33.2) | 7 (36.8) |
| Yes | 23 (5.5) | 09 (4.7) | – |
| Former smoker | 29 (7.0) | 07 (3.7) | – |
| Uninformed | 213 (51.2) | 111 (58.4) | 12 (63.2) |

UC: ulcerative colitis; CD: Crohn's disease; IC: indeterminate colitis.

**TABLE 4.** Montreal classification for Crohn's disease.

| Characteristics | Total of patients n=416 | Sex | |
|---|---|---|---|
| | | Female n=196 (47.1%) | Male n=220 (52.9%) |
| Age at the diagnostic (A) | | | |
| A1: ≤16 years | 45 (10.8) | 17 (8.7) | 28 (12.7) |
| A2: 17–40 years | 303 (72.8) | 146 (74.5) | 157 (71.4) |
| A3: >40 years | 68 (16.4) | 33 (16.8) | 35 (15.9) |
| Location (L) | | | |
| L1: Terminal ileum | 139 (33.4) | 59 (30.1) | 80 (36.4) |
| L2: Colon | 131 (31.5) | 65 (33.2) | 66 (30.0) |
| L3: Ileocolon | 143 (34.4) | 71 (36.2) | 72 (32.7) |
| L4: Upper GI | 03 (0.7) | 01 (0.5) | 02 (0.9) |
| Behavior (B) | | | |
| B1: Inflammatory | 195 (46.9) | 90 (45.9) | 105 (47.7) |
| B2: Stricturing | 99 (23.8) | 49 (25.0) | 50 (22.7) |
| B3: Penetrating | 122 (29.3) | 57 (29.1) | 65 (29.6) |
| Perianal disease modifier (P) | | | |
| Yes | 185 (44.5) | 90 (45.9) | 95 (43.2) |
| No | 231 (55.5) | 106 (54.1) | 125 (56.8) |

GI: gastrointestinal.

Regarding UC, the predominant extension was E2 (left-sided colitis), followed by E3 (pancolitis), with a prevalence of 50.5% and 45.8%, respectively. Concerning the severity of the disease, most patients were in remission (S0, 87.9%) (TABLE 5).

**TABLE 5.** Montreal classification for ulcerative colitis.

| Characteristics | Total of patients n=190 | Sex | |
|---|---|---|---|
| | | Female n=100 (52.6%) | Male n=90 (47.4%) |
| Disease extent (E) | | | |
| E1: Proctitis | 07 (3.7) | 03 (3.0) | 04 (4.4) |
| E2: Left-sided colitis | 96 (50.5) | 55 (55.0) | 41 (45.6) |
| E3: Pancolitis | 87 (45.8) | 42 (42.0) | 45 (50.0) |
| Severity (S) | | | |
| S0: Clinical remission | 167 (87.9) | 91 (91.0) | 76 (84.5) |
| S1: Mild UC | 18 (9.5) | 07 (7.0) | 11 (12.2) |
| S2: Moderate UC | 04 (2.1) | 01 (1.0) | 03 (3.3) |
| S3: Severe UC | 01 (0.5) | 01 (1.0) | – |

UC: ulcerative colitis.

It was possible to verify in 547 medical records the elapsed time between the onset of symptoms and diagnosis of IBD. Among them, in case of five patients it took more than 20 years to establish the correct diagnosis of IBD. Twenty-nine (5.3%) patients had their diagnoses between 11 and 20 years; 67 (12.2%) between 6 and 10 years; 123 (22.5%) between 2 and 5 years; and 323 (59.0%) with less than 2 years after the onset of symptoms. Regarding the IBD EIMs, these were observed in 219 patients, the most frequent being rheumatological diseases, represented by arthritis, arthralgia, and ankylosing spondyloarthritis, which were present in 174 (79.4%) patients, with a prevalence of 57.1% in patients with CD and 21.0% in patients with UC. Subsequently, dermatological and ophthalmological manifestations were observed. Primary sclerosing cholangitis was present in 10 patients, six with UC and four with CD.

Regarding the use of medications, 85.4% of the patients were under pharmacological therapy. Biological therapies and thiopurines for CD and aminosalicylates for UC are the most common. For patients undergoing biological therapy, 55.3% were on combined immunosuppressive therapy (CD=46.4%; UC=8.9%). Among the previously used drugs, the most frequent were aminosalicylates (72.5%). Furthermore, 4.1% of the patients used more than one biological therapy during the course of treatment (TABLE 6).

**TABLE 6.** Pharmacological treatment of inflammatory bowel diseases patients followed-up at Clinical Hospital of the University of Campinas.

| Characteristics | Total n= 625 | Types of IBD | |
|---|---|---|---|
| | | CD: n=416 | UC: n=190 |
| Current medications | | | |
| No | 91 (14.6) | 65 (15.6) | 22 (11.6) |
| Yes | 534 (85.4) | 351 (84.4) | 168 (88.4) |
| Current medications | | | |
| Aminosalicylates | 149 | 38 | 106 |
| Thiopurines | 284 | 217 | 59 |
| Methotrexate | 16 | 13 | 03 |
| Biological* | 338 | 265 | 66 |
| Previous medications | | | |
| Aminosalicylates | 454 | 280 | 162 |
| Thiopurines | 327 | 245 | 70 |
| Methotrexate | 32 | 29 | 03 |
| Biological | 205 | 169 | 31 |
| More than one biological | 26 | 20 | 06 |

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease.
*Combination therapy (immunosuppressant + biological) in 157 with CD and 30 with UC.

Concerning surgeries, only seven patients with UC underwent surgical procedures in other services, while 157 patients with CD had already undergone previous surgeries. In our IBD unit, 35 patients with UC underwent surgical procedures (FIGURE 2), ileal pouch-anal anastomosis in 17 (8.9%) patients, total colectomy and rectal closure with terminal ileostomy in 8 (4.2%) patients, and ileorectal anastomosis in 6 (3.1%) patients. All patients who underwent ileal pouch surgery and two patients with ileorectal anastomosis also had protective ileostomy, which was later closed. Regarding CD, 251 patients required surgery at our IBD unit (FIGURE 2), of which perianal procedures were the most frequently performed. Among the abdominal surgeries, the most commonly performed procedures were enterectomy and ileocectomy/right colectomy. In addition, several patients required more than one surgery, both abdominal and perianal procedures.

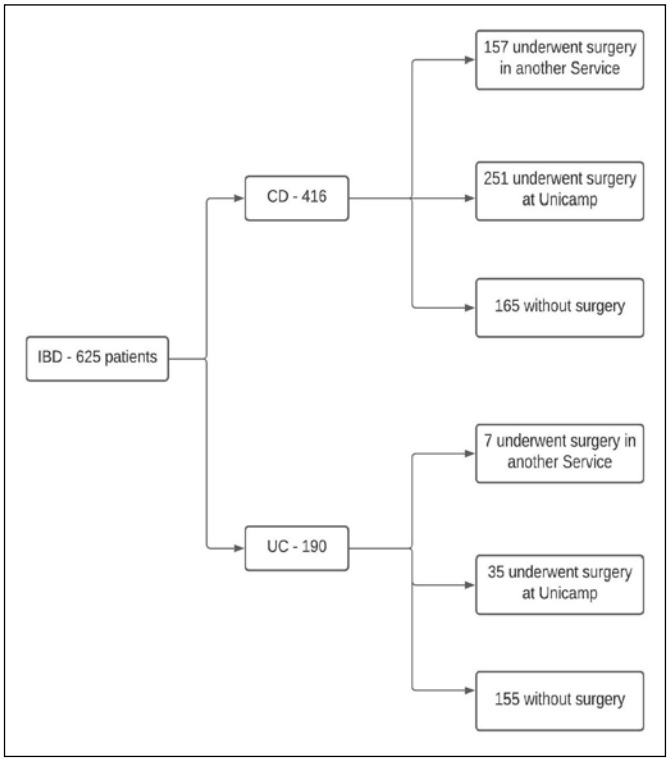



**FIGURE 2.** Total number of patients previously submitted to surgery in other services and in the Clinical Hospital of Unicamp.
CD: Crohn's disease; IBD: inflammatory bowel disease; UC: ulcerative colitis.

## DISCUSSION

Incidence/prevalence: the prevalence of IBD is increasing worldwide. Several studies have sought to identify its origin and factors that in combination lead to its development. IBD results from the interaction between environmental and epigenetic factors, which are not completely known, in predisposed individuals[14].

Until the middle of the 20th century, IBD was found mainly in developed countries in North America, Europe, and Oceania. Further, its incidence increased in newly industrialized countries in Asia, South America, and Africa, especially in the 21st century[7,15]. The highest incidence and prevalence rates were found in the most economically developed countries, as in Northern Europe, Canada, the United States, Australia, and New Zealand[16-19].

The present study is justified by the lack of data on the clinical and demographic characteristics of the Brazilian population, especially in the heartland of São Paulo state. In our study, most of the patients came from the macro-region of Campinas, while 29.2% belonged to other RHDs or even other states. The 7th RHD is composed of 43 municipalities in the Campinas region; thus, presenting an estimated IBD prevalence of 15.59 cases/100,000 inhabitants and an estimated CD prevalence of 10.38/100,000 inhabitants. This prevalence is estimated and not absolute, as not all diagnosed patients use our service. In similar studies, Victoria et al.[20] and Lima-Martins et al.[21] observed an estimated CD prevalence of 5.7/100,000 and 14.1/100,000 inhabitants, respectively. The estimated prevalence of UC was 4.74/100,000 inhabitants, which is lower than that in the study of these two authors, which was 15/100,000 inhabitants and 24.1/100,000 inhabitants, respectively[20,21].

Brazilian and international population studies have demonstrated a higher prevalence rate of UC compared to CD[20,22,23]. Victoria et al.[20] reported that UC represented 65.2% of the diagnoses and 25.2% of CD, while Lima-Martins et al.[21] reported prevalence rates of 63% and 37%, respectively. In contrast to these findings, some studies in IBD reference centers in Brazil have observed the opposite: Parente et al.[24] demonstrated a higher prevalence of CD in relation to UC (60.3% vs 39.7%) in the Piaui state and Santos et al.[25] in Rio de Janeiro (56.4% vs 38.3%), as observed in our study. The follow-up of CD is generally more complex, requiring more frequent therapeutic adjustments to keep the patient in remission, so it is expected that these patients will be referred more regularly to a specialized center, which explains the higher frequency of CD in these units.

In addition to the data on the incidence and prevalence of CD and UC, other factors that contribute to this are the lack of patients' records in the private service and the fact that reporting IBD is not mandatory[10]. Five Brazilian studies that focused on IBD have been published, three in the São Paulo state[9,20,26], one in the Piaui state[24], and another in the Espirito Santo state[21], covering the period from 1986 to 2015. All of these studies were retrospective, with variations in the severity of the patients included, as well as the location of data collection, its scope, type of database used, and form of access to the health system, whether public or private. Although these studies are not comparable owning to their different methodologies, there was a variation in the incidence rate of CD from 3.50 to 6.14/100,000 inhabitants and UC from 2.40 to 7.16/100,000 inhabitants, which demonstrates a chronological increase in the incidence of IBD[9,20,21,24,26].

Selvaratnan et al.[27] in a systematic review involving six studies (three from Brazil, one from Argentina, one from Colombia, and one from Uruguay), described the incidence of IBD in South America, a significantly higher incidence of UC than of CD was reported. A female predominance, which was not statistically significant, for both UC and CD, was demonstrated in most of these studies. There was also a predominance of colonic CD and pancolonic UC. This finding may be associated with differences in genetic polymorphisms or environmental exposure. However, the potential for divergence due to problems, such as notification of the disease, also needs to be considered. Colonic CD and pancolonic UC are generally more symptomatic. Therefore, they are more likely to be reported when compared to CD that involves the small intestine or less extended UC, which is prone to underreporting or misdiagnosis, particularly in the most disadvantaged regions.

Ng et al.[7] in a systematic review involving 119 studies of incidence and 69 of prevalence of IBD observed that most studies showed stability or decrease in incidence in the most prevalent areas, however this incidence remains high. This study also demonstrated that the incidence of IBD was increasing in countries in Africa, Asia, and South America, including Brazil. The current incidence and prevalence rates reported in recently industrialized countries are similar to those reported in Western developed countries 50 years ago.

Symptoms/time for the diagnosis: another important factor is the time interval that some patients require from the beginning of the symptoms until the definitive diagnosis is established and adequate treatment is initiated. In this study, five patients required more than 20 years for their diagnoses, and 29 required more than 10 years. These data are important to highlight that these diseases have often been underreported and are neglected. However, the di-

agnosis can be difficult, especially if the symptoms are nonspecific, and the correct diagnosis and treatment plan are essential to avoid the complications of the disease over the years.

Both CD and UC are diseases that manifest themselves in young individuals, which increases the need to request adequate monitoring to minimize the negative impacts that can occur in the social and professional lives of the patients[12]. In this study, IBD was found in all ages, but it was most often found between 20 and 25 years of age. These data are similar to those of previously published studies that highlight the age between 15 and 40 years for the occurence of IBD[24-26,28]. When assessing the age at diagnosis of patients with IBD separately, CD presents only one peak in the age group, unlike the UC, where a second peak is observed between 40 and 44 years of age[28]. The results found in our study were similar to those already published in the literature, which present the age at the time of diagnosis as bimodal profile, a peak in the young adult, and a second peak in older individuals[21,29,30]. Regarding gender distribution, most studies did not show statistical differences between men and women[21,31], as confirmed in our data. When diseases were analyzed separately, there was a slight female predominance for UC and a male predominance for CD, similar to the findings of Ooi et al. in Asia[28].

Family history and smoking habits: regarding the occurrence of IBD in individuals of the same family, our study demonstrated that approximately 10% had a family history of IBD, values higher than those found by Torres et al.[26], who observed a family history rate in only 2% of patients. These numbers may be underestimated because in a wide country like Brazil, many patients do not have this information or even have no contact with their family. The association between smoking habit and IBD is well established and smoking is a risk factor for CD and a protector for UC, especially in severe forms[8,32,33]. The etiology of these associations is not yet well established, and it is not known whether this relationship is local (nicotine and intestine) or systemic[34]. In this study, this information was partially impaired, as approximately 50% of medical records lacked documented data. Based on the other cases, approximately a quarter of the patients were smokers or former smokers[7,8,34].

Extraintestinal manifestations: one of the characteristics of IBD is the occurrence of manifestations of the disease outside the GIT, the EIMs. Articular manifestations are the most commonly found, followed by dermatological manifestations[35], similar to our findings. Some European studies have indicated the presence of EIMs in 20–40% patients with CD and 15–20% patients with UC. D'Inca et al.[36] after a 1-year follow-up found that joint symptoms were observed in 45.1% of patients with CD and 36.9% of patients with UC. Skin or dermatological lesions are the second most common type of EIMs, with erythema nodosum, pyoderma gangrenosum, and aphthous stomatitis[35]. The frequency of dermatological EIMs in Hungary was 5–15% in CD, with a female predominance and patients with active disease[16,35]. Another study that analyzed this factor was a French cohort with 2,402 patients with IBD, of which 5.6% had erythema nodosum and 0.75% had pyoderma gangrenosum[37]. Lastly, eye manifestations were the third most frequent EIMs found in our study, for both CD and UC, in accordance with data from the international literature, such as the findings of Lakatos et al.[35], who reported a prevalence of 3% for CD and 6% for UC.

Classification: the Montreal classification is the most commonly used classification for IBD. To select the best therapeutic approach and most appropriate follow-up for each patient, it is important to correlate specific disease phenotypes with possible clinical and prognostic outcomes. CD considers the following parameters: age at clinical presentation, location of the disease, and clinical behavior[38]. Regarding the location of ileal (L1), colonic (L2), and ileocolonic (L3) disease, the frequency of involvement observed was similar to that reported in the literature[16] and in the Brazilian study by Lima-Martins et al.[21]. For upper GIT disease (L4), there was a variation between 10% and 15%[39,40], and Lima-Martins et al.[21] observed 8% higher values than this study. This occurred mainly because we considered L4 patients who had only upper GIT involvement. We also observed a higher frequency of the inflammatory form (B1), corroborating the data obtained by Lima-Martins et al.[21], whose study showed 57.7% of patients with this classification. We found 44.5% of perianal involvement, data similar to the studies by Lima-Martins et al.[21] and Parente et al.[24]. However, in other demographic studies, the rate of perianal disease ranged between 10% and 18%[23,41].

In these reference centers, the services are coordinated by colorectal surgeons and the patients receive referrals with surgical treatment proposals. Most patients with perianal disease undergo surgical exploration of the perineum with combined clinical therapy.

Regarding UC, the Montreal classification includes the extent and severity of the disease. In this study, the most frequent location was left-sided colitis, followed by pancolitis. Proctitis was rare, as in other studies in the literature[20,21,26]. The low percentage of patients with proctitis is probably because many of these patients are oligosymptomatic and are treated by physicians who are not IBD specialists. Regarding the severity of the disease, the vast majority of patients were in remission, as in the study by Silva et al.[33], who reported 45.7% of patients in remission, differing from the findings by Parente et al.[24], who had a predominance of patients with moderate disease.

Clinical/surgical treatment: aminosalicylates have been the most widely used class of medication over the years (approximately 72.5% of patients in this study used this drug). Currently, only 23.8% of patients are using this medication. When diseases are analyzed separately, only 9.1% patients with CD use aminosalicylates, while 55.8% patients with UC are under this medication. These data are in accordance with the current management guidelines[16]. Immunosuppressants, represented by thiopurines and methotrexate, are used mainly in combination therapy with biological therapies to reduce the formation of anti-drug antibodies[42]. In our study, 55.3% of the patients who used biological drugs maintained combination therapy. In the Brazilian Unified Health System Service, only anti-tumor necrosis factor-α monoclonal antibodies (infliximab, adalimumab, and certolizumab pegol) were available. Nonetheless, more than half of our patients with CD and approximately one-third of patients with UC were using this class of drugs. These data are superior to those described by Lima-Martins et al.[21].

The rates of patients requiring surgery for CD are higher than for UC. In our CD cohort, more than half of the patients underwent surgery, which was performed both in our hospital and previously in other services, values much higher than those reported by Bernstein et al.[43] and Bechara et al.[44], who observed the rate of surgery ranging from 12% to 26.7%. Regarding UC, 18.4% of our patients had underwent surgery, and the numbers were higher than those found in the literature[44,45]. As mentioned before, these findings are justified because it is an outpatient clinic coordinated by colorectal surgeons, where patients are mostly referred for the purpose of surgical evaluation.

The main limitation of the present study was its retrospective nature, which led to the lack of clinical data, in addition to the inclusion of patients from only a single center. However, further studies must be performed in different regions of Brazil to have a broader view of IBD epidemiology among Brazilian population and to create better strategies to deal with this relevant chronic disease.

## CONCLUSION

Most of the patients belonged to the 7th RHD of Campinas. There was a predominance of patients with CD, young age, and disease onset in the third decade of life. In general, there was no predominance of sex, but more women with UC and men with CD were observed. There was an equivalence of distribution between the locations (L1 = L2 = L3) and a predominance of inflammatory behavior (B1), with almost half of the patients showing perianal involvement in CD patients. In UC, the most common phenotype was left-sided colitis (E2), and most patients had been in remission (S0). Aminosalicylates have been the most widely used drugs over the years, with biological therapy being the most commonly used. More than half of the patients with CD underwent surgical procedures, unlike UC patients who were treated mostly by pharmacological therapy.

## ACKNOWLEDGEMENTS

We thank Professor Tristan Torriani for the English revision of our manuscript and Editage (www.editage.com.br) for English language editing.

### Authors' contribution

Fucilini LMP: data collection, statistical analysis, data interpretation, writing of the manuscript. Genaro LM: data collection, data interpretation, writing of the manuscript. Souza DC: data collection. Coy CSR: final revision of manuscript. Leal RF: planning of the study, design study, manuscript concept, data interpretation, and final revision of manuscript. Ayrizono MLS: planning of the study, design study, manuscript concept, data interpretation, writing and final revision of manuscript.

### Orcid

Luiza Maria Pilau Fucilini: 0000-0001-5475-2230.
Lívia Moreira Genaro: 0000-0002-4640-6554.
Daniela Cunha e Sousa: 0000-0002-3395-2038.
Cláudio Saddy Rodrigues Coy: 0000-0002-0916-4138.
Raquel Franco Leal: 0000-0003-4285-4402.
Maria de Lourdes Setsuko Ayrizono: 0000-0002-7035-2568.

Fucilini LMP, Genaro LM, Sousa DC, Coy CSR, Leal RF, Ayrizono MLS. Perfil epidemiológico e características clínicas das doenças inflamatórias intestinais em um centro de referência Brasileiro. Arq Gastroenterol. 2021;58(4):483-90.

**RESUMO – Contexto –** O aumento na incidência e prevalência das doenças inflamatórias intestinais (DIIs) é evidente em muitos países recém-industrializados da Ásia, África, Europa Oriental e do continente Americano. No Brasil, os registros ainda são bastante escassos, sendo necessários mais estudos sobre este tema. **Objetivo –** Avaliar o perfil epidemiológico e as características clínicas dos doentes com DIIs, acompanhados em um serviço de referência no Estado de São Paulo. **Métodos –** Análise retrospectiva, descritiva, dos prontuários médicos dos pacientes com DIIs acompanhados em um centro de referência no Brasil. **Resultados –** Foram avaliados 625 doentes, sendo 416 com doença de Crohn (DC), 190 com retocolite ulcerativa (RCU) e 19 com colite indeterminada. A média de idade foi de 31,6 anos, sendo a distribuição homogênea entre os sexos. Na DC predominou a classificação de Montreal A2 L3 e B1, com 44,8% dos doentes apresentando doença perianal; na RCU, E2 e S0. A principal manifestação extraintestinal presente foi reumatológica, vindo a seguir as lesões cutâneas e as oftalmológicas. A maioria dos doentes (85,4%) fazia uso de alguma medicação, sendo as mais frequentes, aminossalicilatos na RCU e terapia biológica na DC. Em relação às cirurgias, na DC um significativo percentual de pacientes foi submetido a algum tipo de procedimento cirúrgico, ao contrário da RCU, incluindo fistulotomias e colocação de sedenhos, estomas de derivação, enterectomia, ileotiflectomia/colectomia direita, colectomia total ou parcial e plastias intestinais. Apenas 195 (31,2%) doentes eram da cidade de Campinas, mas 443 (70,9%) eram provenientes do 7º Departamento Regional de Saúde (DRS), que corresponde a Grande Campinas. **Conclusão –** No estudo, houve um predomínio de pacientes do DRS de Campinas; os doentes eram jovens, sem predominância de sexo; a frequência maior foi de doentes com DC (66,6%). A maioria (85,4%) estava em tratamento farmacológico, e um percentual significativo de doentes com DC, havia sido submetido à cirurgia.

**Palavras-chave –** Doença de Crohn; retocolite ulcerativa; epidemiologia; trato gastrointestinal.

## REFERENCES

1. Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology. 2007;133:1670-89.
2. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94:155-65.
3. Cardozo WS, Sobrado CW. Doença Inflamatória Intestinal. 2a Edição ed. São Paulo SP 2015.
4. Mulder DJ, Noble AJ, Justinich CJ, Duffin JM. A tale of two diseases: the history of inflammatory bowel disease. J Crohns Colitis. 2014;8:341-8.
5. Baumgart DC. The diagnosis and treatment of Crohn's disease and ulcerative colitis. Dtsch Arztebl Int. 2009;106:123-33.
6. Vasconcelos RS, Rocha RM, Souza EB, Amaral VRS. Life quality of patients with inflammatory bowel disease: integrative review Calidad de vida de pacientes con enfermedad inflamatoria intestinal: revisión integrativa. [Article in Portuguese]. ESTIMA, Braz J Enterostomal Ther. 2018;16. doi: 10.30886/estima.v16.480_PT
7. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2018;390:2769-78.
8. Vedamurthy A, Ananthakrishnan AN. Influence of Environmental Factors in the Development and Outcomes of Inflammatory Bowel Disease. Gastroenterol Hepatol (NY). 2019;15:72-82.
9. Gasparini RG, Sassaki LY, Saad-Hossne R. Inflammatory bowel disease epidemiology in São Paulo State, Brazil. Clin Exp Gastroenterol. 2018;11:423-9.
10. Quaresma AB, Kaplan GG, Kotze PG. The globalization of inflammatory bowel disease: the incidence and prevalence of inflammatory bowel disease in Brazil. Curr Opin Gastroenterol. 2019;35:259-64.
11. Bernstein CN, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. Gut. 2008;57:1185-91.
12. Kaplan GG, Ng SC. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. Gastroenterology. 2017;152:313-21.e2.

13. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005;19 (Suppl A): 5A-36A.
14. Queiroz DM, Oliveira AG, Saraiva IE, Rocha GA, Rocha AM, das Graças Pimenta Sanna M, et al. Immune response and gene polymorphism profiles in Crohn's disease and ulcerative colitis. Inflamm Bowel Dis. 2009;15:353-8.
15. Buenavida G, Casañias A, Vásquez C, De Souza M, Martínez L, Gardil I, et al. Incidence of inflammatory bowel disease in five geographical areas of Uruguay in the biennial 2007-2008. Acta Gastroenterol Latinoam. 2011;41:281-7.
16. Burisch J, Jess T, Martinato M, Lakatos PL, EpiCom E. The burden of inflammatory bowel disease in Europe. J Crohns Colitis. 2013;7:322-37.
17. Lophaven SN, Lynge E, Burisch J. The incidence of inflammatory bowel disease in Denmark 1980-2013: a nationwide cohort study. Aliment Pharmacol Ther. 2017;45:961-72.
18. Kaplan GG, Bernstein CN, Coward S, Bitton A, Murthy SK, Nguyen GC, et al. The Impact of Inflammatory Bowel Disease in Canada 2018: Epidemiology. J Can Assoc Gastroenterol. 2019;2(Suppl 1):S6-S16.
19. Studd C, Cameron G, Beswick L, Knight R, Hair C, McNeil J, et al. Never underestimate inflammatory bowel disease: High prevalence rates and confirmation of high incidence rates in Australia. J Gastroenterol Hepatol. 2016; 31:81-6.
20. Victoria CR, Sassak LY, Nunes HR. Incidence and prevalence rates of inflammatory bowel diseases, in midwestern of São Paulo State, Brazil. Arq Gastroenterol. 2009;46:20-5.
21. Lima Martins A, Volpato RA, Zago-Gomes MDP. The prevalence and phenotype in Brazilian patients with inflammatory bowel disease. BMC Gastroenterol. 2018;18:87.
22. Simian D, Fluxá D, Flores L, Lubascher J, Ibáñez P, Figueroa C, et al. Inflammatory bowel disease: A descriptive study of 716 local Chilean patients. World J Gastroenterol. 2016;22:5267-75.
23. Burisch J, Pedersen N, Čuković-Čavka S, Brinar M, Kaimakliotis I, Duricova D, et al. East-West gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort. Gut. 2014;63:588-97.
24. Parente JM, Coy CS, Campelo V, Parente MP, Costa LA, da Silva RM, et al. Inflammatory bowel disease in an underdeveloped region of Northeastern Brazil. World J Gastroenterol. 2015;21:1197-206.
25. Santos RMD, Carvalho ATP, Silva KDS, Sá SPC, Santos AHD, Sandinha MR. Inflammatory Bowel Disease: Outpatient Treatment Profile. Arq Gastroenterol. 2017;54:96-100.
26. Torres US, Rodrigues JO, Junqueira MS, Uezato S, Netinho JG. The Montreal Classification for Crohn's disease: clinical application to a Brazilian single-center cohort of 90 consecutive patients. Arq Gastroenterol. 2010;47:279-84.
27. Selvaratnan S, Gullino S, Shim L, Lee E, Lee A, Paramsothy S. Epidemiology of inflammatory bowel disease in South America: A systematic review. World J Gastroenterol. 2019;25:6866-75.
28. Ooi CJ, Makharia G, Hilmi I, Gibson P, Fock KM, Ahuja V. Asia Pacific Consensus Statements on Crohn's disease. Part 1: Definition, diagnosis, and epidemiology (Asia Pacific Crohn's Disease Consensus- Part 1). Gastroenterol Hepatol. 2015;31:45-55.
29. Manninen P, Karvonen AL, Huhtala H, Rasmussen M, Collin P. The epidemiology of inflammatory bowel diseases in Finland. Scand J Gastroenterol. 2010;45:1063-7.
30. Tozun N, Atug O, Imeryuz N, Hamzaoglu HO, Tiftikci A, Parlak E, et al. Clinical characteristics of inflammatory bowel disease in Turkey: a multicenter epidemiologic survey. J Clin Gastroenterol. 2009;43:51-7.
31. Lakatos L, Kiss LS, David G, Pandur T, Erdelyi Z, Mester G, et al. Incidence, disease phenotype at diagnosis, and early disease course in inflammatory bowel diseases in Western Hungary, 2002-2006. Inflamm Bowel Dis. 2011;17:2558-65.
32. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019;94:155-65.
33. Silva BC, Lyra AC, Mendes CM, Ribeiro CP, Lisboa SR, de Souza MT, et al. The Demographic and Clinical Characteristics of Ulcerative Colitis in a Northeast Brazilian Population. Biomed Res Int. 2015;2015:359130.
34. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol. 2015;12:720-7.
35. Lakatos L, Pandur T, David G, Balogh Z, Kuronya P, Tollas A, et al. Association of extraintestinal manifestations of inflammatory bowel disease in a province of western Hungary with disease phenotype: results of a 25-year follow-up study. World J Gastroenterol. 2003;9:2300-7.
36. D'Incà R, Podswiadek M, Ferronato A, Punzi L, Salvagnini M, Sturniolo GC. Articular manifestations in inflammatory bowel disease patients: a prospective study. Dig Liver Dis. 2009;41:565-9.
37. Farhi D, Cosnes J, Zizi N, Chosidow O, Seksik P, Beaugerie L, et al. Significance of erythema nodosum and pyoderma gangrenosum in inflammatory bowel diseases: a cohort study of 2402 patients. Medicine (Baltimore). 2008;87:281-93.
38. Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. J Crohns Colitis. 2012;6:965-90.
39. Cosnes J, Cattan S, Blain A, Beaugerie L, Carbonnel F, Parc R, et al. Long-term evolution of disease behavior of Crohn's disease. Inflamm Bowel Dis. 2002;8:244-50.
40. Jess T, Riis L, Vind I, Winther KV, Borg S, Binder V, et al. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. Inflamm Bowel Dis. 2007;13:481-9.
41. Burisch J, Pedersen N, Cukovic-Cavka S, Turk N, Kaimakliotis I, Duricova D, et al. Environmental factors in a population-based inception cohort of inflammatory bowel disease patients in Europe-an ECCO-EpiCom study. J Crohns Colitis. 2014;8:607-16.
42. Hanauer SB, Sandborn WJ, Lichtenstein GR. Evolving Considerations for Thiopurine Therapy for Inflammatory Bowel Diseases-A Clinical Practice Update: Commentary. Gastroenterology. 2019;156:36-42.
43. Bernstein CN, Loftus EV, Ng SC, Lakatos PL, Moum B. Hospitalisations and surgery in Crohn's disease. Gut. 2012;61:622-9.
44. Bechara CS, Lacerda Filho A, Ferrari MLA, Andrade DAR, Luz MMP, Silva RG. Caracterização de pacientes operados por doença de Crohn pela classificação de Montreal e identificação de fatores preditores de sua recorrência cirúrgica. Rev Col Bras Cir. 2015;42:97-105.
45. Targownik LE, Singh H, Nugent Z, Bernstein CN. The epidemiology of colectomy in ulcerative colitis: results from a population-based cohort. Am J Gastroenterol. 2012;107:1228-35.

ORIGINAL ARTICLE

AG-2021-83
doi.org/10.1590/S0004-2803.202100000-88

# Symptoms associated with different degrees of megaesophagus in Chagas disease

Jaline de Araujo **OLIVEIRA**[1], Aretuza Zaupa Gasparim El **GHARIB**[1] and Roberto Oliveira **DANTAS**[2]

Received: 16 April 2021
Accepted: 15 June 2021

**ABSTRACT** – **Background** – Dysphagia is the most frequent digestive symptom in Chagas disease, although other symptoms are reported. These symptoms can be associated with the degree of radiological impairment of the esophagus and the duration of dysphagia. **Objective** – This investigation aimed to assess the symptoms and the time of dysphagia related to the different degrees of megaesophagus in patients with Chagas disease. **Methods** – A total of 29 patients aged 48 to 73 years participated in this investigation. All of them had dysphagia and a positive serum result for Chagas disease. They were submitted to the assessment of symptoms and radiological examination of the esophagus to assess the degree of megaesophagus, which ranged from I (mild change) to IV (intense change). Dysphagia was quantified with the Eating Assessment Tool (EAT-10). **Results** – Twelve (41%) patients had megaesophagus degree I, 9 (31%) had degree II, and 8 (28%) had degrees III (6) and IV (2). The intensity of dysphagia was not related to the result of the radiological examination, with EAT-10 median of 5.5 for the degree I, 9.0 for degree II, and 5.5 for degrees III and IV ($P>0.25$). Choking (14%), regurgitation (21%), voice complaint (21%), weight loss (17%), and odynophagia (17%) were not related to the degree of megaesophagus. Voice changes and odynophagia were related to the patients' time of dysphagia. Likewise, the frequency of symptoms and EAT-10 values were related to the duration of dysphagia. **Conclusion** – The longer the patient had dysphagia, the more frequent were the symptoms reported by the patients. There was no relationship between the degrees of megaesophagus and the symptoms and intensity of dysphagia.

**Keywords** – Chagas disease; esophageal achalasia; deglutition disorders; esophageal disorders; trypanosomiasis.

## INTRODUCTION

Chagas disease, caused by the flagellate protozoan *Trypanosoma cruzi*, is an infectious condition with great morbimortality potential and impact on the biological, social, economic, and psychological aspects of health[1]. It is widely distributed in Latin America and present in other continents, as well[1,2].

In the chronic and symptomatic phase of the disease, the heart and digestive system suffer significant clinical impairment[1,3]. In the digestive system, esophagopathy causes the most frequent clinical manifestations, a result of the destruction of the myenteric plexus of the esophagus. It triggers functional motility changes, such as hypocontractility, loss of peristalsis, achalasia of the lower esophageal sphincter, and megaesophagus[4,5]. Esophageal motility disorder causes dysphagia and possible regurgitation of the swallowed material, heartburn, and weight loss[4,5].

Dysphagia, which means swallowing difficulty, is the most frequent digestive symptom, occurring during ingestion of both solid and liquid foods – though most often and intense with solid foods[4]. The patient most often manifests the perception of the difficult transit of the bolus through the retrosternal area, yet the difficulty can be perceived in a more proximal location.

Despite the expected relationship between the degree of megaesophagus and dysphagia – in which the greater the intensity of dysphagia perceived by the patient, the greater the radiological impairment of the esophagus –, patients with significant dilation of the esophagus may report discrete dysphagia. It can be either oropharyngeal or esophageal, as changes in the esophagus, pharyngoesophageal transit, and pharynx are observed in the disease[4-8].

The objective of this investigation was to relate dysphagia and other symptoms to the different degrees of megaesophagus in Chagas disease, as well as the time since the patient first perceived they had dysphagia. The hypothesis is that patients whose esophagus is most impaired according to radiological examination and whose dysphagia has lasted longer have more intense dysphagia and more frequent symptoms.

## METHODS

This research was approved by the Human Research Ethics Committee of the University of Maringá (UEM) under Certificate of Presentation for Ethical Consideration (CAAE) number 45350415.0.0000.0104. All participating patients signed the Informed Consent Form (ICF).

This is an observational, non-intervention investigation. The data were collected at UEM, while the radiological examinations were conducted at a radiology clinic in the city of Maringá (Paraná). Chagas disease patients (positive serum result) of both sexes with radiological change in the esophagus and self-reported dysphagia for at least one year were included. Patients with a negative serum result for *Trypanosoma cruzi* infection, with a history of neurological and/or oncological changes, without dysphagia, or submitted to surgical or endoscopic treatment were not included.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade Estadual de Maringá, Maringá, PR, Brasil. [2] Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.
Corresponding author: Roberto Oliveira Dantas. E-mail: rodantas@fmrp.usp.br

It was investigated whether the patients had symptoms other than dysphagia, and for how long they had had dysphagia. The Eating Assessment Tool (EAT-10)[9] translated and validated into Portuguese[10] was used to assess dysphagia. All the patients had EAT-10 results ≥3, which indicates dysphagia according to what has been established in this protocol[9,10].

Radiological examinations of the esophagus were conducted to classify megaesophagus into four degrees based on the technique described in 1960[11], in which degree I is the least impaired and degree IV, the most impaired[11-13]. The descriptions of these degrees are in reference[13].The diagnosis of esophageal involvement by Chagas' disease was done by the radiologic examination.

The data obtained were entered into a Microsoft Excel 2013 spreadsheet and statistically analyzed with Statistical Single User, version 13.2. The chi-square test was used for the statistical analysis, and the multiple quartile regression model, for the EAT-10 assessment[14]. Comparisons with $P \leq 0.05$ were considered significant.

## RESULTS

Altogether, 29 individuals participated in the study – 21 (72%) females and 8 (28%) males, aged 48 to 73 years, mean 63±5 years. Twelve (41%) patients had megaesophagus degree I, 9 (31%) had degree II, and 8 (28%) had either degree III or IV (six with degree III and two with degree IV).

The EAT-10 results related to the degree of megaesophagus are shown in TABLE 1, and the frequency of symptoms in relation to the degree of megaesophagus is shown in TABLE 2. There was no relationship between the quantitative assessment of dysphagia (EAT-10) and the degree of megaesophagus ($P>0.25$), nor between the frequency of symptoms and the degree of megaesophagus ($P=0.55$).

**TABLE 1.** Results of the Eating Assessment Tool (EAT-10) related to the degree of megaesophagus in patients with Chagas disease.

| | 1st quartile | Median | 3rd quartile |
|---|---|---|---|
| Degree I (N=12) | 3.0 | 5.5 | 10.0 |
| Degree II (N=9) | 8.0 | 9.0 | 11.5 |
| Degrees III/IV (N=8) | 4.5 | 5.5 | 13.5 |

$P>0.25$. N: number.

**TABLE 2.** Frequency of the symptoms in relation to the degree of megaesophagus.

| Symptoms | Degree of megaesophagus | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Degree I | | Degree II | | Degrees III/IV | | Total | |
| | N=12 | | N=9 | | N=8 | | N=29 | |
| | N | % | N | % | N | % | N | % |
| Coughing | 1 | 8 | 0 | 0 | 0 | 0 | 1 | 3 |
| Choking | 1 | 8 | 1 | 11 | 2 | 25 | 4 | 14 |
| Voice complaint | 3 | 25 | 2 | 22 | 1 | 13 | 6 | 21 |
| Regurgitation | 2 | 16 | 0 | 0 | 4 | 50 | 6 | 21 |
| Odynophagia | 3 | 25 | 2 | 22 | 0 | 0 | 5 | 17 |
| Weight loss | 2 | 16 | 2 | 22 | 1 | 13 | 5 | 17 |
| Food stuck in the throat | 0 | 0 | 1 | 11 | 0 | 0 | 1 | 3 |
| Vomit | 0 | 0 | 1 | 11 | 0 | 0 | 1 | 3 |

$P=0.55$ (chi-square test). N: number.

Furthermore, there was no relationship between the time of dysphagia and the degree of megaesophagus ($P=0.87$, TABLE 3). On the other hand, there was a relationship between time of dysphagia and EAT-10 ($P=0.02$, TABLE 4), with a tendency of higher EAT-10 scores occurring in patients whose dysphagia had been lasting for longer. The EAT-10 median was 5.0 (limits 3 to 11) in patients with dysphagia for one to ten years and 13.5 (limits 4 to 26) in patients with dysphagia for more than ten years ($P<0.05$). There was a relationship between the time the patient had had dysphagia and the frequency of the symptoms – the longer the dysphagia had been lasting, the more frequent the symptoms ($P=0.02$, TABLE 5).

**TABLE 3.** Time of dysphagia, in years, in patients with Chagas disease in relation to the degree of megaesophagus, expressed in number (N) and percentage (%) of patients.

| Time of dysphagia | | Degree I (N=12) | | Degree II (N=9) | | Degrees III/IV (N=8) | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| 1–5 | (N=9) | 4 | 33 | 3 | 33 | 2 | 25 |
| 6–10 | (N=7) | 3 | 25 | 2 | 22 | 2 | 25 |
| 11–20 | (N=7) | 4 | 33 | 2 | 22 | 1 | 12 |
| 21 | (N=6) | 1 | 8 | 2 | 22 | 3 | 38 |

$P=0.87$. N: number.

**TABLE 4.** Time of dysphagia, in years, in patients with Chagas disease in relation to the results of the Eating Assessment Tool (EAT-10).

| Time of dysphagia | | EAT-10 | | |
|---|---|---|---|---|
| | | 1st quartile | Median | 3rd quartile |
| 1–5 | (N=9) | 2.5 | 3.0 | 3.5 |
| 6–10 | (N=7) | 5.0 | 8.0 | 10.0 |
| 11–20 | (N=7) | 5.0 | 15.0 | 22.0 |
| 21 | (N=6) | 10.0 | 13.5 | 17.0 |

EAT-10: Eating Assessment Tool; $P=0.02$. 1–5 years vs >20 years. N: number.

**TABLE 5.** Frequency of the symptoms of the patients with Chagas disease in relation to the time of dysphagia, in years.

| Symptoms | Time of dysphagia (years) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1–5 (N=9) | | 6–10 (N=7) | | 11–20 (N=7) | | >20 (N=6) | |
| | N | % | N | % | N | % | N | % |
| Coughing | 0 | 0 | 1 | 14 | 0 | 0 | 0 | 0 |
| Choking | 0 | 0 | 2 | 29 | 0 | 0 | 2 | 33 |
| Voice complaint | 1 | 11 | 2 | 29 | 3 | 43 | 0 | 0 |
| Regurgitation | 3 | 33 | 0 | 0 | 0 | 0 | 3 | 50 |
| Odynophagia | 1 | 11 | 0 | 0 | 4 | 57 | 0 | 0 |
| Weight loss | 3 | 3 | 2 | 29 | 0 | 0 | 0 | 0 |
| Food stuck in the throat | 1 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vomit | 1 | 11 | 0 | 0 | 0 | 0 | 1 | 3 |

$P=0.02$. N: number.

## DISCUSSION

Of the patients infected with *Trypanosoma cruzi*, 7% to 10% develop motor changes in the esophagus in the chronic phase – i.e., simultaneous and ineffective contractions of the body of the esophagus and partial or absent relaxation of the lower esophageal sphincter (achalasia)[4]. They cause various symptoms, mainly dysphagia, but also regurgitation, chest pain, and heartburn.

In this study, 12 (41%) out of the patients assessed had megaesophagus degree I, 9 (31%) had degree II, and 8 (28%) had either degree III or IV. We assumed that the longer the dysphagia had been lasting, the more intense the radiological impairment of the esophagus would be. This hypothesis did not prove to be true, as it had been suggested in a previous paper[15]. This indicates that the esophageal impairment caused by the disease occurs mainly in the acute phase, with little or no evolution in the esophageal changes in most of the patients, which must be a consequence of the cellular and humoral response to the *Trypanosoma cruzi*. The association between the loss of neurons in the myenteric plexus caused by Chagas disease and the loss caused by aging[16] can explain both the onset of dysphagia many years after the patient was infected with *T. cruzi* and the greater intensity of dysphagia years after the symptoms appeared. However, a paper with a larger number of Chagas disease patients did not found relationship between age and radiological grade, and described a relation between dysphagia and the degree of megaesophagus[13].

The most reported symptoms associated with dysphagia were voice complaint (in 21% of the patients assessed), regurgitation (21%), odynophagia (17%), weight loss (17%), and choking (14%). The most frequent voice alteration was hoarseness. Previous paper describe the occurrence of multiple swallowings (14%), coughing (18%), phlegm (9%), hoarseness (36%) and, in the videofluoroscopy, pharyngeal residues (18%) and laryngeal penetration (18%)[8]. In a paper including patients without megaesophagus, the described symptoms, besides dysphagia, were heartburn (40%), regurgitation (6%), odynophagia (4%), retrosternal pain (12%), and coughing (6%)[17].

In patients with dysphagia onset over 20 years before, the symptoms were reportedly more frequent. Regurgitation usually appears later than dysphagia and occurs either after ingesting foods or when the patient lies down. It is more frequent when the diameter of the esophagus is increased (megaesophagus degrees III and IV)[13], potentially causing aspiration bronchopneumonia. A modern assessment method revealed that 78% of the patients with megaesophagus due to Chagas disease have aspiration of esophageal content into the airways[18].

The overall analysis of the results revealed no statistically significant data in the association between symptomatology and degree of megaesophagus. However, it has been described that patients with the increased diameter of the esophagus lose weight more frequently than those whose esophagus is not increased in diameter[13,19]. The cultural diversity of the patients influences the manifestation of symptoms[20]. Hence, patients with greater impairment in motility and esophageal transit may have fewer complaints than patients with little functional change in the esophagus. Previous publication evaluated the symptoms of Chagas disease megaesophagus in 500 consecutive prospective patients and found dysphagia (97%), regurgitation (65%), retroesternal pain (59%), odinophagia (56%) and heartburn (32%) as the symptoms[13]. Different of the present investigation there was more intense dysphagia, more frequent chest pain, odynophagia and weight loss in patients with a more intense degree of megaesophagus, and a relation between degree of megaesophagus and time of dysphagia. Different populations and the number of patients included may be the cause of the contradictory results. However, the method of evaluation of dysphagia should be the most important factor that explain the difference, once EAT-10 evaluated dysphagia by the patients perspective.

EAT-10 is a quantitative method to characterize dysphagia by the patients, that has been validated and used in many countries around the world and whose score ranges from 0 to 40 – the higher the score, the more intense the dysphagia in the patient's perception[21].

This investigation has limitations. The number of cases studied was not large, though enough to come to conclusions. The patients' age may have influenced the results, although the effects of aging on swallowing occur more intensely after 70 years old[22].

In conclusion, we found no relationship between the degree of megaesophagus and the frequency of manifestations associated with swallowing in Chagas disease. The time of dysphagia was related to the symptoms – the longer the time of dysphagia, the more frequent the symptoms and the intensity of dysphagia perceived by the patients.

### Authors' contribution

Oliveira JA, Gharib AZGE, and Dantas RO: participated in the project, data acquisition, discussion of the results, writing of the manuscript, and decision to submit for publication.

### Orcid

Jaline A Oliveira: 0000-0002-2056-1716.
Aretuza Z G El Gharib: 0000-0001-6183-2204.
Roberto O Dantas: 0000-0003-2183-0815.

Oliveira JA, Gharib AZGE, Dantas RO. Sintomas associados aos diferentes graus de megaesôfago na doença de Chagas. Arq Gastroenterol. 2021;58(4):491-4.

**RESUMO** – **Contexto** – Disfagia é o mais frequente sintoma digestivo da doença de Chagas; entretanto, outros sintomas podem ser referidos. Esses sintomas podem ser associados ao grau de comprometimento radiológico do esôfago e à duração da disfagia. **Objetivo** – Avaliar os sintomas e o tempo de disfagia relacionados com os diferentes graus de megaesôfago em pacientes com doença de Chagas. **Métodos** – Participaram da investigação 29 pacientes com idades entre 48 e 73 anos, todos com disfagia e teste sorológico positivo para doença de Chagas. Eles foram submetidos à avaliação de sintomas e exame radiológico do esôfago para avaliar o grau de megaesôfago, que variou de I (alteração discreta) a IV (alteração intensa). Disfagia foi quantificada pelo método *Eating Assessment Tool* (EAT-10). **Resultados** – Doze (41%) pacientes apresentaram grau I de megaesôfago, 9 (31%) grau II, e 8 (28%) graus III/IV. A intensidade da disfagia não foi relacionada com o resultado do exame radiológico, com a mediana do EAT-10 de 5,5 para o grau I, 9,0 para o grau II, e 5,5 para os graus III/IV ($P$>0,25). Engasgo (14%), regurgitação (21%), queixa vocal (21%), perda de peso (17%), e odinofagia (17%) não foram relacionados ao grau de megaesôfago. Houve relação entre alteração vocal e odinofagia com o tempo que os pacientes tinham disfagia. Houve relação entre frequência de sintomas e valores do EAT-10 com a duração da disfagia. **Conclusão** – Quanto mais longo o tempo que o paciente tem disfagia maior a frequência de sintomas referidos pelos pacientes. Não há relação entre graus de megaesôfago com os sintomas e a intensidade da disfagia.

**Palavras-chave** – Doença de Chagas, acalasia esofágica, transtornos da deglutição, doenças do esôfago, tripanossomíase.

## REFERENCES

1. Lidani KCF, Andrade FA, Bavia L, Damasceno FS, Beltrame MH, Messias-Reason IJ, et al. Chagas' disease: from discovery to a worldwide health problem. Front Public Health. 2019;7:166. doi: 10.3389/fpubh.2019.00166.
2. Roure S, Valerio L, Vallès X, Morales B, Garcia Diaz MI, Pedro-Botet ML, Serra J. Oesophageal motility disorders in infected immigrants with Chagas disease in a non-endemic European area. United European Gastroenterol J. 2016;4:614-20.
3. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões MV. Pathogenesis of chronic Chagas heart disease. Circulation. 2007;115:1109-23.
4. Oliveira RB, Troncon LEA, Dantas RO, Meneghelli UG. Gastrointestinal manifestations of Chagas' disease. Am J Gastroenterol. 1998;93:884-9.
5. Matsuda NM, Miller SM, Évora PRB. The chronic gastrointestinal manifestations of Chagas' disease. Clinics (São Paulo). 2009;64:1219-24.
6. Dantas RO. The pharyngo-esophageal transition zone in Chagas' disease. Clin Gastroenterol Int. 2020;2:20-3.
7. Santos CM, Cassiani RA, Dantas RO. Videofluoroscopic evaluation of swallowing in Chagas' disease. Dysphagia. 2011;26:361-5.
8. Cabral DMG, Abrahão Junior LJ, Marques CHD, Pereira BB, Pedrosa RC. Oropharyngeal dysphagia in patients with chronic Chagas disease: phonoaudiological, videofluoroscopic, and manometric evauations. Acta Fisiatr. 2015;22:24-9
9. Belafsky PC, Mouadeb DA, Rees CJ, Pryor JC, Postma GN, Allen J, et al. Validity and reliability of the Eating Assessment Tool (EAT-10). Ann Otol Rhinol Laryngol. 2008;117:919-24.
10. Gonçalves MIR, Remaili CB, Behlau M. Cross-cultural adaptation of the Brazilian version of the Eating Assessment Tool EAT-10. CoDAS. 2013;25:601-4.
11. Rezende JM, Lauar KL, Oliveira AR. Aspéctos clínicos e radiológicos da aperistalsis do esôfago. Rev Bras Gastroenterol. 1960;12:247-62.
12. Abud TG, Abud LG, Vilar VS, Szejnfeld D, Reibscheid S. Radiologic findings in megaesophagus secondary to Chagas' disease: chest X-ray and esophagogram. Radiol Bras. 2016;49:358-62.
13. Vaz MGM, Rezende JM, Ximenez CA, Luquetti AO. Correlação entre a sintomatologia e a evolução do megaesôfago. Rev Goiana Med. 1995;41:1-15.
14. Koenker R, Bassett G. Regression quantiles. Econometrica. 1978;46:33-50.
15. Meneghelli UG, Peria FM, Darezzo FMR, Almeida FH, Rodrigues CM, Aprile LRO, et al. Clinical, radiographic and manometric evolution of esophageal involvement by Chagas' disease. Dysphagia. 2005;20:40-5.
16. Köberle F. Chagas' disease and Chagas' syndrome: The pathology of American trypanosomiasis. Adv Parasitol. 1968;6:63-116.
17. Sanchez-Lermen RLP, Dick E, Salas JAP, Fontes CJF. Sintomas do trato digestivo superior e distúrbios motores do esôfago em pacientes portadores da forma indeterminada da doença de Chagas crônica. Rev Soc Bras Med Trop. 2007;40:197-203.
18. Alves LR, Soares EG, Aprile LRO, Elias Junior J, Bras PBV, Baddini-Martinez J. Chlorophyllin-stained macrophages as markers of pulmonary aspiration. Am J Respir Crit Care. 2013;188:1470-2.
19. Santos CM, Cassiani RA, Dantas RO. Clinical evaluation of swallowing in Chagas' disease. Rev Soc Bras Fono. 2011;16:215-20.
20. Koidou I, Kollias N, Sdravou K, Grouios G. Dysphagia: a short review of the current state. Edu Gerontol. 2013;39:812-927.
21. Batista AO, Nascimento WV, Cassiani RA, Silva ACV, Alves LMT, Alves DC, et al. Prevalence of non-obstructive dysphagia in patients with heartburn and regurgitation. Clinics (São Paulo). 2020;75:e1556.
22. Namasivayam-MacDonald AM, Barbon CEA, Steele CM. A review of swallowing timing in the elderly. Physiol Behav. 2018;184:12-26.

ORIGINAL ARTICLE

AG-2021-94
doi.org/10.1590/S0004-2803.202100000-89

# Classical serological markers in pediatric inflammatory bowel disease in Brazil

Maraci **RODRIGUES**[1], Cleonice **BUENO**[2], Elizete Aparecida **LOMAZI**[3], Maria Inez Machado **FERNANDES**[4], Clarice Blaj **NEUFELD**[5], Maria Fernanda Marranghello **D'AMICO**[6] and Fátima Regina De Almeida **PATIÑO**[6]

Received: 5 May 2021
Accepted: 29 June 2021

**ABSTRACT** – **Background** – Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCAs) have long been used to differentiate between Crohn's disease (CD) and ulcerative colitis (UC), more recently having been used as prognostic indicators. **Objective** – To determine the diagnostic accuracy of serological markers in the identification of pediatric CD and UC in Sao Paulo, Brazil, as well as to correlate those markers with characteristics demographic and clinical of these two diseases. **Methods** – Retrospective cross-sectional multi-center study involving pediatric patients with inflammatory bowel disease (IBD). We identified ASCAs serological markers and p-ANCA, correlating their presence with demographic and clinical data, not only in the patients with IBD but also in a group of age-matched gastrointestinal disease-free controls. **Results** – A total of 122 patients, 74 with IBD (46% males), treated at four pediatric gastroenterology referral centers, the mean age of 13±7 years, 49 (66%) with CD, and 25 (34%) with UC. The control Group comprised 48 patients (54% males). The proportion of patients testing positive for p-ANCA was significantly higher in the UC group (69.9%) compared to the CD group (30.4%), as well as being significantly higher in the CD group versus the control Group ($P$<0.001 for both). The proportion of patients testing positive for ASCA IgA (76.2%) and ASCA IgG (94.4%) markers was also significantly higher in the CD group than in the control Group ($P$<0.001), and such positivity correlated significantly with the use of immunomodulatory medications such as azathioprine and anti-tumor necrosis factor agents (azathioprine 38.9%, anti-TNF 55.6%; $P$=0.002). In the CD group, the proportion of patients testing positive for the ASCA IgA was significantly higher among those who underwent surgery than among those who did not (26.86±17.99; $P$=0.032). **Conclusion** – In pediatric patients with IBD in Sao Paulo, Brazil, serological tests proving to be highly specific, although not very sensitive, for the diagnosis of IBD. However, the serological markers showed a positive correlation with the severity of the disease.

**Keywords** – Antibodies; antineutrophil cytoplasmic; antibodies; anti- *Saccharomyces cerevisiae*; inflammatory bowel disease.

## INTRODUCTION

The diagnosis of pediatric inflammatory bowel disease (IBD) is always a challenge and is based on a combination of symptoms and signs at intestinal and extraintestinal sites, endoscopic findings in the upper and lower gastrointestinal tract, as well as radiological or capsule endoscopy findings consistent with the condition in the small bowel, assuming that other diseases with similar clinical manifestations have been excluded[1]. In approximately 25% of patients with IBD, the first symptoms appear before the age of 18[2]. Despite careful clinical evaluation, IBD-unclassified (IBD-U, a form of colonic IBD whose features make it impossible to define as either colitis of Crohn's disease or ulcerative colitis at diagnosis), seemed straightforward and whereas more than 14% are misdiagnosed with ulcerative colitis (UC), consequently undergoing unnecessary colectomy and ileoanal anastomosis[3].

Classical serological markers, such as perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCAs), have been used in pediatric patients since 1993 and 1998, respectively[4,5]. Although they were initially used to differentiate between Crohn's disease (CD) and UC, later they have been used for predicting disease course and outcomes in IBD[6-10].

ANCAs are defined as autoantibodies directed against an unidentified component of the nuclear envelope within neutrophil granules. On indirect immunofluorescence (IIF), these antibodies present staining that is either cytoplasmic (c-ANCA) or perinuclear (p-ANCA); a further subtype comprises those that show the latter type of staining and are sensitive to deoxyribonuclease (DNase). High levels of such p-ANCA are more strongly associated with UC than with CD[6,11].

Although it seems that ASCAs develop as an epiphenomenon during the onset of CD, luminal antigens such as bacteria and fungus play essential roles in perpetuating the inflammatory process. In patients with CD, the loss of immune tolerance in the presence of resident bacterial flora is one of the fundamental aspects of the pathogenesis of the disease[11].

Given the severe clinical repercussions of IBD in children and adolescents, more efforts have been focused on improving the diagnosis and especially identifying patients at risk for a poor prognosis, including serological biomarkers[9,10].

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Faculdade de Medicina da Universidade de São Paulo, Hospital das Clínicas, Departamento de Gastroenterologia, São Paulo, SP, Brasil. [2] Faculdade de Medicina da Universidade de São Paulo, Hospital das Clínicas, Laboratório de Investigação Médica Reumatologia, São Paulo, SP, Brasil. [3] Universidade Estadual de Campinas, Hospital de Clínicas, Departamento de Pediatria, Campinas, SP, Brasil. [4] Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Pediatria, Ribeirão Preto, SP, Brasil. [5] Santa Casa de São Paulo, Departamento de Pediatria, São Paulo, SP, Brasil. [6] Complexo Hospitalar do Mandaqui, Unidade de Gastroenterologia Pediátrica, São Paulo, SP, Brasil.
Corresponding author: Maraci Rodrigues E-mail: maraci@uol.com.br

The lack of studies of the ASCA immunoglobulin (Ig)A, ASCA IgG, and p-ANCA serological markers in pediatric patients with IBD in Brazil motivated us to apply these markers with the following objectives: to determine the diagnostic accuracy of serological antibody tests for the identification of pediatric patients with CD or UC, as well as to determine the prevalence of those serological markers in pediatric patients without gastrointestinal problems; and to correlate those markers with sex, age at symptom onset, age at diagnosis, time from symptom onset to diagnosis, site of the disease, type of treatment (clinical or surgical) and complications.

## METHODS

A cross-sectional multi-center study involving children and adolescents with an established diagnosis of either DC or UC, based on the Porto criteria[1]. Children with atypical UC and IBD-U were excluded. We decided to publish this study which was done between 2005 and 2008 to determine the diagnostic accuracy of serological markers in the identification of pediatric CD and UC in four centers in São Paulo's state, Brazil *Universidade de São Paulo Faculdade de Medicina Hospital das Clínicas, Departamento de Gastroenterologia e Laboratório de Investigação Médica (LIM) Reumatologia; Universidade Estadual de Campinas, Hospital de Clínicas, Departamento de Pediatria; Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Pediatria; Santa Casa de São Paulo, Departamento de Pediatria Complexo Hospitalar do Mandaqui, Unidade de Gastroenterologia Pediátrica* as well as to correlate those markers with characteristics demographic and clinical of the disease because this subject persists with great importance nowadays. Demographic and clinical data of the controls were obtained by reviewing their medical records.

Blood samples (5 mL) to test for ASCAs markers (IgA and IgG) and p-ANCA were collected from patients and controls. We employed an indirect IIF technique to determine if the predominant staining pattern of the ANCAs was perinuclear (p-ANCA) or cytoplasmic (c-ANCA), using human granulocytes fixed in ethanol and formalin as the standard method. The standard dilution of 1:20 was used, and the positive sera were titrated with antinuclear antibodies (ANAs) on human epithelial type 2 cells. Cases testing positive for c-ANCA were confirmed by fixation with an enzyme-linked immunosorbent assay (ELISA) kit (INOVA Diagnostics, Inc., San Diego, CA, USA). Patients who were ANA positive were also p-ANCA positive only if the p-ANCA title was >2 dilutions greater than that of ANA. We also used ELISA (QUANTA Lite ASCA kit; INOVA Diagnostic Inc.) to quantify ASCAs. The pattern was arbitrarily designated negative: values from 0 to 20.0 IU/mL, indeterminate from 20.1 to 24.9 IU/mL, and positive when above or equal to 25 IU/mL. Positivity for ASCA IgG, ASCA IgA, or both were considered a marker for CD. Laboratory technicians were blinded for clinical histories and diagnosis.

The study was approved by the Research Ethics Committees of the respective centers. All participating patients gave written informed consent.

### Statistical analysis

Chi-square tests of homogeneity or Fisher's exact tests were used to evaluate the association between serological markers and categorical variables. Mann-Whitney tests were used to determine the relation of markers and continuous variables. Mann-Whitney tests were used to evaluate the association between ASCAs markers and categorical variables or Kruskal-Wallis tests. Sensitivity and specificity values were calculated in comparison with the control Group. Statistical analyses were performed with the Statistical Package for the Social Sciences, version 13.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

We evaluated 122 patients, 49 with a confirmed diagnosis of CD, of whom 25 were male (CD group). In that group, ages 14±4.6 years (range 1.6–18). We also evaluated 25 patients with a confirmed diagnosis of UC, 11 males (UC group). In the UC group, ages 12.5±4.8 years (range 2–18). Also, we evaluated a group of 48 controls, ages 7.4±4.2 years (range 0.5-18), TABLE 1.

The proportions of patients testing positive for p-ANCA were significantly higher in the UC group (69.6%) compared to the CD group (30.4%; $P<0.001$), as well as being significantly higher in the CD group than in the control Group (0%; $P<0.001$), TABLE 1. TABLE 2 shows that the proportion of patients testing positive for the ASCA IgG marker was significantly higher in the CD group than in the control Group ($P<0.001$) and that positivity for ASCA IgG correlated with the use of immunomodulatory medication (Azathioprine 38.9%; anti-TNF 55.6%; $P=0.002$). As shown in TABLE 3, the proportion of patients testing positive for the ASCA IgA marker was also significantly higher in the CD group (76.2%) than in the control Group (23.8%; $P=0.001$).

There was no significant relationship between the detection of the serological markers and localization of the disease ($P>0.05$ for all), TABLES 1, 2, and 3.

There was no significant relationship between the detection of the serological markers and the age-related measures ($P>0.05$ for all), TABLE 4. It is also noteworthy that the ASCA IgA marker values were statistically higher among individuals who had undergone surgery (26.86±17.99; $P=0.032$), TABLES 5 and 6.

p-ANCA marker is highly specific, albeit not sensitive, for the diagnosis of UC, TABLE 7. ASCAs IgG and IgA markers had high specificity and low sensitivity for the diagnosis of CD, TABLE 7.

Three patients in the UC group tested positive for c-ANCA, as confirmed by ELISA, which showed negativity for anti-myeloperoxidase antibodies and positivity for anti-proteinase three antibodies – 37 IU/mL, 32 IU/mL, and 42 IU/mL, respectively – well above the reference range of ≤25 IU/mL.

## DISCUSSION

The classic biomarkers p-ANCA and ASCA help in the differential diagnosis between pediatric CD and UC, in the prognosis in CD, in the clinical expression of UC, and more recently its corrections with early treatment with anti-TNF, in addition to the association with the variability of the intestinal microbiome and the damage of the intestinal mucosa, justifying the interest in this subject in the present day[7-8,12-17].

Limited data have been reported about serological biomarkers in pediatric IBD in the Brazilian population, but all of them including a cohort or case report in adult patients, except one study that included both adult and pediatric population[18-23].

In the present study, the presence of p-ANCA was found to have a significant association with UC. Our study founded the accuracy of p-ANCA testing for the detection of UC with a sensitivity of 64%, specificity of 100%, and negative predictive value of 84.2%.

**TABLE 1.** Characteristics of pediatrics patients, by the result of perinuclear anti-neutrophil cytoplasmic antibody testing.

| Measure | Category | p-ANCA | | Total | *P* |
|---|---|---|---|---|---|
| | | Negative (n=99) n (%) | Positive (n=23) n (%) | n (%) | |
| Sex | Male | 46 (46.5) | 14 (60.9) | 60 (49.2) | 0.213 |
| | Female | 53 (53.5) | 9 (39.1) | 62 (50.8) | |
| Disease | Controls | 48 (48.5) | 0 (0) | 48 (39.3) | **<0.001** |
| | CD | 42 (42.4) | 7 (30.4) | 49 (40.2) | |
| | UC | 9 (9.1) | 16 (69.6) | 25 (20.5) | |
| Total | | 99 (100) | 23 (100) | 122 (100) | |
| CD location | Ileum | 5 (11.9) | 0 (0) | 5 (10.2) | 0.449 |
| | Small bowel | 6 (14.3) | 0 (0) | 6 (12.2) | |
| | Colon | 1 (2.4) | 0 (0) | 1 (2) | |
| | Ileum+colon | 30 (71.4) | 7 (100) | 37 (75.5) | |
| Total | | 42 (100) | 7 (100) | 49 (100) | |
| UC location | Pancolitis | 8 (88.9) | 12 (75) | 20 (80) | 0.621* |
| | Distal colitis | 1 (11.1) | 4 (25) | 5 (20) | |
| Total | | 9 (100) | 16 (100) | 25 (100) | |
| Medication | Other | 15 (29.4) | 6 (26.1) | 21 (28.4) | 0.762 |
| | Azathioprine | 22 (43.1) | 12 (52.2) | 34 (45.9) | |
| | anti-TNF-α | 14 (27.5) | 5 (21.7) | 19 (25.7) | |
| Surgery | No | 41 (80.4) | 19 (82.6) | 60 (81.1) | >0.999* |
| | Yes | 10 (19.6) | 4 (17.4) | 14 (18.9) | |
| Complication | No | 18 (35.3) | 7 (30.4) | 25 (33.8) | 0.683 |
| | Yes | 33 (64.7) | 16 (69.6) | 49 (66.2) | |
| Total | | 51 (100) | 23 (100) | 74 (100) | |

p-ANCA: perinuclear anti-neutrophil cytoplasmic antibody; anti-TNF-α: anti-tumor necrosis factor-alpha; CD: Crohn's disease; UC: ulcerative colitis. *Fisher's exact test.

**TABLE 2.** Characteristics of pediatrics patients, by the result of anti-*Saccharomyces cerevisiae* antibody immunoglobulin G testing.

| Measure | Category | ASCA IgG | | Total | *P* |
|---|---|---|---|---|---|
| | | Negative (n=104) n (%) | Positive (n=18) n (%) | n (%) | |
| Sex | Male | 51 (49.0) | 9 (50) | 60 (49.2) | 0.940 |
| | Female | 53 (51.0) | 9 (50) | 62 (50.8) | |
| Disease | Controls | 48 (46.2) | 0 (0) | 48 (39.3) | <0.001 |
| | CD | 32 (30.8) | 17 (94.4) | 49 (40.2) | |
| | UC | 24 (23.1) | 1 (5.6) | 25 (20.5) | |
| Total | | 104 (100) | 18 (100) | 122 (100) | |
| CD location | Ileum | 4 (12.5) | 1 (5.9) | 5 (10.2) | 0.652 |
| | Small bowel | 3 (9.4) | 3 (17.6) | 6 (12.2) | |
| | Colon | 1 (3.1) | 0 (0) | 1 (2.0) | |
| | Ileum+colon | 24 (75.0) | 13 (76.5) | 37 (75.5) | |
| Total | | 32 (100) | 17 (100) | 49 (100) | |
| UC location | Pancolitis | 19 (79.2) | 1 (100) | 20 (80) | >0.999* |
| | Distal colitis | 5 (20.8) | 0 (0) | 5 (20) | |
| Total | | 24 (100) | 1 (100) | 25 (100) | |
| Medication | Other | 20 (35.7) | 1 (5.6) | 21 (28.4) | 0.002 |
| | Azathioprine | 27 (48.2) | 7 (38.9) | 34 (45.9) | |
| | anti-TNF-α | 9 (16.1) | 10 (55.6) | 19 (25.7) | |
| Surgery | No | 48 (85.7) | 12 (66.7) | 60 (81.1) | 0.090* |
| | Yes | 8 (14.3) | 6 (33.3) | 14 (18.9) | |
| Complication | No | 20 (35.7) | 5 (27.8) | 25 (33.8) | 0.536 |
| | Yes | 36 (64.3) | 13 (72.2) | 49 (66.2) | |
| Total | | 56 (100) | 18 (100) | 74 (100) | |

anti-TNF-α: anti-tumor necrosis factor-alpha; ASCA: anti-*Saccharomyces cerevisiae* antibody; CD: Crohn's disease; IBD: inflammatory bowel disease; IgG: immunoglobulin G; UC: ulcerative colitis. *Fisher's exact test.

**TABLE 3.** Characteristics of pediatrics patients, by the result of anti-*Saccharomyces cerevisiae* antibody immunoglobulin A testing.

| Measure | Category | ASCA IgA | | | *P* |
|---|---|---|---|---|---|
| | | Negative (n=101) n (%) | Positive (n=21) n (%) | Total n (%) | |
| Sex | Male | 51 (50.5) | 9 (42.9) | 60 (49.2) | 0.524 |
| | Female | 50 (49.5) | 12 (57.1) | 62 (50.8) | |
| Disease | Controls | 43 (42.6) | 5 (23.8) | 48 (39.3) | 0.001 |
| | CD | 33 (32.7) | 16 (76.2) | 49 (40.2) | |
| | UC | 25 (24.8) | 0 (0) | 25 (20.5) | |
| Total | | 101 (100) | 21 (100) | 122 (100) | |
| CD location | Ileum | 4 (12.1) | 1 (6.3) | 5 (10.2) | 0.488 |
| | Small bowel | 4 (12.1) | 2 (12.5) | 6 (12.2) | |
| | Colon | 0 (0) | 1 (6.3) | 1 (2) | |
| | Ileum+colon | 25 (75.8) | 12 (75.0) | 37 (75.5) | |
| Total | | 33 (100) | 16 (100) | 49 (100) | |
| UC location | Pancolitis | 20 (80) | 0 (0) | 20 (80) | N-C |
| | Distal colitis | 5 (20) | 0 (0) | 5 (20) | |
| Total | | 25 (100) | 0 (100) | 25 (100) | |
| Medication | Other | 19 (32.8) | 2 (12.5) | 21 (28.4) | 0.233 |
| | Azathioprine | 26 (44.8) | 8 (50) | 34 (45.9) | |
| | anti-TNF-α | 13 (22.4) | 6 (37.5) | 19 (25.7) | |
| Surgery | No | 48 (82.8) | 12 (75) | 60 (81.1) | 0.486* |
| | Yes | 10 (17.2) | 4 (25) | 14 (18.9) | |
| Complication | No | 21 (36.2) | 4 (25) | 25 (33.8) | 0.401 |
| | Yes | 37 (63.8) | 12 (75) | 49 (66.2) | |
| Total | | 58 (100) | 16 (100) | 74 (100) | |

anti-TNF-α: anti-tumor necrosis factor-alpha; ASCA: anti-*Saccharomyces cerevisiae* antibody; CD: Crohn's disease; IBD: inflammatory bowel disease; IgA: immunoglobulin A; N-C: non-calculable; UC: ulcerative colitis. *Fisher's exact test.

**TABLE 4.** Comparison among serological markers, by age-related measures.

| Marker | Measure | Category | Mean ± SD | Median (range) | N | *P* |
|---|---|---|---|---|---|---|
| ASCA | Age (years) at symptom onset | Negative | 9.88 ± 5.14 | 10.0 (0.5–20.0) | 99 | 0.240 |
| | | Positive | 11.19 ± 4.43 | 11.0 (1.0–19.0) | 23 | |
| | Age (years) at diagnosis | Negative | 13.67 ± 4.83 | 14.4 (1.6–20.0) | 51 | 0.468 |
| | | Positive | 13.08 ± 4.34 | 14.0 (4.0–19.5) | 23 | |
| | Years from onset to diagnosis | Negative | 1.40 ± 1.02 | 1.0 (0.0–5.3) | 51 | 0.136 |
| | | Positive | 1.90 ± 1.64 | 1.0 (0.5–8.0) | 23 | |
| ASCA IgG | Age (years) at symptom onset | Negative | 9.82 ± 4.91 | 10.0 (0.5–20.0) | 104 | 0.106 |
| | | Positive | 11.86 ± 5.41 | 12.9 (0.6–19.0) | 18 | |
| | Age (years) at diagnosis | Negative | 13.53 ± 4.52 | 14.2 (2.0–20.0) | 56 | 0.955 |
| | | Positive | 13.35 ± 5.22 | 13.4 (1.6–20.0) | 18 | |
| | Years from onset to diagnosis | Negative | 1.60 ± 1.35 | 1.0 (0.0–8.0) | 56 | 0.832 |
| | | Positive | 1.43 ± 0.91 | 1.0 (0.2–3.0) | 18 | |
| ASCA IgA | Age (years) at symptom onset | Negative | 9.78 ± 4.91 | 9.5 (0.5–19.0) | 101 | 0.090 |
| | | Positive | 11.76 ± 5.33 | 12.0 (0.6–20.0) | 21 | |
| | Age (years) at diagnosis | Negative | 13.42 ± 4.57 | 14.2 (2.0–20.0) | 58 | 0.703 |
| | | Positive | 13.71 ± 5.13 | 13.9 (1.6–20.0) | 16 | |
| | Years from onset to diagnosis | Negative | 1.62 ± 1.35 | 1.0 (0.2–8.0) | 58 | 0.619 |
| | | Positive | 1.33 ± 0.84 | 1.0 (0.0–3.0) | 16 | |

ASCA: anti-*Saccharomyces cerevisiae* antibody; IgA: immunoglobulin A; IgG: immunoglobulin G.

**TABLE 5.** Characteristics of the anti-*Saccharomyces cerevisiae* antibody immunoglobulin G positive patients.

| Measure | Category | ASCA IgG IU/mL | | N | *P* |
|---|---|---|---|---|---|
| | | Mean ± SD | Median (range) | | |
| Sex | Male | 18.09 ± 32.95 | 10.5 (5.0–258.0) | 60 | 0.982 |
| | Female | 14.68 ± 11.81 | 11.0 (6.0–60.0) | 62 | |
| Disease | Controls | 9.52 ± 3.20 | 9.0 (5.0–16.0) | 48 | **0.002** |
| | CD | 25.38 ± 36.68 | 14.0 (6.0–258.0) | 49 | |
| | UC | 11.80 ± 6.89 | 10.0 (6.0–40.0) | 25 | |
| Total | | 16.36 ± 24.55 | 11.0 (5.0–258.0) | 122 | |
| CD Location | Ileum | 17.30 ± 11.78 | 11.0 (10.0–38.0) | 5 | 0.945 |
| | Small bowel | 25.67 ± 18.78 | 24.5 (7.0–59.0) | 6 | |
| | Colon | 12.00 ± N-C | 12.0 (12.0–12.0) | 1 | |
| | Ileum+colon | 26.78 ± 41.39 | 14.0 (6.0–258.0) | 37 | |
| Total | | 25.38 ± 36.68 | 14.0 (6.0–258.0) | 49 | |
| UC location | Pancolitis | 12.05 ± 7.62 | 10.0 (6.0–40.0) | 20 | 0.717 |
| | Distal colitis | 10.80 ± 2.68 | 12.0 (8.0–14.0) | 5 | |
| Total | | 11.80 ± 6.89 | 10.0 (6.0–40.0) | 25 | |
| Medication | Other | 13.45 ± 6.13 | 11.0 (6.0–30.0) | 21 | **0.016** |
| | Azathioprine | 23.40 ± 43.61 | 11.0 (6.0–258.0) | 34 | |
| | anti-TNF-α | 24.24 ± 14.28 | 26.0 (7.0–60.0) | 19 | |
| Surgery | No | 16.75 ± 12.32 | 12.0 (6.0–60.0) | 60 | 0.110 |
| | Yes | 38.11 ± 64.83 | 13.3 (9.0–258.0) | 14 | |
| Complication | No | 15.12 ± 10.25 | 11.0 (6.0–40.0) | 25 | 0.112 |
| | Yes | 23.68 ± 36.81 | 12.0 (6.0–258.0) | 49 | |
| Total | | 20.79 ± 30.69 | 12.0 (6.0–258.0) | 74 | |

anti-TNF-α: anti-tumor necrosis factor-alpha; ASCA: anti-*Saccharomyces cerevisiae* antibody; CD: Crohn's disease; IgG: immunoglobulin G; N-C: non-calculable; UC: ulcerative colitis.

**TABLE 6.** Characteristics of the anti-*Saccharomyces cerevisiae* antibody immunoglobulin A-positive patients.

| Measure | Category | ASCA IgA IU/mL | | N | *P* |
|---|---|---|---|---|---|
| | | Mean ± SD | Median (range) | | |
| Sex | Male | 16.28 ± 16.21 | 12.0 (4.0–106.0) | 60 | 0.439 |
| | Female | 21.52 ± 29.01 | 12.0 (5.0–169.0) | 62 | |
| Disease | Controls | 10.29 ± 5.53 | 8.0 (4.0–28.0) | 48 | **0.001** |
| | CD | 30.58 ± 33.78 | 19.0 (5.0–169.0) | 49 | |
| | UC | 12.76 ± 3.57 | 12.0 (6.0–21.0) | 25 | |
| Total | | 18.95 ± 23.65 | 12.0 (4.0–169.0) | 122 | |
| CD Location | Ileum | 29.00 ± 30.33 | 20.0 (8.0–82.0) | 5 | 0.656 |
| | Small bowel | 44.33 ± 62.26 | 18.5 (5.0–169.0) | 6 | |
| | Colon | 53.00 ± N-C | 53.0 (53.0–53.0) | 1 | |
| | Ileum+colon | 27.96 ± 28.77 | 17.0 (7.0–150.0) | 37 | |
| Total | | 30.58 ± 33.78 | 19.0 (5.0–169.0) | 49 | |
| Location UC | Pancolitis | 13.00 ± 3.52 | 12.5 (9.0–21.0) | 20 | 0.717 |
| | Distal colitis | 11.80 ± 4.03 | 12.0 (6.0–16.0) | 5 | |
| Total | | 12.76 ± 3.57 | 12.0 (6.0–21.0) | 25 | |
| Medication | Other | 16.38 ± 16.31 | 11.0 (6.0–82.0) | 21 | **0.017** |
| | Azathioprine | 28.03 ± 32.20 | 17.0 (7.0–169.0) | 34 | |
| | anti-TNF-α | 27.39 ± 32.33 | 15.0 (5.0–150.0) | 19 | |
| Surgery | No | 24.03 ± 30.81 | 14.5 (5.0–169.0) | 60 | **0.032** |
| | Yes | 26.86 ± 17.99 | 21.5 (11.0–68.0) | 14 | |
| Complication | No | 19.56 ± 14.26 | 15.0 (6.0–54.0) | 25 | 0.433 |
| | Yes | 27.11 ± 33.69 | 15.0 (5.0–169.0) | 49 | |
| Total | | 24.56 ± 28.75 | 15.0 (5.0–169.0) | 74 | |

anti-TNF-α: anti-tumor necrosis factor-alpha; ASCA: anti-*Saccharomyces cerevisiae* antibody; CD: Crohn's disease; IgA: immunoglobulin A; N-C: non-calculable; UC: ulcerative colitis.

**TABLE 7.** Measures of the accuracy of ANCA) testing for the detection of ulcerative colitis, and ASCA IgA testing, and ASCA IgG testing for the detection of Crohn's disease.

| | Sensitivity Estimate– 95%CI | Specificity Estimate– 95%CI | PPV Estimate – 95%CI | NPV Estimate– 95%CI |
|---|---|---|---|---|
| ANCA | 64% (42.5–82%) | 100% (92.6–100%) | 100% (79.4–100%) | 84.2% (72.1–92.5%) |
| ASCA IgG | 34.7% (21.7–49.6%) | 100% (92.6–100%) | 100% (80.5–100%) | 60.0% (48.4–70.8%) |
| ASCA Ig A | 32.7% (20.0–47.5%) | 89.6% (77.3–96.5%) | 76.2% (52.8–91.8%) | 56.6% (44.7–67.9%) |

IgA: immunoglobulin A; IgG: immunoglobulin G; PPV: positive predictive value; NPV: negative predictive value.

In pediatric IBD, sensitivity/specificity of p-ANCA in UC range between 28.9% to 83% and 65% to 94.2%, respectively[6,12-15,18, 24,25].

Although p-ANCA was present in almost all our pediatric patients with UC, it was also positive around one-third of our CD patients as in others studies, and absent in all our control groups, in contrast with the results from others studies[6,12,14,20].

Recently, one study evaluated the diagnostic utility of anti-neutrophil cytoplasmatic antibodies specific for proteinase-3 (PR3-ANCA), a classical c-ANCA to distinguish pediatric UC from DC, and the authors founded similar results of sensitivity/specificity of this single marker as our study, but when they used a four antibody-panel including PR3-ANCA,x-ANCA (also called "atypical ANCA, with a rim-like perinuclear fluorescence), p-ANCA and ASCA IgG had an area under the ROC curve (AUC) of 0.90 in the training cohort and 0.84 in the external validation cohort for to distinguish between UC and CD, concluding is a superior and utilization of accessible panel can support accurate classification in the work-up of pediatric and adolescent patients with IBD patients[6].

The interpreting and comparing p-ANCA data between studies varying definitions and determination methods made direct comparison difficult. In some IBD studies, the expression "p-ANCA" has been synonymously used for atypical p-ANCA, in others, there is no differentiation between p-ANCA and atypical p-ANCA, and some author has been used the term x-ANCA only for the rim-like staining pattern, and interpreting it as the main ANCA found in UC patients when performing IIF[6,14].

The other aspects we should take into consideration for these differences in the results ranges include an individual serological response to various microbial and autoantigens that can develop in IBD, the loss of mucosal immune tolerance, an increased mucosa permeability, influenced by several distinct genetic determinants and or environmental exposure across various geographic regions, age at diagnosis and the time of the sample at diagnosis or during the follow-up of the disease[12,26,27].

We didn't found any correlation between p-ANCA and sex, the extension of the disease, or drugs using for his treatment as the same of other studies[12,14].

Olbjorn et al. found in their study the majority of pediatric UC patients were ANCA positive and had extensive colitis as the usual phenotype in pediatric UC at diagnosis, like our study[14]. None of the UC patients received early TNF blocker treatment, but this could be due to TNF blocker therapy not being standard of care in pediatric UC patients during the period of study[14].

It is important to remember that in our study we take the simple in the followed of the disease to measure the ANCA biomarker. Interestingly, Olbjorn et al. evaluated their pediatric population at diagnosis, e and 1–2 year later, and founded a decline in p-ANCA titles after treatment, in both CD and UC patients, and they concluded that p-ANCA status should probably be determined early in the disease course to be a prognostic factor[14].

Spencer et al. examined disease phenotype and serology in a cohort of children newly diagnosed with UC, and they didn't find any correlation with the age of diagnosis or a specific phenotype through p-ANCA≥100 was strongly associated with pancolitis, like our study, but in our case no relationship with a higher level of p-ANCA[15].

Recently, Vojdani et al. assessed ASCA and ANCA-positive blood samples and measured antibodies against lipopolysaccharides (LPS) and barrier proteins (zonulin + occluding, S100B, and aquaporin-4) in these samples and compare them with the same antibodies in controls and they founded significant elevation in antibodies in about 30% of ASCA-and ANCA-positive sera and demonstrate positive linear relationships between these antibodies. The findings suggest that individuals positive ASCA and ANCA have increased odds of developing intestinal and blood-brain barrier permeability compared to healthy subjects[28].

In the present study, the presence of ASCA IgG and ASCA IgA was found to have a significant association with DC in our cohort. Our study founded the accuracy of ASCA IgG and ASCA IgA testing for the detection of CD the sensitivity of 34.7% and 32.7%, the specificity of 100% and 89.6%, and negative predictive value of 60% and 56.6% respectively. In pediatric IBD, sensitivity/specificity of ASCA in CD range between 35% to 76% and 88% to 95%, respectively[12,14,24-27].

Our study didn't show a relationship signification between the age of diagnosis or localization and the presence of ASCA IgA or IgG. On the other hand, Markowitz et al. founded ASCA IgA and IgG in their patients less than 20% of those 0–7 years compared to nearly 40% of those 8–15 years ($P$<0.001). By contrast, older age at diagnosis and small bowel CD independently increased the risk of ASCA IgA and IgG expression[27].

Although ASCAs were present in our almost pediatric patients with DC, the studies showed this marker in 0% to15% of UC and 0% to 5.8% of the control group[3,6,12,14].

The appearance of these antibodies reflects a loss of tolerance to different intestinal bacteria. CD appears to rise from a dysregulated immune response to the endogenous enteric microflora in a genetically susceptible host. Defective innate immune responses, cause a cascade of events that ultimately are expressed as chronic intestinal inflammation[29].

Some authors demonstrated that, in pediatric patients with CD, ASCAs positivity correlated with an increased risk of intestinal resection. The detection of children with initial symptoms of CD could identify a risk group for surgery and the need for immunosuppressive therapy from the onset of the disease[8,16,30]. These findings agree with our study, in which most of the ASCAs-positive patients with CD had undergone surgery.

About the durability of this biomarker, Dubinsky and Seidman[31] reported the disappearance of ASCAs after surgery, in contrast with Kim et al.[17] reported durability of the ASCA title over the time, at the diagnosis and the follow-up, the mean duration there was no significant difference in the title of ASCA before and after infliximab treatment.

For to answer the doubt about the durability of this biomarker, we can observe what happened in the IBSEN study, which was investigated serological antibodies in a population-based inception cohort of CD had been followed prospectively for 20 years. No significant change of antibody status (positive versus negative) was found from 10-year to 20-year follow-up. Negative p-ANCA, positive ASCA IgA, and positive ASCA IgG at 10-year follow-up were all individuals associated with increased risk for CD-related surgery[8].

Chandrakumar et al. showed that in comparison to ASCA IgG negative patients, ASCA IgG positive patients who were treated with biologics had a significantly lower relapse rate[7].

Kim et al. related that in patients who have not achieved mucosa healing, ASCA IgG is closely related to mucosal damage and clinical remission[17].

Kansal et al. studied the variation of the gut mucosal microbiome with ASCA status in pediatric CD. They showed that ASCA-positive and ASCA-negative patients with CD have significant differences in gut microbiome composition, with could be influencing the phenotypes of the disease, especially the presence of *Rhuminococcus* torques and *Yersinia enterocolitica*[16].

Haberman et al. studied a program involving eight macrophage and fibroblast activation genes, ASCA, and CBir1 biomarkers associated with risk of stenosis complication in children with pretreatment CD after 5 years of diagnosis[30].

ASCA biomarker was evaluated in another gastrointestinal disease besides IBD. Kotze et al.[21] evaluation of ASCA positivity in three cohorts of patients with CD, celiac disease, and healthy individuals. The author founded that with statistical significance, ASCA IgA was positive in CD, celiac disease at diagnosis, and celiac disease with diet transgression, ASCA IgG in CD, and all groups with celiac disease. The authors suggested that the detection of ASCA is not a specific marker for Crohn's disease, but was associated with the inflammation of the small intestine. The increased levels of positive ASCA may be due to genetic factors and increased intestinal permeability[21].

Grzybowska-Chlebowczyk et al. evaluation of frequency and title of IgA ASCA and IgG ASCA and p-ANCA, c-ANCA in children with IBD and occurrence of ASCA antibodies about the coexistence of food allergy (FA). They did not prove any relation between ASCA antibodies and the occurrence of food allergy in the examined groups of children[32].

Other serological besides the classical biomarkers have been used in IBD patients. One study evaluated the predictive value of the Prometheus panel with seven IBD biomarkers, including classical markers (IBD serology seven; Prometheus Laboratories, San Diego, CA, USA) in children with suspected IBD[33]. The anti-flagellin antibody, a new marker added to the panel, presented a sensitivity of 50% and a specificity of 53%. Besides, the test was not reproducible in four of the ten patients evaluated. The authors concluded that, for IBD screening in pediatric patients, the IBD serology seven panel has a predictive value lower than that of the classic tests in pediatric IBD screening[33].

Recently, the company introduced a new panel with 17 markers, with the addition of new serological markers of inflammation and genetic risk (IBD sgi Diagnostic; Prometheus Laboratories). For this new panel, an algorithm is used to arrive at a score for the diagnosis of IBD. However, an analysis of the efficacy of these results showed that, as the IBD serology seven panel, the results obtained with the IBD sgi panel were not statistically different from those obtained using only three classical markers[34]. Therefore, it is not clinically efficient or cost-effective.

### Strengths and limitations

We acknowledge the small sample size as the main limitation of our study. Also, including patients with a different time lapse from diagnosis and serum sampling. Another strength is the employment of patients from four different centers.

Another aspect to be justified is that the period of data collection of the study was before the publication of the Modified Port Criteria, however, we emphasize that we did not include patients with atypical UC and IBD-U, not compromising the diagnostic criteria.

## CONCLUSION

The results of the classical serological tests for IBD in children in Sao Paulo, Brazil were generally like those in the literature. We have demonstrated that such tests, although highly specific, are not sensitive and therefore do not facilitate the diagnosis. However, the results correlate positively with the severity of the disease, high ASCA IgA titles being seen in patients with CD and requiring treatment with azathioprine, anti-TNF-α, or surgery.

### Authors' contribution

Rodrigues M was the research adviser, wrote the manuscript, contributed to the study design, statistical analysis, interpretation of data and performed the critical review of the manuscript. Bueno C performed the laboratory tests and contributed to the study design. Lomazi EA collected the data, contributed to the study design and performed the critical review of the manuscript. Fermandes MIM collected the data and contributed to the study design. Neufeld CB collected the data and contributed to the study design. D'Amico MFM collected the data and contributed to the study design. Patiño FRA collected the data and contributed to the study design.

### Orcid

Maraci Rodrigues: 0000-0002-1571-5282.
Cleonice Bueno: 0000-0003-3404-0572.
Elizete Aparecida Lomazi: 0000-0001-5504-4746.
Maria Inez Machado Fernandes: 0000-0002-0737-5062.
Clarice Blaj Neufeld: 0000-0003-1922-3143.
Maria Fernanda Marranghello D'Amico: 0000-0002-6133-0308.
Fátima Regina De Almeida Patiño: 0000-0003-2439-3956.

Rodrigues M, Bueno C, Lomazi EA, Fernandes MIM, Neufeld CB, D'Amico MFM, Patiño FRA. Marcadores sorológicos clássicos na doença inflamatória intestinal pediátrica no Brasil. Arq Gastroenterol. 2021;58(4):495-503.

**RESUMO** – **Contexto** – Os anticorpos citoplasmáticos anti-neutrófilos perinuclear (p-ANCA) e anticorpos anti-*Saccharomyces cereviciae* (ASCAs) são utilizados para diferenciar a doença de Crohn (DC) da colite ulcerativa (CU) e mais recentemente para correlacioná-los com o prognóstico da doença. **Objetivo** – 1) Determinar a acurácia diagnóstica dos marcadores sorológicos na identificação de DC e CU pediátrica em São Paulo, Brasil. 2) Correlacioná-los com as características demográficas e clínicas destas duas doenças. **Métodos** – Estudo multicêntrico transversal em pacientes com diagnóstico estabelecido de doença inflamatória intestinal (DII) determinando a presença dos marcadores sorológicos ASCAs e p-ANCA, correlacionando seus resultados com os dados demográficos e clínicos, e também em pacientes controles isentos de doenças gastrointestinal. **Resultados** – 122 pacientes, 74 com DII (46% masculinos) em quatro centros de referência em Gastroenterologia Pediátrica, média de idade 13±7 anos, 49 (66%) com DC e 25 (34%) com CU e 48 controles (54% masculinos). O marcador p-ANCA apresenta maior porcentagem de detecção na CU (69,6%), mas também na DC (30,4%) quando comparado ao grupo controle ($P$<0,001). Os marcadores ASCA IgA (76,2%) e IgG (94,4%) apresentam maiores porcentagens de detecção na DC, quando comparada ao controle ($P$<0,001) e que a positividade do marcador esteve relacionada ao uso de medicações em pacientes portadores de DC que realizaram cirurgia (26,86±17,99; $P$=0,032). **Conclusão** – Os resultados dos testes sorológicos em crianças com DII em São Paulo, Brasil, foram altamente específicos, mas pouco sensíveis para auxiliar no diagnóstico, embora com correlação positiva com a gravidade da doença.

**Palavras-chave** – Anticorpos citoplasmáticos; anti-neutrófilos; anticorpos; anti-*Saccharomyces cerevisiae*; doenças inflamatórias intestinais.

## REFERENCES

1. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). Inflammatory Bowel Disease in Children and Adolescents: Recommendations for Diagnosis – The Porto Criteria. J Pediatr Gastroenterol Nutr. 2005;41:1-7. doi: 10.1097/01.MPG.0000163736.30261.82.
2. Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World J Gastroenterol. 2018,7;24:2741-63. doi:10.3748/ wjg. v24. i25.2741.
3. Birimberg-Schwartz L, Wilson DC, Kolho KL, Karolewska-Bochenek K, Afzal NA. Christine Spray C, et al. p-ANCA and ASCA in Children with IBD-Unclassified, Crohn's Colitis, and Ulcerative Colitis - A Longitudinal Report from the IBD Porto Group of ESPGHAN. Inflamm Bowel Dis. 2016;22:1908-14. doi: 10.1097/MIB.0000000000000784.
4. Proujansky R, Fawcett PT, Gibney KM, Treem W.R, Hyams JS. Examination of anti-neutrophil cytoplasmic antibodies in childhood inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 1993;17:193-97. doi: 10.1097/00005176-199308000-00011.
5. Ruemmele FM, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology. 1998;115:822-29. doi: 10.1016/s0016-5085(98)70252-5.
6. Hom MP, Peter AM, Grunder FR, Leichtle AB, Spalinder J, Schibli S, et al. PR3-ANCA and panel diagnostics in pediatric inflammatory bowel disease to distinguish ulcerative colitis from Crohn's disease. Plos One. 2018;13: e0208974. doi.org/10.1371/journal.pone.0208974.
7. Chandrakumar A, Georgy M, Agarwal P, j'Jong G, El-Matary W. Anti-Saccharomyces cerevisiae antibodies as a prognostic biomarker in children with Crohn's disease. J Pediatr Gastroenterol Nutr. 2019;69:82-7.doi: 10.1097/MPG.0000000000002311.
8. Kristensen VA, Cvancarova M, Hoivik ML, Moum B, Vatn MH. Serological antibodies and surgery in a population-based inception cohort of Crohn´s disease patients – the IBSEN study. Scand J Gastroenterol. 2020,55:436-41. doi:10.1080/00365521.2020.1745879.
9. Ricciuto A, Aardoom M, Orlanski-Meyer E, Navon D, Carman N, Aloi M, et al. Predicting Outcomes in Pediatric Crohn's Disease for Management Optimization: Systemic Review and Consensus Statements From the Pediatric Inflammatory Bowel Disease - Ahead Program. Gastroenterology. 2021;160:403-36. doi:10.1053/j.gastro.2020.07.065.
10. Orlansky-Meyer E, Aardoon M, Ricciuto A, Navon D, Carman N, Aloi M, et al. Predicting Outcomes in Pediatric Ulcerative Colitis for Management Optimization: Systematic Review and Consensus Statements From the Pediatric Inflammatory Bowel Disease. Gastroenterology. 2021;160:378-402. doi:10.1053/j.gastro.2020.07.066.
11. Dotan I. New Serologic Markers for Inflammatory Bowel Disease Diagnosis. Dig Dis. 2010;28:418-23. doi:10.1159/000320396.
12. Saadah OI, Al-Mughales JA. Serological markers of inflammatory bowel disease in children from the Western region of Saudi Arabia. Arab J Gastroenterol. 2013;14:78-82. doi: 10.1016/j.ajg.2013.05.004.
13. Kovács M, Müller KE, Papp M, Lakatos PL, Csöndes M, Veres G. New serological markers in pediatric patients with inflammatory bowel disease. World J Gastroenterol. 2014;20:4873-82. doi: 10.3748/wjg.v20.i17.4873
14. Olbjørn C, Milada Småstuen MC, Thiis-Evensen E, Nakstad B, Vatn MH, Perminow G. Serological markers in the diagnosis of pediatric inflammatory bowel disease and as predictors for early tumor necrosis factor blocker therapy. Scand J Gastroenterol. 2017;52:414-9. doi:10.1080/00365521.2016.1259653.
15. Spencer EA, Davis SM, Mack DR, Boyle BM, Griffiths AM, LeLeiko NS, et al. Serologic Reactivity Reflects Clinical Expression of Ulcerative Colitis in Children. Inflamm Bowel Dis. 2018;24:1335-43. doi:10.1093/ibd/izy009.
16. Kansal S, Catto-Smith AG, Boniface K, Thomas S, Cameron DJ, Oliver M, et al. Variation of Gut Mucosal Microbiome with Anti-Saccharomyces cerevisiae Antibody Status in Pediatric Crohn Disease. J Pediatr Gastroenterol Nut. 2019;69:696-703. doi:10.1097/MPG.0000000000002461.
17. Kim MJ, Kim E, Kang B, Lee Y, Kang ES, Choe YH. Anti- Saccharomyces cerevisiae Antibody in Pediatric Crohn's Disease Patients without Mucosal Healing Is a Useful Marker of Mucosal Damage. Gut Live. 2020;30:1-8. doi: 10.5009/gnl20212.
18. Steinwurtz F, Scheinberg M. Serologic diagnosis of inflammatory bowel disease (ASCA and ASCA): Evaluation of 70 cases. GED. 2001;20:41-42.
19. Cabral VL, Misputen SJ, Catapani WR. Anti-neutrophil cytoplasmatic antibodies in Brazilian patients with inflammatory bowel disease. Hepatogastroenterology. 2003;50:412-5.
20. Nisihara RM, Carvalho WB, Utiyama SRR, Amarante H, Baptista ML. Diagnostic Role and Clinical Association of ASCA and ANCA in Brazilian Patients with Inflammatory Bowel Disease. Dig Dis Sci. 2010;55:2309-15. doi:10.1007/s10620-009-0998-7.
21. Kotze LMS, Nisihara RM, Utiyama SRR, Kotze PG, Theiss PM, Olandoski M. Antibodies Anti-Saccharomyces Cerevisiae do not Differentiate Crohn´s Disease from Celiac Disease. Arq Gastroenterol. 2010;47:242-5. doi.org/10.1590/S0004-28032010000300006.
22. Santos CE, Dal Pizzol VI, Aragão SC, Rachid Filho A, Marques FM. C-ANCA-associated vasculitis in patients with ulcerative colitis: a case report. Rev Bras Reumatol. 2013;53:441-3.
23. Duarte-Silva M, Afonso PC, Souza PR, Peghini BC, Rodrigues-Junior V, Cardoso CRB. Reappraisal of antibodies Against Saccharomyces cerevisiae (ASCA) as persistent biomarkers in quiescent Crohn's disease. Autoimmunity. 2019;52:37-47. doi:10.1080/08916934.2019.1588889.
24. Zhouludev A. Serologic testing with ANCA, ASCA, and Anti-OmpC in children and Young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. Am. J. Gastroenterol. 2004;99:2235-41. doi: 10.1111/j.1572-0241.2004.40369.x.
25. Khan K, Schwarzenberg SJ, Sharp H, Greenwood D, Weisdorf-Schindele S. Role of serology and routine laboratory in childhood inflammatory bowel disease. Inflamm Bowel Dis. 2002;8:325-29. doi: 10.1097/00054725-200209000-00003.

26. Ashorn S, Honkanen T, Kolho KL, Ashorn M, Valineva T, Wei B, et al. Fecal Calprotectin levels and Serological Responses to Microbial Antigens among Children and Adolescents with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2009;15:199-205. doi:10.1002/ibd.20535.
27. Markowitz J, Kugathasan S, Dubinsky M, Mei L, Crandall W, Leleiko N, et al. Age of Diagnosis Influences Serologic Responses in Children with Crohn Disease: A Possible Clue to Etiology? Inflamm Bowel Dis. 2009;15:714-9. doi:10.1002/ibd.20831.
28. Vojdani A, Vojdani E, Herbert M, Kharrazian D. Correlation between Antibodies to Bacterial Lipopolysaccharides and Barrier Protein in Sera Positive for ASCA and ANCA. Int J Mol Sci. 2020;21:1381. doi:10.3390/ijms21041381.
29. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest. 2007;117:514-21. doi: 10.1172/JCI30587.
30. Haberman Y, Minar P, Karns R, Dexheimer PJ, Ghandikota S, Tegge S, et al. Mucosal inflammatory and Wound Healing Gene Programs Reveal Targets for Structuring Behavior in Pediatric Crohn's Disease. J Crohns Colitis. 2020;15:273-86. doi:10.1093/ecco-jcc/jjaa166.
31. Dubinsky MC, Seidman EG. Diagnostic markers of inflammatory bowel disease. Curr Opin Gastroenterol. 2000;16:337-42. doi:10.1097/00001574-200007000-00008.
32. Grzybowska-Chlebowczyk U, Wos H, Sieron AI, Wiecek S, Augusciak-Duma A, Koryciak-Komarska H, et al. Serologic Investigation in Children with Inflammatory Bowel Disease and Food Allergy. Mediators Inflamm. 2009;2009:512695. doi:10.1155/2009/512695.
33. Benor S, Russell GH, Silver M, Israel EJ, Yuan Q, Winter HS. Shortcomings of the inflammatory bowel disease Serology 7 panel. Pediatrics. 2010;125:1230-6. doi: 10.1542/peds.2009-1936.
34. Shirts B, von Roon AC, Tebo AE. The entire predictive value of the Prometheus IBD sgi diagnostic product may be due to the three least expensive and most available components. Am J Gastroenterol. 2012;107:1760-1. doi: 10.1038/ajg.2012.238.

ORIGINAL ARTICLE

AG-2021-100
doi.org/10.1590/S0004-2803.202100000-90

# Growth analysis of preterm newborns with gastroschisis during hospitalization in a Neonatal Intensive Care Unit

Juliana Zoboli Del **BIGIO**, Mário Cícero **FALCÃO** and Ana Cristina Aoun **TANNURI**

Received: 7 May 2021
Accepted: 18 June 2021

**ABSTRACT** – **Background** – Gastroschisis, especially complex type, prematurity and low birth weight are associated with a worse clinical outcome with higher mortality, higher incidence of sepsis and catheter-related infection, cholestasis, short bowel syndrome, greater number of days to achieve full diet, longer time of parenteral nutrition and longer hospitalization time. **Objective** – To evaluate the growth of preterm newborns with gastroschisis during their hospitalization in the neonatal intensive care unit. **Methods** – Descriptive study, based on a retrospective cohort (January 2012 to December 2018), including preterm newborns (gestational age less than 37 weeks) with simple and complex gastroschisis admitted in a tertiary neonatal intensive care unit. The following parameters were analyzed: maternal age, parity, type of delivery, birth weight, gender, gestational age, nutritional adequacy, type of gastroschisis, fasting time, parenteral nutrition time, time until achieving full enteral nutrition, hospitalization time, weight gain and outcome. The results were expressed in percentage, average, and median. **Results** – A total of 101 newborns with gastroschisis were admitted, of which 59.4% were premature (80.7% of late preterm infants). From the maternal data, the mean age was 21.2 years and 68.3% were primiparous. Regarding childbirth: 80% were cesarean sections. From newborns: the average birth weight was 2137 g, 56.6% were female, the average gestational age was 34.8 weeks, the average weight gain was 20.8 g/day during hospitalization and 83.3% were discharged from the hospital. **Conclusion** – The growth analysis by weight gain (grams/day) during hospitalization in the intensive care unit showed that more than 90% of the sample presented acceptable or adequate weight gain.

**Keywords** – Gastroschisis; infant, premature; growth; infant, newborn.

## INTRODUCTION

Gastroschisis is a defect of the abdominal wall, in most cases to the right of the umbilical cord insertion. Intestinal loops and occasionally parts of other abdominal organs burst out the defect of the abdominal wall, without membranes or sac covering the viscera[1]. Genetic polymorphism, interaction with environmental factors such as smoking and maternal age below 20 years may play a role in the pathogenesis of this disease[2].

The prevalence of gastroschisis has been increasing worldwide since 1995, reaching three to four cases for 10,000 live births, with no difference between genders[3]. Hypotheses for the cause of gastroschisis include failure of mesodermal formation in the abdominal wall, rupture of the amniotic membrane around the umbilical ring, sequelae of right umbilical vein involution, or interruption of the right calf artery[4].

Gastroschisis can be classified as simple when it occurs as an isolated defect and complex, when associated with intestinal anomalies such as intestinal atresias, perforations, necrotic segments or volvolus[4].

Gastroschisis, especially complex type, prematurity and low birth weight are associated with a worse clinical outcome with higher mortality, higher incidence of sepsis and catheter-related infection, cholestasis, short bowel syndrome, greater number of days to achieve full diet, longer time of parenteral nutrition and longer hospitalization time[5]. Prolonged dependence on parenteral nutrition can trigger liver disease associated with parenteral nutrition, with possible progression to severe liver failure[6].

Gastroschisis increases the risk of preterm labor, and only 35% of deliveries occur with gestational age equal to or greater than 37 weeks[7].

Parenteral nutrition has become part of the daily clinical treatment of several patients requiring nutritional supplementation. In newborns, parenteral nutrition contributed to improve the survival of preterm infants, including congenital bowel anomalies.

Currently, newborns with gastroschisis, both premature and term, require early and balanced parenteral nutrition to survive periods of fasting, which the underlying disease itself determines, because intestinal dysmotility associated with gastroschisis is well known, in both situations simple and complex defect.

It is also known that newborns receiving parenteral nutrition have lower energy needs than those in enteral nutrition, due to lower intestinal losses, exclusion of digestion and absorption processes and dynamic-specific action of food. A non-protein supply of 60

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Instituto da Criança, São Paulo, SP, Brasil.
Corresponding author: Juliana Zoboli Del Bigio. E-mail: juliana.zoboli@hc.fm.usp.br

kcal/kg, associated with an adequate supply of amino acids, meets the rest metabolic needs of the newborn. With caloric offers of 80–90 kcal/kg and protein of 3 g/kg/day, some increase in weight and growth is already observed. Thus, in newborns with gastroschisis, total parenteral nutrition with 3–4 g/kg/day of protein and 100 kcal/kg/day of non-protein calories, maintaining a non-protein nitrogen/calorie ratio of around 1/150 to 1/200, promotes anabolism and adequate growth of these newborns[8].

Despite this therapeutic arsenal in parenteral nutritional therapy, studies show that newborns, mainly preterm with associated diseases, do not present expected weight gain based on currently growth charts[9].

Potential clinical effects of this growth failure include increased stay in the neonatal intensive care unit and worse outcomes in neurodevelopment. There is also evidence showing a link between inadequate early neonatal nutrition and health outcomes, including increased cardiovascular disease, obesity and diabetes[8].

The numerical methods used to describe the growth rate of preterm newborns in relation to weight, length and head circumference include grams/kg/day (g/kg/d), grams/day (g/d), centimeters/week (cm/w) and z-score evaluation[10].

From the above, there are difficulties in adequately nourishing preterm infants with gastroschisis, as well as there are difficulties in measuring this nutrition, that is, in measuring an adequate growth.

Thus, the aim of the present study is to evaluate the growth of preterm newborns with simple and complex gastroschisis during their hospitalization in the neonatal intensive care unit.

## METHODS

This is a descriptive study, based on a retrospective cohort, including preterm newborns (gestational age less than 37 weeks) with simple and complex gastroschisis, admitted to the Neonatal Intensive Care Center 2 (CTIN-2) of the Child and Adolescent Institute of the Clinics Hospital – Faculty of Medicine – University of São Paulo (HC–FMUSP), Brazil, from January 2012 to December 2018.

CTIN-2 is a Neonatal Intensive Care Unit tertiary-level that provides care to high complexity newborns. The criterion of hospitalization in this center consists of newborns and young infants under 45 days of life who require clinical and surgical intensive care. In recent years, gastroschisis was the second surgical pathology with the highest number of hospitalizations.

The neonatology, child surgery, hospital infection control commission, physiotherapy, nursing and nutrition teams developed a managed protocol to care the newborn with gastroschisis.

Parenteral nutrition is initiated on the first days of life if the newborn has hemodynamic stability. The nutritional target in total parenteral nutrition is 3 to 3.5 g/kg/day of amino acids, 3 g/kg/day of lipids, preferably with fish oil and glucose infusion rate of 12 mg/kg/minute[11]. The volume is titrated from daily water balance and electrolytes and micronutrients are prescribed based on basic needs and serial laboratory evaluation.

The venous access of choice is the peripherally inserted central catheter (PICC) and is maintained with rigorous antisepsis techniques, with standardized dressing switching and proper manipulation, to avoid catheter-associated infection.

The onset of enteral diet occurs when gastric residue is in reduction and the appearance becomes yellow or salivary. Breast milk is the diet of choice and starts with 20 mL/kg, with daily increase of the diet, until reaching the full diet. When fasting is longer than 30 days, it is chosen to start the diet with hydrolyzed formula in the absence of breast milk.

The project was approved by the Ethics Committee of the Department of Pediatrics and the Research Project Analysis Committee (CAPPesq) of HC–FMUSP, protocol 2476188, and the Informed Consent Form was waived because it is a collection of data from the medical records of these newborns.

The following items were collected from medical records: maternal age (years); parity (primiparous or not); type of delivery (vaginal or cesarean); birth weight (grams); gender; gestational age (weeks), calculated by fetal ultrasound up to 20 weeks of gestation[12], for subsequent classification of prematurity; nutritional adequacy (small – below the 10th percentile, adequate – between 10th and 90th percentiles or large for gestational age – above the 90th percentile), using Fenton curves (2013)[13]; type of gastroschisis (simple or complex) – diagnosed during surgery; fasting time (days), time of parenteral nutrition (days); time until reaching full enteral nutrition (minimum volume of 120 mL/kg/day of enteral diet)[14] (days); hospitalization time (days); weight gain from hospitalization to discharge (grams/day), and outcome (hospital discharge or death).

For the evaluation of the growth of these preterm infants with gastroschisis through weight, the criterion of increasing or decreasing daily weight in grams/day was adopted from hospitalization to hospital discharge[10]. Adequate gain was considered values higher than 15 grams/day, acceptable values between 10 and 15 grams/day and inadequate values, less than 10 grams/day. For this study, the Fenton growth curves were chosen to classify the newborn after birth and in the follow-up period, and the weight gain in gram/day criterion was chosen. Newborns are weighed daily on digital scales and calibrated, naked and always by a nurse. The justification for choosing the grams/day criterion to evaluate the growth of these preterm infants was based on a systematic review, published in 2017, where the methods for this evaluation were studied. It was evaluated 373 studies from England, France, Germany and Spain and the most used criteria to calculate the weight gain of preterm newborns were: gram/kg/day, in 40% of the publications, followed by gram/day, 32%, and 29% used changes in the z score in intrauterine and postnatal growth charts, however, the authors do not present definitive conclusions regarding the best method for this evaluation[10].

In a study published in 2017 studying weight gain in preterm infants by the grams/day criterion, daily increments of 12 to 16 g/day were observed[15], justifying the choice, in this research, of inadequate values (less than 10 g/day), acceptable (between 10 and 15 g/day) and adequate (greater than 15 g/day).

The classification of prematurity adopted was very premature (gestational age between 28 and 32 weeks) and, moderate preterm infants (gestational age between 32 and 37 weeks, including late preterm infants – gestational age between 34 and 37 weeks); in relation to birth weight (extreme low birth weight – birth weight less than 1000 g, very low birth weight – birth weight between 1000 and 1500 g, low birth weight between 1500 and 2500 g and birth weight greater than 2500 g), and nutritional adequacy (small, adequate, or large for gestational age, according to the reference curve adopted)[10].

The results are expressed in proportions, average with standard deviation and median, with minimum and maximum values. The sample size was not calculated because it is a convenience sample.

## RESULTS

During the study period, from January 2012 to December 2018, 101 newborns with gastroschisis were admitted, with an admission rate of 14.4 newborns/year. Of these, 60 newborns (59.4%) were premature (gestational age less than 37 weeks).

TABLE 1 shows the variables studied in this group of preterm infants, expressed in proportions, averages and standard deviation or medians, with minimum and maximum values.

TABLE 2 describes the classification of prematurity. 80.7% were late preterm infants, 18.3% were small for gestational age and 71.8% were low birth weight.

TABLE 3 shows the weight gain of this group of preterm infants by the criterion of grams/day, being adequate gain values higher than 15 g/day, acceptable values between 10 and 15 g/day, and inadequate values, less than 10 g/day.

## DISCUSSION

This study presents two extremely important aspects that have not yet been addressed in the literature. The first is to analyze a cohort of gastroschisis composed only of preterm newborns, because published studies on gastroschisis cohorts analyze premature and term infants together[16,17]. The second is to describe the growth of these preterm infants, because there are few studies that analyze growth and not only with preterm newborns[9].

The prematurity rate analyzed in this cohort was 59.4%, with a mean gestational age of 34.8 weeks. A recent study published in 2020 analyzing 566 newborns with gastroschisis in eight tertiary centers showed that gestational age at birth ranged from 28 to 40 weeks, with a median of 36 weeks and 56% were premature[18].

Among the risk factors for gastroschisis, young and primiparous mothers stand out. This study showed an average of maternal age of 21.2 years, compatible with data from Eggink et al. (2006)[19], who had an average of 19.3 years and Raymond et al. (2020)[18], with a median of 21 years. Regarding primiparous women, the findings were also in agreement with the literature, 68.3% in the present study, 68.7% (Calcagnotto et al., 2013)[20] and 60.8% (Friedman et al., 2016)[7].

Another fact that draws attention is the high rate of cesarean deliveries found (80%), because there is no evidence that cesarean delivery improves the result of gastroschisis, especially with complications. The literature shows a rate around 50%[18]. In Brazil, Calcagnotto et al., in Porto Alegre, studying the risk factors associated with mortality in newborns with gastroschisis, found a cesarean section rate of 92.2%[20]. Elective preterm delivery before 37 weeks of gestation has been suggested to protect the externalized intestine from damage due to exposure to amniotic fluid, with consequent serositis and compressions, however, these studies still present conflicting results, therefore, surgical delivery is reserved only for obstetric indications[21].

Of the 60 newborns studied, there was no predominance between genders in the incidence of gastroschisis (female 53.3% and male 46.7%), a fact that is comparable to the literature, according to Mastroiacovo et al., 2007[22]. The incidence of complex gastroschisis was 18.3%, slightly higher when compared to the literature, which average of 14%[18].

**TABLE 1.** Variables studied (n=60).

| Variable | n |
|---|---|
| Maternal age (years) | 21.20±1.41* |
| Primiparous | 41 (68.33%) |
| Type of delivery | |
| Vaginal | 12 (20.0%) |
| Caesarian | 48 (80.0%) |
| Birth weight (grams) | 2137.61±414.46* |
| Gender | |
| Female | 32 (53.33%) |
| Male | 28 (46.67%) |
| Gestational age (weeks) | 34.80±3.90* |
| Type of gastroschisis | |
| Simple | 49 (81.66%) |
| Complex | 11 (18.34%) |
| Fasting time (days) | 27** (minimum 7, maximum 105) |
| Parenteral nutrition time (days) | 30.50** (minimum 7, maximum 125) |
| Time to achieve full enteral diet (days) | 36.50** (minimum 15, maximum 134) |
| Hospital stay (days) | 39.50** (minimum 7, maximum 138) |
| Weight gain (grams/day) | 20.84±6.57 |
| Outcome | |
| Discharged | 50 (83.34%) |
| Death | 10 (16.66%) |

*Average. **Median.

**TABLE 2.** Classification of preterm newborns in relation to gestational age, birth weight and nutritional adequacy (n=60).

| Classification | n |
|---|---|
| Gestational age* | |
| Very premature | 3 (5.0%) |
| Moderate premature | 57 (95.0%) |
| Late premature | 46 (80.70%) |
| Birth weight** | |
| Extreme low birth weight | 0 |
| Very low birth weight | 3 (5.0%) |
| Low birth weight | 43 (71.77%) |
| Birth weight >2500 g | 14 (23.33%) |
| Nutritional adequacy*** | |
| Small for gestational age | 11 (18.33%) |
| Adequate for gestational age | 48 (80.0%) |
| Large for gestational age | 1 (1.67%) |

*Very premature – gestational age between 28 and 32 weeks, moderate preterm infants – gestational age between 32 and 37 weeks) and late preterm infants – gestational age between 34 and 37 weeks). **Extreme low birth weight – birth weight less than 1000 g, very low birth weight – birth weight between 1000 and 1500 g, low birth weight between 1500 and 2500 g and birth weight greater than 2500 g). ***Small ($P$<10), adequate ($P$=10–90), or large for gestational age ($P$>90).

**TABLE 3.** Weight gain by grams/day criterion (n=60).

| Weight gain (grams/day) | n |
|---|---|
| Inadequate gain (<10 grams/day) | 5 (8.33%) |
| Acceptable gain (between 10 and 15 grams/day) | 5 (8.33%) |
| Adequate gain (>15 grams/day) | 50 (83.34%) |

The median fasting time was 27 days, parenteral time, 30.5 days, and to achieve full enteral nutrition, 36.5 days, compatible with recently published data, showing median parenteral nutrition time of 27 to 30 days[18]. Literature data with preterm infants smaller than 1500 g show a median of 67 days for parenteral nutrition and 107 days for full enteral nutrition[18].

The intestinal dysmotility in gastroschisis is a common evolution with important interference in the beginning of enteral diet and need for prolonged parenteral nutrition time[23]. After surgical correction of gastroschisis, a period of intestinal hypomotility usually occurs, with spontaneous regression[24].

The etiology of this transient intestinal dysfunction is not yet fully elucidated and may be related to a defect in the maturation of intestinal neurons. A Brazilian experimental study, published in 2003[24], analyzing fetuses of rabbits with gastroschisis, showed that the intestinal plexus of fetuses with gastroschisis are more immature compared to controls. The irritating action of amniotic fluid is the most plausible explanation for this neuronal disorder of intestinal maturation[24].

The median hospital stay was 39.5 days, like the literature, where the hospitalization time in three studies analyzed was 48 days (mean)[25], 33 days (median)[26] and 37 days (median)[18]. In preterm infants smaller than 1500 g, the hospital stay in a recent publication was 77.5 days[18].

Regarding the outcomes (hospital discharge or death), mortality was 16.6%, higher than the literature that is around 5%[18]. This difference can be explained by inadequate prenatal care for pregnant women and a higher incidence of complex gastroschisis. In two Brazilian studies, one conducted at the Clinics Hospital of the Federal University of Minas Gerais and the other at the Clinics Hospital of Porto Alegre, mortality rates were 14.9%[25] and 23.4%[20], respectively. However, all these mortality rates are from cohorts that included preterm and term newborns.

The average birth weight was 2137.6 g, with 5% very low birth weight, 71.7% low birth weight and 23.3% with birth weight greater than 2500 g. Regarding nutritional adequacy: 18.3% small, 80% adequate and 1.7% large for gestational age, according to Fenton curves[14], standardized in the service where the research was conducted.

It is known that an adequate fetal growth, especially in the third trimester of pregnancy, depends on a satisfactory physiology of the digestive tract, a fact that does not occur in gastroschisis, because there is loss of nutrients, especially proteins, due to intestinal exposure to amniotic fluid. This fact may lead to restriction of intrauterine growth and, consequently, small for gestational age newborns. However, in this study, a relatively small number of newborns small for gestational age were obtained (18.3%), in agreement with another Brazilian study that showed 14.3%, although this percentage included premature and term newborns[25].

The evaluation of growth during hospitalization time, adopting the criterion of grams/day, showed an average weight gain of 20.8 g/day, distributed as well: 8.3% of the neonates had inadequate weight gain, considering less than 10 g/day, 8.3% had acceptable gain, between 10 and 15 g/day and the most (83.3%), adequate gain, greater than 15 g/day.

## CONCLUSION

In conclusion, the growth analysis of preterm newborns with simple and complex gastroschisis through the criterion of weight gain (grams/day) during hospitalization in a neonatal intensive care unit, showed that more than 90% of the sample presented acceptable weight gain (10 to 15 g/day) or adequate (>15 g/day), emphasizing that preterm newborns who do not gain weight in the neonatal intensive care unit remain hospitalized for longer, increasing morbidity and mortality, besides the risk of presenting cognitive deficits in the follow-up. In addition, prolonged hospitalizations substantially increase health costs.

### Authors' contribution

Bigio JZD: data collection, research execution, text writing, statistical analysis. Falcão MC: data collection, research execution, text writing, statistical analysis. Tannuri ACA: research execution, text writing.

### Orcid

Juliana Zoboli Del Bigio: 0000-0002-4326-2575.
Mário Cícero Falcão: 0000-0002-5658-3992.
Ana Cristina Aoun Tannuri: 0000-0002-5481-032X.

Bigio JZD, Falcão MC, Tannuri ACA. Análise do crescimento de recém-nascidos pré-termo com gastrosquise durante a Internação em Unidade de Terapia Intensiva Neonatal. Arq Gastroenterol. 2021;58(4):504-8.

**RESUMO** – **Contexto** – Gastrosquise, principalmente o tipo complexo, prematuridade e baixo peso ao nascer estão associados a um pior desfecho clínico com maior mortalidade, maior incidência de sepse e infecção relacionada ao cateter, colestase, síndrome do intestino curto, maior número de dias para atingir dieta plena, maior tempo de nutrição parenteral e maior tempo de internação. **Objetivo** – Avaliar o crescimento de recém-nascidos pré-termo com gastrosquise durante sua internação na unidade de terapia intensiva neonatal. **Métodos** – Estudo descritivo, baseado em uma coorte retrospectiva (janeiro de 2012 a dezembro de 2018), incluindo recém-nascidos pré-termo (idade gestacional inferior a 37 semanas) com gastrosquise simples e complexa, admitidos em uma unidade de terapia intensiva neonatal de nível terciário. Foram analisados: idade materna, paridade, tipo de parto, peso de nascimento, gênero, idade gestacional, adequação nutricional, tipo de gastrosquise, tempos de jejum, de nutrição parenteral, até atingir nutrição enteral plena e de internação, ganho de peso e desfecho. Os resultados estão expressos em porcentagem, médias e medianas. **Resultados** – Foram admitidos 101 recém-nascidos com gastrosquise, dos quais 59,4% eram prematuros (80,7% prematuros tardios). Dos dados maternos, a idade média foi de 21,2 anos e 68,3% eram primigestas. Com relação ao parto: 80% foram cesarianas. Dos recém-nascidos: o peso médio de nascimento foi de 2137 g, 56,6% eram do sexo feminino, a idade gestacional média foi de 34,8 semanas, o ganho médio de peso de 20,8 g/dia durante a internação e 83,3% receberam alta hospitalar. **Conclusão** – A análise do crescimento por meio de ganho de peso (gramas/dia) durante a internação na unidade de terapia intensiva mostrou que mais de 90% da amostra apresentou ganho aceitável ou adequado de peso.

**Palavras-chave** – Gastrosquise; recém-nascido prematuro; crescimento; recém-nascido.

## REFERENCES

1. Prefumo F, Izzi C. Fetal abdominal wall defects. Best Pract Res Clin Obstet Gynaecol. 2014;28:391-402.
2. Torfs CP, Christianson RE, Iovannisci DM, Shaw GM, Lammer EJ. Selected gene polymorphisms and their interaction with maternal smoking, as risk factors for gastroschisis. Birth Defects Res A Clin Mol Teratol. 2006;76:723-30.
3. Jones AM, Isenburg J, Salemi JL, Arnold KE, Mai CT, Aggarwal D, et al. Increasing Prevalence of Gastroschisis-14 States, 1995-2012. MMWR Morb Mortal Wkly Rep. 2016;65:23-6.
4. Bergholz R, Boettcher M, Reinshagen K, Wenke K. Complex gastroschisis is a different entity to simple gastroschisis affecting morbidity and mortality-a systematic review and meta-analysis. J Pediatr Surg. 2014;49:1527-32.
5. Raymond SL, Hawkins RB, St Peter SD, Downard CD, Qureshi FG, Renaud E, et al. Predicting Morbidity and Mortality in Neonates Born with Gastroschisis. J Surg Res. 2020;245:217-24.
6. Dennison FA. Closed gastroschisis, vanishing midgut and extreme short bowel syndrome: Case report and review of the literature. Ultrasound. 2016;24:170-4.
7. Friedman AM, Ananth CV, Siddiq Z, D'Alton ME, Wright JD. Gastroschisis: epidemiology and mode of delivery, 2005-2013. Am J Obstet Gynecol. 2016;215:348.e1-9.
8. Bishay M, Lakshminarayanan B, Arnaud A, Garriboli M, Cross KM, Curry JI, et al. The role of parenteral nutrition following surgery for duodenal atresia or stenosis. Pediatr Surg Int. 2013;29:191-5.
9. Hall NJ, Drewett M, Burge DM, Eaton S. Growth pattern of infants with gastroschisis in the neonatal period. Clin Nutr ESPEN. 2019;32:82-7.
10. Fenton TR, Chan HT, Madhu A, Griffin IJ, Hoyos A, Ziegler EE, et al. Preterm Infant Growth Velocity Calculations: A Systematic Review. Pediatrics. 2017;139:e20162045.
11. Mihatsch WA, Braegger C, Bronsky J, Cai W, Campoy C, Carnielli V, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition. Clin Nutr. 2018;37:2303-5.
12. Cardoso LE, Falcão MC. Nutritional assessment of very low birth weight infants: relationships between anthropometric and biochemical parameters. Nutr Hosp. 2007;22:322-9.
13. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr. 2013;13:59.
14. Hay WW. Optimizing nutrition of the preterm infant. Zhongguo Dang Dai Er Ke Za Zhi. 2017;19:1-21.
15. Evereklian M, Posmontier B. The Impact of Kangaroo Care on Premature Infant Weight Gain. J Pediatr Nurs. 2017;34:e10-e16.
16. Tannuri AC, Sbragia L, Tannuri U, Silva LM, Leal AJ, Schmidt AF, et al. Evolution of critically ill patients with gastroschisis from three tertiary centers. Clinics (Sao Paulo). 2011;66:17-20.
17. Lund CH, Bauer K, Berrios M. Gastroschisis: incidence, complications, and clinical management in the neonatal intensive care unit. J Perinat Neonatal Nurs. 2007;21:63-8.
18. Raymond SL, Hawkins RB, St Peter SD, Downard CD, Qureshi FG, Renaud E, et al. Predicting Morbidity and Mortality in Neonates Born with Gastroschisis. Journal of Surgical Research. 2020;245:217-24.
19. Eggink BH, Richardson CJ, Malloy MH, Angel CA. Outcome of gastroschisis: a 20-year case review of infants with gastroschisis born in Galveston, Texas. J Pediatr Surg. 2006;41:1103-8.
20. Calcagnotto H, Müller ALL, Leite JCL, Sanseverino MTV, Gomes KW, Magalhães JAA. Associated factors for perinatal mortality in gastroschisis. Rev Bras Ginecol Obstet. 2013;35:549-53.
21. Puligandla PS, Janvier A, Flageole H, Bouchard S, Laberge JM. Routine cesarean delivery does not improve the outcome of infants with gastroschisis. J Pediatr Surg. 2004;39:742-5.
22. Mastroiacovo P, Lisi A, Castilla EE. The incidence of gastroschisis: research urgently needs resources. BMJ. 2006;332:423-4.
23. Fallon EM, Mitchell PD, Potemkin AK, Nehra D, Arsenault DA, Robinson EM, et al. Cholestasis and growth in neonates with gastroschisis. J Pediatr Surg. 2012;47:1529-36.
24. Santos MM, Tannuri U, Maksoud JG. Alterations of enteric nerve plexus in experimental gastroschisis: is there a delay in the maturation? J Pediatr Surg. 2003;38:1506-11.
25. Alves FMS, Iranda ME, De Aguiar MJB, Viana MCFB. Nutritional management and postoperative prognosis of newborns submitted to primary surgical repair of gastroschisis. J Pediatr (Rio). 2016;92:268-75.
26. Redondo AC, Feferbaum R, Vieira RA, Moreira DAR, Tannuri U, Carvalho WB, et al. Characteristics of the clinical development of a newborn with gastroschisis in an intensive care unit in Latin America. J Hum Growth Dev. 2016;26:190-8.

ORIGINAL ARTICLE

AG-2021-111
doi.org/10.1590/S0004-2803.202100000-91

# Difficult biliary cannulation: should we always try a second ERCP after a failed needle-knife fistulotomy?

Victor Kalil **FLUMIGNAN**[1], Marina Garcia **SEIKE**[2], Victória Soares de **SOUZA**[2], Matheus Iguera **CIRQUEIRA**[2], Ana Beatriz **SILVA**[2] and Everson Luiz de Almeida **ARTIFON**[3]

Received: 26 May 2021
Accepted: 14 July 2021

**ABSTRACT** – **Background** – A successful bile duct cannulation is a prerequisite for the realization of endoscopic retrograde cholangiopancreatography (ERCP). When biliary cannulation is not possible, needle-knife fistulotomy (NKF) can be performed. However, when biliary access is not successfully achieved even after performing NKF, it is possible to interrupt the procedure, and repeat the ERCP after a short interval. **Objective –** The aim of this study is to analyze if repeating an ERCP after a short interval of 48 hours is effective in achieving biliary access after an initial NKF was unsuccessfully performed. **Methods –** A total of 1024 patients with a naive papilla, that underwent ERCP between the years of 2009–2019, were retrospectively reviewed. Difficult biliary cannulation was identified in 238 of these cases and NKF was performed. Success of biliary cannulation, NKF success at the first and second ERCPs, the associations between the type of the papilla, biliary dilatation, and overall success of NKF and adverse events rates were evaluated. **Results –** Biliary access was initially achieved in 183 (76.8%) cases. Of the 55 (23.1%) remaining cases a second attempt was performed after 48 hours, and biliary access was successfully achieved in 46 (83.6%) of them. The overall success of NKF after the first and second ERCP, the success rate was 96.2%. Papilla located out of its normal position was related to a minor chance of success at NKF ($P<0.05$). **Conclusion –** We conclude that when biliary access is not achieved after the performance of a NKF, a second attempt is safe and effective and should be attempted.

**Keywords –** ERCP; catheterization; endoscopic sphincterotomy.

## INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is the standard diagnostic and treatment procedure for various pancreatic and biliary diseases[1]. A successful bile duct cannulation is a prerequisite for the realization of the ERCP and even in experienced hands, biliary cannulation can fail in 5–20% of the cases[1-5]. When biliary cannulation is not possible, additional techniques are necessary to continue the procedure and access the common bile duct[6]. Despite the improvements in the gastrointestinal scopes and emergence of new catheters and guide-wires over the last decade, a standard technique has not been defined for difficult biliary cannulation. Needle-knife precut sphincterotomy, needle knife fistulotomy (NKF), transpancreatic sphincterotomy, double-guidewire and rendezvous are the most frequently performed techniques in this situation[5,7].

Pancreatitis is the most common ERCP complication, with overall rates of 5–7%[8]. Some studies suggest that needle knife precut sphincterotomy is related to higher rates of complications including perforation, bleeding, and pancreatitis[6,9,10], and in comparison, it's been reported that NKF is related to a lower risk of PEP[1]. The European Society of Gastrointestinal Endoscopy (ESGE) recommends the NKF be used as the preferred technique for precutting[11].

Although NKF is a safe and effective technique in difficult biliary cannulation, it can also result in cautery related edema and tissue necrosis, that will not allow biliary access[12]. When this occurs, some endoscopists abandon the procedure and change their strategy to percutaneous procedures or EUS access, for example. It has been observed that after a short time, with the resolution of the edema and tissue necrosis, the biliary access becomes feasible in the most part of the cases. In our center, we opted to repeat the ERCP 48 hours after the first NKF. The aims of this study are to analyze NKF for its overall safety, effectiveness, and its use in successful biliary access when it used as a second attempt after primary failure.

## METHODS

A total of 1115 ERCP realized between the years of 2009–2019 in *Hospital Santa Marcelina*, were analyzed. In this observational retrospective study informed consent was obtained from all the patients. The study was also approved by the Ethical Committee of the Institution. From the total, 91 patients were excluded due to: previous ERCP in 37 (40.6%) cases and surgical altered anatomy, such as Roux-en-Y gastrojejunostomy, or Billroth II gastrectomy, in 54 (59.4%) cases. The remaining 1024 cases were then analyzed. The procedures were performed under deep sedation, or general anesthesia, depending on patient status, and all of them were executed by an advanced endoscopy fellow under supervision of an experienced endoscopist.

Declared conflict of interest of all authors: none
Disclosure of funding: none
[1] Hospital Santa Marcelina, São Paulo, SP, Brasil. [2] Faculdade Santa Marcelina, São Paulo, SP, Brasil. [3] Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.
Corresponding author: Victor Kalil Flumignan. E-mail: victor_flumignan@hotmail.com

Difficult biliary cannulation was defined as a failure to achieve biliary access after 10 minutes of attempted cannulation, five contacts to the papilla or five pancreatic duct cannulations. When difficult biliary cannulation was defined, NKF was indicated as the first option. NKF using a Needle-Knife papillotome (Micro-Knife XL, Boston Scientific Corp, Natick, Mass), and pure cutting current, was performed at 11 o'clock position initiating at the top of the infundibulum, with a slowly downward movement, creating an 3–5 mm incision, until bile or a whitish mucosa of bile duct, was seen. Then the catheter was exchanged for a regular sphincterotome and biliary access was performed with a guide wire and confirmed by contrast injection. Following the successful biliary cannulation, the fistulotomy orifice was usually expanded with the regular sphincterotome. When deep cannulation was not possible even after the NKF, the procedure was then suspended, and a new ERCP was performed 48 hours later. At the second ERCP attempt, with the resolution of edema, tissue necrosis, and absence of clots the procedure was usually performed successfully with a regular sphincterotome, and the biliary access was easily achieved with cannulation of the fistulized area. If this was not possible, a second NKF was attempted, usually at the same location where the previous incision was performed.

The primary outcome of this study is to analyze the success rate of biliary access following NKF, and its success at the second attempt when biliary access wasn't achieved during the first ERCP. Secondary outcomes analyze the association between the type of the papilla, biliary dilatation and NKF final success rate. We also compared If the ERCP indications were related to final NKF success, overall procedure complications and CBD success rate cannulation.

Informed consent was obtained from all patients and the study was approved by the Institutional Ethical Committee of Hospital Santa Marcelina.

The statistical analysis was performed using the SPSS 22 for Windows software. All tests were performed with 95% confidence ($\alpha$=0.05). The analysis was performed with chi-square test and Logical Regression.

## RESULTS

Of the 1024 patients, 64.88% were female, and the average age of the patients was ±58 years with standard deviation of 19 years. The most common ERCP indication was choledocholithiasis which occurred in 70.2% of the patients, followed by pancreatic cancer (12.8%), cholangiocarcinoma (4%), papillary neoplasm (3%), gallbladder neoplasm (2.5%), hepatic metastasis (1.4%), and other less frequent etiologies (6.1%).

Cannulation of the biliary ostium was successfully performed in 786 (76.7%) patients. In 238 (23.3%) patients, primary cannulation was not possible and NKF was performed, as shown in TABLE 1. From the 238 NKF performed, biliary access was achieved initially in 183 (76.8%) cases. Regarding the remaining 55 (23.1%) cases where biliary access couldn't be achieved at the initial procedure, a second attempt was performed after an interval of 48 hours. With the resolution of the edema, tissue necrosis, and clots, at the second attempt the biliary access was feasible in the majority of patients. The remaining 55 cases were then submitted to the second ERCP, and success was achieved in 46 (83.6%) of them, as seen at FIGURE 1. When considering the overall success of NKF after the first and second ERCP, the success rate was 96.2%.

**TABLE 1.** Fistulotomy frequency.

| Fistulotomy | Frequency | Percent |
|---|---|---|
| No | 786 | 76.7 |
| Yes | 238 | 23.3 |
| Total | 1024 | 100.0 |

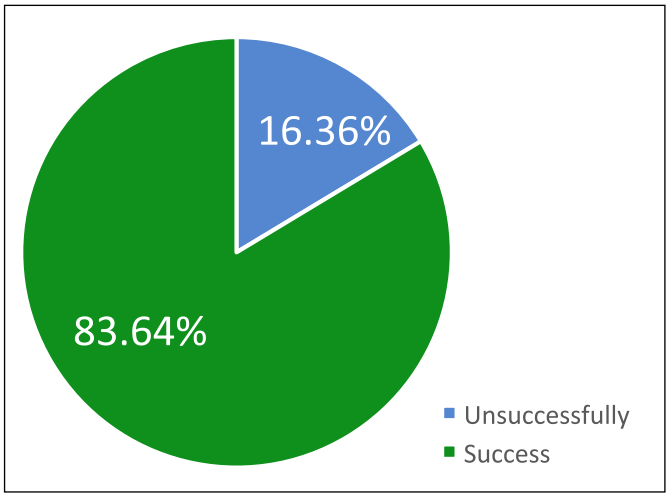



**FIGURE 1.** Success in the second nkf approach.

When applying or testing the chi-square to verify an association between papilla classification and success in fistulotomy, there was a significant association between them ($P$<0.05). We observed in TABLE 2 that regular papilla (polypoid), protruding papilla, peri/intra-diverticular papilla, with greater frequency were found with success at fistulotomy. When we look at the classification "located out of its normal position", we can see that the percentages are much more balanced, different from the other classifications, showing that when the papilla is out of its normal position, there is a greater chance of an unsuccessful fistulotomy. When associating success at fistulotomy with etiology of the ERCP, there was no evidence for an association between them ($P$>0.05), this data can be seen in TABLE 3.

**TABLE 2.** Final success of fistulotomy regarding the type of papilla.

| Classification of the papilla | Sucess at fistulotomy | | Chi-square test (*P*-value*) |
|---|---|---|---|
| | No | Yes | |
| Regular (polypoid) papilla | 5.6% | 94.4% | |
| Protruding papilla | 5.4% | 94.6% | |
| Peri/Intra-diverticular papilla | 0.0% | 100.0% | 22,989 (0.000**) |
| Located in the third duodenal portion | 41.7% | 58.3% | |

*Chi-square test (95%confidence). **Statistical significance.

**TABLE 3.** Final success of fistulotomy in relation to the final diagnosis.

| Final diagnosis | Sucess at fistulotomy | | Chi-square test (*P*-value*) |
|---|---|---|---|
| | No | Yes | |
| Cholelithiasis | 0.0% | 14.0% | |
| Choledocholithiasis | 8.3% | 36.7% | |
| Mirizzi syndrome | 0.0% | 0.5% | |
| Pancreatic cancer | 66.7% | 27.4% | |
| Cholangiocarcinoma | 8.3% | 7.9% | 13,593 (0.093) |
| Papilla neoplasm | 0.0% | 5.6% | |
| Liver metastasis | 0.0% | 2.3% | |
| Gallblader cancer | 8.3% | 3.3% | |
| Others | 8.3% | 2.3% | |

*Chi-square test (95%confidence). **Statistical significance.

When evaluating the presence of biliary dilatation and successful NKF performance, there was no evidence of a relationship between them. The chi-square test and the Logical Regression mode did not show statistical significance in the analysis of the relationship ($P$>0.05), and this is shown in TABLES 4 and 5.

**TABLE 4.** Analysis of the association between the presence of biliary dilation and the success of the procedure.

| Biliary dilatation | Resolution | | Chi-square test (*P*-value*) |
|---|---|---|---|
| | No | Yes | |
| Mild dilatation | 2.3% | 14.6% | |
| Moderate dilatation | 6.2% | 39.6% | |
| Marked dilatation | 2.8% | 12.3% | 3,990 (0.263) |
| Normal diameter | 2.5% | 19.8% | |
| Total | 13.8% | 86.2% | |

*Chi-square test (95%confidence). **Statistical significance.

**TABLE 5.** Association between the presence of biliary dilation and the success of the procedure – logistic regression analysis.

| Biliary dilatation | Resolution | Exp (B) | CI (95%) | *P*-value* |
|---|---|---|---|---|
| | Yes | | | |
| Mild dilatation | 14.6% | 1 | | |
| Moderate dilatation | 39.6% | 1.001 | (0.584–1.714) | 0.998 |
| Marked dilatation | 12.3% | 0.681 | (0.364–1.275) | 0.230 |
| Normal diameter | 19.8% | 1.240 | (0.659–2.334) | 0.505 |

*Wald test (95%confidence).

When analyzing adverse events related to NKF (considering the first and the second procedures), from the 238 NKFs, 13 had complications: 5 (38.4%) of them had pancreatitis, 5 (38.4%) had bleeding, and 3 (23%) had other complications, achieving a post-NKF adverse event rate of 5.4%, as seen in TABLE 6.

**TABLE 6.** Needle-knife fistulotomy (nkf) complications.

| NKF complications | Frequency | Percent |
|---|---|---|
| Acute pancreatitis | 5 | 2.1% |
| Bleeding | 5 | 2.1% |
| Other complications | 3 | 1.26% |
| Total | 13 | 100.0% |

## DISCUSSION

In 1980 Seigel first introduced pre-cutting to improve success rate at ERCP, followed then by the invention of the needle knife by Huibregtse, that promoted different precut techniques in order to achieve bile duct cannulation in difficult ERCPs[6,12]. The needle-knife fistulotomy (NKF) technique is defined as the use of a needle-knife catheter to perform an incision in the roof of the papilla, achieving biliary access, when difficult biliary cannulation is present. Studies showed that NKF is effective in achieving biliary access when difficult biliary cannulation is present[13]. Recent studies defend its early implementation during challenge cases[14]. Another common technique is the needle-knife precut sphincterotomy when the incision is initiated from the papillary orifice. This technique is, otherwise, related to pancreatic duct trauma, and greater rates of PEP[15]. However, it is also well known that the difficult biliary cannulation by itself is an independent risk factor for PEP[16], and there is no consensus on whether or not needle-knife precut results in higher rates of PEP pancreatitis.

The NKF employs an incision at the intraduodenal segment of the CBD creating a choledochoduodenal fistula, avoiding the contact to the papillary orifice and leaving the papillary sphincter partially intact[2]. This segment is usually above the papilla orifice and can be executed with a Needle-Knife catheter with movements in the downward direction in relation to papilla or the upward or cephalic direction, using electric current (cut, coagulation or blend) until the CBD is seen. A guidewire is then introduced into the CBD and after the radiological confirmation of successful biliary access, the fistulotomy orifice can be expanded by using a regular sphincterotome. In FIGURE 2, a classic sphincterotomy after an initial successful cannulation is shown. In FIGURE 3, after failed cannulation, a needle-knife catheter can be seen prior to the start of the incision in the roof of the papilla, in the proper position to perform the fistulotomy NKF, and in FIGURE 4 a scheme showing the steps of a NKF can be seen.

After the recognition of a biliary difficult cannulation, an early NKF was performed. Over the 10-year period of this retrospective study, 1024 cases were analyzed and 237 NKF were performed. These cases were always performed by an advanced endoscopy fellow and supervision was performed by a senior expert endoscopist. Of the 237 cases, success was initially achieved in biliary access in 78.6% of the patients. When biliary access was not possible even after NKF, a new ERCP after a short interval

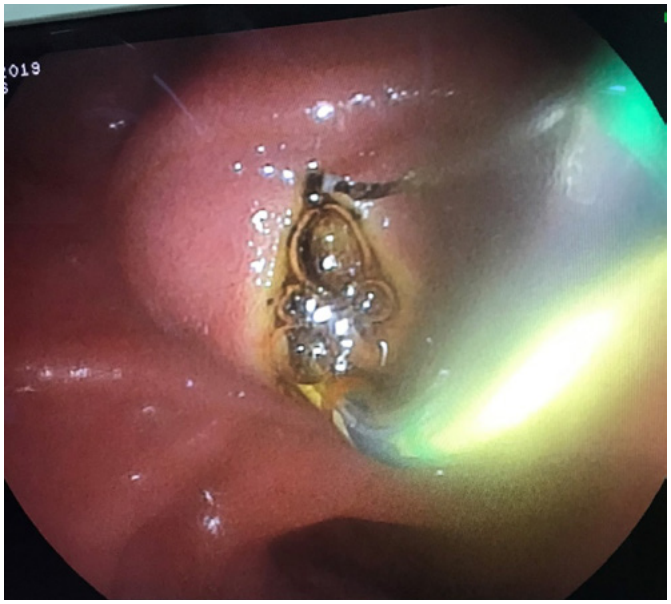


**FIGURE2.** Classic sphincterotomy.

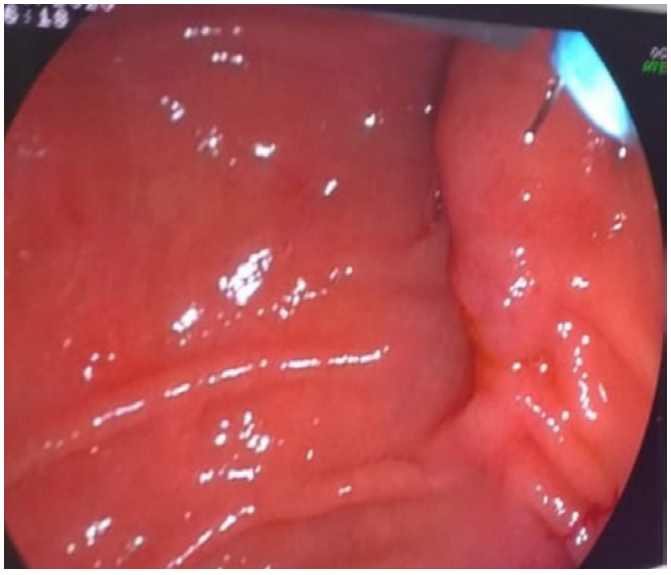


**FIGURE 3.** Needle knife catheter positioned in the roof of the papilla prior to attempting a nkf procedure.

of 48 hours was performed. Success rate of the ERCP after the short interval was 83.6%, reaching an overall NKF success rate of 96.2%. The success of ERCP after a short interval can be explained due to the improvement of the edema and inflammation[17], that allows better visualization of the site, and a safe and effective biliary cannulation.

Cannulation of the papilla ostium rates usually reaches 80–95%[1-5]. In our study a cannulation rate of 76.7% can be justified by the fact that all procedures were performed by an advanced endoscopy fellow, and when cannulation was not possible, usually a NKF was precociously indicated, considering that this could prevent PEP and the fact that the fellows needed to perform the technique to achieve proficiency. There are studies that shows that the presence of a trainee may compromise cannulation success rates[18].

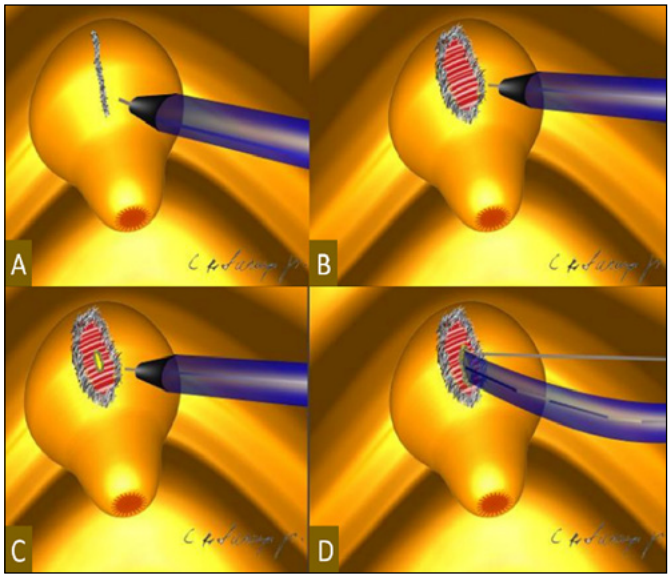



**FIGURE 4.** Needle-knife fistulotomy technique: (A) incision in the roof of the papilla; (B) exposure of the sphincter muscle layer; (C and D) dissection and access to cbd[5].

There are several descriptions of the macroscopic papillary appearance, but there is no consensus on which is the best classification[19]. In the present study they were classified in four types: regular (or polypoid), protruding, peri-diverticular and located out of its normal position (usually at third duodenal portion). In our analysis when the papilla was located out of its normal position, there was a relation with lower success at NKF performance. Periampullary diverticula are present in 10–20% of all ERCP cases and are considered for some authors as an impediment to the ERCP procedure[20,21]. However, in our study the presence of periampullary diverticula did not influenced in the NKF overall success or complication rates.

The standard post-ERCP complication rate is 3–10%[22]. In our study the overall adverse-events rate was 5.4%. Pancreatitis is the most common post ERCP complication and results in an increase in the overall cost of care[16,23]. In this analysis pancreatitis was responsible for 78.6 % of all ERCP complications, followed by bleeding in 12.5% of cases, and other less frequent causes in 8.9%. However, when analyzing the post-NKF complications, we can see that pancreatitis rate was only 38.4%, much less than in the overall post-ERCP complication rate (78.6%), suggesting that an early approach with NKF may prevent pancreatitis. Some studies show that the use of a plastic pancreatic stent is related to a decreased occurrence of PEP in high-risk patients[16]. It can be endoscopically withdrawn after a few days or spontaneously migrate, which can be confirmed by X-ray, and avoid another endoscopic procedure.

Because this is a retrospective study, there are limitations including selection bias. A prospective study analyzing the performance of NKF may be superior but it would take much longer to perform a similar analysis.

## CONCLUSION

In conclusion, NKF is feasible, safe, and can be attempted after a short interval of 48 hours if the first ERCP attempt was not successful.

## ACKNOWLEDGEMENTS

We would like to thank Dr Hélio Tanaka for his help and advice.

## Authors' contribution

Flumignan VK: conceptualization, methodology, project administration, writing-original draft, and writing-review and editing. Seike MG, Souza VS, Silva AB and Cirqueira MI: data curation. Artifon ELA: formal analysis.

## Orcid

Victor Kalil Flumignan: 0000-0003-3591-1237.
Marina Garcia Seike: 0000-0002-1268-955X.
Victória Soares de Souza: 0000-0002-9669-3408.
Matheus Iguera Cirqueira: 0000-0002-1793-3902.
Ana Beatriz Silva: 0000-0001-9815-9121.
Everson Luiz de Almeida Artifon: 0000-0003-1900-8777.

Flumignan VK, Seike MG, Souza VS, Cirqueira MI, Silva AB, Artifon ELA. Canulação biliar difícil: devemos tentar uma segunda CPRE após uma fistulotomia papilar mal-sucedida? Arq Gastroenterol. 2021;58(4):509-13.

**RESUMO** – **Contexto –** A canulação biliar de sucesso é pré-requisito para a realização da colangiopancreatografia retrógrada endoscópica (CPRE). Quando a canulação biliar não é possível, a fistulotomia com auxílio do cateter *Needle-Knife* (NKF) pode ser realizada. Entretanto, quando o acesso biliar não é atingido mesmo após a realização de um NKF, é possível optar-se pela interrupção do procedimento, e pela repetição da CPRE após curto intervalo de 48 horas. **Objetivo –** O objetivo desse estudo é analisar se a repetição da CPRE após um curto intervalo de 48 horas é efetivo em atingir o acesso biliar, quando um NKF foi realizado inicialmente sem sucesso. **Métodos –** Um total de 1024 pacientes com papila virgem de tratamento, submetidos à CPRE entre os anos de 2009–2019, foram retrospectivamente analisados. Canulação biliar difícil foi identificada em 238 deles, e NKF foi então realizado. Foram avaliadas as taxas de sucesso durante a canulação biliar, assim como durante a realização de NKF na primeira e segunda CPREs. A associação entre o tipo de papila, dilatação biliar e o sucesso final na realização do NFK também foi avaliada, assim como a presença de eventos adversos associados à realização do NKF. **Resultados –** Dentre todos os NKF realizados, acesso biliar foi inicialmente atingido em 183 (76,8%) casos. Os 55 (23,1%) casos restantes, foram submetidos a uma segunda CPRE após 48 horas e o acesso biliar foi atingido em 46 (83,6%) deles, resultando em uma taxa final de sucesso, após a primeira e segunda CPREs, de 96,2%. Papila localizada fora da sua posição habitual foi relacionada a menor chance de sucesso durante a realização de NKF ($P$<0,05). **Conclusão –** Concluiu-se que quando o acesso biliar não pode ser atingido após a realização de um NKF, uma segunda CPRE é segura, efetiva e deve ser realizada.

**Palavras-chave** – CPRE; cateterização; esfincterotomia endoscópica.

## REFERRENCES

1. Jin YJ, Jeong S, Lee DH. Utility of needle-knife fistulotomy as an initial method of biliary cannulation to prevent post-ERCP pancreatitis in a highly selected at-risk group: a single-arm prospective feasibility study. Gastrointest Endosc. 2016;84:808-13. doi:10.1016/j.gie.2016.04.011.
2. Ayoubi M, Sansoè G, Leone N, Castellino F. Comparison between needle-knife fistulotomy and standard cannulation in ERCP. World J Gastrointest Endosc. 2012;4:398-404. doi:10.4253/wjge.v4.i9.398.
3. Swan MP, Bourke MJ, Williams SJ, Alexander S, Moss A, Hope R, et al. Failed biliary cannulation: clinical and technical outcomes after tertiary referral endoscopic retrograde cholangiopancreatography. World J Gastroenterol. 2011;17:4993-98. doi:10.3748/wjg.v17.i45.4993.
4. Wen J, Li T, Lu Y, Bie LK, Gong B. Comparison of efficacy and safety of transpancreatic septotomy, needle-knife fistulotomy or both based on biliary cannulation unintentional pancreatic access and papillary morphology. Hepatobiliary Pancreat Dis Int. 2019;18:73-8. doi:10.1016/j.hbpd.2018.11.007.
5. Furuya CK, Sakai P, Marinho FRT, Otoch JP, Cheng S, Prudencio LL, et al. Papillary fistulotomy *vs* conventional cannulation for endoscopic biliary access: A prospective randomized trial. World J Gastroenterol. 2018;24:1803-11. doi:10.3748/wjg.v24.i16.1803.
6. Lopes L, Dinis-Ribeiro M, Rolanda C. Safety and efficacy of precut needle-knife fistulotomy. Scand J Gastroenterol. 2014;49:759-65. doi:10.3109/00365521.2014.898085.
7. Halttunen J, Keränen I, Udd M, Kylänpää L. Pancreatic sphincterotomy versus needle knife precut in difficult biliary cannulation. Surg Endosc. 2009;23:745-9. doi:10.1007/s00464-008-0056-0.
8. Lopes L, Dinis-Ribeiro M, Rolanda C. Early precut fistulotomy for biliary access: time to change the paradigm of "the later, the better"? Gastrointest Endosc. 2014;80:634-41. doi:10.1016/j.gie.2014.03.014.
9. Mavrogiannis C, Liatsos C, Romanos A, Petoumenos C, Nakos A, Karvountzis G. Needle-knife fistulotomy versus needle-knife precut papillotomy for the treatment of common bile duct stones. Gastrointest Endosc. 1999;50:334-9. doi:10.1053/ge.1999.v50.98593.
10. Testoni PA, Mariani A, Aabakken L, Arvanitakis M, Bories E, Costamagna G, et al. Papillary cannulation and sphincterotomy techniques at ERCP: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. Endoscopy. 2016;48:657-83. doi:10.1055/s-0042-108641.
11. Kevans D, Zeb F, Donnellan F, Courtney G, Aftab AR. Failed biliary access following needle knife fistulotomy: is repeat interval ERCP worthwhile? Scand J Gastroenterol. 2010;45:1238-41. doi:10.3109/00365521.2010.495418.
12. Zhang QS, Han B, Xu JH, Gao P, Shen YC. Needle-knife papillotomy and fistulotomy improved the treatment outcome of patients with difficult biliary cannulation. Surg Endosc. 2016;30:5506-12. doi:10.1007/s00464-016-4914-x.
13. Donnellan F, Zeb F, Courtney G, Aftab AR. Suprapapillary needleknife fistulotomy: a safe and effective method for accessing the biliary system. Surg Endosc. 2010;24:1937-40. doi:10.1007/s00464-010-0881-9.
14. Archibugi L, Mariani A, Capurso G, Traini M, Petrone MC, Rossi G, et al. Needle-knife fistulotomy vs. standard biliary sphincterotomy for choledocholithiasis: common bile duct stone recurrence and complication rate. Endosc Int Open. 2019;7:E1733-E1741. doi:10.1055/a-1024-3789.
15. Zagalsky D, Lasa J. Needle-knife fistulotomy and risk of post-ERCP pancreatitis. Gastrointest Endosc. 2017;86:247-8. doi:10.1016/j.gie.2017.01.042.
16. Davee T, Garcia JA, Baron TH. Precut sphincterotomy for selective biliary duct cannulation during endoscopic retrograde cholangiopancreatography. Ann Gastroenterol. 2012;25:291-302.
17. Lee TH, Bang BW, Park SH, Jeong S, Lee DH, Kim SJ. Precut fistulotomy for difficult biliary cannulation: is it a risky preference in relation to the experience of an endoscopist? Dig Dis Sci. 2011;56:1896-03. doi:10.1007/s10620-010-1483-z.
18. Frost JW, Kurup A, Shetty S, Fisher N. Does the presence of a trainee compromise success of biliary cannulation at ERCP? Endosc Int Open. 2017;5:E559-E562. doi:10.1055/s-0043-105579.
19. Haraldsson E, Lundell L, Swahn F, Enochsson L, Löhr JM, Arnelo U, et al. Endoscopic classification of the papilla of Vater. Results of an inter- and intraobserver agreement study. United European Gastroenterol J. 2017;5:504-10. doi:10.1177/2050640616674837.
20. Park CS, Park CH, Koh HR, Jun CH, Ki HS, Park SY, et al. Needle-knife fistulotomy in patients with periampullary diverticula and difficult bile duct cannulation. J Gastroenterol Hepatol. 2012;27:1480-3. doi:10.1111/j.1440-1746.2012.07201.x.
21. Parlak E, Suna N, Kuzu UB, Taşkiran I, Yildiz H, Torun S, et al. Diverticulum With Papillae: Does Position of Papilla Affect Technical Success? Surg Laparosc Endosc Percutan Tech. 2015;25:395-8. doi:10.1097/SLE.0000000000000130.
22. Zhang QS, Han B, Xu JH, Bao WM, Tao JL, Zhang Y. Needle knife precut papillotomy and fistulotomy for difficult biliary cannulation during endoscopic retrograde cholangiopancreatography. Digestion. 2013;88:95-100. doi:10.1159/000352027.
23. Artifon EL, Sakai P, Cunha JE, Halwan B, Ishioka S, Kumar A. Guidewire cannulation reduces risk of post-ERCP pancreatitis and facilitates bile duct cannulation. Am J Gastroenterol. 2007;102:2147-53. doi: 10.1111/j.1572-0241.2007.

ORIGINAL ARTICLE

AG-2021-113
doi.org/10.1590/S0004-2803.202100000-92

# Robotic liver resection. Report of the first 50 cases

Marcel Autran C **MACHADO**, Murillo M **LOBO-FILHO**, Bruno H **MATTOS**, André O **ARDENGH** and Fábio F **MAKDISSI**

Received: 23 May 2021
Accepted: 7 June 2021

**ABSTRACT** – **Background** – Robotic surgery has gained growing acceptance in recent years, expanding to liver resection. **Objective** – The aim of this paper is to report the experience with our first fifty robotic liver resections. **Methods** – This was a single-cohort, retrospective study. From May 2018 to December 2020, 50 consecutive patients underwent robotic liver resection in a single center. All patients with indication for minimally invasive liver resection underwent robotic hepatectomy. The indication for the use of minimally invasive technique followed practical guidelines based on the second international laparoscopic liver consensus conference. **Results** – The proportion of robotic liver resection was 58.8% of all liver resections. Thirty women and 20 men with median age of 61 years underwent robotic liver resection. Forty-two patients were operated on for malignant diseases. Major liver resection was performed in 16 (32%) patients. Intrahepatic Glissonian approach was used in 28 patients for anatomical resection. In sixteen patients, the robotic liver resection was a redo hepatectomy. In 10 patients, previous liver resection was an open resection and in six it was minimally invasive resection. Simultaneous colon resection was done in three patients. One patient was converted to open resection. Two patients received blood transfusion. Four (8%) patients presented postoperative complications. No 90-day mortality was observed. **Conclusion** – The use of the robot for liver surgery allowed to perform increasingly difficult procedures with similar outcomes of less difficult liver resections.

**Keywords** – Liver; robotic surgical procedures; liver resection.

## INTRODUCTION

Minimally invasive liver resection is a feasible and safe technique and has been used to treat several types of liver neoplasms[1,2]. Robotic surgery has gained growing acceptance in recent years, expanding to liver resection[3-6]. The robotic approach, with its added degrees of freedom, improved visualization, stability of the robotic platform, and better ergonomics improve the surgeon's dexterity during complex minimally invasive procedures.

There is a recent interest in robotic liver surgery and the number and complexity of procedures are rapidly increasing[6-10]. The aim of this paper is to report the experience with our first fifty robotic liver resections.

## METHODS

This was a single-cohort, retrospective analysis of a prospective maintained database of all robotic procedures. From May 2018 to December 2020, 50 consecutive patients underwent robotic liver resection in a single center by the senior author (MAM). All patients with indication for minimally invasive liver resection underwent robotic liver resection. The indication for the use of minimally invasive technique followed practical guidelines based on the second international laparoscopic liver consensus conference[11]. Over this period, 38 patients presented contraindication for minimally invasive approach and underwent open liver resection. Main contraindications for the use of a minimally invasive technique were: a) patients with huge tumors which mobilization could result in tumor disruption or jeopardize the oncological aspect of the procedure, b) multiple and small lesions that could be missed with minimally invasive approach, c) lesions in close contact with major hepatic veins that should be preserved (R1 vascular). The patients were informed about the advantages and risks of the robotic technique, and they gave informed consent.

### Surgical technique

#### Patient positioning and port placement

The patient is placed in a supine position and 30º reverse Trendelenburg position. Robotic surgery is performed using the da Vinci Si or Xi robotic platform (Intuitive Surgical Inc., Sunnyvale, CA). This technique uses five trocars. A pneumoperitoneum is created using an open technique in the infra-umbilical area. The pneumoperitoneum is established at 14 mmHg. The remaining trocars are inserted under direct vision and its location will depend on the patient biotype and type of liver resection planned. During this technique, the surgeon is seated at the robotic console and the assistant surgeon stands on the patient's left side. The assistant surgeon performs retraction, suction, clipping, stapling, and changes the robotic instruments.

#### Intraoperative ultrasound and liver mobilization

Right or left liver is mobilized depending on the type of liver surgery planned. Intraoperative ultrasound is used in all cases to locate the tumor, to determine the liver anatomy, and to stablish relationship between the tumor and major liver vessels and screening for other lesions.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
Hospital Nove de Julho, São Paulo, SP, Brasil.
Corresponding author: Marcel Autran Machado. E-mail: dr@drmarcel.com.br

### Pringle Maneuver and hilar dissection

Hepatoduodenal ligament is dissected, and a Foley catheter is passed around to perform intermittent intracorporeal Pringle maneuver in cases where this maneuver was indicated (FIGURE 1.A).

In our initial cases of anatomical liver resection, complete hilar dissection was performed with individual control of the portal vein, hepatic artery, and bile duct (FIGURE 2). As our experience increased, the technique of intrahepatic control of Glissonian pedicle was used more frequently, especially in patients with previous liver resection, previous hilar dissection or need for segmental liver resection. Hilar dissection was restricted to patients with need for hilar lymphadenectomy (FIGURE 2.D) and hilar cholangiocarcinoma. In some patients, no hilar dissection or Pringle maneuver was used. In other patients, selective hepatic artery clamping was used.

### Glissonian approach

Intrahepatic Glissonian approach technique was used according to our previous description[12]. For the intrahepatic Glissonian approach, two small incisions are used following specific anatomical landmarks (FIGURE 3). Removal of liver tissue around the pedicle allows the intrahepatic identification of the Glissonian pedicle. It is then encircled using the Cadière forceps, a robotic wristed instrument, similarly to the open technique of Glissonian approach. FIGURE 4 shows the Machado's points, used as anatomical landmarks for Glissonian approach during anatomical liver resections[12].

### Liver transection

After delineation of the area to be resected by either ischemic discoloration, by negative fluorescence imaging (FIGURE 1.B) after indocyanine green injection (anatomic resections) or by simple cautery demarcation guided by intraoperative ultrasound

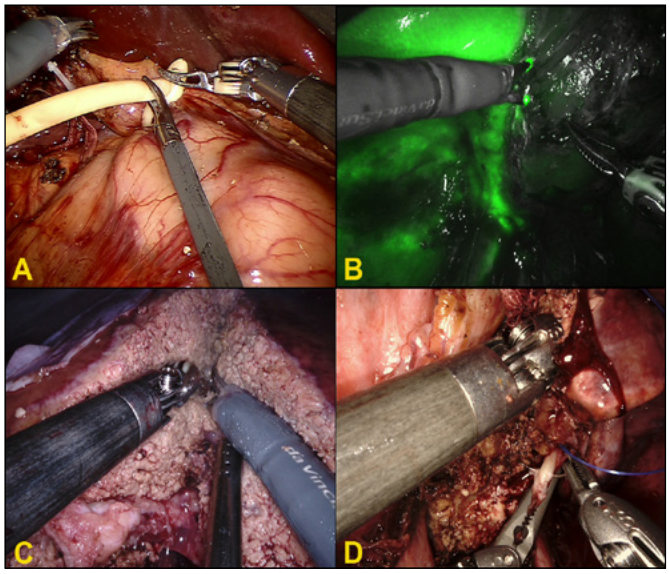



**FIGURE 1.** Robotic liver resection.
A) Foley catheter is used for intermittent intracorporeal Pringle maneuver.
B) Indocyanine green fluorescence imaging during robotic left hepatectomy. Left liver is ischemic.
C) Liver is transected with a combination of robotic bipolar forceps under continuous saline irrigation and robotic scissors.
D) Intraoperative control of major bleeding from inferior vena cava (IVC) branch. IVC is temporary clamped, and a suture is placed.

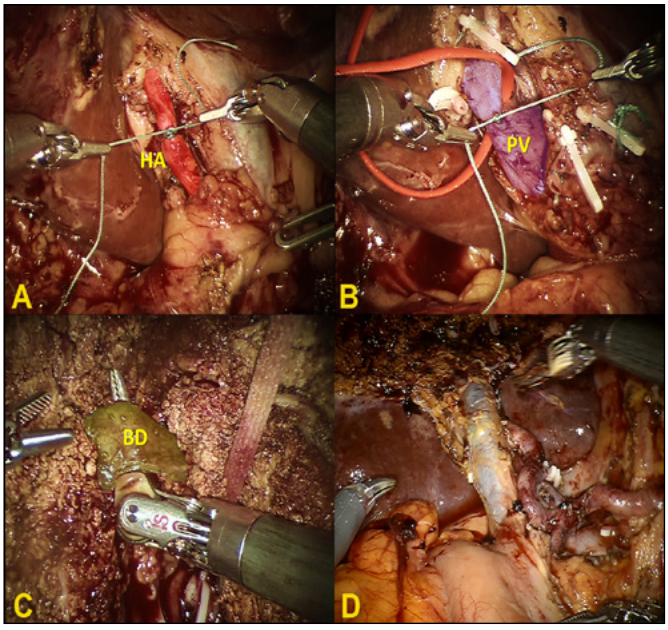



**FIGURE 2.** Robotic liver resection. Hilar dissection.
A) Right hepatic artery (HA) is dissected and ligated during robotic right hepatectomy.
B) Right portal vein (PV) is dissected and ligated during robotic right hepatectomy.
C) Right bile duct (BD) is identified during liver transection, encircled, and ligated.
D) Final aspect of hilar lymphadenectomy during robotic left hepatectomy for intrahepatic cholangiocarcinoma.

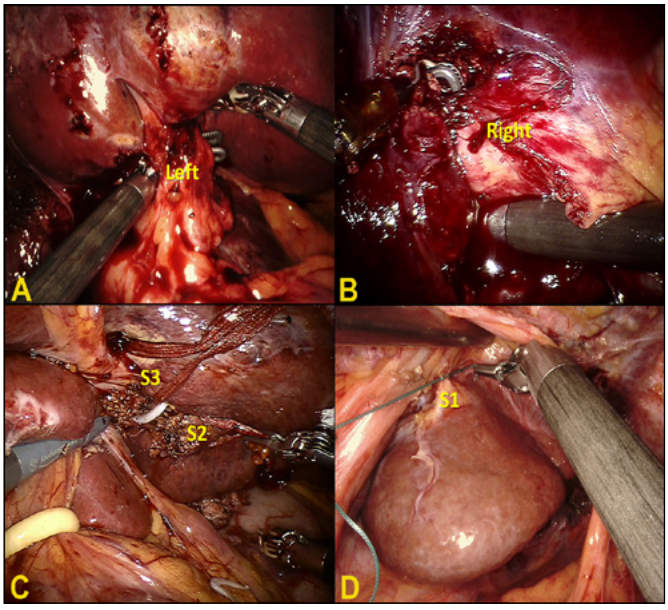



**FIGURE 3.** Robotic liver resection. Intrahepatic Glissonian approach.
A) Left Glissonian pedicle is encircled during robotic left hepatectomy.
B) Right Glissonian pedicle is encircled during robotic right hepatectomy.
C) Glissonian pedicle from segment 3 (S3) and segment 2 (S2) are encircled during bi-segmentectomy 2–3.
D) Glissonian pedicle from segment 1 (S1) is ligated during segmentectomy 1.

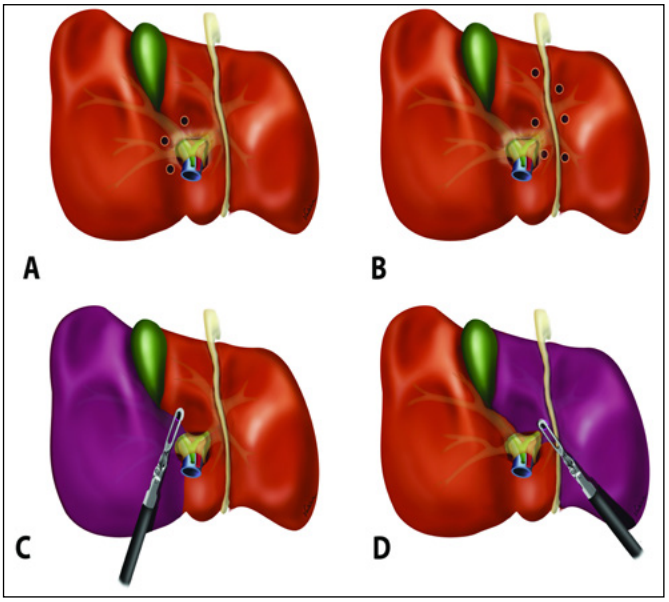



**FIGURE 4.** Schematic drawings of robotic Glissonian approach during liver resection.
A) Machado's points used for intrahepatic access to Glissonian pedicles from right liver.
B) Machado's points used for intrahepatic access to Glissonian pedicles from left liver.
C) Schematic drawing of caudal view for intrahepatic access of right Glissonian pedicle.
D) Schematic drawing of caudal view for intrahepatic access of left Glissonian pedicle.

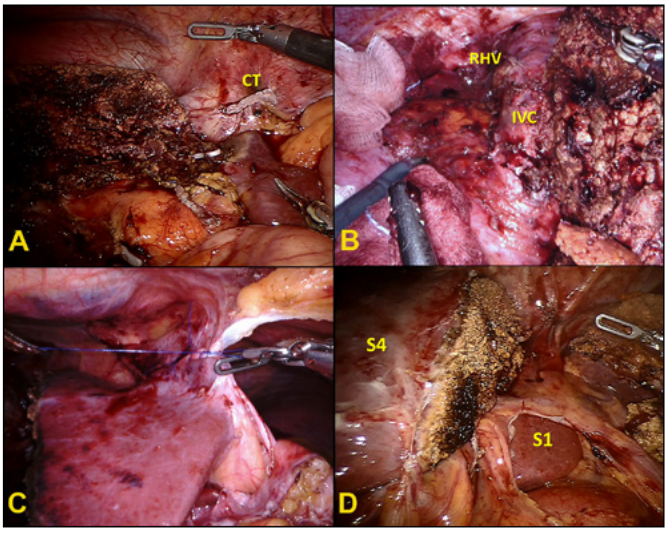



**FIGURE 5.** Robotic liver resection. View after liver resection.
A) Final view after robotic left trisegmentectomy with caudate lobe preservation. CT: common trunk, containing middle and left hepatic veins.
B) Final view after robotic bi-segmentectomy 7–8. IVC: inferior vena cava; RHV: right hepatic vein.
C) Final view after robotic right hepatectomy. Falciform ligament is sutured to the abdominal wall to maintain the left liver (remnant) in its original position avoiding hepatic vein kinking.
D) Final view after robotic bi-segmentectomy 2–3. S1: segment 1; S4: segment 4.

(wedge resections), the liver is transected (FIGURE 5). Our technique of liver transection is the use of the robotic bipolar forceps under continuous saline irrigation (FIGURE 1.C) with or without Pringle or selective inflow control. The liver tissue is then transected with robotic scissors. Large hepatic veins or pedicles encountered during liver transection are divided between hem-o-locks. Main hepatic veins are divided with vascular stapler or suture ligated (FIGURE 1.D).

### Variables

The primary endpoint was safety of the procedures. Safety was assessed as the occurrence of intraoperative events or during hospitalization complications such as biliary fistulas, transfusion, liver failure, infection and 90-day mortality. To further account for the severity of complications, the Clavien-Dindo classification was used[13]. Secondary outcomes were surgical efficacy endpoints such as conversion rate, operative time, blood loss, need for transfusions, and length of hospitalization.

## RESULTS

We have performed robotic liver resection on 50 consecutive patients. Over the same period, 38 patients underwent open liver resection, thus, the proportion of robotic liver resection was 58.8% of all liver resections. Thirty women and 20 men with median age of 61 years (range: 30–88) underwent robotic liver resection (TABLE 1). Forty-two patients were operated on for malignant diseases, 34 for liver metastases, three for hepatocellular carcinoma, three for intrahepatic cholangiocarcinoma, one for hilar cholangiocarcinoma and one for hemangioendothelioma. Robotic liver resection was performed in eight patients for benign diseases, three for intrahepatic lithiasis, four for biliary cystadenoma and one due to a large focal nodular hyperplasia with extrinsic gastric compression (TABLE 1). Major liver resection, defined as resection of three or more adjacent liver segments, was performed in 16 (32%) patients. Anatomical bi-segmentectomies were performed in six patients, anatomical segmentectomies were performed in

**TABLE 1.** Demographics of 50 patients undergoing robotic liver resection.

| Variable of interest | Patients (n=50) |
|---|---|
| Age, years, median (range) | 61 (30–88) |
| Sex, male/female, n (%) | 20/30 (40%/60%) |
| Type of operation | |
| Minor, n (%) | 34 (68%) |
| Major, n (%) | 16 (32%) |
| Malignancy | |
| Benign, n (%) | 8 (16%) |
| Malignant, n (%) | 42 (84%) |
| Tumor type | |
| Primary, n (%) | 16 (32%) |
| Secondary, n (%) | 34 (68%) |
| Liver parenchyma | |
| Normal, n (%) | 46 (92%) |
| Cirrhosis, n (%) | 4 (8%) |

n: number.

11 patients. Associating Liver Partition and Portal vein ligation for Stage hepatectomy (ALPPS) procedure was performed in two patients (TABLE 2).

**TABLE 2.** Types of robotic liver resection.

| Procedure | Patients (n=50) |
|---|---|
| Left liver | |
| S1 | 3 |
| S2 | – |
| S3 | 3 |
| S4 | 4 |
| S2–S3 | 4 |
| Left hemihepatectomy | 5 |
| Right liver | |
| S5–S6 | 1 |
| S6–S7 | 1 |
| S7–S8 | 1 |
| S5 | – |
| S6 | 6 |
| S7 | 2 |
| S8 | 2 |
| Right hemihepatectomy | 6 |
| Bilateral | |
| Right trisectionectomy | 2 |
| Left trisectionectomy | 1 |
| Mesohepatectomy | 2 |
| ALPPS | 2 |
| Other types/combination of segments | 5 |

ALPPS: Associating Liver Partition and Portal vein ligation for Stage hepatectomy.

Intrahepatic Glissonian approach was used in 28 patients for anatomical liver resection. Five major liver resections were performed with dissection of the hilar pedicle for anatomical liver resection, two right and three left hepatectomies. In two of these patients, hepaticojejunostomy was performed and in one case, the portal vein was resected and reconstructed. Hilar lymphadenectomy was performed in three cases.

In 16 patients, the robotic liver resection was a redo hepatectomy. In 13 patients, it was the second hepatectomy, in two patients it was the third and in one patient it was the fourth liver resection. In ten patients, previous liver resection was performed by open approach and in six by minimally invasive technique. In two patients, robotic liver resection was performed after open pancreatoduodenectomy and no hilar dissection or Pringle maneuver was performed due to the presence of hepaticojejunostomy. Simultaneous colon resection was done in three patients.

One patient was converted to open resection due to invasion of the hepatic hilum that preclude a safe and oncological operation. Two patients received blood transfusion (TABLE 3). Four (8%) patients presented postoperative complications, two clinical complications, acute renal failure (Clavien-Dindo IVa) and cardiac arrhythmia (Clavien-Dindo II) were conservatively managed and two surgical complications (subphrenic abscess and biliary fistula). Biliary fistula (Clavien-Dindo II) was managed by late removal of the drain while the abscess needed drainage under general anesthesia (Clavien-Dindo IIIb). Median hospital stay was 4 days. Mortality was nil.

**TABLE 3.** General outcomes of 50 patients undergoing robotic liver resection.

| Variable of interest | Patients (n=50) |
|---|---|
| Operative time, minutes, median (SD) | 293 (143) |
| Patients transfused (RBC), n (%) | 2 (4%) |
| Blood loss | |
| <100 mL, n (%) | 31 (62%) |
| 101–600 mL, n (%) | 17 (34%) |
| 601–1000 mL, n (%) | 2 (20%) |
| >1000 mL, n (%) | 0 (0%) |
| Conversion, n (%) | 1 (2%) |
| Hospital stay, days, median (range) | 4 (1–15) |
| Malignant tumor in pathology, n (%) | 42 (84%) |
| Positive margins*, n (%) | 1 (2.4%) |

SD: standard deviation, RBC: red blood cell. *Benign tumors excluded.

## DISCUSSION

Since May 2018, all our minimally invasive liver surgeries are performed using the robotic platform and their data has been recorded on a prospective maintained database. Our experience with this new system increased over this period and so the indications for more complex cases[7-10]. The robotic approach, with its added degrees of freedom and stability of the robotic platform, may offer options for minimally invasive performance of complex liver resections that were a relative contraindication of laparoscopy[15-17]. Procedures that require excellent accuracy and dexterity are the best candidates for robotic surgery. Vascular structures, such as portal vein[8,9], hepatic artery and hepatic veins, are magnified in robotic vision. The magnified vision camera can expose the anatomic structure of the hilum. The da Vinci robot provides 20x magnified 3D vision, improving the precision of hilar dissection allowing vascular sutures, venous reconstruction[9], and biliary anastomosis[10] at difficult angles with the non-dominant hand, when necessary. An excellent visualization is key to the control of the intraoperative bleeding during mobilization and transection of the liver.

The first robotic liver resection in Brazil was performed by our team in 2008[14]. However, high-cost and absence of specific instruments for this complex procedure paused its use in our center for 10 years. Since May 2018, with the development of new instruments, acquisition of a new robotic platform and a new hospital policy with significant cost reduction for the use of the robotic platform, inspired us to employ the robot in all minimally invasive robotic surgery. Our previous and significant experience in laparoscopic liver surgery was important to decrease our learning curve. In our first 50 consecutive cases, there was just one conversion that occurred in the beginning of our experience. The morbidity rate was low (8%) with no mortality even though almost one third of procedures were major liver resections. The proportion of robotic liver resections was 56.8% among all liver resections. Laparoscopy

was completely replaced by the robotic approach. Open approach was reserved for the patients with contraindications for minimally invasive technique[11]. In brief, every patient with a straightforward liver resection can and should be operated by minimally invasive approach. There is no consensus for contraindication for minimally invasive approach. Patients that until recently had a contraindication for minimally invasive approach are now being operated on by robotic approach[9,10]. Therefore, indication for the use of the robotic technique is changing fast and the proportion of minimally invasive liver resection will certainly increase with more experience with this new technology. The three techniques, open, laparoscopic, and robotic will coexist in the future, but with a different proportion than is seen today. In our opinion, the robotic technique will prevail, at least for liver resection.

Cost has always been considered to be the greatest limitation for the use of the robotic platform. However, recent studies with cost analysis have concluded that robotic hepatectomy has a lower overall cost. Robotic approach has greater intraoperative costs but this is outbalanced by a lower postoperative cost conferred by lower complication rate and shorter hospital stay[15,17-20]. This robotic effect on outcome, according to Luberice et al.[7], is independent of difficulty level of the liver resection. It has been noted by us since the inception of our robotic program and shown by these authors with their analysis of Iwate criteria of laparoscopic liver resection difficulty applied to robotic hepatectomy[7].

Robotic approach is useful for a precise dissection of the hepatic hilum. Individual dissection and identification of the portal triad seems faster and easier than with laparoscopy[3]. The intrahepatic Glissonian approach is our preferred method of inflow control for anatomical resections because it permits a rapid control of the portal pedicles while allowing segmental liver resections[12,21]. The use of this approach in laparoscopic liver resection needed an adaptation in the technique[12,21]. Instead of encircling the Glissonian pedicle for individual control of the correspondent portal pedicle (as in open intrahepatic Glissonian technique), laparoscopic Glissonian approach was achieved with blind insertion of a vascular clamp around the target pedicle using specific anatomical landmarks[12,21]. The use of the robotic platform permitted the safe encircling of the Glissonian pedicle in the same way that it was originally described for open liver resection, precluding the hilar dissection, even though robotic facilitates such dissection. Fluorescence imaging after indocyanine green injection is an important tool to define the limits of liver resection and to check liver perfusion and bile leaks after resection[22]. Individual dissection of the hilar elements was used for inflow control in five major liver resections, two right and three left hepatectomies. In two of these patients, hepaticojejunostomy was performed and in one case, the portal vein was resected and reconstructed. Hilar lymphadenectomy was performed in three patients.

### Lessons learned

Our initial experience with robotic liver surgery raised some issues that may be important for any surgeon who intend to embark in this new technology. Previous experience in both open and laparoscopic liver surgery is essential for better results. Trocar placement may vary depending on the biotype of the patient and it is different from laparoscopic hepatectomy. Correct trocar placement is one of the most important steps for successful robotic procedure. The constant mobilization of the operating table, common during laparoscopic liver resection, is not possible in most robotic platforms. The supine position and reverse Trendelenburg position should be correctly stablished before robot docking. New platforms (da Vinci Xi) have operating tables integrated with the robot, but its availability is still scarce in our country. Another important issue is that there are some surgical instruments commonly used in liver surgery that are not available in the robotic systems so far, such as CUSA, cavitron ultrasonic surgical aspirator (used for liver transection) and other which are expensive and its use may be limited in developing countries, such as integrated staplers and robotic ultrasound. Therefore, we may overcome this issue by using laparoscopic available instruments, CUSA, laparoscopic ultrasound probe and staplers. However, these instruments are controlled by the bedside surgeon, so adequate experience in advanced liver surgery is also a prerequisite for the bedside surgeon. Conversion to open approach is less common than laparoscopy since bleeding is easier to fix with the use of wristed instruments that allows precise suturing whenever necessary (FIGURE 1.D). Indeed, several patients with major bleeding during this initial experience were easily controlled by suture. Nevertheless, if emergency conversion is needed, it may be hazardous once the undocking may take an extra time. Fortunately, the only patient converted in the present series was electively converted due to technical difficulty.

## CONCLUSION

The use of the robot for liver surgery allowed to perform increasingly difficult procedures with similar outcomes of less difficult liver resections.

### Authors' contribution

Machado MAC, Lobo-Filho MM, Mattos BH and Makdissi FF participate in the operative procedures. Ardengh AO collected the data. Machado MA wrote the manuscript draft. Ardengh AO, Lobo-Filho MM, Mattos BH and Makdissi FF supervised and commented on the manuscript. All authors discussed the results and contributed to the final manuscript.

### Orcid

Marcel Autran C Machado: 0000-0002-4981-7607.
Murillo M Lobo Filho: 0000-0002-4716-0082.
Bruno H Mattos: 0000-0002-2849-5717.
André O Ardengh: 0000-0001-6373-5598.
Fábio Ferrari Makdissi: 0000-0001-8202-5890.

Machado MAC, Lobo-Filho MM, Mattos BH, AO Ardengh, Makdissi FF. Hepatectomia robótica. Relato dos primeiros 50 casos. Arq Gastroenterol. 2021;58(4):514-9.

**RESUMO** – **Contexto** – A cirurgia robótica tem tido aceitação crescente nos últimos anos, expandindo-se para a ressecção hepática. **Objetivo** – Relatar a experiência com as primeiras cinquenta ressecções hepáticas robóticas. **Métodos** – Trata-se de análise retrospectiva de dados coletados prospectivamente. De maio de 2018 a dezembro de 2020, 50 pacientes consecutivos foram submetidos à ressecção hepática robótica em um único centro. Todos os pacientes com indicação de ressecção hepática minimamente invasiva foram submetidos à hepatectomia robótica. A indicação de técnica minimamente invasiva seguiu as diretrizes práticas baseadas na segunda conferência internacional de consenso laparoscópico hepático. **Resultados** – A proporção de ressecções hepáticas robóticas foi de 58,8% de todas as ressecções hepáticas. Trinta mulheres e 20 homens com idade mediana de 61 anos foram submetidos à ressecção hepática robótica. Quarenta e dois pacientes foram operados por doenças malignas. Ressecção hepática maior foi realizada em 16 (32%) pacientes. A abordagem Glissoniana intra-hepática foi usada em 28 pacientes para ressecção anatômica. Em 16 pacientes, a ressecção hepática robótica foi uma re-hepatectomia. Em 10, a hepatectomia prévia foi aberta e em seis foi por via minimamente invasiva. Ressecção simultânea do cólon foi feita em três pacientes. Um paciente foi convertido para ressecção aberta. Dois pacientes receberam transfusão sanguínea. Quatro (8%) pacientes apresentaram complicações pós-operatórias. Mortalidade em 90 dias foi nula. **Conclusão** – O uso do robô permitiu realizar procedimentos progressivamente mais complexos com resultados semelhantes às hepatectomias menos complexas.

**Palavras-chave** – Fígado; cirurgia robótica; hepatectomia.

## REFERENCES

1. Wakabayashi G, Cherqui D, Geller DA, Buell JF, Kaneko H, Han HS, et al. Recommendations for laparoscopic liver resection: a report from the second international consensus conference held in Morioka. Ann Surg. 2015;261:619-29.
2. Ciria R, Cherqui D, Geller DA, Briceno J, Wakabayashi G. Comparative Short-term Benefits of Laparoscopic Liver Resection: 9000 Cases and Climbing. Ann Surg. 2016;263:761-77.
3. Giulianotti PC, Sbrana F, Coratti A, Bianco FM, Addeo P, Buchs NC, et al. Totally robotic right hepatectomy: surgical technique and outcomes. Arch Surg. 2011;146:844-50.
4. Swaid F, Sucandy I, Tohme S, Marsh JW, Bartlett DL, Tsung A, Geller DA. Changes in Performance of More Than 1000 Minimally Invasive Liver Resections. JAMA Surg. 2020;155:986-8.
5. Sucandy I, Giovannetti A, Ross S, Rosemurgy A. Institutional First 100 Case Experience and Outcomes of Robotic Hepatectomy for Liver Tumors. Am Surg. 2020;86:200-7.
6. Ciria R, Berardi G, Alconchel F, Briceño J, Choi GH, Wu YM, et al. The impact of robotics in liver surgery: A worldwide systematic review and short-term outcomes meta-analysis on 2,728 cases. J Hepatobiliary Pancreat Sci. 2020;17. doi: 10.1002/jhbp.869.
7. Luberice K, Sucandy I, Modasi A, Castro M, Krill E, Ross S, Rosemurgy A. Applying IWATE criteria to robotic hepatectomy: is there a "robotic effect"? HPB (Oxford). 2020:S1365-182X(20)31196-5. doi: 10.1016/j.hpb.2020.10.008.
8. Machado MA, Surjan RC, Makdissi F. Robotic ALPPS. Ann Surg Oncol. 2020;27:1174-9.
9. Machado MA, Mattos BH, Lobo Filho MM, Makdissi FF. Robotic Right Hepatectomy with Portal Vein Thrombectomy for Colorectal Liver Metastasis (with Video). J Gastrointest Surg. 2021;10. doi: 10.1007/s11605-021-04954-x.
10. Machado MA, Mattos BV, Lobo Filho MM, Makdissi F. Robotic Resection of Hilar Cholangiocarcinoma. Ann Surg Oncol. 2020;27:4166-70.
11. Cho JY, Han HS, Wakabayashi G, Soubrane O, Geller D, O'Rourke N, et al. Practical guidelines for performing laparoscopic liver resection based on the second international laparoscopic liver consensus conference. Surg Oncol. 2018;27:A5-A9.
12. Machado MA, Makdissi F, Surjan R. Laparoscopic glissonean approach: Making complex something easy or making suitable the unsuitable? Surg Oncol. 2020;33:196-200.
13. Dindo D, Clavien PA. What is a surgical complication? World J Surg. 2008;32: 939-41.
14. Machado MA, Makdissi FF, Surjan RC, Abdalla RZ. First robotic-assisted laparoscopic liver resection in Latin America. Arq Gastroenterol. 2009;46:78-80.
15. Wong DJ, Wong MJ, Choi GH, Wu YM, Lai PB, Goh BKP. Systematic review and meta-analysis of robotic versus open hepatectomy. ANZ J Surg. 2019;89: 165-70.
16. Tsung A, Geller DA, Sukato DC, Sabbaghian S, Tohme S, Steel J, et al. Robotic versus laparoscopic hepatectomy: a matched comparison. Ann Surg. 2014;259:549-55.
17. Ziogas IA, Giannis D, Esagian SM, Economopoulos KP, Tohme S, Geller DA. Laparoscopic versus robotic major hepatectomy: a systematic review and meta-analysis. Surg Endosc. 2021;35:524-35.
18. Cortolillo N, Patel C, Parreco J, Kaza S, Castillo A. Nationwide outcomes and costs of laparoscopic and robotic vs. open hepatectomy. J Robot Surg. 2019;13:557-65. doi: 10.1007/s11701-018-0896-0.
19. Daskalaki D, Gonzalez-Heredia R, Brown M, Bianco FM, Tzvetanov I, Davis M, et al. Financial Impact of the Robotic Approach in Liver Surgery: A Comparative Study of Clinical Outcomes and Costs Between the Robotic and Open Technique in a Single Institution. J Laparoendosc Adv Surg Tech A. 2017;27:375-82.
20. Sham JG, Richards MK, Seo YD, Pillarisetty VG, Yeung RS, Park JO. Efficacy and cost of robotic hepatectomy: is the robot cost-prohibitive? J Robot Surg. 2016;10:307-13.
21. Machado MA, Herman P, Machado MC. Anatomical resection of left liver segments. Arch Surg. 2004;139:1346-9.
22. Marino MV, Di Saverio S, Podda M, Gomez Ruiz M, Gomez Fleitas M. The Application of Indocyanine Green Fluorescence Imaging During Robotic Liver Resection: A Case-Matched Study. World J Surg. 2019;43:2595-2606.

ORIGINAL ARTICLE

AG-2021-117
doi.org/10.1590/S0004-2803.202100000-93

# Efficacy of endoscopic balloon dilation in Iranian pediatric patients with esophageal stricture

Mitra **AHMADI**[1], Mohammad **MANZARI-TAVAKOLI**[1], Hazhir **JAVAHERIZADEH**[1,2], Mehran **HAKIMZADEH**[1], Mohammadreza **MIRKARIMI**[3] and Asaad **SHARHANI**[4]

Received: 30 May 2021
Accepted: 6 July 2021

**ABSTRACT** – **Background** – Esophageal stenosis (ES) in children is a fixed intrinsic narrowing of the esophagus due to numerous aetiologies. **Objective** – This study aimed to determine the clinical and nutritional impacts of endoscopic balloon dilation (EBD) in Iranian children with an esophageal stricture. **Methods** – This retrospective study, pediatric patients (aged <18 years) who underwent EBD for esophageal stricture from April 2015 until March 2020 in Abuzar Children's Hospital (Ahvaz, Iran) were enrolled in the study. Outcome parameters were the frequency of dilations, nutritional status, complications, and clinical success rates. EBD was used in children with radiologic evidence of esophageal stenosis. The nutritional status was evaluated by weight-for-age (z-score). Clinical success was considered as no necessity of EBD for a minimum of one year and/or increasing interval among dilation and the frequency of EBD was less than four times per year. **Results** – A total of 53 cases (mean age, 4.72±3.38 years) were enrolled. There were 25 (47.2%) females and 28 (52.8%) males. During follow-up, a total of 331 EBD sessions were performed, with an average of 6.24 sessions per patient. There was one case of perforation and one case of mediastinitis, while there was no other complication or mortality. The clinical success rate of EBD therapy was 62.3% (33/53). The mean standard deviation z-score weight-for-age of patients before and after endoscopic dilation was 2.78 (2.41) and 1.18 (1.87), respectively. The *t*-test showed a significant difference between the weights-for-age (z-score) before and after endoscopic dilation. The majority of the patients had raised weight-for-age (z-score) after EBD treatment. **Conclusion** – EBD attained a good clinical success rate and nutritional improvement in children with an esophageal stricture.

**Keywords** – Endoscopic balloon dilation; esophageal stenosis; esophageal stricture; esophagus.

## INTRODUCTION

Esophageal strictures in pediatric patients have several causes such as eosinophilic esophagitis, esophageal atresia, congenital anomalies, gastro-esophageal reflux disease, inflammatory disorders, and caustic ingestion[1-3]. The occurrence of diverse etiologies differs between studies. In developing countries, caustic injuries are more common[4,5]. The rate of esophageal stricture following caustic ingestion was 20% in a Honar et al. study[6]. It is a severe problem that leads to high morbidity associated with high aspiration risk, failure to thrive, and dysphagia[7]. Failure to thrive is the most significant concern of this clinical condition, as leads to an impaired oral intake[8]. This makes the cure of this severe condition critical.

Besides the outdated procedures, surgical revision, and bougienage, presently esophageal dilation with a balloon catheter is progressively utilized as a treatment modality in esophageal strictures[9]. Meanwhile, balloon dilation has been utilized commonly due to its theoretical benefits and safety, numerous studies started to cope with reasonable and long-term outcomes in pediatric patients. Nevertheless, there is no common consent on some parts of the technique, such as the optimal size of the balloon, intervals between dilations, or other methodological details[7]. In pediatric patients, balloon dilation (BD) is related to fewer dilation sessions and complication rates compared with bougienage[10]. In the study by Raboei et al., esophageal balloon dilation had the lower complication rate compared to savary dilations[11].

The objective of esophageal dilation in patients with esophageal stricture is a clinical success or attaining adequate food intake. The majority of data regarding esophageal dilation are considered successful treatment due to the raised time among dilations and raised tolerance of food intake while assessment of nutritional status has seldom been reported. This present study aimed to evaluate the efficacy and long-term consequences of endoscopic balloon dilation (EBD) on the clinical and nutritional outcomes in children with an esophageal stricture.

## METHODS

In this retrospective study, patients (aged less than 18 years) who underwent therapy with EBD for esophageal stricture from April 2015 until March 2020 in Abuzar Children's Hospital (Ahvaz, Iran) were enrolled in the study. Children's data were collected from the medical records. Patients entered the study at least one year after the first dilation. The children undertook barium esophagography and/or endoscopy to recognize the stricture and its site before EBD.

Declared conflict of interest of all authors: none
Disclosure of funding: none
[1] Department of Pediatric Gastroenterology, Abuzar Children's Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [2] Alimentary Tract Research Center, Clinical Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [3] Department of Pediatric Pulmonology, Abuzar Children's Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [4] Department of Epidemiology and Biostatistics, School of public health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Corresponding author: Hazhir Javaherizadeh. E-mail: hazhirja@yahoo.com

The author's contraindications in this study for EBD incorporated recent perforation, malignant illness of the esophagus, recent upper gastrointestinal surgery, severe cardiorespiratory disease, un-correctable coagulopathy, and instability of vital signs. Anticoagulants had to be withdrawn and coagulopathy modified before dilation.

EBD was implemented cautiously on children under general sedation taking midazolam with serious checking of vital signs; at least 6 hours before EBD, the patients had to have fasted.

Pediatric endoscopy was primarily utilized to evaluate stricture. A guidewire was pass through the stricture to assess the patency and size of it. In addition, a balloon catheter was located through the stricture, which was dilated by water inflation to apply linear pressure on the stricture. The precise placing of the balloon was accepted when it displayed a "waist" as it was centered through the stricture. Manual inflation (held for 30 s) was implemented until the waist was eliminated. Each session of balloon dilation was three 30 seconds totally 90 seconds. Endo-flex balloon dilator (Voerde, Germany) was used for the treatment of oesophageal strictures. Pentax endoscope EG-2790k (Tokyo, Japan) was used in our unit for the treatment. The fluoroscopic method was not used in our setting. We used x-ray 6 hour after esophageal dilation to evaluate perforation not identified during procedure. Patients were observed 24 hours after esophageal dilation.

The goal of EBD was to eliminate the stricture by full development of the waist of the balloon or to upsurge the esophageal diameter, thus permitting the endoscope to go over the stricture. The progress of ingestion ability (dysphagia scale) and nutritional status were used to evaluate the therapeutic efficacy of EBD.

The nutritional status of the patients was evaluated before and after EBD. The nutritional parameter measured was weight-for-age (z-score). Weight-for-age below -3 was considered severely underweight. Weight-for-age below -2 was considered underweight. Weight-for-age above 2 was considered overweight. Outcome parameters were the overall dilations, nutritional status, clinical success rates on EBD, and procedural complications. Furthermore, correlations of the site of the esophageal stricture with the clinical success rate were also assessed. Clinical success after EBD treatment was considered as having no requisite for EBD for at least one year or raising interval among EBD procedures and less than four EBD sessions each year. The clinical failure after EBD treatment was considered as a requirement for EBD for above one year or surgical intervention or enteral nutrition by gastrostomy. In addition, an improvement in nutritional status was considered as the attainment of an increase of weight-for-age (z-score).

The analyzed clinical were gender, clinical symptoms at presentation, age at diagnosis, nutritional status (z-score for body weight), management, sites (single or multiple) location (upper, middle, or lower), and procedural complications.

### Statistical analysis

Data analysis was done using SPSS version 25. Descriptive statistics comprising means, range, percentage, and standard deviation were utilized to describe demographic information. Normality of data and homogeneity of variances was assessed by Kolmogorov-Smirnov and Leven test, respectively. Continuous variables were expressed as mean ± standard deviation. Status changes were also analyzed by Student's *t*-test. Pearson's correlation analysis was used to determine the correlation between two groups. The chi-square test and the Wilcoxon test also were used. $P<0.05$ was considered to indicate statistical significance.

### Ethics statement

Ethical approval was given by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, reference number (No: IR.AJUMS.REC.1398.803). All collected data were kept confidential without any imposed extra cost on the parents.

### Limitation

Limited sample size and single-center study. We could not analyze some correlations due to the lack of some contrast studies.

## RESULTS

A total documents of 53 patients with diagnosis of esophageal strictures were reviewed and the following results were obtained. The patients were 25 (47.2%) females and 28 (52.8%) were males. Ages of the patients ranged from 1 to 13 years with a mean age of 4.72±3.38 years. Among patients, 32 (60.4%) had esophageal atresia, 7 (13.2%) had idiopathic esophageal strictures (IES), 5 (9.4%) achalasia, 4 (7.5%) patients with epidermolysis bullosa (EB), 2 (3.8%) patients with gastroesophageal reflux disease (GERD), 2 (3.8%) people suffering from strictures due to ingestion of corrosive substances), and one person with secondary esophageal strictures due to diaphragmatic hernia repair surgery (TABLE 1).

Correlation between aetiologies of esophageal strictures and clinical success of EBD therapy Among patients, 32 (60.4%) had esophageal atresia, 7 (13.2%) had IES, 5 (9.4%) achalasia, 4 (7.5%) patients with EB, 2 (3.8%) patients with GERD, 2 (3.8%) people suffering from strictures due to ingestion of corrosive substances), and one person with secondary esophageal strictures due to diaphragmatic hernia repair surgery (TABLE 2). There was no significant correlation between aetiologies of esophageal strictures and clinical success of EBD therapy among subjects using the chi-square test ($X^2$) ($P=0.749$ and $X^2=3.462$) (TABLE 2).

**TABLE 1.** Frequency of etiologies of esophageal stricture among patients (n=53).

| Etiologies of esophageal stricture | Number of patients(n) | % |
|---|---|---|
| Esophageal atresia | 32 | 60.4% |
| Idiopathic esophageal stenosis | 7 | 13.2% |
| Achalasia | 5 | 9.4% |
| Epidermolysis bullosa | 4 | 7.5% |
| GERD | 2 | 3.8% |
| Ingestion of corrosive substances | 2 | 3.8% |
| Diaphragmatic hernia repair | 1 | 1.9% |
| Total | 53 | 100% |

GERD: gastroesophageal reflux disease.

**TABLE 2.** Frequency of clinical success of endoscopic balloon dilation therapy based on etiologies of esophageal stricture among subjects (n=53).

| Etiologies of esophageal stricture | Clinical success n (%) | Clinical failure n (%) | Total |
|---|---|---|---|
| Esophageal atresia | 18 (56.3%) | 14 (43.8%) | 32 |
| Idiopathic esophageal stenosis | 4 (57.1%) | 3 (42.9%) | 7 |
| Achalasia | 4 (80%) | 1 (20%) | 5 |
| Epidermolysis bullosa | 3 (75%) | 1 (25%) | 4 |
| GERD | 1 (50%) | 1 (50%) | 2 |
| Ingestion of corrosive substances | 1 (50%) | 1 (50%) | 2 |
| Diaphragmatic hernia repair | 1 (50%) | 0 | 1 |

GERD: gastroesophageal reflux disease.

### Comparison of weight-for-age (z-score) before and after endoscopic dilation (TABLE 3)

The mean standard deviation z-score weight-for-age of patients before and after endoscopic dilation was 2.78 (2.41), 1.18 (1.87) respectively. The independent *t*-test showed a significant difference between the weight- for-age (z-score) of patients before and after endoscopic dilation.

Thirty-three (62.3%) children achieved clinical success and 20 (37.7%) patients experienced clinical failure (TABLE 4). In this study, a total of 331 sessions of balloon dilation were performed in 53 patients with esophageal stricture and mean session of EBD was 6.24 per patient. There was one case of perforation and one case of mediastinitis, while there was no other complication or mortality.

### Correlation between clinical success of EBD therapy and site of esophageal stricture

Among the 53 patients, stricture at the upper third of the esophagus was seen in 16 (30.2%) patients, in the middle third in 20 (37.7 %), and in the lower third in 12 (22.6%) patients. 5 (9.4%) patients had stricture at multiple sites (TABLE 5). There was no significant correlation between the site of esophageal strictures and clinical success of EBD therapy among patients using the chi-square test ($X^2$) ($P$=0.341 and $X^2$=3.346) (TABLE 6).

### Correlation between clinical success of EBD therapy and gender of patients

Of 25 female cases, 15 (60%) showed successful EBD therapy. Of 28 male cases, 18 (64.3%) had successful EBD therapy. There was no significant correlation between patients gender and clinical success using the chi-square test ($P$=0.748 and $X^2$=0.103).

**TABLE 4.** Frequency of Clinical success of endoscopic balloon dilation therapy among patients (n=53).

| Clinical achievement | n | % |
|---|---|---|
| Clinical success | 33 | 62.3% |
| Clinical failure | 20 | 37.7% |

**TABLE 5.** Frequency of sites of esophageal stricture among subjects (n=53).

| Site of esophageal stricture | Number of patients(n) | % |
|---|---|---|
| Upper esophagus | 16 | 30.2% |
| Middle esophagus | 20 | 37.7% |
| lower esophagus | 12 | 22.6% |
| Multiple sites | 5 | 9.4% |
| Total | 53 | 100% |

**TABLE 6.** Frequency of clinical success of endoscopic balloon dilation therapy based on the site of esophageal stricture (n=53).

| Site of esophageal stricture | Clinical success n (%) | Clinical failure n (%) | Total |
|---|---|---|---|
| Upper esophagus | 8 (50%) | 8 (50%) | 16 |
| Middle esophagus | 12 (60%) | 8 (40%) | 20 |
| lower esophagus | 10 (83.3%) | 2 (16.7%) | 12 |
| Multiple sites | 3 (60%) | 2 (40%) | 5 |

### Correlation between the clinical success of EBD therapy and first dilation among patients

The chances of clinical success increased by 1.004 times with each year of age using the chi-square test but it was not significant ($P$=0.668).

## DISCUSSION

The findings of this present study revealed that EBD treatment assisted to get a better enhancement in nutritional status (weight-for-age) in the majority of the patients. This study indicates that EBD has a good clinical impact on esophageal stricture for nutritional support. We could not find any significant correlations of mean age, sites of strictures, gender, aetiologies, and sites of the stricture with clinical success of EBD therapy. Among, 53 documents of children which were reviewed, more than half of patients were males and the majority of patients (60.4%) had esophageal atresia followed by 13.2% who had IES.

**TABLE 3.** Comparison of weight for age (z-score) before and after endoscopic dilation among patients.

| Weight for age (z-score) | Mean | Standard deviation | Minimum | Maximum | *P*-value |
|---|---|---|---|---|---|
| Before dilation | -2.87 | 2.41 | -8.38 | 1.60 | <0.001 |
| After dilation | -1.18 | 1.87 | -7.00 | 3.50 | |

Esophageal stricture is a critical problem that decrease the quality of life of patients. It's main and common complication is failure to thrive due to impaired food intake[10]. Chronic malnutrition and underweight was reported among 66.6% of children with esophageal stricture[12]. The *t*-test showed a significant difference between the weight-for-age (z-score) of patients before and after endoscopic dilation. EBD therapy helped to achieve a greater improvement in nutritional status (weight-for-age) in the majority of the patients similar to the study, which was conducted in Taiwan[13]. Underweight was frequent among children with repaired esophageal atresia[14]. In the current study, weight-for-age z score was improved following EBD. In another study among 50 cases with 268 EBD session, weight-for-age z score showed increment in all cases[13]. Nutritional outcome was improved following EBD therapy[11]. The findings of the mentioned studies were similar in terms of improvement in nutritional status following EBD.

The present study found that balloon dilation had a good clinical success rate (62.3%) while in another similar studies in Taiwan[13] the rate was 72% and in Korea 64% of clinical success rate of EBD treatment was in corrosive injury[15] .

Esophageal perforation is a potential serious complication in patients with esophageal strictures[16]. The perforation of EBD in this study was one case 331 EBD session while another study in China[16] reported a rate of esophageal perforation of 1.5% (4 of 260 EBD sessions) in their sessions as well as in another study in Taiwan[13] that the overall perforation happened in 5 (10%) children and 7 of the 268 (2.6%) sessions. In the study by Geng et al., among 43 patients, 168 sessions of esophageal dilation were done. Among 168 sessions, seven esophageal perforations occurred in six patients[17]. As seen above, the rate of esophageal perforation in our study was lower than in the previous study. Etiology of esophageal strictures and endoscopist experiences may affect the frequency of esophageal perforations following EBD.

A total of 16 (30.2%) patients had upper esophageal stricture, 20 (37.7%) patients had middle esophageal stricture, and 12 (22.6%) patients the lower esophageal stricture, and 5 (9.4%) patients had multiple strictures. In contrast, in another study from Taiwan, stricture at the upper, middle, and lower third of the oesophagus were seen in 15 (30%) patients, 11 (22%), 17 (34%) patients, respectively[13] was reported among 50 patients.

A total of 331 sessions of balloon dilation were carried out and the mean session of EBD was 6.24 per patient. The number of EBDs was higher than the mean five sessions per patient in another study which was conducted in Turkey[7]. Earlier studies stated average intervals of 2–4 weeks among dilation sessions to get a complete clinical response[16,18]. The differences in time intervals depend on the initial stricture size, clinical response to the previous session, and stricture resistance to dilation[19]. Currently, there is no agreement on the ideal procedure of endoscopic dilation (time interval between sessions, frequency of sessions, and targeted lumen diameter). The rate of dilation and time interval between sessions depends mainly on the impacts of the earlier dilation session and the degree of symptomatic improvement[19].

In the current study, there was no difference between site of stricture in terms of clinical success. But in another study, lower stricture had the highest clinical success[11]. This difference may be due to different sample size, number of esophageal dilation, and etiology.

In this study, there was no mortality but two cases of complications. Recently, balloon dilation has been implemented frequently in the cure of esophageal strictures, with a high success rate of 76–100%[7]. In the current study, the weight status differed markedly before and after endoscopic dilation. Our findings indicated that EBD therapy has impacts on nutritional support. The only disadvantage is that a balloon is single-use, therefore it is more expensive than a Savary[20]. In the study by Al Sarkhy et al., on 43 children with esophageal stricture, endoscopic is a safe and effective intervention by an experienced hand[21]. In another study by Bawasir et al., the complication of esophageal dilation are not common and open surgery is not frequently required[22]. In the recent retrospective study on 64 children with repaired esophageal atresia, number; diameter; and length of strictures were the most important predictive factors of the effectiveness of EBD[23]. In the study by Osuga et al., in 14 children with esophageal strictures, they found EBD is a safe modality for the treatment of esophageal stricture in children[24]. In the study by Ten Kate et al. they found large variations in management of strictures in the children with esophageal atresia due to lack of current consensus[25].

The current study had some limitations. First, it was a retrospective study with a small sample size and there were differences in the time of beginning the EBD therapy in patients with different underlying illnesses. Second, to evaluate nutritional status, we tried to use z-scores for all however, some patients did not have weight records. Third, we did not consider the height of the children thus body mass index z-score and the weight-for-height index did not evaluate.

## CONCLUSION

In conclusion, endoscopic dilation is a safe technique with low morbidity and mortality when used by an experienced pediatric gastroenterologist. EBD attained a good clinical success rate in paediatric patients with an oesophageal stricture.

## Orcid

Mitra Ahmadi: 0000-0001-8215-9784.
Mohammad Manzari Tavakoli: 0000-0002-8862-6838.
Hazhir Javaherizadeh: 0000-0001-7898-4589.
Mehran Hakimzadeh: 0000-0002-3210-1776.
Mohammadreza Mirkarimi: 0000-0001-7617-1322.
Assad Sharhani: 0000-0001-9277-7447.

## Authors' contribution

Ahmadi M and Manzari-Tavakoli M: conceptualised the study and drafted the initial study protocol. Ahmadi M, Manzari-Tavakoli M, Javaherizadeh H, Hakimzadeh M, Mirkarimi M and Sharhani A: participated in the design of the protocol. All authors critically reviewed the draft of the manuscript and approved the final version.

Ahmadi M, Manzari-Tavakoli M, Javaherizadeh H, Hakimzadeh M, Mirkarimi M , Sharhani A. Eficácia da dilatação do balão endoscópico em pacientes pediátricos iranianos com estenose esofágica. Arq Gastroenterol. 2021;58(4):520-4.

**RESUMO** – **Contexto** – Estenose esofágica (EE) em crianças é um estreitamento intrínseco fixo do esôfago devido a inúmeras etiologias. **Objetivo** – Este estudo teve como objetivo determinar os impactos clínicos e nutricionais da dilatação do balão endoscópico (DBE) em crianças iranianas com restrição esofágica. **Métodos** – Foram inscritos neste estudo retrospectivo, pacientes pediátricos (com idade <18 anos) submetidos a DBE para restrição esofágica de abril de 2015 a março de 2020 no Hospital Infantil de Abuzar (Ahvaz, Irã). Os parâmetros de desfecho foram a frequência de dilatações, o estado nutricional, complicações e taxas de sucesso clínico. A DBE foi usada em crianças com evidência radiológica de estenose esofágica. O estado nutricional foi avaliado pelo peso-por-idade (escore z). O sucesso clínico foi considerado como não necessidade de DBE por um período mínimo de um ano e/ou aumento de intervalo entre dilatações e frequência inferior a quatro vezes por ano. **Resultados** – Foram incluídos 53 casos (média de idade, 4,72±3,38 anos). Eram 25 mulheres (47,2%) e 28 homens (52,8%). Durante o acompanhamento, foram realizadas 331 sessões de DBE, com média de 6,24 sessões por paciente. Houve um caso de perfuração e um caso de mediastinite, enquanto não houve outra complicação ou mortalidade. A taxa de sucesso clínico da terapia de DBE foi de 62,3% (33/53). A média (escore z) peso-para-idade dos pacientes antes e depois da dilatação endoscópica foi de 2,78 (2,41) e 1,18 (1,87), respectivamente. O teste *t* mostrou uma diferença significativa entre os pesos por idade (escore z) antes e depois da dilatação endoscópica. A maioria dos pacientes havia aumentado o peso por idade (escore z) após o tratamento com DBE. **Conclusão** – A DBE atingiu boa taxa de sucesso clínico e melhora nutricional em crianças com restrição esofágica.

**Palavras-chave** – Dilatação endoscópica por balão, estenose esofágica, esôfago.

## REFERENCES

1. Thomson M, Tringali A, Dumonceau J-M, Tavares M, Tabbers MM, Furlano R, et al. Paediatric gastrointestinal endoscopy: European society for paediatric gastroenterology hepatology and nutrition and European society of gastrointestinal endoscopy guidelines. J Pediatr Gastroenterol Nutr. 2017;64:133-53.
2. Pearson EG, Downey EC, Barnhart DC, Scaife ER, Rollins MD, Black RE, et al. Reflux esophageal stricture-a review of 30 years' experience in children. J Pediatr Surg. 2010;45:2356-60.
3. Ghiselli A, Bizzarri B, Ferrari D, Manzali E, Gaiani F, Fornaroli F, et al. Endoscopic dilation in pediatric esophageal strictures: a literature review. Acta Biomed. 2018;89:27-32.
4. Özdemir R, Bayrakci B, Teksam Ö, Yalçin B, Kale G. Thirty-three-year experience on childhood poisoning. Turk J Pediatr. 2012;54:251.
5. Urganci N, Usta M, Kalyoncu D, Demirel E. Corrosive substance ingestion in children. Indian J Pediatr. 2014;81:675-9.
6. Honar N, Haghighat M, Mahmoodi S, Javaherizadeh H, Kalvandi G, Salimi M. Caustic ingestion in children in south of Iran: Retrospective study from Shiraz-Iran. Rev Gastroenterol Peru. 2017;37:22-5.
7. Cakmak M, Boybeyi O, Gollu G, Kucuk G, Bingol-Kologlu M, Yagmurlu A, et al. Endoscopic balloon dilatation of benign esophageal strictures in childhood: a 15-year experience. Dis Esophagus. 2016;29:179-84.
8. Alessia G, Barbara B, Daniela F, Elisabetta M, Federica G, Fabiola F, et al. Endoscopic dilation in pediatric esophageal strictures: a literature review. Acta Biomed. 2018;89:27.
9. Youn BJ, Kim WS, Cheon J-E, Kim W-Y, Shin S-M, Kim I-O, et al. Balloon dilatation for corrosive esophageal strictures in children: radiologic and clinical outcomes. Korean J Radiol. 2010;11:203-10.
10. Chang CF, Kuo S-P, Lin HC, Chuang CC, Tsai T-K, Wu SF, et al. Endoscopic balloon dilatation for esophageal strictures in children younger than 6 years: experience in a medical center. Pediatr Neonatol. 2011;52:196-202.
11. Raboei E, Alabdali A, Sayed MH, Yousef Y, Bawazir O, Alsaggaf A, et al. The Outcome of Pediatric Esophageal Strictures Managed with Endoscopic Balloon Dilation in Saudi Arabia. J Laparoendosc Adv Surg Tech A. 2021;31:210-5.
12. Sag E, Bahadir A, Imamoglu M, Sag S, Reis GP, Erduran E, et al. Acquired noncaustic esophageal strictures in children. Clin Exp Pediatr. 2020;63:447-50.
13. Chang CH, Chao HC, Kong MS, Chen SY, Chen CC, Lai MW. Clinical and nutritional outcome of pediatric esophageal stenosis with endoscopic balloon dilatation. Pediatr Neonatol. 2019;60:141-8.
14. Askarpour S, Peyvasteh M, Dashtyan M, Javaherizadeh H, Ahmadi M, Ali-Samir M. Incidence of malnutrition, esophageal stenosis and respiratory complications among children with repaired esophageal atresia. Arq Bras Cir Dig. 2020;33:e1486.
15. Doo E-Y, Shin J, Kim J, Song H-Y. Oesophageal strictures caused by the ingestion of corrosive agents: effectiveness of balloon dilatation in children. Clin Radiol. 2009;64:265-71.
16. Lan L, Wong K, Lin S, Sprigg A, Clarke S, Johnson P, et al. Endoscopic balloon dilatation of esophageal strictures in infants and children: 17 years' experience and a literature review. J Pediatr Surg. 2003;38:1712-5.
17. Geng LL, Liang CP, Chen PY, Wu Q, Yang M, Li HW, et al. Long-Term Outcomes of Caustic Esophageal Stricture with Endoscopic Balloon Dilatation in Chinese Children. Gastroenterol Res Pract. 2018;2018:8352756.
18. Alshammari J, Quesnel S, Pierrot S, Couloigner V. Endoscopic balloon dilatation of esophageal strictures in children. Int J Pediatr Otorhinolaryngol. 2011;75:1376-9.
19. Dall'Oglio L, Caldaro T, Foschia F, Faraci S, Di Abriola GF, Rea F, et al. Endoscopic management of esophageal stenosis in children: new and traditional treatments. World J Gastrointest Endosc. 2016;8:212.
20. Khanna S, Khanna S. Management of benign oesophageal strictures in children. Indian J Otolaryngol Head Neck Surg. 2008;60:218-22.
21. Al Sarkhy AA, Saeed A, Hamid YH, Al Asmi MM, Altokhais TI, Ullah AA, et al. Efficacy and safety of endoscopic dilatation in the management of esophageal strictures in children. Saudi Med J. 2018;39:787-91.
22. Bawazir O, Almaimani MO. Complications of esophageal strictures dilatation in children. A tertiary-center experience. Saudi Med J. 2020;41:720-5.
23. Dai DL, Zhang CX, Zou YG, Yang QH, Zou Y, Wen FQ. Predictors of outcomes of endoscopic balloon dilatation in strictures after esophageal atresia repair: A retrospective study. World J Gastroenterol. 2020;26:1080-7.
24. Osuga T, Ikura Y, Hasegawa K, Hirano S. Use of endoscopic balloon dilation for benign esophageal stenosis in children: our 11 year experience. Esophagus. 2018;15:47-51.
25. Ten Kate CA, Tambucci R, Vlot J, Spaander MCW, Gottrand F, Wijnen RMH, et al. An international survey on anastomotic stricture management after esophageal atresia repair: considerations and advisory statements. Surg Endosc. 2021;35:3653-61.

ORIGINAL ARTICLE

AG-2021-122
doi.org/10.1590/S0004-2803.202100000-94

# Gastroesophageal reflux disease: a practical approach

Gerson **DOMINGUES**[1] and Joaquim Prado P de **MORAES-FILHO**[2]

Received: 20 May 2021
Accepted: 26 June 2021

**ABSTRACT** – Gastroesophageal reflux disease (GERD) presents typical manifestations such as heartburn and/or regurgitation as well as atypical manifestations such as throat symptoms, laryngitis, hoarseness, chronic cough, asthma, and sleep alterations. There are two phenotypes of the disease: erosive GERD, when erosions are identified by upper digestive endoscopy, and non-erosive GERD, when the esophageal mucosa presents a normal endoscopic aspect. Relevant clinical findings are usually absent in the physical examination, but it should be highlighted that obesity is an important aggravating factor of reflux. The treatment is established based on clinical findings and, according to the clinical situation, on complementary exams such as upper digestive endoscopy. In dubious cases where a precise diagnosis is required, the indicated test is esophageal pHmetry or impedance-pHmetry. Clinical treatment is divided into behavioral/dietary measures and pharmacological measures. Most patients benefit from clinical treatment, but surgical treatment may be indicated in the presence of a larger hiatal hernia and complications of the disease.

**Keywords** – Gastroesophageal reflux disease; diagnosis; treatment.

## INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic condition that develops when reflux of the gastric contents causes troublesome symptoms and/or complications[1]. It is one of the most common diagnoses among outpatients of the gastroenterology clinic[1].

A population-based study covering approximately 14,000 people in 22 cities in different regions identified a prevalence of approximately 12% to 20% in the urban Brazilian population[2].

A very important aspect of the disease is the reduction in the quality of life of the affected individuals. For example, the quality of sleep of the affected individuals might be compromised since nocturnal reflux is relatively frequent, causing awakenings, REM sleep interruptions, and the shortening of the individual's sleep time[3]. In addition to health as a whole, the reduction in quality of life has negative consequences on social activities and labor productivity, with a resulting financial impact (absenteeism, cost of medication, medical consultations, loss of working hours, etc).

Traditionally, when GERD is suspected, an acid-suppressive medication is started, particularly proton pump inhibitors (PPIs). However, up to 40% of these patients treated with PPIs may show incomplete or no response to therapy[4-6].

## METHODS

In order to achieve a comprehensive narrative review, the relevant literature was assessed in Pubmed, Medline, and other sources using the following search terms: heartburn, pyrosis, gastroesophageal reflux, GERD, refluxate, endoscopy of gastroesophageal reflux disease, esophagitis, and esophageal motility.

## 1. Etiopathogenesis and pathophysiology

The process by which gastroesophageal reflux causes GERD consists of a sequence of events that involve the esophagogastric junction (EGJ) and esophageal body, as well as mechanisms of visceral sensitivity regulation mediated by the central and peripheral nervous system. The imbalance between protective and aggressive factors is responsible for the development of GERD.

The main protective factors are the lower esophageal sphincter (LES), saliva, peristalsis, and the angle of esophageal passage through the hiatus (angle of Hiss). The aggressive factors are represented by the transient lower esophageal sphincter relaxations, hypotension of the LES, refluxed gastric acid pH, increased distensibility of the LES, prolonged esophageal clearance, reduced gastric emptying speed, and hiatus hernia[7].

### Anti-reflux barrier

### Esophagogastric junction and angle of Hiss

The EGJ involves the superposition of the lower esophageal sphincter and the diaphragmatic crura (DC) and represents the anti-reflux barrier[7,8], the main defensive factor. The LES maintains a zone of high pressure due to the tone of the intrinsic muscles and the excitatory stimulation of cholinergic neurons[9]. The diaphragmatic crura provides extrinsic compression to the LES, contributing to the resting pressure, with approximately 5 to 10 mmHg[10].

The oblique entry of the esophagus into the stomach creates an acute angle with the great gastric curvature, called Hiss angle, which creates a valve effect that contributes to the competence of the EGJ[8].

Declared conflict of interest: Domingues G: lecture for AstraZeneca, Takeda Advidory Board. Moraes-Filho JP: publication for Reckitt-Benkiser, Takeda Advirory Board.
Disclosure of funding: no funding received
[1] Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil. [2] Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.
Corresponding author: Gerson Domingues. E-mail: gersondomingues62@gmail.com

## 2. Mechanisms that favor gastroesophageal reflux

### a. Transient lower esophageal sphincter relaxations

The main pathophysiological mechanism of GERD is the transient relaxation of the LES (TLESR)[11,12]. The TLESR is mediated by the vagus nerve and strongly influenced by the proximal gastric distension, food, or gas, which precipitate the activation of mechanoceptors adjacent to the cardia. Patients with GERD do not present a higher number of TLESR episodes than control individuals; however, in patients with GERD, TLESRs are more associated with acid reflux[12].

Acid reflux is much more common than non-acid reflux in the etiopathogenesis of GERD: in less than 10% of the patients, the reflux may be weakly acid or even alkaline[13].

The so-called "gastric reflux" contains harmful material that is capable of injuring the esophagus and/or generating symptoms. Esophageal exposure to gastric reflux is therefore the primary determinant of disease severity. The greater or lesser intensity of esophagitis is related to the time of acid exposure, pH of the refluxed gastric content, and resistance of the esophageal mucosa[13].

### b. Hiatal hernia

Hiatal hernia (HH) is defined by the proximal migration of the LES in relation to the DC, which occurs mainly by the weakening or rupture of the phrenoesophageal ligament and results in a more incompetent anti-reflux barrier[14]. The Brazilian Consensus on GERD based on Evidence quotes HH as a risk factor for the persistence of symptoms to clinical treatment[15]. HH with a size larger than 3 cm or more is related to significantly higher levels of acid exposure in the distal esophagus and erosive esophagitis[14]. Patients with HH have more reflux episodes and higher acid exposure than normal patients[15]. Moreover, the gastric content that is retained in the hernial sac (between the LES and the diaphragmatic crura) refluxes when the LES relaxes during swallowing, characterizing the phenomenon of superimposed reflux[16,17].

### c. Acid pocket

After meals, it was observed that the content that refluxes into the esophagus has comparatively more acid than the gastric body and stems from a region near the EGJ where an acid "pouch" is found, resulting from the neoformation of acidic supernatant from the parietal cells in the proximal gastric region[18,19]. The acid pouch volume is larger in patients with GERD than in controls, and larger acid pouches are found in patients with GERD and large HH (>3 cm)[20]. It has been shown that the location of the acid pouch is more important than the volume: supradiaphragmatic pouches result in 74% to 85% of acid reflux during TLESR, while more distal pouches result in 7% to 20% of acid reflux during TLESR[18].

### d. Esophageal clearance

After the reflux reaches the esophagus, the main defense against the refluxed acid content is the mechanical esophageal clearance, done by peristalsis and the chemical clearance, promoted by the alkaline pH of saliva[15,21].

### e. Slow gastric emptying

The rationale for slow gastric emptying rely on the hypothesis that this phenomenon generates gastric distension, which could trigger TLESR episodes[22].

### f. Gastroesophageal valve

Patients with GERD have more obtuse insertion angles than controls, resulting in a higher degree of opening of the EGJ and a higher number of reflux events[15,17].

### g. Impairment of reflux clearance

Under normal conditions, the primary peristalsis induced by swallowing brings down the esophageal bicarbonate-rich saliva, promoting chemical clearance and pH normalization. However, it is important to mention that the chemical clearing of reflux is impaired by the reduced amplitude of peristaltic muscle contraction, as observed in patients with erosive esophagitis compared to normal controls[23]. Moreover, volumetric clearing in the distal esophagus is aided by the action of secondary peristalsis, which is a reflex and is mediated by the stimulation of mechanical receptors that respond to distension of the distal esophageal walls caused by reflux[24].

## 3. Factors that influence symptom perception

There may be a low correlation between the presence of acid reflux and symptoms. This is sometimes observed in patients with more severe GERD symptoms who may have low esophageal acid exposure, while those with mild symptoms often have a high acid exposure in the distal esophagus. This observation suggests that there are other factors besides the acid reflux that influence the perception of symptoms, such as the sensitivity of each individual in the perception of stimuli, which is related to the psycho-emotional aspect. Stress, tension, anxiety, and depression may be factors that influence sensitivity, i.e., the generation of symptoms[18] (visceral hypersensitivity).

### a. Characteristics of reflux

With the implementation of impedance-pHmetry, it was possible to characterize the reflux as to its acidity and composition, as well as to identify the level of its rise, allowing the study of the relationship between these variables and GERD symptoms. The reflux may be classified as acid (pH <4) and non-acid, which is subdivided into mildly acid (pH 4–7) and mildly alkaline (pH >7).

Reflux episodes with pH <4 are capable of generating symptoms, but impedance-pHmetry studies suggest that a certain percentage of patients with symptomatic episodes are associated with non-acid reflux[25]. In this context, a recent meta-analysis drew attention to the importance of non-acidic reflux episodes, although in low incidence, as a cause of symptoms, especially in patients who are non-responsive to proton pump inhibitors (PPIs)[25].

Reflux may contain other harmful constituents besides gastric hydrochloric acid, such as pepsin, trypsin, or bile acids. Studies have shown that biliary reflux and mixed (acid-biliary) reflux account for only 6–9% and 12% of symptomatic reflux, respectively, in patients not using PPIs[26,27].

### b. Contraction of the longitudinal muscle of the esophagus

The presence of acid in the esophagus may also produce symptoms by inducing the spasm of the longitudinal muscles of the esophagus, which is one of the causes of heartburn. This finding suggests that a motor disorder may be the cause of heartburn, associated or not with acid reflux[28].

### c. Integrity of the esophageal mucosa

In patients with erosive esophagitis, a clear breakdown of the squamous epithelium barrier is observed, which allows the reflux components to stimulate nociceptors in the lamina propria, causing symptoms. In patients with non-erosive GERD, it has been suggested that a microscopic damage to the mucosa may be a factor associated with the development of symptoms[29]. A microscopic change in the mucosa to be considered is the presence of dilated intercellular spaces (DIS), which increase the esophageal mucosa permeability. It is worth mentioning, however, that the relationship between the presence of these changes and symptom perception has not been proven[30].

### d. Hypersensitivity

The perception of symptoms in GERD is related) to the increased sensitivity of the esophagus to several stimuli. This visceral hypersensitivity could be a consequence of the positive regulation of peripheral afferent nerve receptors by the acid-induced inflammation[31].

In addition to peripheral sensitization, sensitization of a central nature plays an essential role in esophageal hypersensitivity. The hypothesis is that acid stimulation in the esophagus promotes sensitization of the cingulate and insular cortex, which are part of the limbic system and process and modulate the sensory signals of the gastrointestinal tract. Consequently, there is a reduction in the pain threshold, which starts to occur with non-painful stimuli[32,33].

## DIAGNOSIS

### 1. Clinical presentation

The typical symptoms of GERD are heartburn and regurgitation. atypical manifestations of the disease, which may not be accompanied by any of the typical symptoms, include: chest pain and otorhinolaryngological and pulmonary manifestations such as cough, laryngitis, asthma, hoarseness, hawking, and globus sensation[34]. Chronic cough, chronic laryngitis, and asthma are multifactorial processes that may have reflux as a potential aggravating factor and, therefore, GERD may not be the only cause of these manifestations[35].

Studies have shown that GERD may interfere in the quality of sleep, and heartburn may occur during the sleep period in 25% of the cases[36]. Therefore it is important to note that nighttime heartburn and complaints related to sleep quality are more frequent in patients with GERD and that the treatment of these patients with PPIs may improve sleep quality[37].

The diagnosis based only on the anamnesis and physical examination for patients aged less than 45 years, with typical GERD symptoms, without warning signs (anemia, digestive hemorrhage, weight loss, dysphagia, and odynophagia), and without further investigation, is a conduct recommended by many authors, including the Latin American GERD Consensus[38]. The III Brazilian Consensus on GERD, however, recommended that every patient with suspected or diagnosed GERD should undergo an upper digestive endoscopy (UDE) before the beginning of treatment[9]. This guideline is based on the fact that the UDE is a safe and an easily performed procedure, widely available, and of low cost in our environment, besides excluding eventual diseases that may occur with non-characteristic symptoms.

### 2. Non-erosive reflux disease (NERD)

It is the most frequent form of the disease, defined by the presence of classic or atypical GERD symptoms associated with reflux and the absence of lesions in the endoscopic examination[1]. The patients diagnosed with GERD constitute a heterogeneous group, since a portion of these patients present symptoms related to the esophageal exposure to abnormal acid, while others are symptomatic, presenting symptoms associated with reflux, but with a normal total time of acid exposure, which is called esophageal reflux hypersensitivity[39]. On the other hand, some patients with reflux symptoms neither present evidence of the presence of abnormal acid reflux nor of the association with reflux. It has been suggested as a differential diagnosis that the latter present symptoms as a consequence of inflammation, gastrointestinal motility abnormalities, or visceral hypersensitivity (functional pyrosis)[39,40].

The diagnosis of laryngo-pharyngeal reflux (LFR) is more difficult than in typical GERD because of the lack of definitive diagnostic methodology, since most diagnostic tests for LFR, such as laryngoscopy, UDE, and esophageal pHmetry, have shown low sensitivity in detecting that reflux may be the cause of laryngeal symptoms. Therefore, there is no diagnostic test that unequivocally characterizes any extra-esophageal symptom to GERD[41].

### 3. Erosive reflux disease

It consists of the classic presentation of the disease with the occurrence of suggestive symptoms and the presence of erosions in the UDE, although the exam does not have a high specificity[42]. The most frequently used classification nowadays is the Los Angeles classification[43] (FIGURE 1).

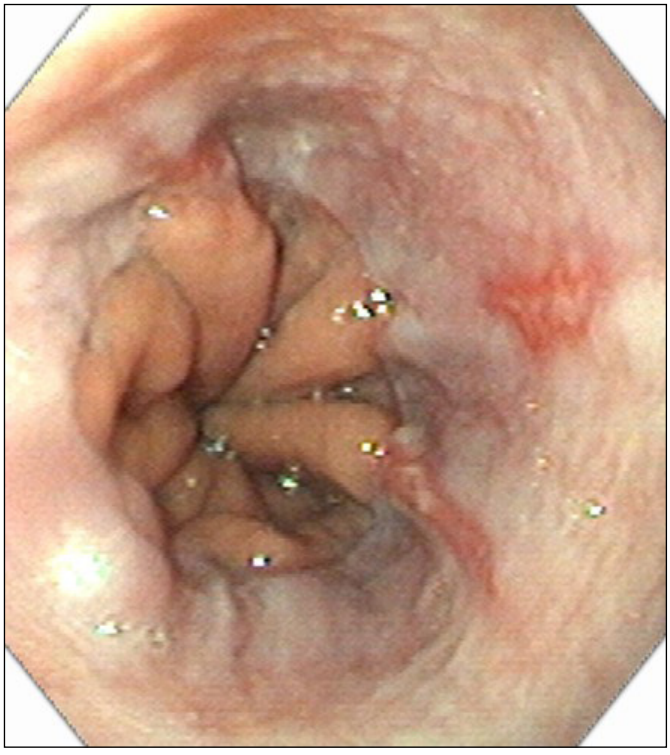


**FIGURE 1.** Erosive GERD. Presence of mucosal erosions. Esophagitis grade A according to the Los Angeles classification[44].

### 4. PPI testing

As above mentioned, the empirical therapeutic test with standard-dose PPIs is satisfactory in some cases, allowing the inference of the GERD diagnosis[45]. The test has, however, limitations due to its low specificity and because there is no standardization regarding the drug to be used, the dose, or the observation time. Due to these considerations, the Brazilian GERD Consensus does not recommend the use of the therapeutic test[9].

### 5. Endoscopic examination and esophageal biopsy

The endoscopic examination is indicated for cases in which it might contribute to the diagnosis of lesions caused by the reflux, such as in the characterization of erosions and enabling the performance of biopsies, which are indispensable for the diagnosis of complications such as Barrett's esophagus, esophageal ulcers, stenosis, and esophageal adenocarcinoma[46,47].

### 6. 24-hour pHmetry or prolonged ambulatory pHmetry

The test evaluates the acidic pH inside the esophagus. It is particularly indicated for patients with the non-erosive form of the disease, for the evaluation of patients refractory to PPIs, and in situations in which the diagnosis of GERD is questionable. It must be remembered that the 24-hour pH measurement is the only test that allows us to verify the association between reflux and the presence of symptoms[46].

Very satisfactory results in the determination of esophageal acid pH have been described with the use of Bravo® capsule (Medtronics Inc., USA), which is an improvement of extended pHmetry. The capsule allows the period of assessment of intra-esophageal pH to be extended from 24 to 48 hours and up to 96 hours, which may represent an increase of approximately 25% in the diagnostic capacity. Extending the monitoring time may be especially useful for patients with infrequent symptoms and to optimize the reflux-symptom association[48,49].

### 7. pH-impedance

It is currently considered the gold standard test for the diagnosis of GERD[50,51]. It allows the identification of the food bolus direction, in anterograde (deglutition) or retrograde (reflux), differentiating between the physical (liquid, gaseous or mixed) and chemical properties of the food bolus, characterizing the reflux episodes in acid and non-acid. The 24-hour monitoring by the impedance-pHmetry system has greater sensitivity than pHmetry alone in the detection of gastroesophageal reflux[40] (FIGURE 2). The indications are the same as those of 24-hour pHmetry.

pH-impedance can be performed with or without PPI therapy. Basically, when the diagnosis of GERD has not yet been established, the study should be better performed without the use of PPIs. On the other hand, the study with the use of PPIs is reserved for patients whose GERD diagnosis has already been established, but there is the persistence of symptoms, or in the evaluation of the treatment in patients with Barrett's esophagus[48].

Recently, it has been sugggested a role to non-acid (or weakly acidic) reflux in the genesis of otorhinolaryngological symptoms, especially cough, hoarseness and globus, in patients being treated with PPIs[25]. In these patients with LFR, especially those in whom there was no response to the test with PPIs or the response was partial, pH-impedance may reveal the association of symptoms with acid and non-acid reflux[48]. New parameters in esophageal pH-impedance analysis were recently introduced: the post-reflux swallow-induced peristaltic wave (PSPW) index and mean nocturnal basal impedance (MNBI)[52,53]. With the use of these new parameters, it was possible to increase the spectrum of GERD diagnosis in patients whose association between reflux and symptoms was not evident during the test[52,53].

PSPW evaluates the effectiveness of esophageal chemical clearance, referring to a swallow that occurs within 30 seconds of a reflux event. The PSPW index is defined as the number of events followed by a PSPW divided by the total number of reflux events, being very useful in the distinction between GERD and functional heartburn. This parameter is an independent predictor of refractoriness to IBP[54]. The percentage value of PSPW is lower in patients with abnormal acid exposure compared to healthy volunteers and patients with functional heartburn[52].

The nocturnal basal impedance of the esophageal mucosa MNBI is a measure of permeability and, therefore, translates the integrity of the esophageal mucosa in the distal esophagus. This parameter is measured during sleep, where we observe a lower number of swallows that can impact the result of the evaluation. MNBI increases the diagnostic range of pHmetry because, like PSPW, it helps in the differential diagnosis between NERD and functional heartburn[54]. A prospective study showed that MNBI has a predictive value for symptomatic response to anti- reflux therapies[55]. Patients with abnormal acid exposure time have lower baseline impedance values in the distal esophagus compared to healthy controls and patients with functional heartburn[45].

### 8. Lyon Consensus

According to the Lyon Consensus[50], the main modifications proposed for the diagnosis of GERD are as follows:

In the endoscopic examination, confirmatory evidence for GERD must be considered: esophagitis grades C and D (Los Angeles classification) and peptic stenosis. Esophagitis grades A and B may be nonspecific, provided that they may occasionally be present in normals. Barrett's epithelium must be confirmed by biopsy.

On pH-impedance, esophageal acid exposure time <4% is normal (physiologic) and >6% abnormal. Intermediate values: inconclusive.

Reflux monitoring (pHmetry/impedance-pHmetry) should be performed without the use of PPIs.

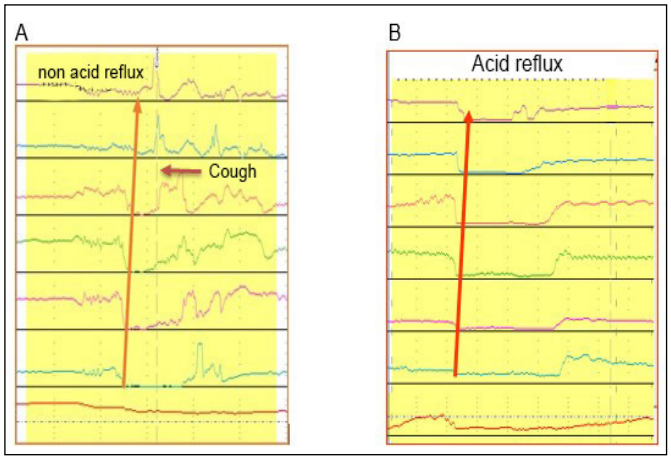



**FIGURE 2.** pH-impedance tracing demonstrating the presence of: A. An episode of non-acid reflux (slightly acidic) associated with a cough episode. B. An episode of acid reflux.

## TREATMENT

The clinical therapy aims to minimize the harmful effects to the esophagus of the refluent material (reflux), whose pH in the absolute majority of times is acidic. It aims, therefore, to relieve symptoms, heal lesions when present, avoid recurrences, and prevent complications. Behavioral, pharmacological, endoscopic, and surgical therapeutic modalities are available for the treatment of GERD. The endoscopic treatment, although quite promising, is still not consensually indicated and therefore, it must not be recommended as an alternative[56].

The ideal therapy should aim at correcting the motor defects of the disease, particularly the TLESR. With this objective, products such as Baclofen, a type B agonist of the gamma-aminobutyric acid (GABA) receptor, showed some satisfactory therapeutic results; however, it presents side effects that limit its use[57].

### 1. Clinical treatment

The great majority of patients benefit from clinical treatment that should encompass behavioral and pharmacological measures.

Apparently, patients with erosive GERD may respond better to treatment than those with the non-erosive form[58]. However, it is worth highlighting that a well conducted meta-analysis showed that, when the diagnosis is accurately established with the inclusion of functional tests, patients with the non-erosive form respond in a similar manner as those with the erosive form[59].

#### a. Behavioral measures

Change in lifestyle is part of the GERD treatment, comprising the so-called behavioral measures, which aim to avoid situations that promote or facilitate gastroesophageal reflux (FIGURE 3).

The reduction in body weight is important. There is evidence of a significant association between symptomatology improvement and body mass index (BMI) reduction in obese patients who have lost at least 2 kg/m$^2$ in BMI[60]. So, the reduction in body weight is important.

| Behavioral measure | Note |
|---|---|
| Elevation of the head of the bed (15 cm). Preference for right lateral decubitus. | Indicated in more severe cases, especially when there is nocturnal reflux. |
| Avoid lying down after meals: wait for 2–3 hours. | Important measure particularly in nocturnal reflux. |
| Body weight reduction in obese people and overweight patients. | Elevated intragastric pressure and fatty infiltration of the esophagogastric junction in these cases are factors that favor gastroesophageal reflux. |
| Avoid: fatty foods, alcohol, chocolate, tomatoes, coffee, tea, carbonated drinks. | Selective elimination, particularly when there is a food/symptom correlation. |

**FIGURE 3.** Behavioral measures in the treatment of GERD[17,69].

No consistent associations in the relationship between GERD and certain diets have been demonstrated, with varying levels of evidence and degrees of recommendation. However, from a practical point of view, instructions regarding the diet such as eating slowly, avoiding liquids at meals, and avoiding foods that notoriously trigger symptoms (such as fatty foods, chocolate, tomatoes, etc.) may be recommended according to the clinical conditions and the patient's acceptance, with satisfactory results.

#### b. Pharmacological treatment

Pharmacologic options are: I) proton pump inhibitors, II) antacids, III) histamine H2 receptor blockers, IV) alginate.

#### I. Proton pump inhibitors (PPIs)

In clinical practice, the treatment of choice are the PPIs, which have high healing rates and are safe and effective drugs. PPIs inhibit acid secretion by inactivating the hydrogen-ATPase molecules (proton pump) of parietal cells (TABLE 1).

**TABLE 1.** Proton pump inhibitors. Daily doses.

| Proton pump inhibitor | Daily standard dose/mg |
|---|---|
| Omeprazole | 40 |
| Lansoprazole | 30 |
| Pantoprazole/pantoprazole | 40 |
| Rabeprazole | 20 |
| Esomeprazole | 40 |
| Dexlansoprazole | 30–60 |

PPIs have been widely used for decades, but the number of recent publications questioning their safety has increased dramatically, causing considerable patient anxiety. Evidence has shown that they are risk-free drugs with relatively rare adverse effects and very low mortality[61,62]. There are certainly cases where there is a risk of vascular complications, regardless of the use of PPIs, such as in elderly patients with diabetes, hypertension, and history of strokes, who often require aspirin for prophylaxis of cardio-cerebrovascular events[63].

International guidelines have indicated that young patients with typical symptoms should first undergo a therapeutic trial with PPIs[64]. The test consists of administering a full dose of PPI for 4 to 8 weeks and observing whether the clinical outcome is satisfactory. However, some restrictions should be remembered because there is no standardization regarding which PPI to use, the dose, and effective observation time, in addition to limitations regarding the diagnostic establishment for this type of conduct: 78% sensitivity and 54% specificity[65]. On the other hand, in young patients with typical complaints and without other comorbidities, the therapeutic test may be the initial option.

PPIs should be prescribed for eight-week treatment periods (although healing can often occur in four weeks) for symptomatic relief and healing of the erosive form of the disease. In general, there are no major differences between the various PPIs, but in certain cases, they may determine different clinical responses. Hence the recommendation that: if the response with one PPI is unsatisfactory, it should be replaced by another[12].

Pharmacological treatment with PPIs should be started with the standard dose once a day, approximately 30–40 minutes before breakfast, except for dexlansoprazole, which can be taken regardless of food and has a longer plasma concentration time[66,67].

There are no major differences between the use of one daily morning dose or two half-doses (morning and before dinner) and, therefore, when there is a predominance of nocturnal symptoms, the PPI (half dose) can be prescribed twice a day[9]. In cases in which there is a partial response to treatment, the double dose of the product should be considered, and eventually the change to another PPI[68].

In patients under treatment with PPIs, the recovery of nocturnal gastric acid secretion characterizes nocturnal acid escape, which leads to the occurrence of symptoms during the night. Acid leak is frequently observed with the most currently used PPIs, whose pharmacokinetic characteristic involve the release of the drug at a single time point when used in a single daily dose in the morning. Although increasing the PPI dose to two daily doses is a common medical procedure, patients may eventually continue to experience symptoms related to nocturnal acid leak[69].

It is important to mention that in up to 40% of patients, the result might not be totally satisfactory due to the perpetuation of symptoms or only partial resolution of symptoms[5]. In these cases, it is relevant to carefully observe patient compliance with the medical prescription, since the level of patient adherence to treatment may be low, impairing the therapeutic outcome[70,71].

Other less potent agents can be used in cases where symptoms are mild or intermittent or as supplemental therapy to PPIs[9]. These are:

#### II. Alkalines (or antacids)

Alkalines (or antacids) are used to neutralize gastric acid secretion, but they have a low neutralization capacity and short duration, which leads to low compliance by patients, serving for immediate relief of symptoms.

#### III. $H_2$ histamine receptor blockers

$H_2$ histamine receptor blockers (cimetidine, nizatidine, ranitidine, and famotidine). They were very important as the first inhibitors of acid secretion since the launch of cimetidine in the late 1970 s. Currently, they correspond to the second line of PPIs. They can eventually be used in the control of nocturnal acid secretion, although the tachyphylaxis that they present after a few days of use is a restrictive factor that must be considered[72].

#### IV. Alginate

Extracted from seaweed, it forms a real barrier on the esophageal mucosa and is associated with sodium bicarbonate and calcium carbonate[73]. The formulation containing alginate, sodium bicarbonate and calcium carbonate relief of symptoms irrespective of the nature of the stimulus (acid, pepsin, bile). They can be administered in association with PPIs, achieving satisfactory therapeutic results[73,74].

### c. Maintenance treatment

GERD is a chronic condition, and the improvement of symptoms observed with the acid suppression, promoted by the full (or possibly doubled) dose of PPIs, may be followed by the return of symptoms when treatment is discontinued, since the pathophysiological defect persists. The return of clinical manifestations may occur particularly in cases of more intense reflux, and in this sense, up to 80% of patients with erosive esophagitis, within 12 months of treatment discontinuation, and after the satisfactory response of the first phase of treatment, have a major or minor relapse of symptoms[15,75].

Thus, at the end of the acute phase of treatment, certain patients require maintenance care: PPIs can be prescribed in half-daily doses or on-demand (administration when the patient presents symptoms)[76]. Interestingly, approximately 20% of patients remain asymptomatic with the use of antacids, or alginate and behavioral measures after standard IBP treatment[17].

The risk of adverse events from long-term PPI use is relatively modest[77], although publications (some of which have been criticized for inadequacies) have reported that these can occur with prolonged PPI use. A well-conducted randomized controlled trial showed no association between adverse events and the use of PPIs administered for three years, with the possible exception of an increased risk of enteric infections[78]. In summary, frail elderly patients on chemotherapy should be more carefully monitored, particularly regarding the dosages of calcium, vitamin B12, and magnesium as well as enteric infections.

## 2. Surgical treatment

Surgical treatment (fundoplication) is indicated in the presence of complications such as stenosis, ulcerations, and hiatal hernias larger than 3 cm, being an option for long-term therapy, or for cases where symptoms are refractory to clinical treatment. In general, however, surgical intervention is not recommended for patients who do not respond satisfactorily to clinical treatment[79].

There is an equivalence between clinical treatment with IBP (93%) and surgical Nissen fundoplication treatment (90%) among patients who have been in remission for three years[79]. Long-term surgical follow-up studies are not yet available, in which case patients should be informed that over time, reintroduction of clinical treatment or reoperation is often necessary[79].

## 3. New therapies

Despite the therapeutic success of PPIs in the pharmacological treatment of GERD, there are still areas with unmet needs such as: severe erosive esophagitis (grades C and D), with possible therapeutic failure in 20–40%, and non-erosive reflux disease, which may have up to 40% of unsatisfactory therapeutic response. In this context, new options of clinical, endoscopic, and surgical therapeutic modalities have been developed, some still in the research area[5].

### a. Pharmacological treatment

#### I. PCABs (potassium competitive acid blockers)

An innovative approach was the development of PCABs, which are reversible proton pump blockers (H+/K+-ATPase) that block the K+ exchange channel, resulting in rapid, competitive, and reversible inhibition of gastric acid secretion. Among the PCABs in clinical use in Brazil, Japan and South Korea, Vonoprazan stands out with important clinical data available[80]. Considering the limitations of the currently available anti-secretory compounds, particularly PPIs, this new class of drugs achieves faster, more potent, and prolonged acid suppression, with the possibility of solving many of the clinical needs not achieved by PPIs[81].

#### II. Mucous membrane protector

Rebamipide is an amino derivative of quinolinone that serves as a mucosal protector by increasing the production of prostaglandin EP4 and epidermal growth factor, with antioxidant properties. It is marketed in south-east Asian countries and is indicated for the treatment of acid-related diseases of the oesophagus, including after endoscopic submucosal resection procedures[82].

### III. Prokinetics

As mentioned above, there is no indication for the use of prokinetics in the treatment of GERD. Recently, however, preliminary studies have shown that acotiamide and prucalopride[83,84] may play a role in the clinical treatment of GERD. Acotiamide[83,84] is indicated for the treatment of functional dyspepsia and prucalopride in the treatment of chronic constipation, and both drugs have shown beneficial effects on esophageal function such as an increase in the EGJ pressure and in the distal esophageal contraction, with an improvement in primary peristalsis, reduction in the number of reflux episodes, decrease in the acid exposure time, and acceleration of gastric emptying[83,85].

### b. Endoscopic therapy

Minimally invasive endoscopic or endoluminal procedures for GERD treatment have been proposed as alternative therapeutic strategies to medical or surgical treatment. Cases with indication for endoscopic procedures include patients with typical GERD symptoms, presence of erosive esophagitis grades A and B (Los Angeles), normal endoscopic examination and abnormal acid exposure, and HH of dimensions <3 cm with partial response to IBP treatment[86].

Of the various endoscopic techniques proposed for the treatment of GERD, we highlight:

(I) Stretta® procedure with radiofrequency. The system applies radiofrequency energy to the LES and cardia. The motor response responsible for the effect depends on the increased thickness of the EGJ consequent to the modulation of local muscles and the decrease in the frequency of transient relaxations of the LES. Meta-analysis involving 28 studies and 2,468 patients indicated that the Stretta procedure significantly improved GERD-related symptoms and decreased the percentage of patients using PPIs[86]. Future long-term follow-up studies are needed.

(II) TIF procedure (transoral incisionless fundoplication). It is an endoscopic technique performed with EsophyX® to create an anterior fundoplication. The procedure is performed under general anesthesia and a 3 to 5 cm long valve with a circumference of 200 to 300 degrees is constructed to prevent gastroesophageal reflux. Studies have characterized the efficacy and safety of the procedure, showing improvement of symptoms, quality of life, and reduction of acid exposure time and the use of PPIs[87]. The durability of the effectiveness of the procedure is a point to be better defined[88]: follow-up studies are needed.

### c. Surgical therapy

Laparoscopic fundoplication is the traditional recommendation for patients who do not wish to use PPIs chronically and eventually do not respond to the doubled dose of daily PPIs. It has an initial success rate of over 90% in centers with good esophageal surgical training[89]. It is worth mentioning, however, that fundoplication is technically difficult to perform and is associated with some adverse effects during the post-procedure, such as the patient's inability to eructate or vomit, and distension sensation present in up to 25% of patients (gas bloat syndrome)[89]. Recurrence is also relatively high with a 10-year average of 10–15%[90].

Recently, LINX® (magnetic sphincter augmentation) was introduced as a surgical alternative for GERD treatment aiming at reducing adverse events observed in the traditional laparoscopic fundoplication[91]. The device consists of interconnected magnets wrapped in a titanium cover forming a ring like a bracelet, positioned circumferentially around the esophagus near the JEG[92]. The procedure is safe and minimally invasive with good results in many centers, with contraindication for patients with allergy to titanium and nickel[93]. Future studies should determine the long-term efficacy.

### Authors' contribution

Domingues G and Moraes-Fiho JPP collected and analyzed data, drafted, critically revised de manuscript and approved the final manuscript draft.

### Orcid

Gerson Domingues: 0000-0003-0431-451X.
Joaquim Prado P de Moraes-Filho: 0000-0003-1280-6047.

Domingues G, Moraes-Filho JPP. Doença do refluxo gastroesofágico: uma abordagem prática. Arq Gastroenterol. 2021;58(4):525-33.

**RESUMO** – A doença do refluxo gastroesofágico (DRGE) apresenta manifestações típicas, pirose e/ou regurgitação, assim como, manifestações atípicas, pigarro, laringite, rouquidão, tosse crônica, asma, alterações do sono. Existem dois fenótipos da doença: a DRGE erosiva, quando são identificadas erosões pela endoscopia digestiva alta (EDA) e a DRGE não-erosiva, com mucosa esofágica de aspecto endoscópico normal. Ao exame físico não costumam ser encontrados achados relevantes, mas a obesidade deve ser destacada como importante fator agravante do refluxo. O tratamento é estabelecido com base nos achados clínicos e, conforme a situação clínica, em exames complementares como a EDA. Nos casos duvidosos onde o diagnóstico preciso se impõe, o exame indicado é a pHmetria esofágica ou a impedância-pHmetria. O tratamento clínico é dividido em medidas comportamentais/dietéticas e medidas farmacológicas. A maioria dos pacientes se beneficia com o tratamento clínico, mas o tratamento cirúrgico pode estar indicado como na presença de hérnia hiatal de maior dimensão e nas complicações da doença.

**Palavras-chave** – Doença do refluxo gastroesofágico; diagnóstico; tratamento.

## REFERENCES


1. Vakil N, Zanten SV, Kahrilas P, Dent J, Jones R, Global Consensus Group. The Montreal Definition and Classification of Gastroesophageal Reflux Disease: a Global Evidence-Based Consensus. Am J Gastroenterol. 2006;101:1900-20.
2. Moraes-Filho JPP, Chinzon D, Eisig E, Hashimoto CL, Zaterka S. Prevalence of heartburn and Gastroesophageal Reflux Disease in the urban Brazilian population. Arq Gastroenterol. 2005;42:122-7.
3. Fass R. Effect of Gastroesophageal Reflux Disease on sleep. J Gastroenterol Hepatol. 2009;25 (Suppl. 1):S41-S44.
4. Bytzer P, Jones R, Vakil N, Junghard O, Lind T, Wernersson B, et al. Limited ability of the proton-pump inhibitor test to identify patients with gastroesophageal reflux disease. Clin Gastroenterol Hepatol. 2012;10:1360-6.
5. Domingues G, Moraes-Filho JPP, Fass R. Refractory Heartburn: A Challenging Problem in Clinical Practice. Dig Dis Sci. 2018;63:577-82.
6. Yadaplati R, Pandolfino JE. Personalized approach in the work-up and management of gastroesophageal reflux disease. Gastrointest Endoscopy Clin N Am. 2020;30:227-38.
7. Mittal RK, Balaban DH, Epstein FH. The esophagogastric junction. N Engl J Med. 1997;336:924-32.
8. Mendes-Filho AM, Moraes-Filho JPP, Nasi A, Eisig JN, Rodrigues TN, Barbutti RC, et al. Influence of exercise testing in Gastroeesophageal Reflux in patients with Gastroesophageal Reflux Disease. Arq Bras Cir Dig. 2014;27:3-8.
9. Moraes-Filho JPP, Navarro-Rodriguez T, Barbuti R, Eisig J, Chinzon D, Bernardo W, et al. Guidelines for the diagnosis and management of Gastroesophageal Reflux Disease: an evidence-based consensus. Arq Gastroenterol. 2010;47:99-115.
10. Katz PO, Gerson LB, Vela MF. Guidelines for the diagnosis and management of Gastroesophageal Reflux Disease. Am J Gastroenterol. 2013;108:308-28.
11. Roman S, Holloway R, Keller J, Herbella F, Zerbib F, Xiao Y, et al. Validation of criteria for the definition of transient lower esophageal sphincter relaxations using high-resolution manometry. Neurogastroenterol Motil. 2017;29(2). doi. 10.1111/nmo.12920.
12. Moraes-Filho JPP. Doença do Refluxo Gastroesofágico. Rev Bras Med. 2007;64:1-8.
13. Tack J, Pandolfino JE. Phathophysiology of Gastroesophageal Reflux Disease. Gastroenterology. 2018;154:277-288.
14. Pandolfino JE, Shi G, Trueworthy B, Kahrilas PJ. Esophagogastric junction opening during relaxation distinguishes nonhernia reflux patients, hernia patients, and normal. Gastroenterology. 2003;125:1018-24.
15. Zachariah RA, Goo T, Lee RH. Mechanism and pathophysiology of Gastroesophageal Reflux Disease. Gastrointest Endoscopy Clin N Am. 2020;30:209-26.
16. van Herwaarden MA, Samsom M, Smout AJ. The role of hiatus hernia in gastro-oesophageal reflux disease. Eur J Gastroenterol Hepatol. 2004;16:831-5.
17. Bredenoord AJ, Pandolfino JE, Smout AJ. Gastro-oesophageal reflux disease. Lancet. 2013;381:1933-42.
18. Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE. The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. Gut. 2010;59:441-51.
19. Pandolfino JE, Zhang Q, Ghosh SK, Post J, Kwiatek M, Kahrilas PJ. Acidity surrounding the squamocolumnar junction in GERD patients: "acid pocket" versus "acid film". Am J Gastroenterol. 2007;102:2633-41.
20. Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KEL. Severe reflux disease is associated with an enlarged unbuffered proximal gastric acid pocket. Gut. 2008;57:292-7.
21. Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. Gastroenterology. 1987;92:130-5.
22. Gourcerol, G. Influence of gastric emptying on gastro-esophageal reflux: a combined pH-impedance study. Neurogastroenterol Motil. 2013;25:800-7.
23. Frazzoni M, Manta R, Mirante VG, Conigliaro R, Frazzoni L, Melotti G. Esophageal chemical clearance is impaired in gastro-esophageal reflux disease-a 24-h impedance-pH monitoring assessment. Neurogastroenterol Motil. 2013;25:399-406.
24. Savarino E, Gemignani L, Pohl D, Zentilin P, Dulbecco P, Assandri L, et al. Oesophageal motility and bolus transit abnormalities increase in parallel with the severity of gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2011;34:476-86.
25. Boeckxstaens GE, Smout A. Systematic review: role of acid, weakly acidic and weakly alkaline reflux in gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2010;32:334-43.
26. Koek GH, Tack J, Sifrim D, Lerut T, Janssens J. The role of acid and duodenal gastroesophageal reflux in symptomatic GERD. Am J Gastroenterol. 2001;96:2033-40.
27. Marshall RE, Anggiansah A, Owen WA, Owen WJ. The relationship between acid and bile reflux and symptoms in gastro-oesophageal reflux disease. Gut. 1997;40:182-7.
28. Pehlivanov N, Liu J, Mittal RK. Sustained esophageal contraction: a motor correlate of heartburn symptom. Am J Physiol Gastrointest Liver Physiol. 2001;281:G743-51.
29. Tobey NA, Gambling TM, Vanegas XC, Carson JL, Orlando RC. Physicochemical basis for dilated intercellular spaces in non-erosive aciddamaged rabbit esophageal epithelium. Dis Esophagus. 2008;21:757-64.
30. Calabrese C, Bortolotti M, Fabbri A, Areni A, Cenacchi G, Scialpi C, et al. Reversibility of GERD ultrastructural alterations and relief of symptoms after omeprazole treatment. Am J Gastroenterol. 2005;100:537-42.
31. Bhat YM, Bielefeldt K. Capsaicin receptor (TRPV1) and non-erosive reflux disease. Eur J Gastroenterol Hepatol. 2006;18:263-70.
32. Ma J, Altomare A, Rieder F, Behar J, Biancani P, Harnett KM. ATP: a mediator for HCl-induced TRPV1 activation in esophageal mucosa. Am J Physiol Gastrointest Liver Physiol. 2011;301:G1075-82.
33. Lawal A, Kern M, Sanjeevi A, Antonik S, Mepani R, Rittmann T, et al. Neurocognitive processing of esophageal central sensitization in the insula and cingulate gyrus. Am J Physiol Gastrointest Liver Physiol. 2008;294:G787-94.
34. Moraes-Filho, JPP, Ceconnello I, Gama-Rodrigues JJ, Castro LP, Maria Henry MA, Meneghelli UG, et al. Brazilian Consensus on Gastroesophageal Reflux Disease: proposals for assessment, classification and management. Am J Gastroenterol. 2002;97:241-8.
35. Smith JA, Woodcock A. Chronic cough. N Engl J Med. 2016;375:1544-51.
36. Fass R, Quan SF, O'Connor GE, Ervin A, Iber C. Predictors of heartburn during sleep in a large prospective cohort study. Chest. 2005;127:1658-66.
37. Chen CL, Robert JJT, Orr WC. Sleep symptoms and gastroesophageal reflux. J Clin Gastroenterol. 2008;42:13-17.
38. Cohen H, Moraes-Filho JPP, Cafferata ML, Tomasso G, Salis G, González O, et al. A Latin-American Evidence Based Consensus on Gastroesophageal Reflux Disease. Europ J Gastroenterol Hepatol. 2006;18:349-68.
39. Aziz Q, Fass R, Gyawalli CP, Miwa H, Pandolfino JE, Zerbib F. Functional esophageal disorders. Gastroenterology. 2016:15;S0016-5085(16)00178-5. doi: 10.1053/j.gastro.2016.02.012.
40. Roman S, Gyawali CP, Savarino E, Yadlapati R, Zerbib F, Wu J, et al. Ambulatory reflux monitoring for diagnosis of gastro-esophageal reflux disease: update of the Porto consensus and recommendations from an international consensus group. Neurogastroenterol Motil. 2017;29:1-15.
41. Moore JM, Vaezi MF. Extraesophageal manifestations of gastroesophageal reflux disease: real or imagined? Curr Opin Gastroenterol. 2010;26:389-94.
42. Kahrilas PJ, Shaheen NJ, Vaezi MF. American Gastroenterological Association medical position statement on the management of gastroesophageal reflux disease. Gastroenterology. 2008;135:1383-91.
43. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut.* 1999;45: 172-180.
44. Yadaplati R, Vaezi MF, Vela MF, Spechler SJ, Shaheen NJ, Richter J, et al. Management options for patients with GERD and persistent symptoms on proton pump inhibitors: recommendations from an expert panel. Am J Gastroenterol. 2018;113:980-6.
45. Strand DS, Kim D, Peura DA. 25 years of proton pump inhibitors: a comprehensive review. Gut and Liver. 2017;11:27-37.
46. Weusten BL, Akkermans LM, van Berge-Henegouwen GP, Smout AJ. Symptom perception in gastroesophageal reflux disease is dependent on spatio temporal reflux characteristics. Gastroenterology. 1995;108:1739-44.
47. Velanovich V, Hollingsworth J, Suresh P, Ben-Menachem T. Relationship of gastroesophageal reflux disease with adenocarcinoma of the distal esophagus and cardia. Dig Surg. 2002;19:349-53.
48. Domingues G, Moraes-Filho JPP, Domingues AGL. Impact of prolonged 48-h wireless capsule esophageal pH monitoring on diagnosis of gastroesophageal reflux disease and evaluation of the relationship between symptoms and reflux episodes. Arq Gastroenterol. 2011;48:24-9.
49. Penagini R, Sweis R, Mauro A, Domingues G, Vales A, Sifrim D. Inconsistency in the diagnosis of functional heartburn: usefulness of prolonged wireless pH monitoring in patients with proton pump inhibitor refractory gastroesophageal reflux disease. J Neurogastroenterol Motil. 2015;21:265-72.
50. Gyawali CP, Kahrilas PJ, Savarino E, Zerbib F, Mion F, Smout AJPM, et al. Modern diagnosis of GERD: the Lyon consensus. Gut. 2018;67:1351-62.
51. Domingues G. Multichannel intraluminal impedance and pH. Arq Gastroenterol. 2016;53:129-37.
52. Wang AM, Wang G, Huang N, Zheng YY, Yang F, Qiu X, et al. Association between laryngopharyngeal reflux disease and autonomic nerve dysfunction. Eur Arch Otorhinolaryngol. 2019;276:2283-7.

53. Herregods TV, Bredenoord AJ, Smout AJ. Pathophysiology of gastroesophageal reflux disease: new understanding in a new era. Neurogastroenterol Motil. 2015;27:1202-13.
54. Lipham JC, Taiganides PA, Louie BE, Ganz RA, DeMeester TR. Safety analysis of first 1000 patients treated with magnetic sphincter augmentation for gastroesophageal reflux disease. Dis Esophagus. 2105;28:305-11.
55. Ganz RA, Edmundowicz SA, Taiganides PA, Lipham JC, C Smith D, Vault KR, et al. Long-term outcomes of patients receiving a magnetic sphincter augmentation device for gastroesophageal reflux. Clin Gastroenterol Hepatol. 2016;14:671-7.
56. Hirano I, Pandolfino JE, Boeckxstaens GE. Functional lumen imaging probe for the management of esophageal disorders: expert review from the clinical practice updates committee of the AGA Institute. Clin Gastroenterol Hepatol. 2017;15:325-34.
57. Scarpignato C, Sloan JA, Wang DH, Hunt RH. Gastrointestinal pharmacology: practical tips for the esophagologist. Ann N Y Acad Sci. 2020;1481:1-18. doi:10.1111/nyas.14447.
58. Scarpignato C. Poor effectiveness of proton pump inhibitors in non-erosive reflux disease: the truth in the end! Neurogastroenterol Mot. 2012;24:697-704.
59. Weijenborg PW, Cremonini F, Smout AJPM, Bredenoord AJ. PPI therapy is equally effective in well-defined non-erosive reflux disease and in reflux esophagitis: a meta-analysis. Neurogastroenterol Motil. 2012;24:747-e350.
60. Park SK, Lee T, Yang HJ, Park JH, Sohn CI, Ryu S, et al. Weight loss and waist reduction is associated in improvement of gastroesopghageal disease reflux symptoms: alongitudinal study of 15,295 subjects undergoing health checkups. Neurogastroenterol Motil. 2017;29(5). doi: 10.1111/nmo. 1309.
61. Scarpignato C, Hongo M, Wu JCY, Lottup C, Lazarescu A, Stein E, Hunt R. Pharmacologic treatment of GERD: Where are we now, and where are we going? Ann NY Acad Sci. 2020;1482:193-212.
62. Baik SH, Fung K-W, McDonald CJ. The mortality risk of Proton Pump Inhibitors in 1.9 Million US Seniors: an extended Cox survival analysis. Clin Gastroenterol Hepatol. 2021:13;S1542-3565(21)00017-3. doi: https://doi.org/10.1016/j.cgh.2021.01.014.
63. Batchelor R, Kumar R, Gilmartin-Thomas JFM, Hopper I, Kemp W, Liew D. Systematic review with meta-analysis: risk of adverse cardiovascular events with proton pump inhibitors independent of clopidrogel. Aliment Pharmacol Ther. 2018;48:780-96.
64. Young A, Kumar MA, Thota PN. GERD: a practical approach. Cleveland Clin J Med. 2020;87:223-30.
65. Numans ME, Lau J, de Wit NJ, Bonis PA. Short-term treatment with proton pump inhibitors as a test for gastroesophageal reflux disease: a meta-analysis of diagnostic test characteristics. Ann Intern Med. 2004;140:518-27.
66. Behm BE, Peura DA. Dexlansoprazole MR for the management of gastroesophageal reflux disease. Expert Rev Gastroenterol Hepatol. 2011;5:439-45.
67. Fass R, Frasier R. The role of dexlansoprazole modified-release in the management of gastroesophageal reflux disease. Therap Adv Gastroenterol. 2017;10:243-51.
68. Katz PO, Gerson LB, Vela MF. Guidelines for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol. 2013;108:308-28.
69. Khan BA, Sodhi JS, Zargar SA, Javid G, Yattoo GN, Shah A, et al. Effect of bed head elevationduring sleep in symptomatic patients of nocturnal gastroesophageal reflux. J Gastroenterol Hepatol. 2012;27:1078-82.
70. Tucker E, Sweis R, Anggiansah A, Wong T, Telakis E, Knowles K, et al. Measurement of esophago-gastric junction cross-sectional area and distensibility by na endolumenal functional lumen imaging probe for the diagnosis of gastro-esophageal reflux disease. Neurogastroenterol Motil. 2013;25:904-10.
71. Dal-Paz K, Moraes-Filho JPP, Navarro-Rodriguez T, Eisig JN, Barbuti R, Quigley EMM. Low levels of adherence with proton pump inhibitor therapy contribute to therapeutic failure in gastroesophageal reflux disease. Esophagus Dis. 2011;107-13.
72. McRorie J, Kirby JA, Miner PB. Histamine2 - receptor antagonists: rapid development of tachyphylaxis with repeated dosing. World J Gastrointest Pharmacol Ther. 2014;5:57-62.
73. Fass R, Chey WD, Zakko SF, Andhivarothai N, Palmer RN, Perez MC, et al. Clinical trial: the effects of the proton pump inhibitor dexlanzoprazole MR on daytime and nighttime heartburn in patients with non-erosive reflux disease. Aliment Pharmacol Ther. 2009;29:1261-72.
74. Leiman DA, Riff BP, Morgan S, et al. Alginate therapy is effective treatment for GERD symptoms: a systematic review and meta-analysis. Esophagus Dis 2017;30:1-9.
75. Brunner G, Athmann C, Schneider A. Long-term, open-label trial: safety and efficacy of continuous maintenance treatment with pantoprazole for up to 15 years in severe acid-peptic disease. Aliment Pharmacol Therap. 2012;36:37-47.
76. Pace F, Tonini M, Pallota S, Molteni P, Porro GB. Systematic review: maintenance of treatment of gastro-oesophageal reflux disease with proton pump inhibitors taken "on demand". Aliment Pharmacol Therap. 2007;26:195-204.
77. Sandhu DS, Fass R. Current trends in the management of gastroesophageal reflux disease. Gut and Liver. 2018;12:7-16.
78. Moayyedi P, Eikelboom JW, Bosch J, Connolly SJ, Dyal L, Shestakovska O, et al. Safety of proton pump inhibitors based on a large, multi-year, randomized trial of patients receiving rivaroxaban or aspirin. Gastroenterology. 2019;157:682-91.
79. Spechler SJ. Refractory gastroesophageal reflux and functional heartburn. Gastrointest Endosc Clin N Am. 2020;30:325-42.
80. Yang X, Li Y, Sun Y, Zhang M, Guo C, Mirza IA, et al. Vonoprazan: a novel and potent alternative in the treatment of acid-related diseases. Dig Dis Sci. 2018;63:302-311.
81. Sugano K. Vonoprazan fumarate, a novel potassium-competitive acid blocker, in the management of gastroesophageal reflux disease: safety and clinical evidence to date. Therap Adv Gastroenterol. 2018;11:1756283X17745776.
82. Araki H, Kato T, Onogi F, Ibuka T, Sugiyama A, Nakanishi T, et al. Combination of proton pump inhibitor and rebamipide, a free radical scavenger, promotes artificial ulcer healing after endoscopic submucosal dissection with dissection size >40 mm. J Clin Biochem Nutr. 2012;51:185-8.
83. Yamashita H, Okada A, Naora K, Hongoh M, Kinoshita Y. Adding acotiamide to gastric acid inhibitors is effective for treating refractory symptoms in patients with non-erosive reflux disease. Dig Dis Sci. 2019;64:823-31.
84. Shibli F, Kitayama Y, Fass R. Novel therapies for gastroesophageal reflux disease: beyond proton pump inhibitors. Curr Gastroenterol Rep. 2020;22:16-28.
85. Kessing BF, Smout AJ, Bennink RJ, Kraaijpoel N, Oors JM, Bredenoord AJ, et al. Prucalopride decreases esophageal acid exposure and accelerates gastric emptying in healthy subjects. Neurogastroenterol Motil. 2014;26:1079-86.
86. Fass R, Cahn F, Scotti DJ, Gregory DA. Systematic review and metaanalysis of controlled and prospective cohort efficacy studies of endoscopic radiofrequency for treatment of gastroesophageal reflux disease. Surg Endosc. 2017;31:4865-82.
87. Huang X, Chen S, Zhao H, Zeng X, Lian J, Tseng Y, et al. Efficacy of transoral incisionless fundoplication (TIF) for the treatment of GERD: a systematic review with meta-analysis. Surg Endosc. 2017;31:1032-44.
88. Trad KS, Barnes WE, Prevou ER, Simoni G, Steffen JA, Shughoury AB, et al. The TEMPO trial at 5 years: transoral fundoplication (TIF 2.0) is safe, durable, and cost-effective. Surg Innov. 2018;25:149-57.
89. Castelijns PSS, Ponten JEH, Vd Poll MCG, Bouvy ND, Smulders JF. Quality of life after Nissen fundoplication in patients with gastroesophageal reflux disease: comparison between long- and short-term follow-up. J Minim Access Surg. 2018;14:213-20.
90. Kessing BF, Broeders JA, Vinke N, Schijven MP, Hazebroek EJ, Broeders IA, et al. Gas-related symptoms after antireflux surgery. Surg Endosc. 2013;27:3739-47.
91. Dunn C, Bildzukewicz N, Lipham J. Magnetic sphincter augmentation for gastroesophageal reflux disease. Gastrointest Endoscopy Clin N Am. 2020;30:325-42.
92. Ganz RA, Peters JH, Horgan S. Esophageal sphincter device for gastroesophageal reflux disease. N Engl J Med. 2013;368:719-27.
93. Dunn C, Bildzukewicz N, Lipham J. Magnetic sphincter augmentation for gastroesophageal reflux disease. Gastrointest Endoscopy Clin N Am. 2020;30:325-42.

ORIGINAL ARTICLE

AG-2021-123
doi.org/10.1590/S0004-2803.202100000-95

# Comparison of non-endoscopic scores for the prediction of outcomes in patients of upper gastrointestinal bleed in an emergency of a tertiary care referral hospital: a prospective cohort study

Anurag **SACHAN**[1], Deba Prasad **DHIBAR**[2], Ashish **BHALLA**[3], Ajay **PRAKASH**[4], Sunil **TANEJA**[5] and Vishal **SHARMA**[6]

Received: 8 June 2021
Accepted: 29 June 2021

**ABSTRACT – Background** – Traditionally peptic ulcer disease was the most common cause of upper gastrointestinal (UGI) bleed but with the changing epidemiology; other etiologies of UGI bleed are emerging. Many scores have been described for predicting outcomes and the need for intervention in UGI bleed but prospective comparison among them is scarce. **Objective** – This study was planned to determine the etiological pattern of UGI bleed and to compare Glasgow Blatchford score, Pre-Endoscopy Rockall score, AIMS65, and Modified Early Warning Score (MEWS) as predictors of outcome. **Methods** – In this prospective cohort study 268 patients of UGI bleed were enrolled and followed up for 8 weeks. Glasgow Blatchford score, Endoscopy Rockall score, AIMS65, and MEWS were calculated for each patient, and the area under the receiver operating characteristic (AUC-ROC) curve for each score was compared. **Results** – The most common etiology for UGI bleed were gastroesophageal varices 150 (63.55%) followed by peptic ulcer disease 29 (12.28%) and mucosal erosive disease 27 (11.44%). Total 38 (15.26%) patients had re-bleed and 71 (28.5%) patients died. Overall, 126 (47%) patients required blood component transfusion, 25 (9.3%) patients required mechanical ventilation and 2 (0.74%) patients required surgical intervention. Glasgow Blatchford score was the best in predicting the need for transfusion (cut off – 10, AUC-ROC= 0.678). Whereas AIMS65 with a score of ≥2 was best in predicting re-bleed (AUC-ROC=0.626) and mortality (AUC-ROC=0.725). **Conclusion** – Gastrointestinal bleed was most commonly of variceal origin at our tertiary referral center in Northern India. AIMS65 was the best & simplest score with a score of ≥2 for predicting re-bleed and mortality.

**Keywords** – Upper gastrointestinal bleed; rebleed; mortality; Glasgow Blatchford score; pre- endoscopy Rockall score; AIMS65; Modified Early Warning Score.

## INTRODUCTION

Upper gastrointestinal (UGI) bleed is a common presentation in a medical emergency. UGI bleed is anatomically defined as any gastrointestinal (GI) bleed originating proximal to the ligament of Treitz[1].

Patients generally present with hematemesis or melena. Incidence and etiology vary from region to region and the level of the health care center, ranging from 48–160 cases per 100,000 adults per year[2]. India has a huge burden of UGI bleed with nearly 4.6% of hospital admissions due to UGI bleed[3]. The etiology of UGI bleed is generally divided into variceal and non-variceal in origin[4]. Non-variceal bleed includes peptic ulcer disease (PUD), erosive disease, esophagitis, Mallory Weiss tears, vascular malformation, and malignancies. Variceal bleed is generally due to esophageal varices but rarely can be due to gastric and even ectopic varices in the duodenum[5]. Most available data suggests PUD as the most common cause of UGI bleed in western countries and variceal bleed constitutes only a minor fraction[4]. Few studies have shown an increasing incidence of variceal bleed in recent times[6,7]. This rising trend may be due to increased alcohol consumption, the rise of chronic viral hepatitis B and C and non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) cases around the globe. The clinical severity of UGI bleed may vary from being insignificant to fatal. Mortality from UGI bleed varies from 2 to 26 % whereas 10–30% have re-bleed[2,8,9]. Many scoring systems have been used to identify high-risk and low-risk patients to predict outcomes and the need for intervention. The scoring systems which are widely used are Glasgow Blatchford Score (GBS), Pre-Endoscopy Rockall score (PRS), AIMS65, and Modified Early Warning Score (MEWS), formulated by using basic clinical data, blood investigations, and endoscopic parameters[10-12]. The higher score predicts the need for intensive care and intervention. But the utility of these scoring systems as a predictor of outcome is still controversial.

Declared conflict of interest of all authors: none
Disclosure of funding: none
[1] Post Graduate Institute of Medical Education and Research, Department of Gastroenterology, Chandigarh, India. [2] Post Graduate Institute of Medical Education and Research, Department of Internal Medicine, Chandigarh, India. [3] Post Graduate Institute of Medical Education and Research, Department of Internal Medicine, Chandigarh, India. [4] Post Graduate Institute of Medical Education and Research, Department of Pharmacology, Chandigarh, India. [5] Post Graduate Institute of Medical Education and Research, Department of Hepatology, Chandigarh, India. [6] Post Graduate Institute of Medical Education and Research, Department of Gastroenterology, Chandigarh, India.
Corresponding author: Deba Prasad Dhibar. E-mail: drdeba_prasad@yahoo.co.in

## METHODS

Study design and study site: this prospective cohort study was conducted at the Postgraduate Institute of medical education and research (PGIMER), a tertiary care center in Chandigarh, Northern India. The study was conducted from December 2017 to December 2018 with the collaboration of the Department of Internal medicine, gastroenterology, and hepatology, after prior approval from the institutional Ethics Committee.

Screening and enrollment: the patients presenting with a history of UGI bleed at the emergency medical outpatients department were screened for enrollment in the study as per inclusion and exclusion criteria after getting informed consent.

### Inclusion and exclusion criteria

Irrespective of gender and with age of ≥18 years, all patients with UGI bleed were included in the study.

Patients with a lower GI bleed and not willing to give consent were excluded from the study.

### Management protocol

Patient presenting with hematemesis, melena or hematochezia were considered as having UGI bleed. A detailed history and clinical characteristics were noted down to rule out other causes such as hemoptysis or lower GI bleed. The study was community-based however the study population consisted more of referral patients than primary presentation as ours is a referral center. Once recruited, a blood sample was drawn for assessing baseline hemogram, coagulation profile, electrolytes, renal function, and liver functions. Ultrasonogram (USG) of the abdomen or Fibroscan was performed when required. Data was collected for each patient in a predesigned proforma. All patients were risk stratified by using the GBS, PRS, AIMS65 and MEWS. All patients were managed using standard emergency protocols. For the management-airway, breathing and circulation were given initial priority. A crystalloid infusion was given as and when required. Blood transfusion was given to maintain the target Hb of 7–9 g/dL or with signs of hemodynamic instability despite fluid resuscitation. All patients were subjected to UGI endoscopy within 12–24 hrs of presentation. All patients with non-variceal UGI bleed received endoscopic therapy according to the European Society of Gastrointestinal Endoscopy (ESGE) guideline 2015, which included the use of dual modalities (injection and mechanical) when feasible. Re-bleed was defined as significant UGI bleed anytime post endoscopy leading to repeat endoscopy, hemodynamic instability, a significant drop in hemoglobin (Hb), or requiring blood transfusions during the follow-up period. In case of a re-bleed, another attempt on endoscopic hemostasis was taken and in cases of failure, surgical intervention was advised. The patients were observed during the hospital course and were followed up to 8 weeks via phone call or OPD basis.

### Outcome assessment

The outcomes were assessed as etiology of UGI bleed, the incidence of re-bleed, need for surgical intervention, blood transfusion, mechanical ventilation, duration of hospital stays (>3 days was taken as a prolonged hospital stay), and mortality within 8 weeks. GBS, PRS, AIMS65, and MEWS were calculated for each patient, and the area under the receiver operating characteristic (AUC-ROC) curve for each score was compared. [Risk factors of each score given in supplementary TABLE 1–4].

**TABLE 1.** Baseline clinical profile of patients.

| Clinical feature (n=268) | Frequency (percentage) |
|---|---|
| Gender | |
| Male | 222 (82.8%) |
| Female | 46 (17.2%) |
| Age group in years | |
| 18–44 | 92 (34.33%) |
| 45–60 | 131 (48.88%) |
| >60 | 45 (16.79%) |
| Presentation | |
| Hematemesis | 127 (47.38%) |
| Melena | 80 (29.85%) |
| Hematemesis and melena | 61 (22.76%) |
| Associated symptoms | |
| Abdominal pain | 103 (38.4%) |
| Syncope | 81 (30.2%) |
| Respiratory difficulty | 50 (18.7%) |
| Altered sensorium | 53 (19.77%) |
| Bleeding from other sites | 13 (4.9%) |
| Comorbidity and risk factor | |
| Chronic liver disease | 172 (64.2%) |
| Diabetes mellitus | 38 (14.2%) |
| Hypertension | 32 (11.9%) |
| Chronic hepatitis C or anti-HCV positive | 19 (7.1%) |
| Long-term NSAID intake | 9 (3.4%) |
| Antiplatelet intake | 9 (3.4%) |
| Cardiovascular disease | 8 (3.0%) |
| Malignancy | 6 (2.2%) |
| Chronic renal disease | 5 (1.9%) |
| Chronic hepatitis B | 3 (1.1%) |
| Cerebrovascular disease | 3 (1.1%) |
| Chronic respiratory illness | 3 (1.1%) |
| HIV positive status | 2 (0.7%) |
| Anticoagulant intake | 1 (0.4%) |
| Clinical findings | |
| Tachycardia (pulse rate ≥100) | 147 (54.85%) |
| Hypotension (systolic BP ≤90) | 88 (32.8%) |
| Hypoxia (SpO2 ≤90) | 28 (10.4%) |
| Pallor | 202 (75.4%) |
| Icterus | 82 (30.6%) |
| Pedal edema | 95 (35.4%) |

NSAID: nonsteroidal anti-inflammatory drug.

**TABLE 2.** Endoscopic etiological distribution and frequency of therapeutic procedures done in study patients.

| Parameter | Frequency (percentage) | Parameter | Frequency (percentage) |
|---|---|---|---|
| Etiology – endoscopic diagnosis | (n=236) | Therapeutic procedure | (n=236) |
| Variceal bleeding (including gastroesophageal and esophageal variceal bleeds) | 150 (63.55%) | Endo-variceal ligation | 115 (48.72%) |
| Peptic ulcer disease, including esophageal, duodenal and gastric ulcer | 29 (12.28%) | Glue injection | 13 (5.50%) |
| Mucosal erosive disease, including esophagitis, gastritis, and duodenitis | 27 (11.44%) | Adrenaline injection | 7 (2.96%) |
| Mallory-Weiss tear | 6 (2.54%) | Hemostatic clip | 4 (1.69%) |
| Gastric antral vascular ectasia | 2 (0.84%) | Multimodal therapy | 7 (2.96%) |
| Diverticulum | 2 (0.84%) | None | 90 (38.13%) |
| Malignancy | 1 (0.42%) | | |
| Arteriovenous malformation | 1 (0.42%) | | |
| Esophageal Web | 1 (0.42%) | | |
| Corrosive ingestion | 1 (0.42%) | | |
| Normal UGI endoscopy | 16 (6.77%) | | |

UGI: upper gastrointestinal.

**TABLE 3.** Table showing AUROC curve, cut off value and sensitivity of all scores at the cut off value for predicting outcomes.

| Parameters | Score(S) | Area | 95%CI | | Cut off values | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| 8–week mortality | GBS | **0.670** | 0.597 | 0.744 | >10 | 77.5% | 48.3% |
| | PRS | **0.605** | 0.530 | 0.681 | >2 | 90.1% | 26% |
| | AIMS65 | **0.725** | 0.656 | 0.794 | >1 | 80.3% | 53.9% |
| | MEWS | 0.593 | 0.512 | 0.675 | >2 | 62.0% | 51.1% |
| Rebleeding | GBS | 0.552 | 0.462 | 0.642 | – | – | – |
| | PRS | 0.517 | 0.418 | 0.616 | – | – | – |
| | AIMS65 | **0.626** | 0.546 | 0.707 | >1 | 78.9% | 48.3% |
| | MEWS | 0.530 | 0.435 | 0.626 | – | – | – |
| >3 days of hospital stay | GBS | 0.553 | 0.448 | 0.659 | – | – | – |
| | PRS | 0.482 | 0.378 | 0.585 | – | – | – |
| | AIMS65 | 0.579 | 0.460 | 0.697 | – | – | – |
| | MEWS | 0.466 | 0.367 | 0.565 | – | – | – |
| Need for blood component transfusion | GBS | **0.678** | 0.612 | 0.743 | >9 | 80.7% | 46.9% |
| | PRS | 0.597 | 0.526 | 0.667 | – | – | – |
| | AIMS65 | **0.643** | 0.574 | 0.711 | >1 | 68.1% | 55.4% |
| | MEWS | 0.532 | 0.460 | 0.604 | – | – | – |
| Need for mechanical ventilation | GBS | **0.746** | 0.656 | 0.837 | >11 | 86.4% | 51.1% |
| | PRS | **0.658** | 0.551 | 0.765 | >3 | 72.7% | 46.7% |
| | AIMS65 | **0.738** | 0.624 | 0.853 | >1 | 81.8% | 46.7% |
| | MEWS | **0.748** | 0.643 | 0.852 | >2 | 86.4% | 50.7% |
| Need for surgical intervention | GBS | **0.681** | 0.589 | 0.773 | >12 | 100% | 59.5% |
| | PRS | 0.451 | 0.061 | 0.842 | – | – | – |
| | AIMS65 | **0.914** | 0.810 | 1.000 | >2 | 100% | 75.5% |
| | MEWS | **0.753** | 0.677 | 0.829 | >3 | 100% | 68.4% |

GBS: Glasgow Blatchford Score; PRS: Pre- Endoscopy Rockall score; MEWS: Modified Early Warning Score; CI: confidence interval. AUC: >0.6 – **significant marked in bold**.

**TABLE** 4. Table showing comparison of AUROC cut off value and sensitivity of all scores at the cut off value for predicting outcomes in variceal and non-variceal bleed patients.

| Parameters | Scores | Variceal bleed | | | Non-variceal bleed | | |
|---|---|---|---|---|---|---|---|
| | | Area | 95%CI | | Area | 95%CI | |
| | | | Lower bound | Upper bound | | Lower bound | Upper bound |
| Blood transfusion | GBS | **0.611** | 0.517 | 0.706 | **0.759** | 0.657 | 0.861 |
| | PRS | **0.615** | 0.521 | 0.708 | 0.527 | 0.397 | 0.657 |
| | AIMS65 | **0.602** | 0.508 | 0.697 | **0.645** | 0.523 | 0.767 |
| | MEWS | 0.472 | 0.374 | 0.569 | 0.569 | 0.440 | 0.697 |
| Need for mechanical ventilation | GBS | **0.716** | 0.498 | 0.935 | **0.619** | 0.506 | 0.731 |
| | PRS | **0.687** | 0.484 | 0.889 | **0.644** | 0.492 | 0.796 |
| | AIMS65 | **0.817** | 0.640 | 0.995 | 0.465 | 0.029 | 0.901 |
| | MEWS | **0.817** | 0.667 | 0.967 | 0.596 | 0.276 | 0.916 |
| Rebleed | GBS | 0.528 | 0.412 | 0.645 | **0.677** | 0.509 | 0.845 |
| | PRS | 0.478 | 0.349 | 0.607 | 0.590 | 0.417 | 0.763 |
| | AIMS65 | **0.618** | 0.510 | 0.726 | **0.742** | 0.615 | 0.870 |
| | MEWS | 0.536 | 0.417 | 0.654 | 0.588 | 0.384 | 0.792 |
| Death | GBS | **0.631** | 0.513 | 0.748 | **0.639** | 0.492 | 0.787 |
| | PRS | 0.589 | 0.475 | 0.704 | **0.626** | 0.487 | 0.765 |
| | AIMS65 | **0.704** | 0.606 | 0.801 | **0.659** | 0.502 | 0.815 |
| | MEWS | 0.578 | 0.459 | 0.697 | 0.505 | 0.354 | 0.656 |
| >3 days of hospital admission | GBS | 0.434 | 0.198 | 0.670 | 0.524 | 0.360 | 0.687 |
| | PRS | 0.373 | 0.154 | 0.592 | 0.391 | 0.119 | 0.663 |
| | AIMS65 | 0.519 | 0.302 | 0.736 | 0.271 | 0.059 | 0.484 |
| | MEWS | 0.593 | 0.357 | 0.829 | 0.365 | 0.111 | 0.620 |

CI: confidence interval. AUC: >0.6 – **significant marked in bold**.

### Statistical analysis

The data was analyzed using SPSS (22.0) after compilation of data in a spreadsheet. Descriptive data distribution was presented with the mean and standard deviation. Categorical data were presented as proportions. Nominal variables were evaluated using either Pearson's $x^2$-test or Fisher's exact test. All the correlations between continuous variables were assessed using Pearson's correlation coefficient and chi-square test to look for significant differences. The difference in the distribution of variables between variceal and nonvariceal bleed subgroups was done using multivariate regression analysis. The *P*-value of less than 0.05 was taken as statistically significant (95%CI). The area under the receiver operating characteristic (AUC-ROC) curve was calculated for the GBS, PRS, AIMS65, and MEWS and the predictive accuracy of each scoring system was measured. Pairwise AUC-ROC comparisons were performed between combinations of two different scoring systems using the nonparametric approach developed by DeLong et al.[13]. The AUC-ROC curve of >0.6 was taken as acceptable and the higher the AUC-ROC curve the better is the predictor of outcome.

## RESULTS

The clinical and demographic data was recorded of total 268 patients (TABLE 1). The mean age of the patients enrolled in the study was 48.49±13.23 years. The maximum number of patients was in the age group 45–60 years and males constituted 82.83% of the patients. The most common comorbid condition was chronic liver disease (CLD) seen in 64.17% of patients followed by diabetes mellitus (14.2%) and hypertension (11.9%). Alcohol (79.06%) was the most common etiology for CLD. The most common presentation was hematemesis (47.38%) followed by melena (29.85%), while 22.76% presented with both (hematemesis and melena). The most common clinical finding was pallor (75.4%) followed by tachycardia (54.85%) (TABLE 1).

### Etiology and endoscopic findings

Out of 268 patients, only 236 could undergo endoscopy for definitive therapy and etiology of UGI bleed due to poor clinical condition or not giving consent for endoscopy. (FIGURE 1) The most common cause of UGI bleed was due to variceal pathology

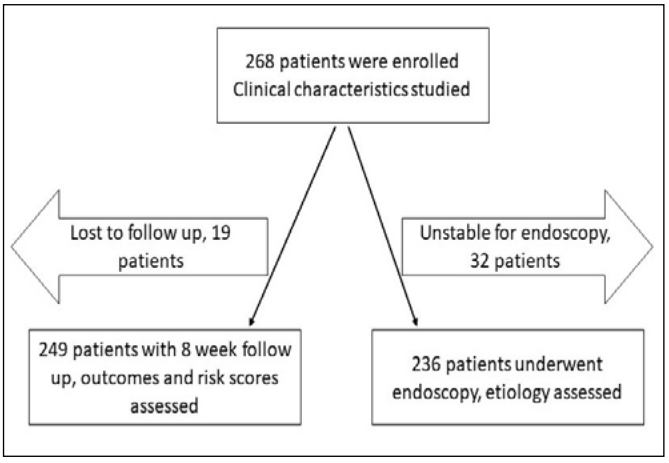



**FIGURE 1.** Flowchart explaining patient distribution while recording results.

seen in 150 (63.55%) patients followed by PUD (12.28%) and mucosal erosive disease (11.44%). Endoscopic interventions were required in 146 (61.86%) of 236 patients who underwent endoscopy. Endoscopic variceal ligation (EVL) was the most common intervention (48.72%) done followed by Glue injection (5.50%) in patients having fundal varices. Multimodal therapy (adrenaline instillation followed by hemostatic clips) was needed in 2.96% of patients (TABLE 2).

### Outcomes

Out of 268 patients, 249 patients completed follow-up. Rebleed occurred in 38 (15.26%) cases out of which 28 cases were of variceal etiology. Out of 249 patients, 126 (50.60%) patients required blood component transfusion and 25 (10.04%) patients required mechanical ventilation and 2 (0.80%) patients required surgical intervention for control of bleed. The mean duration of hospital stay was 3.0±1 day with 35 (14.05%) patients having a hospital stay of >3 days. Out of 249 patients, overall mortality was 71 (28.51%) patients, out of which, 33 (46.5%) patients had variceal bleed, 15 (21.1%) patients had non-variceal bleed and 23 (32.4%) could not undergo endoscopy to establish the etiology of UGI bleed.

### Pre endoscopy scores as a predictor of outcome

Pre-endoscopy Scores (GBS, AIMS65, MEWS & PRS) were calculated for 249 patients who completed follow up. These scores were calculated at initial presentation (TABLE 3). AIMS65 was the best in predicting re-bleed with cut-off value of ≥2 which achieved sensitivity and specificity of 79% and 48% respectively. None of the scores were predictive for duration of hospital stay. GBS and AIMS65 were predictive for the need for blood component transfusion while PRS and MEWS had insignificant AUC-ROC curves. For the need for mechanical ventilation; MEWS, AIMS65 and GBS had good predictive ability. The AUC-ROC for AIMS65, MEWS, and GBS were significant for the need for surgical intervention and was found to be 0.914, 0.753 and 0.681 respectively. For predicting mortality AUC-ROC for AIMS65, GBS, and PRS was significant. Further, we separately compared the AUC-ROC curves for each outcome in variceal and non-variceal patients. AIMS65 was better in both groups in predicting re-bleed and mortality as compared to PRS (TABLE 4).

## DISCUSSION

With the changing epidemiology, the most common etiology for UGI bleed was variceal bleed replacing the PUD in our study. This was consistent with the recent studies done across Northern India where variceal etiology appeared as the predominant etiology[8,14,15]. Studies in Nepal also suggested variceal bleeding to be the predominant cause of UGI bleed followed by peptic ulcer disease[7,16]. Etiological spectrum and clinical data were compared with regional studies across India and results were similar to the studies done in tertiary care centers in Northern, Southern and Western India, whereas peptic ulcer disease was more commonly seen in Eastern India[3,8,14,15,17,18].

Various studies in the South Asian regions have shown a higher incidence of variceal bleed in males as compared to females which were consistent with our study where 66.83% of males had variceal bleed whereas non-variceal etiologies were predominant (52.5%) in the female population[7,8,19]. This could be due to higher alcohol consumption in males and increased incidence of CLD. As expected, variceal bleed was the commonest etiology of UGI bleed in CLD patients, however, it was also important to look for nonvariceal causes. We found that 10.96% of our CLD patients had a nonvariceal bleed. It is important because mortality is higher in patients having CLD with non-variceal bleed than patients without CLD[20]. Endoscopic band ligation was the most common therapeutic procedure done due to esophageal varices being the commonest cause of UGI bleed.

The need for mechanical ventilation occurred in 9.3% of patients which was lower than the previous study[21]. Our study showed mortality (28.51%) on the higher side when compared with other similar studies (2.6–33.5%) done across the world[7,17,19,22,23]. This could be attributed to a higher number of variceal bleeds in our study population as compared to the Western population and because of CLD related complications. Re-bleed was seen in 15.26% of patients which was comparable to the Western as well as the Indian studies[8,22,23].

Risk stratification is an important strategy for the management of patients with UGI bleed regarding in-patient or out-patient care, the need for intervention and early discharge[24,25]. As the epidemiology of UGI bleed varies from region to region**,** a well-validated scoring system needs to be in place for a regional set up. Commonly used validated scoring systems for predicting patient outcomes are GBS, AIMS65, PRS and MEWS[10,21,26,27].

In our study population, AIMS65 was the best in predicting mortality as seen in the previous studies[21,26]. The cut-off value was ≥2, whereas it was ≥3 in other studies[21,26]. GBS and PRS had higher cut-off of ≥14 and ≥3 respectively in studies by Nagaraja et al. and Bozkurt et al. respectively as in comparison to our study having a cut-off of 11[10,28]. AIMS65 was the only predictor for rebleed with a score of ≥2 whereas Robertson et al. kept a cut-off of ≥3. For GBS as a predictor of rebleed, there is conflicting data with a cut-off from 1–13[29,30]. Bozkurt et al. demonstrated the utility of PRS at a cut-off value of four. However, PRS was not effective in our study for predicting rebleed and mortality but gained significance when analyzed separately for the non-variceal population for predicting mortality. Another study from our center showed that PRS works better in non-variceal bleed in predicting outcomes and is similar to our findings[16]. In another large retrospective study done only in variceal bleed patients, AIMS65 was shown to have predictive ability (AUROC >0.8) in predicting mortality but not rebleed[31]. In our

study population, none of the scores were predictive of prolonged hospital stay of >3 days which was similar to another study[21]. GBS was the best in predicting the need for blood transfusion as seen by Robertson et al. and Goncalves et al.[21,27]. The cut-off score for the need for blood transfusion was found ≥10 which was consistent with Robertson et al.[21]. AIMS65 with a cut-off of two was also predictive of the need for blood transfusion in our study, which was similar to Robertson et al.[21]. MEWS was the best in predicting the need for Mechanical Ventilation with a cut-off value of ≥3. To the best of our knowledge, this was the first study comparing MEWS with the need for mechanical ventilation. GBS and AIMS65 were also fairly predictive for need intensive care unit admission with cut off at 12 and 2 respectively. AIMS65 followed by MEWS were predictive for the need for surgical intervention at a cutoff score of ≥3 and ≥4 respectively whereas Goncalves et al. have reported GBS as the only score predictive for surgical intervention[27]. However, patients undergoing surgical intervention were very few (only two); hence predictive ability may not be clinically acceptable.

A single score could not predict all outcomes. AIMS65 emerged as a simple score that was able to predict interventions such as the requirement of blood transfusion and surgery along with outcome variables of rebleed and mortality. AIMS65 with a cut-off of 2–3 can be routinely used in emergency for the need of intensive management (TABLE 5).

This study was conducted in a tertiary care institute in Northern India which caters to a diverse population from Jammu and Kashmir, Punjab, Haryana, Uttarakhand, Uttar Pradesh, Rajasthan, and Bihar. The sample population is small and might not have been representative of the whole Indian population. A multicenter multiregional study with a larger sample should be conducted for a better evaluation of the complete epidemiology of UGI bleed in our nation.

## CONCLUSION

With changing epidemiology, variceal etiology for UGI bleed has become the predominant diagnosis replacing peptic ulcer disease in Northern India and many other Southeastern Asian regions. Rebleed and mortality were more commonly seen in variceal bleed patients as compared to non-variceal bleed patients. Pre-endoscopy Rockall score was not effective in predicting outcomes in variceal bleed patients. AIMS65 was the best & simplest score for predicting mortality and re-bleed in UGI bleed patients.

**TABLE 5.** Table showing the best score for predicting each outcome according to AUROC and their respective cutoffs from the curve.

| Outcome | Score best predictor | Auroc | Sensitivity/ specificity | Cut off | Range |
|---|---|---|---|---|---|
| Need for blood component transfusion | GBS | 0.678 | 80.7/46.9 | ≥10 | 0–23 |
| Need for mechanical ventilation | MEWS | 0.748 | 86.4/50.7 | ≥3 | 0–14 |
| Need for surgical intervention | AIMS65 | 0.914 | 100/25.5 | ≥3 | 0–5 |
| Rebleed | AIMS65 | 0.626 | 78.9/21.1 | ≥2 | 0–5 |
| Mortality | AIMS65 | 0.725 | 80.3/53.9 | ≥2 | 0–5 |
| >3 days hospital stay | None | | | | |

GBS: Glasgow Blatchford Score; MEWS: Modified Early Warning Score.

## Authors' contribution

Sachan A, Dhibar DP and Sharma V: were involved in the drafting of the manuscript. Prakash A: helped with the statistical analysis. Taneja S and Bhalla A: reviewed the manuscript.

## Orcid

Anurag Sachan: 0000-0003-1701-6829.
Deba Prasad Dhibar: 0000-0002-0201-0160.
Ashish Bhalla: 0000-0001-5210-1012.
Ajay Prakash: 0000-0002-3487-8482.
Sunil Taneja: 0000-0003-3901-6969.
Vishal Sharma: 0000-0003-2472-3409.

Sachan A, Dhibar DP, Bhalla A, Prakash A, Taneja S, Sharma V. Comparação dos escores não endoscópicos para a previsão de resultados em doentes com sangramento gastrointestinal alto na emergência de um hospital de referência em cuidados terciários: um estudo de coorte prospectivo. Arq Gastroenterol. 2021;58(4):534-40.

**RESUMO – Contexto** – Tradicionalmente, a doença úlcera péptica era a causa mais comum de sangramento digestivo alto, mas com a mudança da epidemiologia, outras etiologias do sangramento do trato digestivo alto estão emergindo. Muitas pontuações têm sido descritas para prever resultados e a necessidade de intervenção na hemorragia gastrointestinal superior, mas a comparação prospectiva entre elas é escassa. **Objetivo** – Este estudo foi planeado para determinar o padrão etiológico de pacientes com hemorragia digestiva alta e comparar os escores de Glasgow Blatchford, o Rockall pré-endoscopia, o AIMS65 e o Early Warning modificado (MEWS) como preditores do resultado. **Métodos** – Neste estudo prospetivo de coorte, 268 pacientes com sangramento digestivo alto foram acompanhados durante 8 semanas. Os escores Glasgow Blatchford, Rockall pré-endoscopia, AIMS65 e MEWS foram calculados para cada paciente, e a área sob a curva (AUC-ROC) para cada pontuação foi comparada. **Resultados** – A etiologia mais comum para a hemorragia gastrointestinal alta foi varizes gastroesofágicas 150 (63,55%), seguida de úlcera péptica 29 (12,28%) e de doença erosiva de mucosa 27 (11,44%). No total, 38 (15,26%) doentes voltaram a sangrar e 71 (28,5%) doentes morreram. No total, 126 (47%) doentes necessitaram de transfusão de componentes sanguíneos, 25 (9,3%) necessitaram de ventilação mecânica e 2 (0,74%) destes doentes necessitaram de intervenção cirúrgica. O escore de Glasgow Blatchford foi o melhor na previsão da necessidade de transfusão (corte – 10, AUC-ROC =0,678). Enquanto o AIMS65 com uma pontuação de ≥2 foi o melhor na previsão de ressangramento (AUC-ROC =0,626) e mortalidade (AUC-ROC =0,725). **Conclusão** – O sangramento gastrointestinal alto mais comum é de origem varicosa em centro de referência terciária. O AIMS65 é o melhor escore simples, com uma pontuação de ≥2 para prever o ressangramento e a mortalidade.

**Palavras-chave** – Sangramento digestivo alto; ressangramento; mortalidade; escore de Glasgow Blatchford, escore Rockall pré-endoscopia; AIMS65; *Modified Early Warning Score*.

## REFERENCES


1. Khamaysi I, Gralnek IM. Acute upper gastrointestinal bleeding (UGIB) – Initial evaluation and management. Best Pract Res Clin Gastroenterol. 2013;27:633-8.
2. Rotondano G. Epidemiology and Diagnosis of Acute Nonvariceal Upper Gastrointestinal Bleeding. Gastroenterol Clin North Am. 2014;43:643-63.
3. Singh SP, Panigrahi MK. Spectrum of upper gastrointestinal hemorrhage in coastal Odisha. Trop Gastroenterol. 2013;34:14-7.
4. Jairath V, Desborough MJR. Modern-day management of upper gastrointestinal haemorrhage. Transfus Med. 2015;25:351-7.
5. Parikh K, Ali MA, Wong RCK. Unusual Causes of Upper Gastrointestinal Bleeding. Gastrointest Endosc Clin N Am. 2015;25:583-605.
6. Dursun M, Yilmaz S, Yükselen V, Canoruç F, Tuzcu A. Analysis of 1242 cases with upper gastrointestinal system bleeding in Southeastern Turkey: a different etiologic spectrum. Hepatogastroenterology. 2005;52:1456-8.
7. Shrestha UK, Sapkota S. Etiology and Adverse Outcome Predictors of Upper Gastrointestinal Bleeding in 589 Patients in Nepal. Dig Dis Sci. 2014;59: 814-22.
8. Mahajan P, Chandail VS. Etiological and Endoscopic Profile of Middle Aged and Elderly Patients with Upper Gastrointestinal Bleeding in a Tertiary Care Hospital in North India: A Retrospective Analysis. J Midlife Health. 2017;8:137-41.
9. Stanley AJ, Dalton HR, Blatchford O, Ashley D, Mowat C, Cahill A, et al. Multicentre comparison of the Glasgow Blatchford and Rockall Scores in the prediction of clinical end-points after upper gastrointestinal haemorrhage. Aliment Pharmacol Ther. 2011;34:470-5.
10. Bozkurt S, Köse A, Arslan ED, Erdoğan S, Üçbilek E, Çevik İ, et al. Validity of modified early warning, Glasgow Blatchford, and pre-endoscopic Rockall scores in predicting prognosis of patients presenting to emergency department with upper gastrointestinal bleeding. Scand J Trauma Resusc Emerg Med. 2015;23:109.
11. Rockall TA, Logan RF, Devlin HB NT. Selection of patients for early discharge or outpatient care after acute upper gastrointestinal haemorrhage. National Audit of Acute Upper Gastrointestinal Haemorrhage. Lancet (London, England). 1996;347:1138-40.
12. Blatchford O, Murray WR, Blatchford M. A risk score to predict need for treatment for upper-gastrointestinal haemorrhage. Lancet (London, England). 2000;356:1318-21.
13. DeLong ER , DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44:837-45.
14. Parvez MN, Goenka M, Tiwari I, Goenka U. Spectrum of upper gastrointestinal bleed: An experience from Eastern India. J Dig Endosc. 2016;7:55.
15. Anand D, Gupta R, Dhar MAV. Clinical and endoscopic profile of patients with upper gastrointestinal bleeding at tertiary care center of North India. J Dig Endosc. 2014;139-43.
16. Sharma V, Jeyaraman P, Rana SS, Gupta R, Malhotra, S,Bhalla A, et al. Utility of clinical and complete Rockall score in Indian patients with upper gastrointestinal bleeding. Trop Gastroenterol. 2016;37:276-82.
17. Shyamsundar CM, Sharma GD, Rana BS. Profile of acute upper gastro intestinal bleed: a referral hospital-based study in sub Himalayan region. Int J Adv Med. 2018;5:849-53. doi.org/10.18203/2349-3933.ijam20182994.
18. Kashyap R, Mahajan S, Sharma B, Jaret P, Patial RK, Rana SP, LS. A Clinical Profile of Acute Upper Gastrointestinal Bleeding at Moderate Altitude. JIACM. 2005;224-8.
19. Gado AS, Ebeid BA, Abdelmohsen AM, Axon AT. Clinical outcome of acute upper gastrointestinal hemorrhage among patients admitted to a government hospital in Egypt. Saudi J Gastroenterol. 2012;18:34-9.
20. Leontiadis GI, Molloy-Bland M, Moayyedi P, Howden CW. Effect of comorbidity on mortality in patients with peptic ulcer bleeding: systematic review and meta-analysis. Am J Gastroenterol. 2013;108:331-45
21. Robertson M, Majumdar A, Boyapati R, Chung W, Worland T, Terbah R, et al. Risk stratification in acute upper GI bleeding: comparison of the AIMS65 score with the Glasgow-Blatchford and Rockall scoring systems. Gastrointest Endosc. 2016;83:1151-60.
22. Rathi P, Abraham P, Rajeev Jakareddy PN. Spectrum of upper gastrointestinal bleeding in Western India. Indian J Gastroenterol. 2001;20 (Suppl 2) (A37).
23. Venkatesh PGK, Parasa S, Njei B, Sanaka MR, Navaneethan U. Increased mortality with peptic ulcer bleeding in patients with both compensated and decompensated cirrhosis. Gastrointest Endosc. 2014;79:605-14.e3.
24. Chalasani N, Kahi C, Francois F, Pinto A, Marathe A, Bini EJ, et al. Improved patient survival after acute variceal bleeding: a multicenter, cohort study. Am J Gastroenterol. 2003;98:653-9.
25. Vreeburg EM, Snel P, de Bruijne JW, Bartelsman JF, Rauws EA, Tytgat GN. Acute upper gastrointestinal bleeding in the Amsterdam area: incidence, diagnosis, and clinical outcome. Am J Gastroenterol. 1997;92:236-43.
26. Stanley AJ, Laine L, Dalton HR, Ngu JH, Schultz M, Abazi R, et al. Comparison of risk scoring systems for patients presenting with upper gastrointestinal bleeding: international multicentre prospective study. BMJ. 2017;356:i6432.
27. Cúrdia Gonçalves T, Barbosa M, Xavier S, Boal Carvalho P, Firmino Machado J, Magalhães J, et al. Optimizing the Risk Assessment in Upper Gastrointestinal Bleeding: Comparison of 5 Scores Predicting 7 Outcomes. GE Port J Gastroenterol. 2018;25:299-307.
28. Nagaraja BS, Vinay K, Rao AK, Umesh KJ, Prashant BC. Comparison of prediction of outcomes in upper GI bleed using non-endoscopic scoring systems. Int J Adv Med. 2018;5:838-44.
29. Anchu AC, Mohsina S, Sureshkumar S, Mahalakshmy T, Kate V. External validation of scoring systems in risk stratification of upper gastrointestinal bleeding. Indian J Gastroenterol. 2017;36:105-12.
30. Wang CH, Chen YW, Young YR, Yang CJ, Chen IC. A prospective comparison of 3 scoring systems in upper gastrointestinal bleeding. Am J Emerg Med. 2013;31:775-8.
31. Tantai XX, Liu N, Yang LB, Wei ZC, Xiao CL, Song YH, Wang JH. Prognostic value of risk scoring systems for cirrhotic patients with variceal bleeding. World Journal of Gastroenterology. 2019;25:6668.

ORIGINAL ARTICLE

AG-2021-141
doi.org/10.1590/S0004-2803.202100000-96

# Health-related quality of life in adolescents and young adults with inflammatory bowel disease is associated with reduction in school and work productivity rather than physical impairment: a multidisciplinary study

Jane **OBA**[1], Carlos W **SOBRADO**[2,3], Aderson O M C **DAMIÃO**[3], Matheus **AZEVEDO**[3], Alexandre **CARLOS**[3], Natália **QUEIROZ**[2], Claudio A **LEN**[4], Ricardo K **TOMA**[1], Mariana **DEBONI**[1], Marcos J **OZAKI**[1], Flair José **CARRILHO**[3], Sergio **NAHAS**[2] and Clovis A **SILVA**[5]

Received: 8 July 2021
Accepted: 13 July 2021

**ABSTRACT – Background –** Inflammatory bowel diseases (IBD), comprising Crohn's disease and ulcerative colitis, are chronic inflammatory diseases of the gastrointestinal tract that often have their onset among adolescents and young adults (AYA). IBD are characterized by episodes of active disease interspersed with periods of remission, and its activity is inversely correlated with health-related quality of life (HRQL). **Objective –** This study aimed to determine whether AYA in remission or with low IBD activity would exhibit HRQL similar to that of age-matched healthy individuals, and whether demographic and disease factors could affect HRQL using a 'patient-reported outcome' instrument. **Methods –** This study enrolled only AYA with IBD, with low activity. This research included five multidisciplinary clinics of two academic hospitals: Paediatric Gastroenterology, Gastroenterology, Coloproctology, Paediatric Rheumatology and Adolescent divisions, São Paulo, Brazil. A total of 59 AYA with IBD (age, 13–25 years) and 60 healthy AYA (age, 13–25 years) completed the Pediatric Quality of Life Inventory 4.0 and 36-Item Short-Form Health Survey questionnaires and the visual analogue scale (VAS) for pain. Demographic data, extra-intestinal manifestations, treatment, and outcomes regarding CD and UC were evaluated. **Results –** AYA with IBD and healthy controls were similar with respect to median ages (18.63 [13.14–25.80] years vs 20.5 [13.68–25.84] years, *P*=0.598), proportion of female sex (42% vs 38%, *P*=0.654), and percentage of upper middle/middle Brazilian socioeconomic classes (94% vs 97%, *P*=0.596). The school/work score was significantly lower in AYA with IBD than in healthy controls (70 [10–100] vs 75 [5–100], *P*=0.037). The 'general health-perception' score was significantly lower in AYA with IBD than in healthy controls (50 [10–80] vs 0 [25–90], *P*=0.0002). The median VAS, FACES pain rating scale, and total VAS scores were similar between the two groups (2 [0–10] vs 3 [0–9], *P*=0.214). No association between HRQL and clinical and demographic parameters was identified among IBD patients. **Conclusion –** AYA with low IBD activity reported poor HRQL in school/work and general health perception domains, which highlights a disability criterion in this vulnerable population.

**Keywords –** Inflammatory bowel diseases; Crohn's disease; colitis; ulcerative; health-related quality of life; quality of life; disability; adolescent; young adult.

## INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), represents a collection of chronic idiopathic conditions of the gastrointestinal tract that are most often diagnosed in adolescents and young adults (AYA)[1]. IBD is characterised by episodes of remission and active disease that adversely affect health-related quality of life (HRQL) in AYA[2]. Disease activity is strongly and inversely correlated with HRQL; nonetheless, this important issue is rarely assessed in AYA as an endpoint in clinical trials[3].

The most common signs and symptoms of IBD include rectal bleeding, diarrhoea, and particularly abdominal pain, which are recognised as important causes of poor HRQL[4,5]. Furthermore, IBD in adolescents is often more extensive and dynamic in progression than the adult-onset disease, leading to growth retardation, delayed puberty, weight loss, and surgical resections[1]. All of these have an adverse impact on the physical, psychosocial, and body image aspects of young adults, thereby worsening their HRQL[1,6,7].

The International Organization for the Study of Inflammatory Bowel Diseases has recently issued guidelines on 'threat-to-target' strategies (STRIDE-II) that incorporated paediatric and adults

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Faculdade de Medicina da Universidade de São Paulo, Gastroenterologia Pediátrica, São Paulo, SP, Brasil. [2] Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Disciplina de Coloproctologia, São Paulo, SP, Brasil. [3] Faculdade de Medicina da Universidade de São Paulo, Divisão de Gastroenterologia e Hepatologia Clínica, São Paulo, SP, Brasil. [4] Universidade Federal de São Paulo, Reumatologia Pediátrica, São Paulo, SP, Brasil. [5] Universidade de São Paulo, Instituto da Criança e do Adolescente, São Paulo, SP, Brasil.
Corresponding author: Clovis Silva. E-mail: clovis.silva@hc.fm.usp.br

patients[8]. In addition to clinical remission and endoscopic healing, the proposed long-term treatment targets encompass normalization of HRQL, absence of disability, and normal growth in adolescents. In this setting, these new targets are very appropriate to AYA with IBD (age, 13–25 years) because the transition period to adulthood is characterised by a highly unstable period and they often struggle in coping with a lifelong chronic disease[8]. Furthermore, adolescents exhibit more similarities in their HRQL judgments to young adults than to children[9], and young adults show more resemblance to adolescents than to older groups[10].

The World Health Organization defines HRQL as 'those aspects of self-perceived well-being that are related to or affected by the presence of disease or treatment'[11]. HRQL has become an important outcome measure for the evaluation of an individual's adaptation to a chronic medical condition. A patient-reported outcome (PRO) is any report that directly comes from a patient, without interpretation by a clinician or anyone else. In this regard, HRQL is often evaluated using a PRO instrument to provide means for measuring treatment benefits by capturing concepts related to how a patient feels with respect to his/her health[12]. Generic and disease-specific tools have been developed to assess PROs in AYA with IBD.

In this context, we hypothesised that AYA with low IBD activity would exhibit HRQL similar to that of age-matched healthy individuals. The present study aimed to compare HRQL and pain disability between AYA with low IBD activity and age-matched healthy AYA and to identify the demographics and clinical characteristics associated with low HRQL in the IBD population of five multidisciplinary clinics.

## METHODS

### Patients

Only AYA with IBD in remission or low disease activity from July 2019 to February 2020 were selected for this cross-sectional study. This research included five multidisciplinary clinics of two academic hospitals followed in Paediatric Gastroenterology, Gastroenterology, Coloproctology, Paediatric Rheumatology and Adolescent divisions, São Paulo, Brazil. As our hospital is a quaternary center, patients come from all parts of the country. The healthy control group consisted of AYA selected from healthy students in the community. IBD classification was established in adolescents according to the Paris classification and the ESPGHAN revised Porto criteria for the diagnosis of IBD[13] and in adults according to the Montreal classification[14].

All patients with IBD, healthy controls, and legal guardians of adolescents signed an informed consent form. This study was approved by the Ethics Committee of our university hospital (CAAE: 82679518.2.0000).

### HRQL parameters

HRQL assessment was performed using the Pediatric Quality of Life Inventory 4.0 (PedsQL™ 4.0). The PedsQL™ 4.0 is a generic, multidimensional, self-administered questionnaire that was validated in Brazilian Portuguese language for adolescents (age, 13–18 years) and young adults (age, 19–25 years)[15,16] and consists of 23 items divided into four domains: physical capacity, emotional issues, social aspects, and school/work activities, systematically assessed in the last month of study entry. These measures provided a total HRQL score and two summary scores: physical health (comprising the physical health domain score) and psychosocial health (comprising the emotional, social, and school functioning domain scores). A five-point Likert scale ranging from 'never' to 'almost always' was used, and items were scored on a 0–100 metric scale. High scores indicated the best HRQL parameters; if >50% of scale items were missing, the scale score was not computed[15]. Finally, for qualitative analysis, the patients could express their thoughts regarding their disease and treatment at the end of the questionnaire, which were written in their own words.

The 36-Item Short-Form Health Survey (SF-36) is also a generic, multidimensional, self-administered questionnaire for adults. It is validated in Brazilian Portuguese language and consists of 36 items divided into eight domains: physical functioning, role physical (i.e., role limitations due to physical difficulties), role emotional (i.e., role limitations due to emotional difficulties), vitality/energy, mental health, social functioning, bodily pain, and general health perception. This validated instrument was applied only to young adults with IBD for HRQL evaluation[17]. Additionally, one single-item 'health change' evaluated differences in the state of health over the past year. The final score ranged from 0 to 100, with higher scores indicating the best HRQL parameters.

### Functional pain disability

The visual analogue scale (VAS) and FACES pain rating scale (FPRS), which are validated subjective measures of acute and chronic pain intensity, were used to evaluate AYA with IBD. VAS scores were recorded by making a handwritten mark on a 10-cm line at 1-cm intervals, which represents a continuum between 'no pain' and 'worst pain'. The FPRS presents five hand-drawn faces that gradually increase pain expression from neutral to higher pain levels[18].

### Demographic and clinical data

Demographic data, clinical features, laboratory findings, treatments, and outcomes were carefully evaluated according to an extensive standardised protocol. Demographic data included current age, sex, school years, body mass index (BMI), disease duration, and Brazilian socioeconomic class[19]. The following extra-intestinal manifestations were systematically assessed: uveitis, episcleritis, erythema nodosum, pyoderma gangrenosum, and arthritis/arthralgia. The erythrocyte sedimentation rate and C-reactive protein (CRP) level were evaluated using the Westergren method and nephelometry, respectively.

Only patients in remission or with mild activity were selected, and disease activity was defined using the following four indices: (i) Pediatric Crohn's Disease Activity Index (PCDAI), with scores ranging from 0 to 100 points (remission, ≤10; mild activity, 10≤30)[20]; (ii) Pediatric Ulcerative Colitis Activity Index (PUCAI), with scores ranging from 0 to 85 points (remission, ≤10; mild activity, 10≤34)[20]; (iii) Harvey–Bradshaw index for adult CD (remission, <5; mild disease activity, 5–7)[21]; and (iv) partial Mayo index for adult UC (remission, <2; mild disease activity, 2–4)[22].

Additionally, the following treatments for IBD were recorded: 5-ASA drugs, corticosteroids, immunomodulator drugs (azathioprine, 6-mercaptopurine, and methotrexate), and biologic agents (infliximab, adalimumab, and vedolizumab). Outcomes, previous gut surgery, and malignancy were also analysed.

### Statistical analyses

Statistical analyses were performed using SPSS software version

22.0 (IBM Corp., Armonk, NY, USA). The Mann–Whitney test was used to compare continuous variables with asymmetric distribution. Scores are presented as medians (maximum and minimum values), whereas nominal variables are expressed as frequencies. Fisher's exact test was used to compare the data between the two groups. The *P*-values were set at 5% ($P<0.05$) for all statistical tests. A multivariate analysis was performed to evaluate IBD activity, treatments, and outcomes with respect to HRQL in AYA with IBD.

## RESULTS

TABLE 1 summarises the demographic data of patients with IBD and healthy controls. The median current ages of AYA with IBD and healthy controls were similar (18.63 [13.14–25.80] years vs 20.53 [13.68–25.84] years, $P=0.598$). The proportion of female sex was similar between the two groups (42% vs 38%, $P=0.654$). Less than 8% out of 26 adolescents with IBD were below the third percentile in height. IBD patients were similar in terms of height and BMI to the healthy controls. No differences in school years (11 [8–17] vs 15 [6–15] years, $P=0.072$) and upper middle/middle Brazilian socioeconomic classes (94% vs 97%, $P=0.596$) were observed between the two groups (TABLE 1).

TABLE 2 presents the PedsQL™ 4.0 and SF-36 domains, VAS, and FPRS scores of patients with IBD, as compared to those of healthy controls. The school/work domain score with PedsQL™ 4.0 was significantly lower in AYA with IBD than in healthy controls (70 [10–100] vs 75 [5–100], $P=0.037$). Overall, 80% of AYA with IBD provided additional information regarding their complaints about long-term treatment. The main reported reasons for the impaired QoL were frequent medical appointments, time-consuming biological infusions, long-term treatment, and multiple procedures that affect patients' frequency in school or at permanent work. As for SF-36, the general health perception domain score (50 [10–80] vs 70 [25–90], $P=0.0002$) was significantly lower in the adult IBD group than in the healthy control group. The score for the additional item perception of 'health change' was significantly higher in the IBD group than in the healthy control group (75 [25–100] vs 50 [25–100], $P=0.042$]. No differences in other parameters were observed between AYA with IBD and healthy controls ($P>0.05$, TABLE 2).

Among patients with CD and UC, young adult with CD had a significantly higher perception of 'health change' item in SF-36 (75 [25–100) vs 62 [25–100], $P=0.042$; TABLE 3). All the domains in SF-36 were similar between both groups, as were the PedsQL™ 4.0 domain, VAS, FPRS, and total VAS scores ($P>0.05$).

Demographic and clinical data of patients with CD and UC are shown in TABLE 4. Patients with CD had a significantly shorter disease duration (42.5 [1.0–247.0] months vs 78.0 [12.0–209.0] months, $P=0.014$) and lower proportion of female patients (32% vs 62%, $P=0.025$) than patients with UC. The frequency of overlap syndrome with autoimmune sclerosing cholangitis was significantly lower in patients with CD than in patients with UC (0% vs 19%, $P=0.013$). Anaemia was diagnosed in 6% of all patients, hypoalbuminaemia (albumin <3.5 g/dL) in 1%, and high CRP level (>5 mg/dL) in 30%. The frequencies of prednisone and 5-ASA treatments were significantly lower in patients with CD than in patients with UC (5% vs 28%, $P=0019$ and 8% vs 81%, $P=0.001$, respectively). The rate of previous gut surgery was significantly higher in patients with CD than in patients with UC (47% vs 9%, $P=0.004$).

**TABLE 1.** Demographic data of inflammatory bowel disease patients and healthy controls.

| Variables | IBD patients (n=59) | Healthy controls (n=60) | *P* |
|---|---|---|---|
| Demographic data | | | |
| Current age, years | 18.63 (13.14–25.80) | 20.53 (13.68–25.84) | 0.598* |
| Female sex | 25 (42) | 23 (38) | 0.654† |
| BMI, kg/m$^2$ | 20.05 (14.50–30.88) | 21.60 (15.90–28.10) | 0.659* |
| School, years | 11 (8–17) | 15 (6–15) | 0.072* |
| Upper middle/middle socio-economic classes | 33/35 (94) | 39/40 (97) | 0.596† |

BMI: body mass index, *Mann-Whitney test, †Fisher exact test. Results are presented in median (minimum and maximum values) or n (%).

**TABLE 2.** Pediatric Quality of Life Inventory 4.0 (PedsQL™ 4.0), Pain VAS, Faces Pain Rating Scale and Short-Form Health Survey (SF-36) scores according to reports of inflammatory bowel disease patients and healthy controls.

| Variables | AYA with IBD (n=59) | Healthy controls (n=60) | *P** |
|---|---|---|---|
| **PedsQL™ 4.0 score** | | | |
| Physical health score | 81.3 (12.5–100) | 87.5 (34.3–100) | 0.229 |
| Psychosocial health score | 71.7 (18.3–100) | 73.3 (25–100 | 0.6014 |
| Emotional | 60.0 (0–100) | 65 (20–100) | 0.773 |
| Social | 90.0 (40–100) | 82.5 (10–100) | 0.275 |
| School/work | 70.0 (10–100) | 75 (5–100) | 0.037 |
| Total score | 74.4 (20–100) | 77.2 (41.3–100) | 0.756 |
| Pain VAS scale | 2.0 (0–10) | 1.0 (0–7) | 0.111 |
| Faces pain rating scale | 2.0 (0–10) | 2.0 (0–8) | 0.191 |
| Total VAS scale | 2.0 (0–10) | 1.5 (0–8) | 0.190 |
| **SF-36 score** | **Young adults with IBD (n=33)** | **Heathy controls (n=39)** | ***P**** |
| Physical functioning | 95 (10.0–100) | 95 (30–100) | 0.255 |
| Role limitation due to physical health | 100 (0–100) | 100 (0–100) | 0.186 |
| Role limitation due to emotional problems | 67 (0–10) | 67 (0–100) | 0.971 |
| Energy/vitality | 60 (0–100) | 60 (15–90) | 0.918 |
| Mental health | 64 (4–88) | 76 (20–96) | 0.077 |
| Social functioning | 75 (0–100) | 88 (13–100) | 0.106 |
| Bodily pain | 80 (0–100) | 80 (33–100) | 0.927 |
| General health perceptions | 50 (10–80) | 70 (25–90) | 0.0002 |
| Total score | 552 (97–748) | 648 (243–763) | 0.122 |

VAS: visual analogue scale; AYA: adolescents and young adults. *Mann-Whitney test. Results are presented in median (minimum and maximum values).

**TABLE 3.** Pediatric Quality of Life Inventory 4.0 (PedsQL™4.0) and Medical Outcomes Short-Form Health Survey (SF-36) scores according to the reports of Crohn disease (CD) and ulcerative colitis (UC) patients.

| Variables | AYA with CD (n=38) | AYA with UC (n=21) | *P** |
|---|---|---|---|
| **PedsQL™4.0 score** | | | |
| Physical | 82.8 (31.3–100) | 81.3 (12.50–100) | 0.993 |
| Psychosocial Health | 75.3(29–100) | 73.3(16–100) | 0.456 |
| Emotional | 62.5 (0–100) | 60.0 (0–100) | 0.680 |
| Social | 90.0 (45.0–100.0) | 90.0 (40.0–100) | 0.783 |
| School/work | 70 (25–100) | 65 (10–100) | 0.165 |
| Total score | 76.1 (37.5–100) | 73.9 (20–100) | 0.825 |
| Pain VAS scale | 2.0 (0–10) | 2.0 (0–8) | 0.894 |
| Faces pain rating scale | 2.0 (0–10) | 2.0 (0–8) | 0.756 |
| Total VAS scale | 4.0 (0–10) | 4 (0–10) | 0.896 |
| **SF-36 score** | **Young adults with CD (n=19)** | **Young adults with UC (n=14)** | ***P**** |
| Physical functioning | 95 (25–100) | 97.5 (10–100) | 0.369 |
| Role limitation due to physical health | 75 (0–100) | 100 (0–100) | 0.641 |
| Role limitation due to emotional problems | 67 (0–100) | 67 (0–100) | 0.674 |
| Energy/vitality | 60 (0–85) | 60 (20–100) | 0.465 |
| Mental health | 72 (4–88) | 62 (32–88) | 0.454 |
| Social functioning | 75 (25–100) | 88 (13–100) | 0.094 |
| Bodily pain | 80 (33–100) | 80 (0–100) | 0.839 |
| General health perceptions | 50 (15–80) | 50 (10–80) | 0.522 |
| **Total score** | 575 (107–711) | 551 (97–748) | 0.941 |

CD: Crohn disease; UC: ulcerative colitis; AYA: adolescents and young adults. *Mann-Whitney test. Results are presented in median (minimum and maximum values).

**TABLE 4.** Demographic data, cumulative extra-intestinal (EI) manifestations, overlap autoimmune diseases, disease activity parameters, treatments and outcomes in CD and UC patients.

| Variables | AYA with CD (n=38) | AYA with UC patients (n=21) | *P* |
|---|---|---|---|
| Demographic data | | | |
| Current age, years | 17.9 (13.5–25.8) | 19.2 (13.1–25.5) | 0.680* |
| Disease duration, months | 42.5 (1.0–247.0) | 78.0 (12.0–209.0) | 0.014* |
| Female sex (%) | 12 (32) | 13 (62) | 0.025† |
| BMI, kg/m$^2$ | 19.9 (14.5–28.0) | 20.08 (15.30–30.8) | 0.447 |
| School, years | 10 (8–17) | 12 (8–15) | 0.184 |
| Upper middle/middle socio-economic classes | 21/22 (95) | 12/13 (92) | 1.000 |
| Cumulative EI manifestations | | | |
| Uveitis/episcleritis | 3 (8) | 2 (9) | 1.000† |
| Erythema nodosum/ pyoderma gangrenosum | 3 (8) | 2 (9) | 1.000† |
| Arthritis/arthralgia | 10 (26) | 5 (24) | 1.000† |
| Overlap syndromes | | | |
| Autoimmune hepatitis | 0 (0) | 1 (5) | 0.356† |
| Autoimmune sclerosing cholangitis | 0 (0) | 4 (19) | 0.013† |
| Current disease activity parameters Harvey-Bradshaw score for aCD | | | |
| Remission and mild activity n (%) | 19 (32%) | | |
| PCDAI score for pCD | | | |
| Remission and mild activity n (%) | 19 (32%) | | |
| Mayo score for aUC | | | |
| Remission and mild activity n (%) | | 14 (24%) | |
| PUCAI score for pUC | | | |
| Remission and mild activity n (%) | | 7 (12%) | |
| Current treatments | | | |
| Prednisone | 2 (5) | 6 (28) | 0.019† |
| 5-aminosalicylic acid | 3 (8) | 17 (81) | 0.0001† |
| Azathioprine | 24 (63) | 15 (71) | 0.775† |
| Methotrexate | 5 (13) | 1 (5) | 0.407† |
| Infliximab | 19 (50) | 5 (24) | 0.059† |
| Adalimumab | 9 (24) | 4 (19) | 0.754† |
| Vedolizumab | 0 (0) | 1 (5) | 0.356† |
| Outcomes | | | |
| Previous gut surgery | 18 (47) | 2 (9) | 0.004† |
| Malignancy | 0 (0) | 0 (0) | 1.000† |

BMI: body mass index; EI: extra-intestinal; CD: Crohn disease; UC: ulcerative colitis; AYA: adolescents and young adults; PCDAI: Pediatric Crohn's Disease Activity Index; PUCAI: Pediatric Ulcerative Colitis Activity Index; Disease severity: Harvey-Bradshaw, PCDAI, Mayo. a: adult, p: pediatric, *Mann-Whitney test, †Fisher exact test. Results are presented in n (%) or median (minimum and maximum values).

Further analysis of PedsQL™ 4.0 scores in adolescents with IBD aged ≤18 years compared to young adults with IBD aged >19–25 years showed similar medians in both groups for physical (81.3 [12.5–100] vs 87.5 [34.3–100], *P*=0.229), emotional (65 [0–100] vs 65 [25–100], *P*=0.857), social (95 [40–100] vs 80 [45–100], *P*=0.502), school/work scores (70 [15–100] vs 65 [10–100], *P*=0.810), and total score (78 [43–100] vs 73 [20–100], *P*=0.727). The median VAS (2 [0–10] vs 3 [0–9], *P*=0.238], FPRS (2 [0–10] vs 2 [0–8], *P*=0.435], and total VAS scores (2 [0–10] vs 3 [0–9], *P*=0.214] were similar between both groups. Furthermore, multivariate analysis revealed no association between HRQL and clinical and demographic parameters.

## DISCUSSION

We reported the impact of low IBD activity on the HRQL of AYA population at a large referral centre in Brazil. We identified that AYA with well-controlled IBD had significantly lower HRQL than healthy controls with respect to psychosocial scores, specifically in the school/work and general health perception domains. In line with the findings of other studies in the literature, lower HRQL scores in the school/work domain were also observed and described among adolescents and adults with IBD[6,23,24]. These issues reflect the challenges in integrating AYA with IBD into the society, affect their job opportunities and future aspirations, lead to personal absenteeism in school, reduce work productivity, and cause financial burden on the healthcare system[7,25,26]. Moreover, the qualitative sampling assessment provided additional relevant insights to this study when patients reported that, frequent medical appointments, time-consuming biological infusions, long-term treatment, and multiple procedures unquestionably impair their frequency in school or at permanent work. These observations highlighted a disability criterion and emphasised the importance of developing validated disability questionnaires for AYA with IBD in the future. Other domains of PedsQL™ 4.0 and SF-36 were similar between both groups, as were the VAS, FPRS, and total VAS scores.

General health perception refers to patients' assessment of their own personal health[17]. Lower scores for this domain among young adults with IBD, as compared to healthy controls, should reflect patients' experience with the disease and its treatment. No differences in all domains of PedsQL™ 4.0 and SF-36 as well as in the pain scales were observed between patients with CD and UC, suggesting that they had similar HRQL in our study. Analysis of the additional item 'health change over the past year' showed that AYA with UC had lower scores than AYA with CD. This perception should be influenced by the predominance of female sex, longer disease duration, and use of steroids, as observed by other authors[27,28]. Interestingly, previous gut surgery did not negatively influence the HRQL of AYA with CD, suggesting that resection for the treatment of severe complications such as strictures and appropriate drainage of fistulas may improve a previous disabling condition.

In the present study, pain scores (VAS and FPRS) were similar between AYA with IBD and healthy controls and also between the CD and UC groups. Abdominal pain is often associated with disease flares. Furthermore, pain is a category of disability and is consistently associated with emotional distress, anxiety, depression, and cognitive illness that impairs patients with IBD across all ages[5,29,30].

The age limit for paediatric care is debated and has recently been extended. The American Academy of Pediatrics has suggested an upper age limit of 21 years for adolescence[31]. In contrast, other authors have suggested that the adolescent age should range from 10 to 25 years, based on brain growth and contemporary patterns of neurocognitive myelin synthesis, following the recommendations of the Institute of Medicine and National Research Council of the United States[32]. In the present study, we used a wide cut-off age range (13–25 years) because this period also has similar socio-emotional vulnerability to biological, psychological, social, and cognitive development. Moreover, at the beginning of this stage, young adults are nearly finishing high school, living with their parents, and engaging in romantic relationships. These milestones may be affected in patients with chronic conditions such as IBD. Our hospital is establishing the model recommended by "European Crohn's and Colitis Organisation", with a joint adolescent–adult clinic, enrolling five multidisciplinary clinics (Paediatric Gastroenterology, Gastroenterology, Coloproctology Rheumatology and Adolescents) as part of a transition program[33].

It is important to emphasise that most patients enrolled in our study had good control of inflammatory activity, probably due to the implementation of effective therapies for IBD management. Disease activity is not the only factor that influences HRQL; patients' knowledge and concerns about the disease as well as their coping may also positively influence HRQL[34]. It is noteworthy that less than 8% of adolescents exhibited growth failure (height for age below the third percentile) and were similar to healthy controls with respect to growth and BMI. Considering that school/work comprises an important part of disability, general health perception is the patients' own experience, and pain scales are more related to disease activity, there is an urgent need for future studies to explore disabilities in this vulnerable population. Moreover, it is essential to underline that IBD symptoms in AYA are not only driven by disease activity; assessment of differential diagnosis, including irritable bowel syndrome and small intestinal bacterial overgrowth, was outside the scope of the present study.

The present study had some limitations. The small populations for both diseases affecting young patients may preclude the analysis of the impact of HRQL and disability parameters, particularly in specific populations with UC and CD. Additionally, disease-specific IBD instruments for adolescents and adults with IBD were not assessed. At study entry, a disease-specific health-related quality-of-life questionnaire developed for use in pediatric inflammatory bowel disease (IMPACT-III) was not a validated tool available in Brazilian Portuguese language. Disease activity was assessed using clinical scores only because faecal calprotectin, a non-invasive objective inflammatory biomarker, is not included as part of routine screening at our hospital; hence, we did not include it in this study. It is noteworthy that 20% of patients in our sample underwent colonoscopy or cross-sectional imaging at within one month of the questionnaire's application. It was not feasible to frequently repeat these procedures owing to their cost and/or invasive nature. We did not consider these data despite being a relevant target because of the insufficient sample size.

Notwithstanding these limitations, the strength of the present study was the systematic assessment of HRQL and self-reported pain disability parameters in AYA with IBD from five multidisciplinary clinics of two academic hospitals: paediatric gastroenterology, gastroenterology, coloproctology, paediatric rheumatology and adolescent divisions. Considering 'patient-centred care', PROs

have become the current standard for the assessment of HRQL and disabilities in AYA with IBD. PedsQL™ 4.0, SF-36, and pain scales are self-assessment tools for PROs that are feasible and easy to use to capture disease control from the patients' perspective[15-17]. Furthermore, generic questionnaires enabled us to compare AYA with IBD and healthy controls. In particular, PedsQL™ 4.0 showed good correlation with IMPACT-III, a specific tool, in previous study[35]. Additionally, the healthy controls with similar age, sex, school years, and socioeconomic class to AYA with IBD were relevant herein, as these demographic data may influence HRQL parameters. These findings indicate that multidisciplinary teams are recommended for all patients with IBD, irrespective of their current disease activity, in order to clarify the patients' situation and identify aspects that require action.

In conclusion, AYA with IBD constitute a distinct group; given their complex disease phenotype and specific concerns about medications, they find themselves at a period of transition from childhood to adulthood, which is characterised by considerable psychosocial changes. Our multidisciplinary study showed that effective control of inflammatory activity in AYA with IBD is insufficient to improve HRQL. Lower HRQL in school/work and general health perception domains highlights the need to further explore disabilities in this vulnerable population. Additionally, international multicentre studies that include global comparisons of HRQL and disabilities among AYA with IBD using validated questionnaires are warranted to better clarify this issue.

## ACKNOWLEDGEMENTS

The authors acknowledge Dr. Ulysses Doria Filho for his statistical analysis supervisor, and Luisa Leite Barros and Luciane Milani for physician supervision.

### Authors' contribution

Oba J, Sobrado CW and Silva CA contributed to study conception and design. All authors critically reviewed the manuscript, contributed important intellectual content, and approved the final manuscript version for submission. All authors agree to be accountable for all aspects of the work, and Oba J holds overall responsibility for the content and integrity of this paper.

### Orcid

Jane Oba: 0000-0003-4993-6515.
Carlos W Sobrado: 0000-0003-4486-9894.
Aderson O M C Damião: 0000-0001-7584-7351.
Matheus Azevedo: 0000-0001-5487-9418.
Alexandre Carlos: 0000-0001-6485-7968.
Natália Queiroz: 0000-0003-2857-0825.
Claudio Len: 0000-0001-8636-1744.
Ricardo Toma: 0000-0003-3792-8075.
Mariana Deboni: 0000-0002-9702-189X.
Marcos J Ozaki: 0000-0001-9478-7630.
Flair José Carrilho: 0000-0002-7682-3105.
Sergio Nahas: 0000-0002-2268-4146.
Clovis Silva: 0000-0001-9250-6508.

Oba J, Sobrado CW, Damião AOMC, Azevedo M, Carlos A, Queiroz N, Len CA, Toma RK, Deboni M, Ozaki MJ, Carrilho FJ, Nahas S, Silva CA. A qualidade de vida relacionada à saúde em adolescentes e adultos jovens com doença inflamatória intestinal está mais associada à redução na produtividade escolar e no trabalho do que ao comprometimento físico: um estudo multidisciplinar. Arq Gastroenterol. 2021;58(4):541-7.

**RESUMO – Contexto** – As doenças inflamatórias intestinais (DII), que englobam a doença de Crohn e a colite ulcerativa, são doenças inflamatórias crônicas do trato gastrointestinal que frequentemente se manifestam em adolescentes e adultos jovens (AAJ). As DII são caracterizadas por episódios de doença ativa intercalados com períodos de remissão, e sua atividade se correlaciona inversamente com a qualidade de vida relacionada à saúde (QVRS). **Objetivo** – Este estudo teve como objetivo determinar se AAJ em remissão ou com baixa atividade de DII exibiria QVRS semelhante à de indivíduos saudáveis pareados por idade, e se fatores demográficos da doença poderiam afetar a QVRS usando um instrumento de medidas de desfecho relatadas pelo paciente. **Métodos** – Este estudo envolveu apenas AAJ com DII, com baixa atividade. Esta pesquisa incluiu cinco clínicas multidisciplinares de dois Hospitais Universitários: Divisões de Gastroenterologia Pediátrica, Gastroenterologia, Coloproctologia, Reumatologia Pediátrica e Adolescentes, São Paulo, Brasil. Um total de 59 AAJ com DII (13–25 anos de idade) e 60 AAJ controle saudáveis (13–25 anos de idade) responderam os questionários *Pediatric Quality of Life Inventory 4.0* e *36-Item Short-Form Health Survey* e as escalas visuais de dor. Dados demográficos, manifestações extra intestinais, tratamentos e desfechos da doença de Crohn e a colite ulcerativa foram avaliados. **Resultados** – AAJ com DII e os controles saudáveis foram grupos semelhantes com relação à média de idade (18,63 [13,14–25,80] vs 20,5 [13,68–25,84] anos, *P*=0,598), quanto à proporção de pacientes do sexo feminino (42% vs 38%, *P*=0,654), e quanto à porcentagem da classe socioeconômica brasileira média elevada/ média (94% vs 97%, *P*=0,596). Os escores escola/trabalho foram significativamente mais baixos nos AAJ com DII do que nos controles saudáveis (70 [10–100] vs 75 [5–100], *P*=0,037). O escore 'percepção geral de saúde' foi significativamente mais baixo nos AAJ com DII do que no agrupamento controle saudável (50 [10–80] vs 0 [25–90], *P*=0,0002). As escalas de avaliação visual de dor foram semelhantes entre os dois grupos (2 [0–10] vs 3 [0–9], *P*=0,214). Nenhuma associação entre QVRS e parâmetros clínicos e demográficos foi identificada entre os pacientes com DII. **Conclusão** – AAJ com baixa atividade das DII relataram baixa QVRS nos domínios da escola/trabalho e percepção geral da saúde, o que destaca um critério de incapacidade nesta vulnerável população.

**Palavras-chave –** Doenças inflamatórias intestinais; doença de Crohn; colite ulcerativa; qualidade de vida relacionada à saúde; qualidade de vida; incapacidade; deficiência; adolescente; adulto jovem.

## REFERENCES


1. Rosen MJ, Dhawan A, Saeed SA. Inflammatory bowel disease in children and adolescents. Vol. 169, JAMA Pediatrics. 2015;169:1053-60.
2. Moradkhani A, Beckman LJ, Tabibian JH. Health-related quality of life in inflammatory bowel disease: Psychosocial, clinical, socioeconomic, and demographic predictors. J Crohn's Colitis. 2013;7:467-73.
3. Gumidyala AP, Greenley RN. Correlates of health-related quality of life in pediatric inflammatory bowel disease: A cumulative risk model approach. J Pediatr Psychol. 2014;39:55-64.
4. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant R V, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol. 2015;110:1324-38.
5. Greenley RN, Kunz JH, Schurman JV, Swanson E. Abdominal pain and health related quality of life in pediatric inflammatory bowel disease. J Pediatr Psychol. 2013;38:63-71.
6. Eloi C, Foulon G, Bridoux-Henno L, Breton E, Pelatan C, Chaillou E, et al. Inflammatory Bowel Diseases and School Absenteeism. J Pediatr Gastroenterol Nutr. 2019;68:541-6.
7. de S B Fróes R, Carvalho ATP, Antonio AJ, de Barros Moreira AMH, Moreira JPL, Luiz RR, et al. The socio-economic impact of work disability due to inflammatory bowel disease in Brazil. Eur J Heal Econ. 2018;19:463-70.
8. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. Gastroenterology. 2021;160:1570-83.
9. van den Brink G, Stapersma L, Vlug LE, Rizopolous D, Bodelier AG, van Wering H, et al. Clinical disease activity is associated with anxiety and depressive symptoms in adolescents and young adults with inflammatory bowel disease. Aliment Pharmacol Ther. 2018;48:358-69.
10. Trivedi I, Keefer L. The Emerging Adult with Inflammatory Bowel Disease: Challenges and Recommendations for the Adult Gastroenterologist. Gastroenterol Res Pract. 2015;2015:260807.
11. Lippmann J, Fock A, Arulanandam S. Cerebral arterial gas embolism with delayed treatment and a fatal outcome in a 14-year-old diver. Diving Hyperb Med. 2011;41:31-4.
12. Williet N, Sandborn WJ, Peyrin-Biroulet L. Patient-reported outcomes as primary end points in clinical trials of inflammatory bowel disease. Clin Gastroenterol Hepatol. 2014;12:1246-1256.e6.
13. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, De Ridder L, et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J Pediatr Gastroenterol Nutr. 2014;58:795-806.
14. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: Controversies, consensus, and implications. Gut. 2006;55:749-53.
15. Varni JW, Seid M, Kurtin PS. PedsQLTM 4.0: Reliability and Validity of the Pediatric Quality of Life InventoryTM Version 4.0 Generic Core Scales in Healthy and Patient Populations. Med Care. 2001;39:800-12.
16. Varni JW, Limbers CA. The PedsQLTM 4.0 generic core scales young adult version: Feasibility, reliability and validity in a university student population. J Health Psychol. 2009;14:611-22.
17. Jenkinson C, Coulter A, Wright L. Short form 36 (SF 36) health survey questionnaire: Normative data for adults of working age. Br Med J. 1993;306:1437-40.
18. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales. Pain. 2011;152:2399-404.
19. Brazilian Association of Research Companies - ABEP. Economic classification criterion Brazil. Abep. 2018;1:1–5.
20. Turner D, Otley AR, Mack D, Hyams J, de Bruijne J, Uusoue K, et al. Development, Validation, and Evaluation of a Pediatric Ulcerative Colitis Activity Index: A Prospective Multicenter Study. Gastroenterology. 2007;133:423-32.
21. Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation Between the Crohn's Disease Activity and Harvey-Bradshaw Indices in Assessing Crohn's Disease Severity. Clin Gastroenterol Hepatol. 2010;8:357-63.
22. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated Oral 5-Asa for Mildely To Moderately Active Ulcerative Colitis. N Engl J Med. 1987;24;317:1625-9.
23. Leso V, Gervetti P, Macrini MC, Russo F, Iavicoli I. Inflammatory bowel diseases and work disability: A systematic review of predictive factors. Eur Rev Med Pharmacol Sci. 2021;25:165-81.
24. Knowles SR, Keefer L, Wilding H, Hewitt C, Graff LA, Mikocka-Walus A. Quality of Life in Inflammatory bowel disease: a systematic review and meta-analyses - Part II. Vol. 24, inflammatory bowel diseases. Lippincott Williams and Wilkins; 2018. p. 966-76.
25. Ryan JL, Mellon MW, Junger KWF, Hente EA, Denson LA, Saeed SA, et al. The clinical utility of health-related quality of life screening in a pediatric inflammatory bowel disease clinic. Inflamm Bowel Dis. 2013;19:2666-72.
26. Stawowczyk E, Kawalec P, Kowalska-Duplaga K, Mossakowska M. Productivity Loss Among Parents of Children With Inflammatory Bowel Diseases in Relation to Disease Activity and Patient's Quality of Life. J Pediatr Gastroenterol Nutr. 2020;71:340-5.
27. McDermott E, Mullen G, Moloney J, Keegan D, Byrne K, Doherty GA, et al. Body image dissatisfaction: Clinical features, and psychosocial disability in inflammatory bowel disease. Inflamm Bowel Dis. 2015;21:353-60.
28. Karwowski CA, Keljo D, Szigethy E. Strategies to improve quality of life in adolescents with inflammatory bowel disease. Inflamm Bowel Dis. 2009;15:1755-64.
29. De Carlo C, Bramuzzo M, Canaletti C, Udina C, Cozzi G, Pavanello PM, et al. The Role of Distress and Pain Catastrophizing on the Health-related Quality of Life of Children with Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr. 20191;69:E99-104.
30. Peyrin-Biroulet L, Cieza A, Sandborn WJ, Coenen M, Chowers Y, Hibi T, et al. Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health. Gut. 2012;61:241-7.
31. Hardin AP, Hackell JM, Simon GR, Boudreau ADA, Baker CN, Barden GA, et al. Age limit of pediatrics. Pediatrics. 2017;140:e20172151.
32. Silva CA, Terreri MT, Bonfa E, Saad-Magalhães C. Pediatric rheumatic disease patients: time to extend the age limit of adolescence? Adv Rheumatol. 2018;58. DOI: https://doi.org/10.1186/s42358-018-0031-y
33. van Rheenen PF, Aloi M, Biron IA, Carlsen K, Cooney R, Cucchiara S, et al. European Crohn's and Colitis Organisation topical review on transitional care in inflammatory bowel disease. J Crohns Colitis. 20171;11:1032-8.
34. Chouliaras G, Margoni D, Dimakou K, Fessatou S, Panayiotou I, Roma-Giannikou E. Disease impact on the quality of life of children with inflammatory bowel disease. World J Gastroenterol. 2017;23:1067-75.
35. Abdovic S, Pavic AM, Milosevic M, Persic M, Senecic-Cala I, Kolacek S. The IMPACT-III (HR) Questionnaire: A valid measure of health-related quality of life in Croatian children with inflammatory bowel disease. J Crohn's Colitis. 2013;7:908-15.

REVIEW

AG-2021-32
doi.org/10.1590/S0004-2803.202100000-97

# Surgical techniques for the treatment of rectal endometriosis: a systematic review of randomized controlled trials and observational studies

Pedro **POPOUTCHI**[1], Oswaldo Wiliam **MARQUES JUNIOR**[1,2], Pedro **AVERBACH**[3], Celso Augusto Milani **CARDOSO FILHO**[1,2] and Marcelo **AVERBACH**[1]

Received: 11 February 2021
Accepted: 18 June 2021

**ABSTRACT** – **Background** – Endometriosis is a common disease in reproductive-age women and it is estimated to occur in up to 50% of those with infertility. Intestinal involvement is reported in up to a third of the cases. This condition is related to chronic pain and loss of quality of life, resulting in emotional, social and economic costs. Treatment consists of hormonal block and surgical resection, with variable side effects and efficacy. The best choice for surgical treatment for rectal endometriosis is a matter of discussion regarding the indication and the best technique to be employed. **Objective** – To summarize data on indications, results and complications of surgical techniques for the treatment of rectal endometriosis. **Methods** – This comprehensive systematic review is a compilation of the available literature and discussion, carried out by a team with experience in the surgical treatment of intestinal endometriosis. Data regarding indications, results and complications of conservative and radical techniques for the surgical treatment of rectal endometriosis was carefully reviewed. Searches of PubMed, EMBASE, and CENTRAL up to May 2021 were performed to identify randomized controlled trials (RCTs) and observational studies that compared at least two of the three surgical techniques of interest (i.e., shaving, discoid resection, segmental resection). **Results** – One RCT and nine case series studies with a total of 3,327 patients met the eligibility criteria. Participants ages ranged from a mean of 30.0 to 37.9 years old. Mean follow-up ranged from 1.2 to 42.76 months. With regards the methodological quality, overall the included studies presented a low risk of bias in the majority of the domains. Surgical treatment of rectal endometriosis is indicated for patients with obstructive symptoms and those with pain scores above 7/10. Patients with disease involving beyond muscularis propria of the rectum, documented in magnetic resonance imaging or transvaginal pelvic ultrasound with intestinal preparation, are candidates for discoid or segmental resection. The presence of multifocal disease, extension greater than 3 cm and infiltration greater than 50% of the loop circumference favor the radical technique. The distance from the lesion to the anal verge, age, symptoms and reproductive desire are other factors that influence the choice of the technique to be employed. The risk of complications and unfavorable functional results seems to be directly related to the complexity of the procedure. **Conclusion** – The choice of surgical technique performed for the treatment of rectal endometriosis is a matter of discussion and depends not only on the preoperative staging, but also on the patient's expectations, risks and potential complications, recurrence rates and the expertise of the multidisciplinary team.

**Keywords** – Treatment; rectal; endometriosis.

## INTRODUCTION

Deep endometriosis is defined by the presence of endometrial implants located more than 5 mm below the peritoneal surface. It may cause changes in pelvic anatomy and can invade the uterosacral ligaments, the vagina, the bladder, the ureters and various segments of the intestines. FIGURE 1 shows a nodule causing angulation of the rectum wall. Intestinal endometriosis is characterized whenever the muscularis propria on the involved loop is compromised, and its prevalence is estimated at 3 to 37% of all endometriosis cases. The intestinal segments most often affected are the rectum and the sigmoid colon, by up to 90%[1]. FIGURE 2 shows surgical resection specimens of infiltrative rectal endometriosis. The disease progresses slowly and has a heterogeneous clinical presentation that is often neglected, characterized mainly by pelvic pain, gastrointestinal symptoms, and infertility. These symptoms may be severe enough to significantly impact their quality of life and cause physical disabilities[2].

Although clinical treatment of endometriosis can reduce the size of the lesions and the intensity of the symptoms, the response is incomplete and recurrence after treatment discontinuation is high, with a failure rate around 40%[3-6]. Additionally, hormonal treatment for endometriosis is associated with erratic bleeding, increased weight, reduced libido, and headaches[7]. Another important aspect to consider is that patients may not adhere to the clinical treatment due to a reproductive desire. Thus, surgical treatment of intestinal endometriosis is an efficient option for symptomatic female patients[8]. Various studies have shown that surgical resection

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Hospital Sírio-Libanês, Instituto de Ensino e Pesquisa, São Paulo, SP, Brasil. [2] Fundação Antônio Prudente – A.C.Camargo Hospital, São Paulo, SP, Brasil. [3] Disciplina de Coloproctologia, Departamento de Gastroenterologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.
Corresponding author: Pedro Popoutchi. E-mail: pedropopoutchi@gmail.com

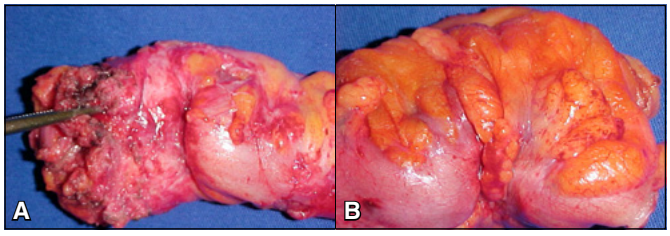



**FIGURE 1, A and B –** Nodule causing rectal angulation.

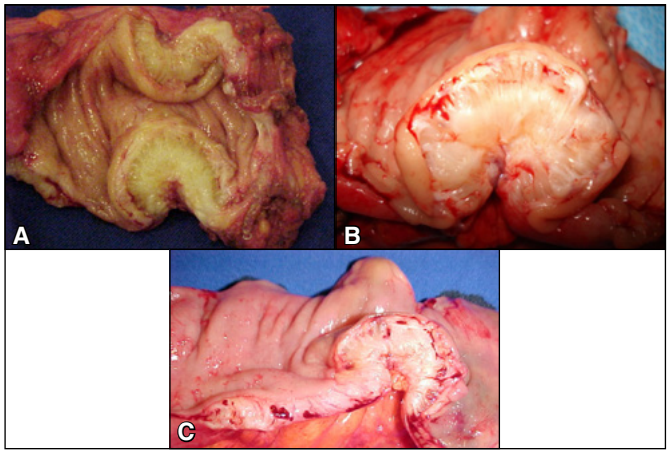



**FIGURE 2. A, B, and C –** Infiltrating endometriosis extrinsic to the rectal wall.

of all lesions, including those in the intestines, is linked to lower recurrence, significant improvement of gastrointestinal symptoms, improvement in quality of life, and satisfactory results in gestation rates[9-11]. Therefore, surgical treatment should be recommended to women who accept the risks and would rather avoid a prolonged clinical treatment, as well as those for whom hormonal treatment has failed or who present contraindications to hormonal treatment, and those at risk of urinary or intestinal obstruction.

The surgical approach to rectal endometriosis is subject to discussion when it comes to indication and the best technique to be applied. The preoperative staging of the disease through clinical exam, transvaginal ultrasound with bowel preparation, magnetic resonance imaging, and rectal ecoendoscopy, when available, presents sensitivity and specificity above 90% and is important in defining the best therapeutic strategy. Support by a multi-disciplinary team is essential to the success of the treatment[12-14]. Implementing an algorithm for the surgical team that is based on supplementary examinations is associated with higher rates of full resection of the disease, more conservative surgeries, and lower incidence of complications[15].

The most important information for surgical planning are: size and number of lesions, depth of intestinal wall infiltration, rate of rectal circumferential involvement, and distance from the anal verge. These elements are required for the definition of the surgical technique, which must be individualized and discussed with each patient, taking into account three important parameters: symptom control, recurrence rate, and desire to reproduce[14,15].

Laparoscopy is an effective and safe method for accessing the abdominal cavity in high volume centers[16,17]. Robotic surgery has been used in Brazil since 2010 to treat intestinal endometriosis[18]. However, studies still show similar results, at a higher cost[19,20]. We therefore conducted an updated systematic review of all randomized controlled trials (RCTs) and observational studies that assessed the effects of different surgical techniques (i.e., shaving, discoid resection, segmental resection) in patients with rectal endometriosis.

## METHODS

The Cochrane Handbook of Systematic Reviews of Interventions[21] guided our choice of methods. Our reporting adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement[22].

### Eligibility criteria

The inclusion criteria were:

- Study design: all RCTs and observational studies (i.e., case series, case-control, and cohort studies) that compared at least two of the three surgical techniques of interest (i.e., shaving, discoid resection, segmental resection).
- Patients: adults with rectal endometriosis.
- Interventions: surgical techniques (i.e., shaving, discoid resection, segmental resection).
- Comparisons: we compared the surgical techniques against each other.
- The patient-important outcomes that we were interested in were: mortality; recurrence; surgical major complications (e.g., pelvic abscess, rectal vaginal fistulae, dehiscence) and minor complications (e.g., stenosis of colorectal anastomoses, transitory bladder atony); fertility.

### Data source and searches

No restrictions were placed on language, year of publication or publication status. We searched Cochrane Central Register of Controlled Trials (CENTRAL, issue 5, 2021), US National Library of Medicine (PubMed, from 1966 to 2021), and Excerpta Medica Database (EMBASE, from 1980 to 2021). Search terms describing rectal endometriosis and surgical techniques were combined (Appendix TABLE 1). The last date was May 5th, 2021.

### Searching other resources

In addition to an electronic database search, we made a manual search in the reference lists of every study deemed eligible in order to identify additional trials that were later included; all potentially eligible studies were screened in duplicate.

### Selection of studies

Pairs of reviewers independently screened all titles and abstracts identified by the search through the Covidence online software. Full-text articles for potentially eligible studies were obtained and screened independently by reviewer pairs using the same eligibility criteria as with title and abstract screening. Consensus for stages of screening, data extraction, and risk of bias assessments were established by discussion and adjudication by a third reviewer, as necessary.

### Data extraction and risk of bias assessment

Once a final set of eligible studies were identified, reviewer pairs independently extracted data for the following variables from each study using a pre-standardized data extraction form with: characteristics of the study design; participants; description of interventions; and duration of follow-up.

Popoutchi P, Marques Junior OW, Averbach P, Cardoso Filho CAM, Averbach M
Surgical techniques for the treatment of rectal endometriosis: a systematic review of randomized controlled trials and observational studies

**TABLE 1.** Characteristics of included studies related to setting, study design, number of participants, mean age, gender, eligibility criteria, criteria used and follow-up.

| Author, year | Country | Multicentre or single-centre | Scenario | # of participants in the whole sample | Inclusion criteria | Exclusion criteria | Surgical technique | Age, mean (SD), y | % Male whole sample | Mean (SD), follow-up (months) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Case series studies** | | | | | | | | | | |
| **Abo, 2018** | France | Single-centre | Department of Obstetrics and Gynecology at Rouen University Hospital, Rouen, France | 364 | Patients with deep endometriosis infiltrating the rectosigmoid had a laparoscopic surgical procedure to treat bowel endometriosis. | Both rectal disc excision and segmental resection of the sigmoid colon. | Shaving<br>Discoid<br>Segmental | 36.1 (7.1)<br>30.0 (4.7)<br>31.4 (5.2) | NA | 40 (22) |
| **Abrão, 2019** | Brazil | Single-centre | Hospital Beneficencia Portuguesa of Sao Paulo | 172 | Women with significant pain (visual analog scale >7) who were diagnosed with bowel endometriosis from preoperative imaging and underwent laparoscopic surgery for bowel endometriosis at a large referral center between 2014 and 2017. | Any previous bowel surgery, pregnant or menopausal, diagnosed with inflammatory bowel disease, or had a current or previous malignancy. | Shaving<br>Discoid<br>Segmental | 37.9 (4.8)<br>36.1 (5.6)<br>36.7 (5.0) | NA | 1.2 |
| **Ballester, 2016** | France | Multicentre | Tenon University Hospital and Rouen University Hospital | 60 | Women who underwent complete surgical removal of colorectal endometriosis and required postoperative ICSI–IVF. | NR | Shaving or discoid<br>Segmental | NR<br>NR | NA | NR |
| **Fanfani, 2010** | Italy | Single-centre | Departments of Obstetrics and Gynecology, Ospedale Sacro Cuore of Negrar, Verona, and Catholic University of the Sacred Heart, Rome, Italy | 136 | DIE with rectosigmoid involvement, nodules with maximum diameter ≤3 cm with bowel stenosis ≤60%, and presence of endometriosis-related symptoms. | NR | Discoid<br>Segmental | 33[c]<br>32[c] | NA | 33 |
| **Gutiérrez, 2019** | Spain | Single-centre | Department of Gynaecology of "La Paz" University Hospital | 143 | Endometriosis of the recto-sigma and eventual other intestinal. localization (ileum, cecum, appendix) with histological confirmation; correct possibility and disposition to follow-up. | Patients with DIE which not affected the bowel, previous bowel resection, no monitoring possible. | Segmental<br>Discoid<br>Shaving | 36.3 (5.6)<br>34.9 (6.8)<br>36.6 (5.8) | NA | 42.76 (1.3) |
| **Hudelist, 2018** | Austria | Single-centre | Department of Gynecology, Hospital St. John of God | 134 | Women underwent surgical treatment for DIE out of which all showed involvement of the rectum and/or sigmoid colon involving at least the serosal and muscular layer confirmed by histological analysis. | Women under the age of 18 years, patients with psychiatric disorders or a history of malignancy as well as virgins. | Segmental<br>Discoid | 34.5 (7.0)<br>34.0 (0.7) | NA | 35.4 (23.1) |
| **Millochau, 2017** | France | Single-centre | Department of Gynecology and Obstetrics of Rouen University Hospital, Rouen, France | 21 | Deep endometriosis of the low or midrectum along with concomitant infiltration of the sigmoid colon or rectosigmoid junction, at least 5 cm of healthy bowel between nodules, and separate surgical procedures requiring bowel sutures to be performed on multiple colorectal nodules with preservation of a healthy normal vascularized bowel. | Patients managed for multifocal colorectal endometriosis by two surgical procedures including at least one bowel shaving. | Discoid<br>Segmental | NR<br>NR | NA | 30 (25.4) |
| **Roman, 2017** | France | Multicentre | 56 public and private healthcare facilities in France | 1135 | Patients with DIE involving muscularis, submucosa or mucosa, operated on from January 1st to December 31st 2015. | Patients presenting with only superficial involvement of bowel serosa. | Shaving<br>Discoid<br>Segmental | NR<br>NR<br>NR | NA | NR |
| **Roman, 2020** | France | Multicentre | Rouen University Hospital, the Clinique Mathilde, Rouen and the Endometriosis Center, Clinique Tivoli-Ducos, Bordeaux, France | 1102 | All patients with deep endometriosis infiltrating the muscular layer or deeper of the rectum and sigmoid colon, who had benefitted from surgical management from October 2009 to May 2019. | NR | Shaving<br>Discoid<br>Segmental | NR<br>NR<br>NR | NA | 4 to 124[§] |
| **Randomized controlled trial** | | | | | | | | | | |
| **Roman, 2019** | France | Multicentre | Rouen University Hospital, Tenon University Hospital and Lille University Hospital | 60 | Patients over 18 and under 45 years and managed for deep endometriosis infiltrating the rectum up to 15 cm from the anus, measuring more than 20 mm in length, involving at least the muscular layer in depth and up to 50% of rectal circumference. | NR | Shaving or discoid<br>Segmental | 31[c] (27–36[§])<br>28[c] (27–36[§]) | NA | 729[c] (726–743[§])<br>727[c] (722–736[§]) |

DIE: deep infiltrating endometriosis; ICSI: intra-cytoplasmic sperm injection; IVF: in vitro fertilization; NR: nor reported; NA: not applicable.
[§]Range. [c]Median. [§]Interquartile.

**APPENDIX TABLE** 1. Search strategy.

| |
|---|
| (Rectal endometriosis OR deep endometriosis OR colorectal endometriosis OR rectovaginal endometriosis OR rectosigmoid endometriosis OR deep infiltrating endometriosis OR bowel endometriosis) AND (Shaving OR nodule shaving OR rectal shaving OR discoid resection OR Disc resection OR disc excision OR segmental resection OR bowel resection OR full thickness resection OR rectosigmoidectomy) |

For RCTs, reviewers independently assessed risk of bias by using a modified version of the Cochrane Collaboration's tool. The tool includes nine domains: adequacy of sequence generation, allocation sequence concealment, blinding of participants and caregivers, blinding of data collectors, blinding for outcome assessment, blinding of data analysts, incomplete outcome data, selective outcome reporting, and the presence of other potential sources of bias not accounted for in the previously cited domains[23,24].

For cohort and case-control studies, we planned to assess risk of bias with a modified version of the Ottawa-Newcastle instrument[25] that includes confidence in assessment of exposure and outcome; however, there was no included study classified as either cohort or case-control study.

For case series, we used the single tool from the Joanna Briggs Institute (JBI) critical appraisal checklist[26]. However, in our view, the structure of the response options in both AXIS and JBI instruments leaves much to be desired. Therefore, we modified the response options to "definitely yes" (low risk of bias), "partially yes" (not all information needed available), "unclear" (no information to judge), and "definitely no" (high risk of bias), and applied it to our form for risk of bias in case series studies.

## RESULTS

### Study selection

Our initial searches identified 898 citations through database searches. After we removed duplicates from different databases, we retained 821 potentially relevant articles for further assessment. Based on title and abstract screening, we obtained full-paper copies for 78 citations that were potentially eligible for inclusion in the review (FIGURE 3). We excluded 64 studies for the following reasons reviews, case reports, off-topic, letter to the editor, diagnostic studies, and observational studies with only a single arm. The remaining one RCT[27] with further four publications and nine observational studies[15,28-35] with a total of 3,327 patients met the minimum requirements and were included in this review. The reasons for exclusion are listed in the PRISMA flow diagram (FIGURE 3).

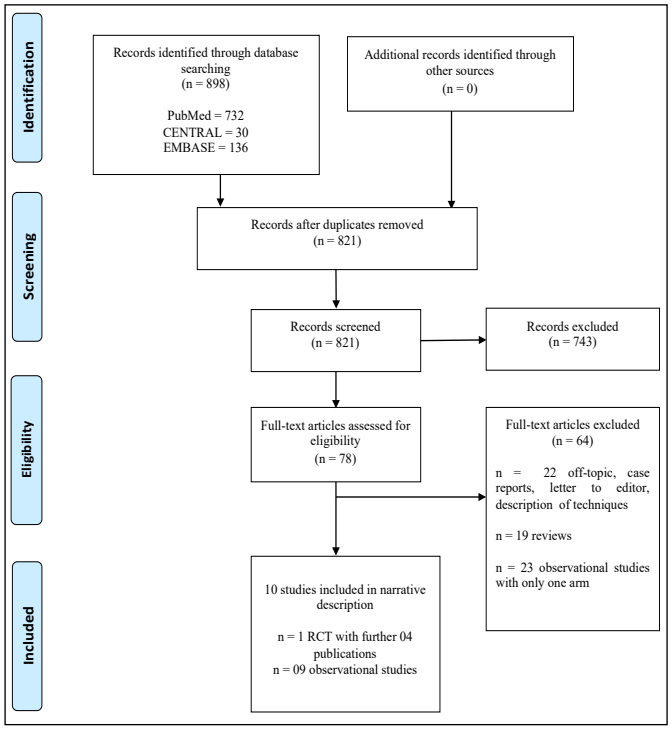



**FIGURE 3.** PRISMA flowchart.

### Study characteristics

TABLE 1 describes study characteristics related to setting, study design, number of participants, mean age, gender, eligibility criteria, criteria used and follow-up. Nine studies (90%) were conducted largely in Europe[27-35] and only one (10%) in South America (Brazil)[15]. Four studies (40%) out of ten were multicenter[27,29,34,35].

The RCT and case series studies sample size ranged from 21[33] to 1,135 patients[35]. Participants ages ranged from a mean of 30.0[28] to 37.9[15] years old (TABLE 1).

Six studies[15,28,29,31,34,35] (60%) compared all three techniques amongst them (i.e., shaving, discoid, and segmental). Mean follow-up ranged from 1.2[15] to 42.76 months[31].

### Risk of bias

FIGURE 4, TABLE 2 (Appendix) and TABLE 3 describe the risk of bias assessment. In the RCT study (FIGURE 4, panel A), only two domains blinding of participants and blinding of

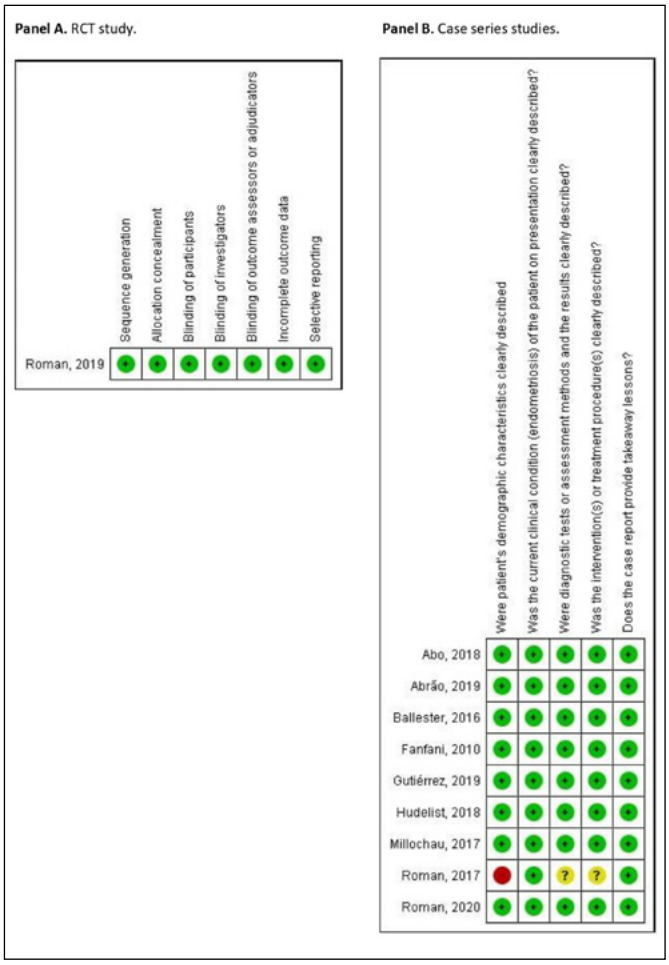



**FIGURE 4.** Risk of bias assessment. Panel A: RCT study. Panel B: case series studies.

**TABLE 2.** Comparative analysis of surgical techniques for rectal endometriosis considering various variables.

| | Superficial resection (shaving) | Discoid resection | Segmental resection |
|---|---|---|---|
| Technical complexity | + | ++ | +++ |
| Cost | + | ++ | +++ |
| Hospitalization period | + | ++ | +++ |
| Complications | + | ++ | +++ |
| Improvement in pain and quality of life indices | +++ | +++ | +++ |
| Risk of unfavorable functional results | + | ++ | +++ |
| Recurrence | +++ | ++ | + |

**Appendix TABLE 2.** Risk of bias for randomized controlled study.

| Autor, year | Sequence generation | Allocation concealment | Blinding of participants | Blinding of investigators | Blinding of outcome assessors or adjudicators | Incomplete outcome data | Selective reporting | Conflict of interest |
|---|---|---|---|---|---|---|---|---|
| **Roman, 2019** | Definitely low risk | Definitely low risk | Probably low risk | Probably low risk | Definitely low risk | Definitely low risk | Definitely low risk | Declared and no |

Definitely yes: low risk of bias; partially yes: probably low risk of bias; partially no: probably high risk of bias; definitely no: high risk of bias.

**TABLE 3.** Risk of bias for case series studies.

| Author, year | Were patient's demographic characteristics clearly described? | Was the current clinical condition (endometriosis) of the patient on presentation clearly described? | Were diagnostic tests or assessment methods and the results clearly described? | Was the intervention(s) or treatment procedure(s) clearly described? | Does the case report provide takeaway lessons? |
|---|---|---|---|---|---|
| Abo, 2018 | Definitely yes | Partially yes | Definitely yes | Definitely yes | Definitely yes |
| Abrão, 2019 | Definitely yes | Partially yes | Definitely yes | Definitely yes | Definitely yes |
| Ballester, 2016 | Definitely yes | Definitely yes | Definitely yes | Definitely yes | Definitely yes |
| Fanfani, 2010 | Definitely yes | Partially yes | Definitely yes | Definitely yes | Definitely yes |
| Gutiérrez, 2019 | Definitely yes | Definitely yes | Definitely yes | Definitely yes | Definitely yes |
| Hudelist, 2018 | Partially yes | Definitely yes | Definitely yes | Definitely yes | Definitely yes |
| Millochau, 2017 | Definitely yes | Partially yes | Definitely yes | Definitely yes | Definitely yes |
| Roman, 2017 | Definitely no | Definitely yes | Unclear | Unclear | Partially yes |
| Roman, 2020 | Definitely yes | Definitely yes | Definitely yes | Definitely yes | Definitely yes |

Definitely yes: low risk of bias; partially yes: probably low risk of bias; partially no: probably high risk of bias; definitely no: high risk of bias; unclear: not enought information for a judgment.

investigators were rated as "probably low risk of bias". In the case series studies (FIGURE 4, panel B), one domain (i.e., demographic characteristics clearly described) was rated as "high risk of bias" in one study[34], and two domains (i.e., results and procedures clearly described) were rated as "unclear" also at the same study[34]. Overall, the included studies presented a low risk of bias in the majority of the domains.

## DISCUSSION

### Surgical techniques for rectal endometriosis

Resection techniques can be classified as conservative and radical. Conservative techniques are those in which the endometriotic nodule is resected, saving the organ and most of the adjacent healthy tissue. Examples of these approaches are the discoid resection, with excision of the full thickness of the anterior wall of the rectum, and the superficial resection, when the rectal lumen is not opened. The so-called radical options include rectal segmental resections with primary anastomosis. The choice of technique depends on the characteristics of the lesion, as shown in FIGURE 5, and must be discussed with the patient and the multi-disciplinary team.

### Superficial resection (shaving)

Superficial resections can be done safely by experienced laparoscopic surgeons. First described in 1991, the use of this technique is becoming more common, as it is technically easier and presents a lower rate of complications; it is reserved to patients with superficial infiltration[36,37]. A recent study shows that shaving is indicated in infiltrations up to 5.2 mm in depth[38]. Patient should undergo bowel preparation in the day before the procedure, due to the possibility of opening the rectal lumen during the surgery.

With the patient under general anesthesia in the Lloyde-Davies position, a 12 mm incision is made on the umbilical scar in order to insert the laparoscope. Two other incisions, of 5 mm, are normally used in the iliac fossa, and a third 5 mm incision on the right flank may help in dissecting the nodule. A uterine manipulator and a rectal probe allow for the individual manipulation of these

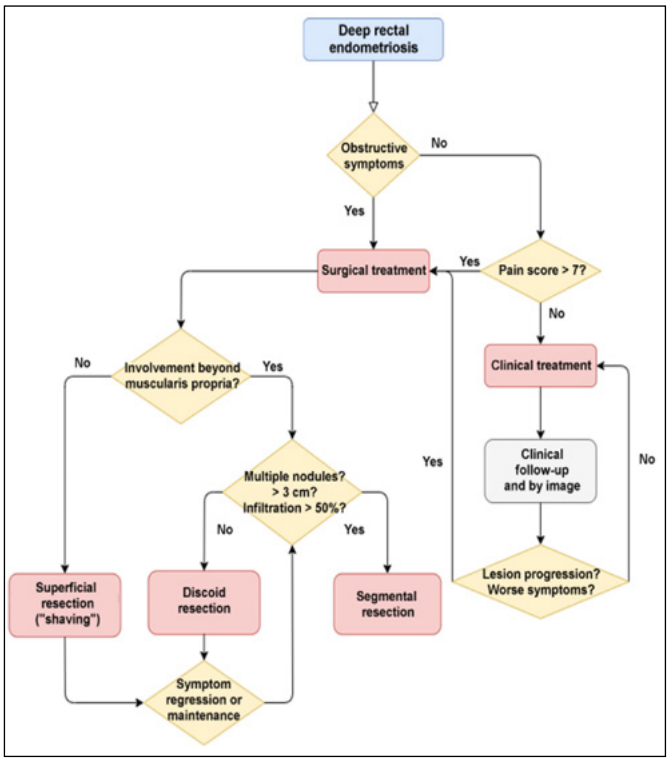



**FIGURE 5.** Flow diagram for therapeutic choice in rectal endometriosis.

structures. The procedure begins by dissecting the nodule from adjacent tissue and leaving it on the anterior wall of the rectum. The pararectal spaces are opened medially to the utero-sacral ligaments and next to the intestinal wall, in order to avoid hypogastric nerve lesion[39]. The lesion is then suspended and dissected, using cold, monopolar or bipolar instruments, blunt or sharp, at the junction of the white fibrous tissue with the normal tissue. In this manner, the superficial lesions involving the serous layer and/or the external muscular layer can be easily removed from the normal intestinal wall. Monopolar electric current must be used with care, since a thermal bowel injury may result in a fistula in the postoperative period. CO2 laser is a viable energy alternative for the superficial resection of rectal endometriosis[40].

In cases involving larger lesions, it may be necessary to separate the ureters in order to protect them. When the disease presents with extensive involvement of the retrocervical region and the anterior rectal wall, the lesion can be divided in order to leave one part on the anterior rectal wall and the other on the retrocervical region, allowing for the separate treatment of each region. The resection of deeper lesions may result in the opening of the intestinal lumen; in such cases, the defect may be corrected with sutures in one or two layers[41].

A prospective study by Donnez et al. with 500 patients with rectovaginal endometriosis and treated by superficial resection showed low complication rates and an 84% pregnancy rate after an average follow-up period of 3 years (from 2 to 6 years). Recurrence was significantly lower in patients who became pregnant naturally (3.6%) in comparison with those who underwent in-vitro fertilization (15%) ($P<0.05$)[42]. Roman et al., reported an improvement in gastrointestinal complaints, low complication rates (two patients with fistulas), and 4% relapse, in a group of 122 superficial resection patients[43]. The recurrence rates reported by Roman et al. and Gutiérrez et al. for patients who underwent superficial resection were 8.7% and 12.7%, with follow-up periods of 60 and 39 months, respectively[31,44].

In comparison with segmental resection, superficial resection has the benefits of technical simplicity, shorter operative times, lower complication rates, shorter hospitalization times, and better functional results in terms of continence and constipation[37]. The main disadvantage of the technique, as reported in a recent systematic revision and meta-analysis, is an increased recurrence rate in comparison with segmental resection (chance rate [OR] 5.53; $P=0.001$) and with discoid resection (OR 3.83, $P=0.01$)[45].

## Discoid resection

The discoid resection is the technique in which a full thickness resection is performed, restricted to the anterior rectal wall, followed by a sutured closure of the defect created. In cases of unifocal, deep and smaller than 3 cm lesions, discoid resection is considered the first option by many specialists[46,47].

First described for treating deep endometriosis over 25 years ago[48], variations of the technique may or not involve the use of mechanical staplers. When staplers are not used, resection of the full thickness of the rectal wall is performed, including the lesion; the defect created on the wall is then closed by sutures in one or two layers.

One option for the disc resection is the technique described by Gordon in 2001, which uses a circular stapler to repair the defect created by the excision of the lesion[49]. With the person under anesthesia, in the Lloyd-Davies position, and with a urinary catheter in place, the nodule resection is performed, creating a defect on the anterior rectal wall. Two stiches are applied at the extremities of the defect for better handling of the segment. The circular staple is then inserted through the anus and the area of the rectum that contains the defect is placed between the stapler's anvil and cartridge. Once the stapler is fired, the defect is repaired and a resection of the perilesional tissue takes place.

A variation of the procedure uses the circular stapler to simultaneously resect and repair the defect. In this technique, a transfixing stich is applied in the central of the lesion, after its dissection. When the lesion is large, a partial or total resection is recommended, followed by repair of the extremities with a transfixing stich, so that the remaining tissue or defect will be fully included in the treated region. The circular stapler is introduced through the rectum. The nodule is inserted in the stapler's chamber, between the anvil and the cartridge, using the repair stich previously applied to facilitate the positioning. The surgeon handling the stapler must anteriorize the device before completing closure. That way, when the device is fired, the nodule will be resect and the defect on the wall corrected by the stapling line, as illustrated in FIGURE 6.

We have routinely preformed endoscopic evaluation after the stapling, in order not only to verify the integrity of the anastomosis, but also to identify and endoscopically treat any bleeding.

The prospective data analysis of patients who underwent discoid resection in two endometriosis reference centers revealed good short-term results and low complication rates. According to the authors, the average size of the lesions was 30 mm (7 to 70 mm)[50]. Despite the possibility, after a disc resection, of the margins being compromised in up to 40% of cases[51], there are few prospective and controlled studies comparing the technique with segmental resection in regard to late postoperative period, quality of life,

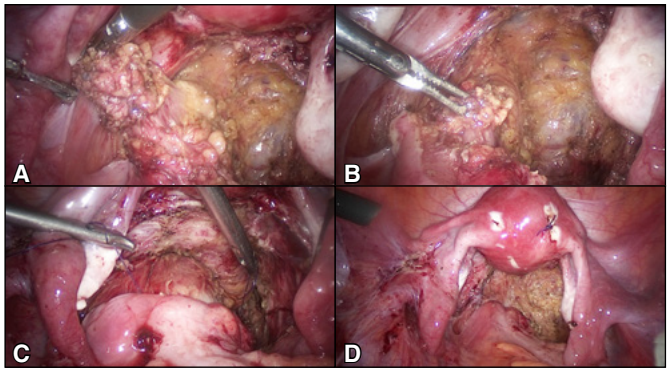



**FIGURE 6.** Discoid resection technique, with stapler. **A)** Dissection of the nodule. **B)** Partial resection of the nodule. **C)** Positioning of the nodule in the stapler by using the suture. **D)** Aspect after stapling.

and persistent remission[27,52]. Retrospective series and control case studies report recurrence rates between 1.8% and 10.4% for patients who underwent discoid resection[30,31,53].

A further possibility in patients with more extensive lesions is the technique of double-transanal circular stapling[54]. Initially, the circular stapling technique described above is performed. After the first firing with a 33 mm stapler, a second firing is performed, with a 29 mm stapler. The aim of the second firing would be to remove residual tissue. Oliveira et al. performed double stapling in 11 out of 120 patients who underwent surgery for rectal endometriosis, with low complication rates, and consider the technique as an option to segmental resection in patients with unifocal lesions measuring between 2 and 4 cm[55]. In our view, since this technique resects a larger area of the anterior rectal wall, it might distort the organ's axis and lead to symptoms such as tenesmus and a sensation of incomplete evacuation, and we find it essential to wait for study results that would support is application from an anatomical and physiological perspective.

Roman et al. described a modification of the discoid resection procedure, called "Rouen technique." This procedure, which also allows for treating lesions bigger than 3 cm, begins with the superficial resection of the nodule, followed by ablation with PlasmaJet®, via laparoscopy. This technique is based on the properties of argon plasma, which presents less lateral heat dissipation, making the resection in contact with the rectal wall safer. Next, stiches are applied to the lesion through the anus, for traction of the target area, and a stapled trans-anal rectal resection is performed using Contour Transtar®[56].

## Segmental resection

Segmental resection is generally indicated in cases of unifocal lesions greater than 3 cm in diameter, two or more infiltrative lesions, or a unifocal lesion infiltrating more than 50% of the intestinal wall[38,57].

Segmental resection for the treatment of endometriosis is often not as extensive, and lymph node draining is not a concern when comparing to oncological resections; therefore, there is rarely the need to mobilize the splenic flexure. Nezhat et al. described for the first time, in 1992, the use of the technique for treating deep endometriosis with intestinal and rectovaginal septum involvement[58].

Bowel preparation for colorectal surgery is controversial. When performed it usually consists of an anterograde preparation, as well as antibiotic prophylaxis – given orally on the day before, and intravenously both intraoperatively and postoperatively, for 24 hours. With the patient under anesthesia, in the Lloyd-Davies position, and with a urinary catheter in place, the uterine manipulator is inserted to facilitate the pelvic approach.

Positioning of the trocars is usually determined by the surgeon, usually with an 11 mm trocar in the umbilical scar, a 12 mm trocar in the right iliac fossa for the linear stapler, and two 5 mm trocars in the left iliac fossa and right flank.

The surgery begins by inspecting the abdominal cavity to identify the affected places and structures; it is important to remember that all segments of the digestive tract may be compromised. In addition to the pelvic organs, the appendix, the terminal ileum, the diaphragm, and the peritoneal surface must also be evaluated.

The next step is the mobilization of the sigmoid colon and identification and preservation of the ureters. The pararectal space is opened, medially to the uterosacral ligament and preserving the nerves and the inferior hypogastric plexus. In segmental resections in endometriosis patients, there is no need for a block resection and sometimes dissection of the rectum is performed through the lesion, leaving endometriotic tissue to be removed after the stapling. This way, the nodule is dissected, and the rectum is sectioned with a linear stapler, ensuring a distal margin of 1 to 2 cm[37]. Afterwards, the eventual residual lesions, which may involve pelvic structures, are dissected and removed.

After the section, the colon can be externalized by extending the incision of one of the trocars, or via a Pfannenstiel incision. The piece may also be removed vaginally, as described by Redwine et al., via an incision in the posterior cul-de-sac[59]. Mechanical anastomosis is performed via double laparoscopic stapling with a circular stapler. Air leak testing is routinely performed, either by insufflating the rectum with air or by intraoperative colonoscopy, in order to assess the quality of the suture. Drainage, whether closed-system or via a Penrose drain, is optional. FIGURE 7 illustrates the surgical steps of the segmental resection.

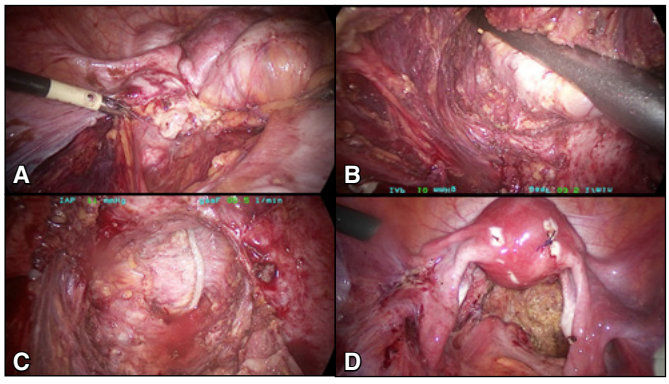



**FIGURE 7.** Segmental resection technique. **A)** Initial aspect. **B)** Linear stapling. **C)** Aspect after linear stapling. **D)** Final aspect after colorectal anastomosis.

Ruffo et al. reviewed 900 colorectal resections for deep endometriosis. The average follow-up period was of 54 months (1 to 120). They observed significant improvement in dyspareunia, constipation, pelvic pain ($P$=0,0001) and diarrhea ($P$=0.004). Non-significant improvement was observed in dysuria and anal bleeding ($P$=0.452 and $P$=0.097, respectively)[60]. Approximately 40% of patients presented postoperative fecal thinning, with gradual improvement over the first year, in most cases[61].

A systematic review of 1889 colorectal resections and 961 rectal resections reported surgery times varying from 101 to 436 minutes. The hospitalization period varied between 4 and 14 days. Pain relief occurred in 71.4% to 93.6% of cases. Symptom recurrence, in the follow-up period of 2 to 5 years, varied between 4 and 54%. Pain recurrence requiring reoperation varied between 0 and 34%. Verified recurrence of endometriosis varied between 0 to 25%. The cumulative rate of spontaneous pregnancy varied between 10% and 13%. The cumulative pregnancy rate, including in-vitro fertilization, was of 18% to 100%[9].

The complete removal of endometriosis foci is more commonly associated with segmental resection, as compared to more conservative techniques[37]. A recent systematic review about histology-verified recurrence at least 12 months after intestinal endometriosis surgery covered 41 studies and selected four for the meta-analysis. According to the authors, the risk of recurrence was significantly lower in segmental or discoid resection when compared to superficial resection[45]. Histopathologic findings, such as the presence of satellite lesions, marginal positivity, and thickness of infiltration, seem to have no relation to recurrence rates or to impact on quality of life[62].

Since segmental resection is associated to higher rates of complete resection and lower recurrence, there is doubt in the literature about the inferiority of intestinal or urinary functional results, compared to techniques that preserve the rectum[37,45]. Roman et al. conducted a randomized study to compare conservative techniques with colorectal resection in 60 patients who presented with lesions varying between 25 and 40 mm, infiltrating the muscle layer, and with 50% of the rectal circumference compromised. The patients who underwent segmental resection presented increased risk of colorectal anastomotic stenosis, requiring dilation. However, conservative surgeries were not superior to the radical surgeries, in the population studied regarding digestive and urinary functions[52]. Data recently published by the same group, with a 5-year follow-up period, also did not show long-term differences in functional results between conservative surgeries and segmental resection[27]. A fertility rate study was conducted with 55 of the 60 patients involved in that study. At the end of the follow-up period, which varied from 50 to 79 months, 81% of the patients were able to get pregnant. The gestation rate after surgical treatment was of 74% for patients considered unfertile, of which 53% were by natural conception[11].

The treatment of multifocal intestinal lesions is subject for debate in the literature. Segmental resection is the treatment recommended by most of the groups and protocols[38,57]. However, nodules that present in the rectum close to the anal verge may lead to functional long-term sequelae in patients who underwent the radical surgery. One option in these cases is to treat the distal lesion by discoid resection, in order to avoid a low colorectal anastomosis. In this scenario, it is important that the resection of the proximal lesion must be done before the discoid resection of the distal lesion, in order to minimize the consequences of manipulating the discoid resection area and reduce the occurrence of fistulas. Additionally, the minimum distance of 5 cm must be observed between the stapling lines to preserve the vascularization of the segment between them. However, this technique is associated with a high incidence of rectovaginal fistulas[33].

### Robotic-assisted resection techniques

Laparoscopic surgery is considered the gold standard in comparison to conventional laparotomy, due to its proven benefits, such as faster recovery, lower hospitalization period, better cosmetic results, and improved conception rates[17]. However, the minimally invasive method presents a series of practical limitations, such as reduced mobility, two-dimensional vision, instability and dependence on the assistant surgeon for the camera, and a narrow angle of work in the pelvis. Robotic surgery, in this context, is a promising option to overcome these hurdles[63].

Soto et al. published a multi-center, prospective, and randomized study with 73 patients, comparing the laparoscopic and robotic-assisted approaches, and did not find a statistical difference between the techniques in regard to operative time, blood loss, conversion to laparotomy, or intra- or postoperative complications. Other studies point in the same direction, suggesting that the higher cost associated with the robotic procedure does not reflect into better surgical outcomes[19,20].

On the opposite direction, there is a discussion about the impact of new robotic platforms and the advancing learning curve in regard to the cost/benefit in colorectal surgery[64]. Therefore, it is possible that technological development and cost reductions may lead to increased use of robotic-assisted methods in greater complexity procedures.

## COMPLICATIONS

The main complications in the surgical treatment of intestinal endometriosis can be categorized into intraoperative, postoperative, and those related to the abdominal laparoscopic procedure.

Complications associated with video laparoscopy are generally rare and are related to establishing the pneumoperitoneum, in 2% of the cases, and to the inadvertent lesion of vessels and organs, or herniations arising from the passage of the trocars, in less than 1.5% of cases.

As in other colorectal surgeries, the incidence of colitis by *Clostridium difficile* has been documented[65].

Potential intra and postoperative complications in intestinal endometriosis are well established and must be considered when choosing between the conservative and the radical surgical approach. Endometriosis is a benign disease that affects young, productive patients that have high expectations of a cosmetic result. Complication rates for the different techniques are hard to estimate, due to the different studies methodology, and to the reports, which mostly mention only immediate outcomes and do not describe late functional results. However, complications seem to be significantly more frequent in the more complex procedures, and with low anastomosis. Case series studies report immediate complications in 15% to 38% of segmental resection patients, 12% to 23% of discoid resection patients, and 3% to 6% of superficial excision patients[66-68].

Renner et al. reported 15% minor complications, and 15.9% major complications, including fistulas, peritonitis, and bleeding requiring transfusion, in 113 patients who underwent segmental resection for deep endometriosis. Transitory urinary dysfunction was described in 22% of the cases, and sexual alterations, such as lessened lubrication during intercourse, were reported in 36% of the operated patients[67]. The same authors published their results for 134 patients operated without intestinal resection and reported lower rates of minor (12.7%) and major (3.7%) complications. Among the minor complications, urinary and sexual alterations were also frequent in patients who underwent conservative surgery[68].

More serious complications, such as urinary and intestinal fis-

tulas, are rare. A French multi-centric study included 1,135 patients who underwent surgery for intestinal endometriosis in 2015. The most common procedure was superficial resection (48.1%), followed by segmental resection (40.4%) and discoid resection (7.3%). Among the most serious complications reported are anastomotic fistulas (0.8%), pelvic abscesses (3.4%), and rectovaginal fistulas, which were more frequent in segmental resections (3.9%) when compared to discoid (3.6%) and superficial resections (1.3%)[34]. A systematic review published by De Cicco et al. reported that 11% of patients who underwent deep endometriosis surgery presented major complications, with anastomotic dehiscence, intestinal fistulas, and severe obstruction occurring in 1.9%, 1.8%, and 2.7% of the patients, respectively. Among minor complications, transitory urinary and intestinal dysfunction were reported in 8.1% and 3.6% of the cases[9].

Ruffo et al., from a cohort of 750 patients of segmental resection, reported incidences of anastomotic dehiscence (3%), rectovaginal fistulas (2%), ureteral lesions (0.7%), and a high number of protective ileostomies (14%). The incidence of unfavorable functional results, such as bowel urgency and incontinence, found in colorectal resections with low anastomosis, were significantly lower[60]. Patients submitted to segmental resection for rectal endometriosis have low reported incidence of low anterior resection syndrome (LARS) compared to surgery for low rectal cancer[10,33]. Advances in technology and in nerve-sparing dissection techniques aim to improve short and long-term functional results.

A study including 364 patients compared complication rates of segmental, discoid, and superficial resection, using the Clavien-Dindo classification[28,69]. More serious complications, such as fistulas and abscesses, classified as Clavien 3b, were reported in 11.8% of the patients, 2/3 of which in the segmental resection group. The main criticism to the retrospective study is the lack of uniformity in extension and severity of the disease among the groups. The clinical presentation of endometriosis was more complex in cases submitted to segmental resection. The authors suggest that, when it is feasible, superficial resection is associated with lower complication rates[28].

Segmental resection is a standardized technique for treating rectal endometriosis that is safe and results in lower residual disease; however, depending on the distance of the nodule in the rectal wall, it is the technique most associated with potentially serious complications and unfavorable intestinal and urinary results. Discoid resection, on the other hand, has a higher probability of residual disease and a smaller incidence of fistulas, but, as it doesn't require mesenteric dissection and ligation, it can present greater intraoperative and immediate postoperative bleeding. Bleeding can be controlled via a low insufflation colonoscopy and clipping or adrenaline injection on the staple line[70]. Superficial resection is associated with the lowest complication rates, both immediate and late, and with higher recurrence rates[31].

Compared to superficial and discoid resections in a retrospective study, more extensive segmental resections had the worst performance rates in various quality of life and bowel function indices, which assessed the occurrence of postoperative constipation, incomplete evacuation, abdominal pain, and straining and pain during defecation[66]. However, the initial and long-term results of two prospective randomized studies carried out by the same group did not show segmental resection as inferior in regard to digestive and urinary outcomes when compared to conservative surgeries[27,52]. Despite the difficulty in analyzing the techniques for treating deep endometriosis separately, a recent systematic review and meta-analysis showed improved quality of life in patients who underwent surgical treatment[8]. TABLE 2 compares the results of different surgical techniques for treating rectal endometriosis, based on different variables.

## CONCLUSION

Surgical treatment of pelvic endometriosis with rectal involvement represents a challenge for surgeons and gynecologists. Choosing which technique to use is still a matter for debate and depends on a perfect preoperative staging and a thorough knowledge of the therapeutic strategies. The complete laparoscopic eradication of the endometriotic tissue is associated with the best results. The decision between a more conservative or radical approach must be made by a multi-disciplinary team, and must be made for each patient, individually. The proposed surgery must take into account the patient's symptoms and expectations, as well as risks, potential complications, recurrence rates, and the surgeon's expertise. Although arguments exist that favor the conservative approach, randomized and controlled studies comparing the various treatment options must inform the decision about what is the best technique for each patient.

### Authors' contribution

Popoutchi P designed the study, collected and analyzed data, drafted the manuscript, revised the article for important medical content and approved the final manuscript draft. Marques Junior OW collected and analyzed data, drafted the manuscript and approved the final manuscript draft. Averbach P designed the study, collected and analyzed data, drafted the manuscript, revised the article for important medical content and approved the final manuscript draft. Cardoso Filho CAM collected and analyzed data. Averbach M designed the study, collected and analyzed data, drafted the manuscript, revised the article for important medical content and approved the final manuscript draft.

### Orcid

Pedro Popoutchi: 0000-0002-2731-278X.
Oswaldo Wiliam Marques Junior: 0000-0002-4589-2749.
Pedro Averbach: 0000-0003-4024-9436.
Celso Augusto Milani Cardoso Filho: 0000-0001-7738-4602.
Marcelo Averbach: 0000-0002-3491-5781.

Popoutchi P, Marques Junior OW, Averbach P, Cardoso Filho CAM, Averbach M. Técnicas cirúrgicas para o tratamento da endometriose do reto: revisão sistemática de ensaios clínicos randomizados e estudos observacionais. Arq Gastroenterol. 2021;58(4):548-59.

**RESUMO** – **Contexto** – A endometriose é uma doença prevalente em mulheres em idade reprodutiva e estimada em até 50% daquelas com infertilidade. O acometimento intestinal é reportado em até um terço dos casos. A doença é relacionada a dor crônica e perda de qualidade de vida, implicando em custos emocionais, sociais e econômicos. O tratamento consiste em bloqueio hormonal e ressecção cirúrgica, com efeitos colaterais e eficácia variáveis. A abordagem cirúrgica da endometriose do reto, conservadora ou radical, é motivo de discussão no que tange a indicação e a melhor técnica a ser empregada. **Objetivo** – Resumir os dados da literatura sobre as indicações, resultados e complicações das técnicas cirúrgicas para o tratamento da endometriose do reto. **Métodos** – Esta revisão sistemática abrangente é uma seleção de estudos da literatura e sua discussão, realizada por equipe com experiência no tratamento cirúrgico da endometriose intestinal, sobre as indicações, resultados e complicações das técnicas conservadoras, ressecção superficial e discoide, e radical para o tratamento cirúrgico da endometriose do reto. Foi realizada uma estratégia de busca nas bases de dados PubMed, EMBASE, e CENTRAL até maio de 2021 para identificar ensaios clínicos randomizados e estudos observacionais que compararam pelo menos duas das três técnicas cirúrgicas de interesse (i.e., shaving, ressecção discóide, ressecção segmental). **Resultados** – Um ensaio clínico randomizado e nove séries de casos, com um total de 3.327 pacientes, preencheram os critérios de elegibilidade da revisão. A idade dos participantes variou de uma média de 30,0 a 37,9 anos. O seguimento médio variou de 1,2 a 42,76 meses. Referente à qualidade metodológica, no geral os estudos incluídos apresentaram baixo risco de viés na maioria dos domínios avaliados. O tratamento cirúrgico das pacientes com endometriose do reto está indicado para as pacientes com sintomas obstrutivos e naquelas com escores de dor acima de 7/10. As pacientes com doença além da camada muscular própria do reto, documentada por meio de ressonância magnética ou ultrassonografia pélvica transvaginal com preparo intestinal, são candidatas a ressecção discoide ou segmentar. A presença de doença multifocal, extensão maior de 3 cm e infiltração maior 50% da circunferência da alça favorecem a técnica radical. A altura da lesão em relação a borda anal, idade, sintomatologia e desejo reprodutivo são outros fatores que podem influenciar na escolha da técnica a ser empregada. O risco de complicações e resultados funcionais desfavoráveis parecem estar relacionados diretamente a complexidade do procedimento. **Conclusão** – A escolha da técnica cirúrgica, conservadora ou radical, realizada pela via laparoscópica, para o tratamento da endometriose do reto é motivo de discussão e depende não somente do estadiamento pré-operatório, mas também das expectativas da paciente, dos riscos e potenciais complicações, das taxas de recorrência e da expertise da equipe multidisciplinar.

**Palavras-chave** – Tratamento; reto; endometriose.

## REFERENCES

1. Wolthuis AM, Meuleman C, Tomassetti C, D'Hooghe T, de Buck van Overstraeten A, D'Hoore A. Bowel endometriosis: colorectal surgeon's perspective in a multidisciplinary surgical team. World J Gastroenterol. 2014;20:15616-23.
2. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med. 2020;382:1244-56.
3. Vercellini P, Crosignani PG, Somigliana E, Berlanda N, Barbara G, Fedele L. Medical treatment for rectovaginal endometriosis: what is the evidence? Hum Reprod. 2009;24:2504-14.
4. Vercellini P, Pietropaolo G, De Giorgi O, Pasin R, Chiodini A, Crosignani PG. Treatment of symptomatic rectovaginal endometriosis with an estrogen-progestogen combination versus low-dose norethindrone acetate. Fertil Steril. 2005;84:1375-87.
5. Ferrero S, Camerini G, Ragni N, Venturini PL, Biscaldi E, Remorgida V. Norethisterone acetate in the treatment of colorectal endometriosis: a pilot study. Hum Reprod. 2010;25:94-100.
6. Vercellini P, Somigliana E, Consonni D, Frattaruolo MP, De Giorgi O, Fedele L. Surgical versus medical treatment for endometriosis-associated severe deep dyspareunia: I. Effect on pain during intercourse and patient satisfaction. Hum Reprod. 2012;27:3450-9.
7. Vercellini P, Buggio L, Berlanda N, Barbara G, Somigliana E, Bosari S. Estrogen-progestins and progestins for the management of endometriosis. Fertil Steril. 2016;106:1552-71.
8. Arcoverde FVL, Andres MP, Borrelli GM, Barbosa PA, Abrão MS, Kho RM. Surgery for Endometriosis Improves Major Domains of Quality of Life: A Systematic Review and Meta-Analysis. J Minim Invasive Gynecol. 2019;26:266-78.
9. De Cicco C, Corona R, Schonman R, Mailova K, Ussia A, Koninckx P. Bowel resection for deep endometriosis: a systematic review. BJOG. 2011;118:285-91.
10. Riiskjaer M, Greisen S, Glavind-Kristensen M, Kesmodel US, Forman A, Seyer-Hansen M. Pelvic Organ Function Before and After Laparoscopic Bowel Resection for Rectosigmoid Endometriosis: A Prospective, Observational Study. BJOG. 2016;123:1360-7.
11. Roman H, Chanavaz-Lacheray I, Ballester M, Bendifallah S, Touleimat S, Tuech JJ, et al. High Postoperative Fertility Rate Following Surgical Management of Colorectal Endometriosis. Hum Reprod. 2018;33:1669-76.
12. Bazot M, Lafont C, Rouzier R, Roseau G, Thomassin-Naggara I, Daraï E. Diagnostic accuracy of physical examination, transvaginal sonography, rectal endoscopic sonography, and magnetic resonance imaging to diagnose deep infiltrating endometriosis. Fertil Steril. 2009;92:1825-33.
13. Guerriero S, Ajossa S, Orozco R, Perniciano M, Jurado M, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in the rectosigmoid: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2016;47:281-9.
14. Roman H, Vassilieff M, Gourcerol G, Savoye G, Leroi AM, Marpeau L, et al. Surgical management of deep infiltrating endometriosis of the rectum: pleading for a symptom-guided approach. Hum Reprod. 2011;26:274-81.
15. Abrão MS, Andres MP, Barbosa RN, Bassi MA, Kho RM. Optimizing Perioperative Outcomes with Selective Bowel Resection Following an Algorithm Based on Preoperative Imaging for Bowel Endometriosis. J Minim Invasive Gynecol. 2020;27:883-91. doi: 10.1016/j.jmig.2019.06.010.
16. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, Bie B, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29:400-12.
17. Touboul C, Ballester M, Dubernard G, Zilberman S, Thomin A, Daraı E. Long-term symptoms, quality of life, and fertility after colorectal resection for endometriosis: extended analysis of a randomized controlled trial comparing laparoscopically assisted to open surgery. Surg Endosc. 2015;29:1879-87.
18. Averbach M, Popoutchi P, Marques OW Jr, Abdalla RZ, Podgaec S, Abrão MS. Robotic rectosigmoidectomy - pioneer case report in Brazil. Current scene in colorectal robotic surgery. Arq Gastroenterol. 2010;47:116-8.
19. Berlanda N, Frattaruolo MP, Aimi G, Farella M, Barbara G, Buggio L, Vercellini P. 'Money for nothing'. The role of robotic-assisted laparoscopy for the treatment of endometriosis. Reprod Biomed Online. 2017;35:435-44.
20. Soto E, Luu TH, Liu X, Magrina JF, Wasson MN, Einarsson JI, et al. Laparoscopy vs. Robotic Surgery for Endometriosis (LAROSE): a multicenter, randomized, controlled trial. Fertil Steril. 2017;107:996-1002.
21. Higgins JPT, Green S, eds. Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011: The Cochrane Collaboration, 2011. [Internet]. Available from: http://handbook.cochrane.org/.

22. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. BMJ. 2009;339:b2535.
23. Guyatt GH, Busse JW. Modification of Cochrane Tool to assess risk of bias in randomized trials. [Internet]. Available from: http://distillercer.com/resources/.
24. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE Working Group. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336:924-6.
25. Guyatt GH, Busse JW. Modification of Ottawa-Newcastle to assess risk of bias in nonrandomized trials. [Internet]. Available from: http://distillercer.com/resources/.
26. Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D, CARE Group. The CARE guidelines: consensus-based clinical case report guideline development. J Clin Epidemiol. 2014;67:46-51.
27. Roman H, Tuech JJ, Huet E, Bridoux V, Khalil H, Hennetier C, et al. Excision Versus Colorectal Resection in Deep Endometriosis Infiltrating the Rectum: 5-year Follow-Up of Patients Enrolled in a Randomized Controlled Trial. Hum Reprod. 2019;34:2362-71.
28. Abo C, Moatassim S, Marty N, Saint Ghislain M, Huet E, Bridoux V, et al. Postoperative Complications After Bowel Endometriosis Surgery by Shaving, Disc Excision, or Segmental Resection: A Three-Arm Comparative Analysis of 364 Consecutive Cases. Fertil Steril. 2018;109:172-8.
29. Ballester M, Roman H, Mathieu E, Touleimat S, Belghiti J, Daraï E. Prior colorectal surgery for endometriosis-associated infertility improves ICSI-IVF outcomes: results from two expert centres. Eur J Obstet Gynecol Reprod Bio. 2016;209:95-9.
30. Fanfani F, Fagotti A, Gagliardi ML, Ruffo G, Ceccaroni M, Scambia G, et al. Discoid or segmental rectosigmoid resection for deep infiltrating endometriosis: a case-control study. Fertil Steril. 2010;94:444-9.
31. Hernández Gutiérrez A, Spagnolo E, Zapardiel I, Zapardiel I, Seivane RGA, Carrasco AL, et al. Post-operative complications and recurrence rate after treatment of bowel endometriosis: Comparison of three techniques. Eur J Obstet Gynecol Reprod Biol X. 2019;4:100083.
32. Hudelist G, Aas-Eng MK, Birsan T, Berger F, Sevelda U, Kirchner L, et al. Pain and fertility outcomes of nerve-sparing, full-thickness disk or segmental bowel resection for deep infiltrating endometriosis-A prospective cohort study. Acta Obstet Gynecol Scand. 2018;97:1438-46.
33. Millochau JC, Stochino-Loi E, Darwish B, Abo C, Coget J, Chati R, et al. Multiple Nodule Removal by Disc Excision and Segmental Resection in Multifocal Colorectal Endometriosis. J Minim Invasive Gynecol. 2018;25:139-46.
34. Roman H. A National Snapshot of the Surgical Management of Deep Infiltrating Endometriosis of the Rectum and Colon in France in 2015: A Multicenter Series of 1135 Cases. J Gynecol Obstet Hum Reprod. 2017;46:159-65.
35. Roman H, Bridoux V, Merlot B, Resch B, Chati R, Coget J, et al. Risk of bowel fistula following surgical management of deep endometriosis of the rectosigmoid: a series of 1102 cases. Hum Reprod. 2020;35:1601-11.
36. Reich H, McGlynn F, Salvat J. Laparoscopic treatment of cul-de-sac obliteration secondary to retrocervical deep fibrotic endometriosis. J Reprod Med. 1991;36:516-22.
37. Donnez O, Roman H. Choosing the right surgical technique for deep endometriosis: shaving, disc excision, or bowel resection? Fertil Steril. 2017;108:931-42.
38. Desplats V, Vitte RL, du Cheyron J, Roseau G, Fauconnier A, Moryoussef F. Preoperative rectosigmoid endoscopic ultrasonography predicts the need for bowel resection in endometriosis. World J Gastroenterol. 2019;25:696-706.
39. Nisolle M, Brichant G, Tebache L. Choosing the Right Technique for Deep Endometriosis. Best Pract Res Clin Obstet Gynaecol. 2019;59:56-65.
40. Fatehchehr S, Macik P, Sinervo K. CO2 Laser Shaving Technique for Resection of Rectosigmoid Endometriosis. J Minim Invasive Gynecol. 2015;22:S138.
41. Donnez J, Squifflet J. Laparoscopic excision of deep endometriosis. Obstet Gynecol Clin North Am. 2004;31:567-ix.
42. Donnez J, Squifflet J. Complications, pregnancy and recurrence in a prospective series of 500 patients operated on by the shaving technique for deep rectovaginal endometriotic nodules. Hum Reprod. 2010;25:1949-58.
43. Roman H, Moatassim-Drissa S, Marty N, Milles M, Vallée A, Desnyder E, et al. Rectal shaving for deep endometriosis infiltrating the rectum: a 5-year continuous retrospective series. Fertil Steril. 2016;106:1438-45.
44. Roman H, Milles M, Vassilieff M, Resch B, Tuech JJ, Huet E, et al. Long-term functional outcomes following colorectal resection versus shaving for rectal endometriosis. Am J Obstet Gynecol. 2016;215:762.
45. Bendifallah S, Vesale E, Daraï E, Thomassin-Naggara I, Bazot M, Tuech JJ, et al. Recurrence after Surgery for Colorectal Endometriosis: A Systematic Review and Meta-analysis. J Minim Invasive Gynecol. 2020;27:441-51.
46. de Almeida A, Fernandes LF, Averbach M, Abrão MS. Disc resection is the first option in the management of rectal endometriosis for unifocal lesions with less than 3 centimeters of longitudinal diameter. Surg Technol Int. 2014;24:243-8.
47. Jayot A, Bendifallah S, Abo C, Arfi A, Owen C, Darai E. Feasibility, Complications, and Recurrence After Discoid Resection for Colorectal Endometriosis: A Series of 93 Cases. J Minim Invasive Gynecol. 2020;27:212-9.
48. Nezhat C, Nezhat F, Pennington E, Nezhat CH, Ambroze W. Laparoscopic disk excision and primary repair of the anterior rectal wall for the treatment of full-thickness bowel endometriosis. Surg Endosc. 1994;8:682-5.
49. Gordon SJ, Maher PJ, Woods R. Use of the CEEA stapler to avoid ultra-low segmental resection of a full-thickness rectal endometriotic nodule. J Am Assoc Gynecol Laparosc. 2001;8:312-6.
50. Abo C, Bendifallah S, Jayot A, Timoh KN, Tuech J-J, Roman H, et al. Discoid Resection for Colorectal Endometriosis: Results From a Prospective Cohort From Two French Tertiary Referral Centres. Colorectal Dis. 2019;21:1312-20.
51. Roman H, Opris I, Resch B, Tuech JJ, Sabourin JC, Marpeau L. Histopathologic features of endometriotic rectal nodules and the implications for management by rectal nodule excision. Fertil Steril. 2009;92:1250-2.
52. Roman H, Bubenheim M, Huet E, Bridoux V, Zacharopoulou C, Daraï E, et al. Conservative Surgery Versus Colorectal Resection in Deep Endometriosis Infiltrating the Rectum: A Randomized Trial. Hum Reprod. 2018;33:47-57.
53. Roman H, Darwish B, Bridoux V, Chati R, Kermiche S, Coget J, et al. Functional outcomes after disc excision in deep endometriosis of the rectum using transanal staplers: a series of 111 consecutive patients. Fertil Steril. 2017;107:977-86.
54. Kondo W, Ribeiro R, Zomer MT, Hayashi R. Laparoscopic Double Discoid Resection With a Circular Stapler for Bowel Endometriosis. J Minim Invasive Gynecol. 2015;22:929-31.
55. Oliveira MA, Crispi CP, Oliveira FM, Junior PS, Raymundo TS, Pereira TD. Double Circular Stapler Technique for Bowel Resection in Rectosigmoid Endometriosis J Minim Invasive Gynecol. 2014;21:136-41.
56. Roman H, Tuech JJ. Laparoscopic and transanal excision of large lower- and mid-rectal deep endometriotic nodules: the Rouen technique. Fertil Steril. 2014;102:e7.
57. Kho RM, Andres MP, Borrelli GM, Neto JS, Zanluchi A, Abrão MS. Surgical treatment of different types of endometriosis: Comparison of major society guidelines and preferred clinical algorithms. Best Pract Res Clin Obstet Gynaecol. 2018;51:102-10.
58. Nezhat C, Nezhat F, Pennington E. Laparoscopic treatment of infiltrative rectosigmoid colon and rectovaginal septum endometriosis by the technique of videolaparoscopy and the CO2 laser. Br J Obstet Gynecol. 1992;99:664-7.
59. Redwine DB, Koning M, Sharpe DR. Laparoscopically assisted transvaginal segmental resection of the rectosigmoid colon for endometriosis. Fertil Steril. 1996;65:193-7.
60. Ruffo G, Sartori A, Crippa S, Partelli S, Barugola G, Manzoni A, et al. Laparoscopic rectal resection for severe endometriosis of the mid and low rectum: technique and operative results. Surg Endosc. 2012;26:1035-40.
61. Bassi MA, Andres MP, Bassi CM, Neto JS, Kho RM, Abrão MS. Postoperative Bowel Symptoms Improve over Time after Rectosigmoidectomy for Endometriosis. [published online ahead of print, 2019 Oct 24]. J Minim Invasive Gynecol. 2019;S1553-4650(19)31261-0.
62. Mabrouk M, Spagnolo E, Raimondo D, Malvi D, Catena F, Ferrini G, et al. Segmental bowel resection for colorectal endometriosis: is there a correlation between histological pattern and clinical outcomes? Hum Reprod. 2012;27:1314-9.
63. Gianardi D, Giannini A. Minimally invasive surgery for deep-infiltrating endometriosis and its impact on fertility: can robotic surgery play a role? J Robot Surg. 2019;13:789-90.
64. Palmeri M, Di Franco G, Furbetta N, Morelli L. Comment on: 'Money for nothing'. The role of robotic-assisted laparoscopy for the treatment of endometriosis. J Robot Surg. 2019;13:529-30.
65. Damle RN, Cherng NB, Flahive JM, Davids JS, Maykel JA, Sturrock PR, et al. Clostridium difficile infection after colorectal surgery: a rare but costly complication. J Gastrointest Surg. 2014;18:1804-11.
66. Roman H, Vassilieff M, Tuech JJ, Huet E, Savoye G, Marpeau L, et al. Postoperative digestive function after radical versus conservative surgical philosophy for deep endometriosis infiltrating the rectum. Fertil Steril. 2013;99:1695-704.
67. Renner SP, Kessler H, Topal N, Proske K, Adler W, Burghaus S, et al. Major and Minor Complications After Anterior Rectal Resection for Deeply Infiltrating Endometriosis. Arch Gynecol Obstet. 2017;295:1277-85.
68. Lermann J, Topal N, Adler W, Hildebrandt T, Renner SP, Beckmann MW, et al. Major and Minor Complications After Resection Without Bowel Resection for Deeply Infiltrating Endometriosis. Arch Gynecol Obstet. 2018;298:991-9.
69. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240:205-13.
70. Shamiyeh A, Szabo K, Ulf Wayand W, Zehetner J. Intraoperative endoscopy for the assessment of circular-stapled anastomosis in laparoscopic colon surgery. Surg Laparosc Endosc Percutan Tech. 2012;22:65-7.

REVIEW

AG-2021-95
doi.org/10.1590/S0004-2803.202100000-98

# Management of ileocecal Crohn's disease during surgical treatment for acute appendicitis: a systematic review

Abel Botelho **QUARESMA**[1], Eron Fabio **MIRANDA**[2] and Paulo Gustavo **KOTZE**[2,3]

Received: 1 May 2021
Accepted: 23 June 2021

**ABSTRACT** – **Background** – In many patients, the diagnosis of Crohn's disease (CD) is made during surgery for appendicitis in urgent settings. Intraoperative diagnosis can be challenging in certain cases, especially for less experienced surgeons. **Objective** – Review of the literature searching for scientific evidence that can guide surgeons through optimal management of ileocecal CD found incidentally in surgery for acute appendicitis (AA). **Methods** – Included studies were identified by electronic search in the PubMed database according to the Preferred Items of Reports for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The quality and bias assessments were performed by Methodological Index for Non-Randomized Studies (MINORS) criteria for non-randomized studies. **Results** – A total of 313 studies were initially identified, six of which were selected (all retrospective) for qualitative assessment (two studies were comparative and four only descriptive case series). Four studies identified a high rate of complications when appendectomy or ileocolectomy were performed and in only one, there was no increased risk of postoperative complications with appendectomy. In the sixth study, diarrhea, previous abdominal pain, preoperative anemia and thrombocytopenia were independent predictors for CD in patients previously operated for suspected AA. **Conclusion** – Despite the paucity of data and low quality of evidence, a macroscopically normal appendix should be preserved in the absence of complicated disease when CD is suspected in surgery for AA. Ileocecal resections should be reserved for complicated disease (inflammatory mass, ischemia, perforation or obstruction). Further prospective studies are needed to confirm these claims.

**Keywords** – Crohn disease; colorectal surgery; appendectomy; systematic review.

## INTRODUCTION

Crohn's disease (CD) is associated to an individual and phenotypic heterogeneity[1] despite various diagnostic methods (clinical, endoscopic, radiological and histological), its definitive diagnosis can be complex in many situations. In urgent settings, the challenge can be more significant, as patients with ileocecal CD not previously diagnosed often have right lower quadrant (RLQ) pain as an initial manifestation and clinical and laboratory parameters which are often compatible with acute appendicitis (AA)[2] unexpected inflammatory masses of uncertain etiology and indistinguishable appearance can also be found in emergency surgical procedures, further complicating surgeons' intraoperative decisions[3].

In many patients, the diagnosis of CD is made during emergency surgery on suspicion of AA. Often an inflammatory condition reaching the appendix and the ileocecal region can place CD as a differential diagnosis. Intraoperative diagnosis can become a challenge in some cases, especially for less experienced surgeons[3-5].

Intraoperative surgical decisions can be controversial regarding the management of acute presentation of CD in the ileocecal region, mimicking AA. Paradoxically, in a British survey, surgeons seemed more conservative in their approach as compared to gastroenterologists in CD management under these conditions[6].

In this scenario, surgeons need to develop the ability to decide what to do and not to do in order to minimize postoperative complications. The aim of this systematic review is to analyze the scientific evidence wich can guide surgeons towards optimal management of ileocecal CD found incidentally in surgical procedures of suspected AA.

## METHODS

### Search strategy

This qualitative systematic review (SR) was performed according to the Reporting Preferred Item Guidelines for Systematic Reviews and Meta-Analysis (PRISMA). A complete (unregistered) protocol for SR was performed to meet the objectives. Included studies were identified by electronic search in the Medline database via PubMed [https://www.ncbi.nlm.nih.gov/pubmed/], a comprehensive bibliographic search was carried out on August 19, 2020 followed by an update on March 12, 2021, by title and summary (title and abstract) using Health Sciences Descriptors (DeCS) developed from the Medical Subject Headings (MeSH) of the US National Library of Medicine (NLM) in the following sequence name: ((appendicitis [title/abstract]) OR (appendicectomy [title/abstract])) AND (Crohn's disease [title/abstract]).

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
[1] Universidade do Oeste de Santa Catarina, Joaçaba, SC, Brasil. [2] Pontifícia Universidade Católica do Paraná, Unidade de Cirurgia Colorretal, Curitiba, PR, Brasil. [3] Pontifícia Universidade Católica do Paraná, Faculdade de Medicina, Programa de Pós-Graduação em Ciências da Saúde, Curitiba, PR, Brasil.
Corresponding author: Abel Botelho Quaresma. E-mail: abel@proctoclinic.com.br

### Eligibility and inclusion/exclusion criteria

All terms were searched as keywords, when available. The search results were selected for potentially relevant studies by title and abstract, followed by the full-text review of the pre-selected publications.

References to relevant publications such as reviews have been searched and cross-referenced manually to appropriate inclusion of additional publications. Studies which were published in full in the peer-reviewed literature were included and review articles, editorials, guidelines, errata and case reports were excluded.

### Selection of studies and data collection process

The citations generated by electronic searches on Pubmed were imported into another specific database. After removal of duplicates, the title and abstract of all identified citations were reviewed (first check). The full publications of potentially relevant citations included in the first check were subsequently examined (second check) for inclusion/exclusion, applying the eligibility criteria listed above. Any disagreements related to eligibility or interpretation were resolved by consensus.

Data extraction and quality control were performed independently by two reviewers (Quaresma AB, Miranda EF), with additional corroboration by an expert reviewer (Kotze PG). Any disagreements were resolved by consensus.

### Evaluation of the methodological quality of the studies

The methodology of the selected studies was assessed using the Methodological Index for Non-Randomized Studies (MINORS)[7] for non-randomized clinical trials and observational studies. The risk of bias was judged to be "0" (not reported), "1" (reported, but inappropriately) or "2" (reported appropriately). The ideal final score would be 16 for non-comparative studies and 24 for comparative studies.

## RESULTS

The PRISMA flowchart derived from the search is illustrated in FIGURE 1. A total of 298 articles were found in the PubMed database, with another 15 articles being added with internet search and analysis of citations, with a total of 313 articles initially evaluated. From those, four were excluded for being duplicates and 254 for not having a direct relationship with the subject. From the 55 that were initially considered, 49 were excluded after reading the full text (44 for not focusing on the proposed topic and five for being case reports). Six studies were finally included for the qualitative analysis. TABLE 1 summarizes the included studies with their main characteristics.

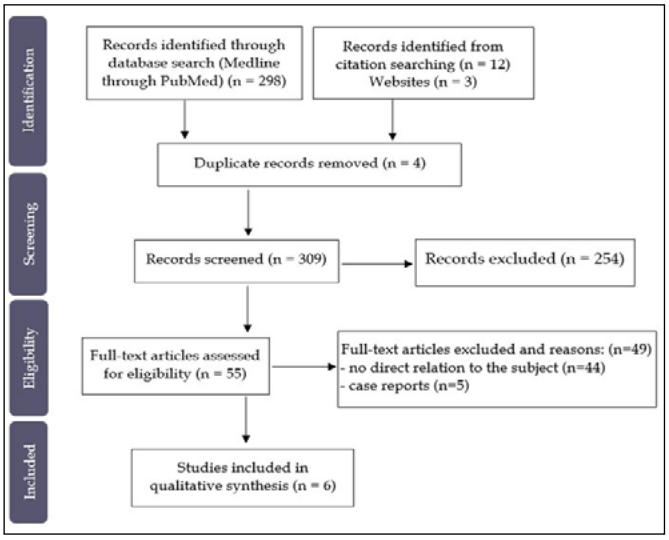



**FIGURE 1.** Systematic review PRISMA flow diagram.

**TABLE 1.** Included studies.

| Author | Journal | Year | Type of study | Cohort / Treated | Main objectives |
|---|---|---|---|---|---|
| Agha et al.[5] | Am J Roentgenol | 1987 | Retrospective single-center | 25/25 | To correlate the characteristics for preoperative diagnosis and postoperative complications |
| Riseman et al.[8] | Arch Surg | 1989 | Retrospective single-center | 1445/13 | Assess management of unexpected ileocecal mass during AA operation |
| Oren et al.[9] | J Clin Gastroenterol | 1992 | Retrospective single-center | 12/12 | To find preoperative factors and postoperative complications |
| Amaral et al.[10] | Rev. Bras. Colo-Proct | 1992 | Retrospective single-center | 94/11 | To analyze postoperative surgical outcomes |
| Smida et al.[11] | Tubis Med | 2016 | Retrospective single-center | 38/4 | To analyze postoperative surgical outcomes |
| Chen et al.[12] | Z Honghua Medical Journal | 2016 | Retrospective single-center | 112/28 | To analyze characteristics of surgical management |

AA: acute appendicitiss.

Agha et al.[5], analyzed 25 patients with AA as the initial manifestation of CD, being responsible for 1.8% of all patients with AA who underwent surgery. Preoperative radiological studies showed abnormalities in 18/25 (72%) cases, indicating the presence of AA or periapendicular abscess, but not the specific diagnosis of CD as the main cause for surgical indication. Histopathological evidence of isolated, transmural or granulomatous AA was found in 20 patients; two of them had local recurrence within 3 years after surgery, while another 18 remained asymptomatic during follow-up of up to 8 years. In the other five patients, CD caused AA with concomitant inflammation of the cecum or terminal ileum; three of these cases were complicated by progressive granulomatous ileocolitis in 2 years.

Riseman et al.[8], identified 13 patients who had a right hemicolectomy performed for unexpected inflammatory masses in which neoplasms, diverticular disease or inflammatory bowel disease could not be differentiated from severe AA during laparotomy. Seven patients had a diagnosis of appendicular phlegmon on the final pathological examination. The other patients had CD, typhlitis or neoplasia. The right hemicolectomy was performed with 7% morbidity and 7% mortality in all patients.

Oren et al.[9], studied 12 patients who underwent laparotomy for suspected AA in which CD was found in the terminal ileum. Appendectomy was performed on all, although only four patients had a severely inflamed appendix. Postoperative complications, abscesses or fistula, occurred in 4 (33%) patients. Detailed investigation of the records revealed some preoperative diagnostic clues for a diagnosis of CD: history of recurrent abdominal pain and/or diarrhea (83%), physical examination revealing normal temperature (50%) and laboratory results compatible with a chronic process such as microcytic anemia (33%) and hypoproteinemia/hypoalbuminemia/hypocholesterolemia (50%).

Amaral et al.[10], studied 94 patients with CD, of which 11 (11.7%) had a suggestive picture of AA who were compared to controls with an incidence of appendicitis of 11%. Three patients presented with AA prior to the diagnosis of CD. All were submitted to appendectomy. In three patients the condition of AA was simultaneous to the diagnosis. Two of these patients underwent appendectomy and one underwent resection of the terminal ileum and cecum with primary anastomosis. In five patients, AA occurred on average 5.8 years after the diagnosis of CD. Four patients underwent appendectomy and one underwent resection of the terminal ileum and cecum with primary anastomosis. All operated patients for suspected AA, regardless of the surgical procedure, presented uneventful evolution.

Smida et al.[11], retrospectively evaluated 38 patients, who underwent a surgical resection for CD. The indications, the type of intervention, duration of preoperative and postoperative complications and the general prognosis of the disease were studied. Of the 38 patients with CD who needed surgery, 17 underwent emergency surgery, and in 11 surgery was the reason which made the diagnosis possible. The average duration of symptoms before surgery was 1.5 years. The most common indication for emergency surgery was acute intestinal obstruction (n=6) followed by perforation and peritonitis (n=5). A misdiagnosis of appendicitis was found in four patients and severe acute colitis complicated by undiagnosed CD was found in two cases. Conventional open surgery was performed for 15 patients. Ileocolic resection was the most common intervention. There was one death and five cases with postoperative complications. The average postoperative hospital stay was 14 days (range, 4 to 60 days). Six patients required a second operation during follow-up.

Chen et al.[12], in the study with the largest sample of patients, described the clinical manifestations and independent diagnostic predictive factors for CD in patients initially diagnosed as appendicitis and treated by surgery. Twenty eight patients were identified (Group CD) and, for each case of CD, three controls with a confirmed diagnosis of appendicitis were matched [Group appendicitis (n=84)]. Clinical manifestations and results of laboratory tests of the two groups were analyzed with multivariable logistic regression to determine independent diagnostic predictive factors for CD initially misdiagnosed as AA. A total of 112 patients were included, with a male/female ratio of 1.04:1 (57:55 and average age of 36 years. Multivariate analysis demonstrated that the change in bowel habits and stool consistency (OR=36.712, 95%CI: 1.672–806,103, *P*=0.022), medical history of chronic abdominal pain or diarrhea (OR=60.142, 95%CI: 4.501–803.573, *P*=0.002), lower preoperative hemoglobin level (OR=0.909, 95%CI: 0.858–0.963, *P*=0.001) and higher platelet count (OR=1.027, 95%CI: 1.007–1.047, *P*=0.008) were independent predictive factors for CD.

TABLE 2 describes the categorization by the MINORS criteria

**TABLE 2.** Methodological Index for non-randomized studies[13] for evaluation of non-randomized clinical trials and observational studies included in the systematic review.

| MINORS criteria | Agha 1987[5] | Riseman 1989[8] | Oren 1992[9] | Amaral 1992[10] | Smida 2016[11] | Chen 2016[12] |
|---|---|---|---|---|---|---|
| A clearly stated aim | 2 | 2 | 2 | 2 | 2 | 2 |
| Inclusion of consecutive patients | 2 | 2 | 2 | 2 | 2 | 2 |
| Prospective collection of data | 0 | 0 | 0 | 0 | 0 | 0 |
| Endpoints appropriate to the aim the study | 2 | 2 | 1 | 2 | 2 | 2 |
| Unbiased assessment of the study endpoint | 2 | 1 | 1 | 2 | 1 | 2 |
| Follow-up period appropriate to the aim the study | 2 | 2 | 2 | 2 | 2 | 2 |
| Loss to follow-up less than 5% | 0 | 0 | 0 | 0 | 0 | 0 |
| Prospective calculation of the study size | 0 | 0 | 0 | 0 | 0 | 1 |
| **Additional criteria in the case of comparative study** | | | | | | |
| An adequate control group | – | – | – | 1 | – | 2 |
| Contemporary groups | – | – | – | 2 | – | 2 |
| Baseline equivalence of groups | – | – | – | 2 | – | 2 |
| Adequate statistical analyses | – | – | – | 0 | – | 2 |
| Total | 10 | 9 | 8 | 15 | 7 | 19 |

The items are scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate). The global ideal score being 16 for non-comparative studies and 24 for comparative studies.

for the six included studies. As noted, most studies have a significant bias, demonstrating the controversy of the topic. Comparative studies had higher rates, but far from the maximum score for quality evidence.

## DISCUSSION

Acute appendicitis remains the most common cause of emergency abdominal surgery[14]. CD commonly affects a similar age group and has a similar initial clinical condition[15]. Thus, it is essential to quickly establish the diagnosis so that the correct treatment can be started as early as possible. According to a review evaluating 74 articles with 2007 patients, rates of incorrect diagnoses and postoperative complications in the past two decades have remained high[16]. The percentage of patients with CD diagnosed incorrectly before surgery was 50.8±30.9% (578/1,268). Authors reported that large reference centers have a relatively better capacity for surgical treatment than centers with less experience. When surgical treatment is indicated for suspected AA, physicians should be aware of the possibility of diagnostic difficulties and complications due to poorly indicated or not properly conducted surgery[17,18]. Thus, postponing surgery in 12/24 h in clinical conditions which are compatible with uncomplicated appendicitis does not increase complication rates and may eventually help in reducing errors in differential diagnosis[19].

This systematic review identified only 6 articles dealing specifically with the subject, most of them with limited sample and low level of evidence. Chen et al.[12], aimed to describe independent diagnostic predictive factors for the correct diagnosis of CD misdiagnosed as appendicitis and treated by surgery. From the 112 patients included, there were no differences related to sex, age and body temperature between the CD and the appendicitis groups (all $P>0.05$). However, changes in bowel habits and stool consistency, medical history of chronic abdominal pain or diarrhea, anemia and increased platelet count have been implicated as predisposing diagnostic factors. These findings can guide surgeons towards a greater suspicion of a CD diagnosis, which can assist in better surgical planning in the preoperative period.

Even with the preoperative diagnosis of AA, intraoperative findings of an inflammatory process in the cecal region is sometimes difficult to differentiate from other causes, given that complicated AA can also cause an intense inflammatory process in this topography. It is up to the surgeon to consider the diagnostic hypothesis of CD, as well as other diseases which affect the terminal ileum, such as infectious or parasitic enteritis (cytomegalovirus, salmonellosis, tuberculosis, actinomycosis, yersiniosis)[2,20]. The presence of a mass in the ileocecal and appendicular region should make the surgeon think equally about the possibility of inflammation and neoplasia from the cecum or the appendix[21]. Periapendicular abscesses can also be found in neoplasms[22]. In such controversial situations, (especially in the presence of sepsis or perforation), intestinal resection by laparoscopy or even the need for conversion to laparotomy may be necessary and the diagnosis will only be established in the histopathological report after a complete analysis of the resected surgical specimen[23].

Complicated appendicitis can be managed laparoscopically by experienced surgeons, with significant advantages including lower overall complications, readmission and small bowel obstruction rates, surgical site infections and faster recovery. Laparoscopy for complicated appendicitis can be performed with low-cost equipment, leading to significantly lower overall costs as compared to open surgery, in association with shorter duration of hospital admission[19].

According to the "2020 update to WSES Jerusalem guideline"[24], the 2010 guidelines of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) and the 2016 guideline of the European Association of Endoscopic Surgery (EAES), an appendectomy is recommended in the case of a normal-looking appendix during surgery for suspected AA. On the other hand, Sørensen et al., performed a retrospective analysis of patients undergoing diagnostic laparoscopy due to the clinical suspicion of AA where no other pathology was found, and the appendix was not removed[25]. From 271 included patients, 56 (20.7%) were readmitted with RLQ pain after an average time of 10 months. Twenty-two (8.1%) patients underwent a new laparoscopic procedure, and the appendix was removed in 18, of which only one had histological signs of inflammation. Based on the results of this study, authors did not consider it necessary to remove a macroscopically normal appendix during laparoscopy for clinical suspicion of AA. Despite this, authors of the "Jerusalem guideline" of 2020, recommended the removal of the appendix if it appears "normal" during surgery and no other disease is found in symptomatic patients [Quality of evidence: low; recommendation strength: weak; 2C][24]. It is important to note that the aforementioned considerations refer to overall clinical scenarios, and not to suspected CD, which can alter the recommendations according to the severity of associated inflammation.

In the European consensus on CD surgery by the European Crohn's and Colitis Organization (ECCO), the authors suggest that when ileitis is identified without associated complications and the appendix is not affected by an inflammatory process, the terminal ileum or even the appendix should not be resected, since in uncomplicated CD, with no signs of dilation or penetrating disease, medical treatment is usually indicated. Appendectomy in these cases is strongly contraindicated, due to a high risk of intra-abdominal septic complications and fistulas[26].

CD is often associated with characteristic macroscopic findings, such as the proliferation of mesentery fat involving the intestinal loops ("creeping fat"), hardened consistency and areas of proximal dilation, among others. In cases of phlegmons, blocked perforations or abscesses, or in cases with associated intestinal partial or total obstruction, an ileocecal resection is indicated. Whenever possible, a primary anastomosis should be performed (mostly in patients with good nutritional status and adequate local conditions). However, proximal diversion with ileostomy or double-stoma may be necessary in patients with poor clinical conditions (sepsis, hemodynamic instability, previous use of corticosteroids, suspected malnutrition) and unfavorable intraoperative findings (peritonitis and local technical difficulties)[27].

The diversity and heterogeneity of the recommendations demonstrate the controversy of the topic and the complex dilemma that surgeons face when finding a normal appendix in these cases. It is also worth mentioning the scarcity of studies with solid evidence on the topic, such as those captured in this systematic review, in addition to the lack of specific guidelines on how to proceed surgically in an emergency situation in the close AA x CD relationship. Thus, a therapeutic algorithm based on the current evidence is suggested, which can serve as a guide for surgeons who may face this scenario in daily practice (FIGURE 2).

This qualitative SR is associated to some limitations which must

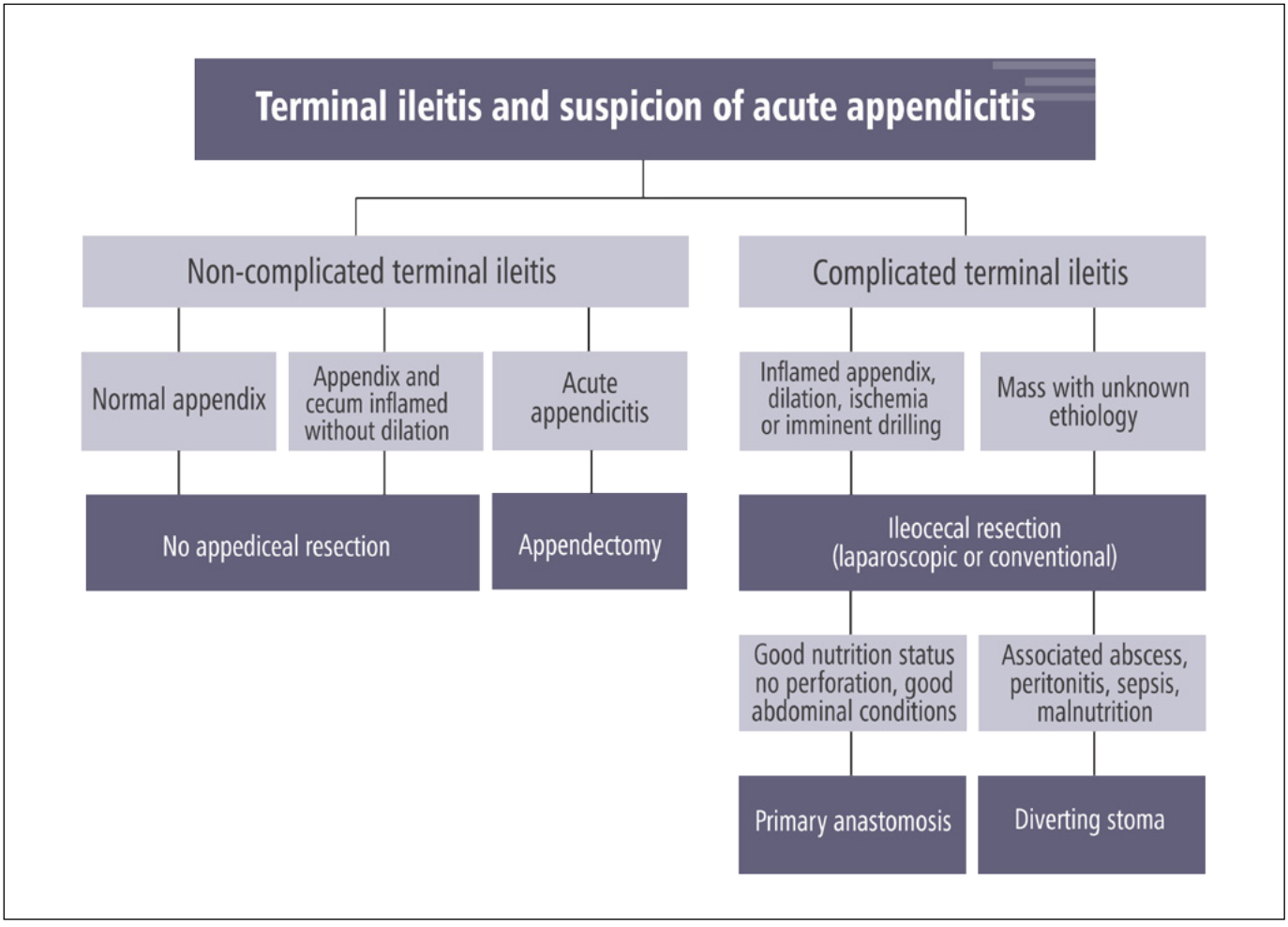



**FIGURE 2**. Therapeutic algorithm based on the current evidences.

be considered when interpreting the results. The literature search demonstrated a scarcity of articles with good quality of evidence. Most studies are retrospective and purely descriptive, with limited patient samples. Only two studies compared groups, but were associated with important biases. Thus, a quantitative study with meta-analysis was not recommended due to the extreme diversity in methodologies and in included variables in the studies. In addition, there is a considerable number of case reports and reviews over the topic of surgery in AA and CD, but the correlation is difficult because they have considerable diversity in relation to numerous differential diagnoses, making it impossible to correctly assess a cause and effect relationship.

In summary, there is a scarce number of specific studies which analyzed results related to the management of ileocecal CD in situations of suspected AA, with small samples and low quality evidence. More comparative studies with a larger number of cases are needed to better elucidate the issue. It is suggested that in the presence of an uncomplicated inflammatory process in the RLQ with suspected CD, the appendix should not be removed. In the presence of a mass, ischemia, fistula or obstruction, an ileocecal resection is recommended and the decision between a primary anastomosis or a diverting stoma is individualized, considering intraoperative conditions, the nutritional status and the presence of septic conditions. Individualization of treatment must be performed according to the surgeons' experience, the patients' condition and the degree of inflammatory involvement.

## Authors' contribution

Quaresma AB, Miranda EF and Kotze PG designed the review and drafted the paper. All authors gave important intellectual contribution and reviewed the final version of the manuscript.

## Orcid

Abel Botelho Quaresma: 0000-0002-3985-7402.
Eron Fabio Miranda: 0000-0003-4011-5112.
Paulo Gustavo Kotze: 0000-0002-2053-5315.

Quaresma AB Miranda EF, Kotze PG. Manejo da doença de Crohn ileocecal como achado incidental em cirurgia de urgência para apendicite aguda: uma revisão sistemática. Arq Gastroenterol. 2021;58(4):560-5.

**RESUMO** – **Contexto** – Em muitos pacientes, o diagnóstico da doença de Crohn (DC) é feito durante uma cirurgia de urgência por suspeita de apendicite. O diagnóstico intraoperatório pode ser desafiador em certos casos, especialmente para cirurgiões menos experientes. **Objetivo** – Revisar a literatura em busca de evidências científicas que possam orientar os cirurgiões no manejo otimizado da DC ileocecal encontrada incidentalmente na cirurgia de apendicite aguda (AA). **Métodos** – Os estudos incluídos foram identificados por busca eletrônica no banco de dados PubMed de acordo com as diretrizes Itens Preferidos de Relatórios para Revisões Sistemáticas e Meta-Análise (PRISMA). As avaliações de qualidade e viés foram realizadas pelos critérios Índice Metodológico para Estudos Não Randomizados (MINORS). **Resultados** – Foram identificados inicialmente 313 estudos, dos quais seis foram selecionados (todos retrospectivos) para avaliação qualitativa (dois estudos eram comparativos e quatro apenas séries de casos descritivos). Quatro estudos encontraram uma alta taxa de complicações quando a apendicectomia ou ileocolectomia foram realizadas e em apenas um, não houve aumento do risco de complicações pós-operatórias com a apendicectomia. No sexto estudo, diarreia, dor abdominal prévia, anemia pré-operatória e trombocitopenia foram fatores preditivos independentes para DC em pacientes operados previamente por suspeita de AA. **Conclusão** – Apesar da escassez de dados e da baixa qualidade das evidências, recomenda-se que um apêndice macroscopicamente normal seja preservado na ausência de doença complicada quando há suspeita de DC na cirurgia de AA. As ressecções ileocecais devem ser reservadas para doenças complicadas (massa inflamatória, isquemia, perfuração ou obstrução). Mais estudos prospectivos são necessários para confirmar essas afirmações.

**Palavras-chave** – Doença de Crohn; cirurgia colorretal; apendicectomia; revisão sistemática.

## REFERENCES

1. Roda G, Ng SC, Kotze PG, Argollo M, Panaccione R, Spinelli A, et al. Crohn's disease. Nat Rev Dis Prim. 20202;6:22.
2. Gionchetti P, Dignass A, Danese S, Dias FJM, Rogler G, Lakatos PL, et al. 3 rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 2: Surgical management and special situations. J Crohn's Colitis. 2017;11: 135-49.
3. Guven H, Koc B, Saglam F, Bayram IA, Adas G. Emergency right hemicolectomy for inflammatory cecal masses mimicking acute appendicitis. World J Emerg Surg. 2014;9:1-5.
4. Yang SS, Gibson P, McCaughey RS, Arcari FA, Bernstein J. Primary Crohn's disease of the appendix. Report of 14 cases and review of the literature. Ann Surg. 1979;189:334-9.
5. Agha FP, Ghahremani GG, Panella JS, Kaufman MW. Appendicitis as the initial manifestation of Crohn's disease: Radiologic features and prognosis. Am J Roentgenol. 1987;149:515-8.
6. Shariff U, Narula H, Speake W, Brown S. Terminal ileal Crohn's disease: Conservative surgeon and aggressive physician? Color Dis. 2009;11:522-3.
7. Slim K, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (Minors): Development and validation of a new instrument. ANZ J Surg. 2003;73:712-6.
8. Riseman JA, Wichterman K. Evaluation of Right Hemicolectomy for Unexpected Cecal Mass. Arch Surg. 1989;124:1043-4.
9. Oren R RD. Preoperative clues to Crohn's disease in suspected, acute appendicitis. Report of 12 cases and review of the literature. J Clin Gastroenterol. 1992;306-10.
10. Amaral E, Ribeiro M, Larangeira L, Teixeira MG, Brunetti C, Habr-gama A. Doença de cröhn e apendicite. Rev.bras.Colo-Proct. 1993;13:91-3.
11. Smida M, Miloudi N, Hefaiedh R, Zaibi R. Les urgences chirurgicales dans la maladie de Crohn. Tunis Med. 2016;94:210-5.
12. Chen F, Wu H, Wu Y Mao R, Zhang S, Feng T, et al. [Clinical manifestations of Crohn's disease misdiagnosed as appendicitis]. Zhonghua yi xue za zhi. 2016;96:792-5. doi: 10.3760/cma.j.issn.0376-2491.2016.10.009.
13. Slim K, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (Minors): Development and validation of a new instrument. ANZ J Surg. 2003;73:712-6.
14. Cheluvappa R, Thomas DG, Selvendran S. The role of specific chemokines in the amelioration of colitis by appendicitis and appendectomy. Biomolecules. 2018;8:1-13.
15. Bass J, Goldman J, Jackson M, Gasior A, Sharp S, Drews A, et al. Pediatric crohn disease presenting as appendicitis: Differentiating features from typical appendicitis. Eur J Pediatr Surg. 2012;22:274-8.
16. Yu Q, Mao R, Lian L, Ng SC, Zhang S, Chen Z, et al. Surgical management of inflammatory bowel disease in China: A systematic review of two decades. Intest Res. 2016;14:322-32.
17. Hsu WF, Wu CS, Wu JM, Chung CS. Ileal Crohn's disease with perforation misdiagnosed as ruptured appendicitis: A case report. J Formos Med Assoc. 2013;112:652-3.
18. Millet I, Alili C, Pages E, Curros Doyon F, Merigeaud S, Taourel P. Infection of the right iliac fossa. Diagn Interv Imaging. 2012;93:441-52.
19. Di Saverio S, Birindelli A, Kelly MD, Catena F, Weber DG, Sartelli M, et al. WSES Jerusalem guidelines for diagnosis and treatment of acute appendicitis. World J Emerg Surg. 2016;11:1-25.
20. Sung Y-N, Kim J. Appendiceal actinomycosis mimicking appendiceal tumor, appendicitis or inflammatory bowel disease. J Pathol Transl Med. 2020;1-6. doi: 10.4132/jptm.2020.05.17.
21. West NE, Wise PE, Herline AJ, Muldoon RL, Chopp WV., Schwartz DA. Carcinoid tumors are 15 times more common in patients with Crohn's disease. Inflamm Bowel Dis. 2007;13:1129-34.
22. Mällinen J, Rautio T, Grönroos J, Rantanen T, Nordström P, Savolainen H, et al. Risk of appendiceal neoplasm in periappendicular abscess in patients treated with interval appendectomy vs follow-up with magnetic resonance imaging: 1-year outcomes of the peri-appendicitis acuta randomized clinical trial. JAMA Surg. 2019;154:200-7.
23. Shindholimath V, Thinakaran K, Rao T, Veerappa Y. Laparoscopic management of appendicular mass. J Minim Access Surg. 2011;7:136-40.
24. Di Saverio S, Podda M, De Simone B, Ceresoli M, Augustin G, Gori A, et al. Diagnosis and treatment of acute appendicitis: 2020 update of the WSES Jerusalem guidelines. World J Emerg Surg. 2020;15:1-42.
25. Sørensen AK, Bang-Nielsen A, Levic-Souzani K, Pommergaard HC, Jørgensen AB, Tolstrup MB, et al. Readmission and reoperation rates following negative diagnostic laparoscopy for clinically suspected appendicitis: The "normal" appendix should not be removed – A retrospective cohort study. Int J Surg. 2019;64:1-4.
26. Bemelman WA, Warusavitarne J, Sampietro GM, Serclova Z, Zmora O, Luglio G, et al. ECCO-ESCP consensus on surgery for Crohn's disease. J Crohn's Colitis. 2018;12:1-16.
27. Benoist S, Panis Y, Beaufour A, Bouhnik Y, Matuchansky C, Valleur P. Laparoscopic ileocecal resection in Crohn's disease: A case-matched comparison with open resection. Surg Endosc Other Interv Tech. 2003;17:814-8.

BRIEF COMMUNICATION

AG-2021-103
doi.org/10.1590/S0004-2803.202100000-99

# Technical aspects of endoscopic submucosal dissection (ESD). From lateral to longitudinal dissection: a new approach to treat colonic tumors

Gianmattia **DEL GENIO**, Francesco Saverio **LUCIDO** and Claudio **GAMBARDELLA**

Received: 17 May 2021
Accepted: 11 June 2021

## INTRODUCTION

Treatment of colonic neoplasia has been driven towards a minimally invasive approach to reduce postoperative discomfort and co-morbidity[1,2]. In the last two decades, several randomized trials showed laparoscopic approach to reach similar or even superior results compared to standard open surgery[3,4]. Given the diffusion of accurate endoscopic screening for colonic neoplasia, a higher percentage of colonic tumors are currently discovered at an early stage, before further anatomical alterations become clinically evident (i.e., altered defecation and/or rectal bleeding)[5,6]. In the effort of reducing postoperative complications and discomfort, as well as achieving a faster return to normal activity preserving the physiologic colorectal function, endoscopic excision has been proposed to address colonic lesions at this early stage[7,8]. This natural orifice trans-luminal endoscopic surgery (NOTES) established in Japan gained soon widespread popularity due to excellent long-term survival associated to an extremely reduced invasiveness[9,10]. This is particularly evident for middle and low rectal lesions that classically required temporary ileostomy or abdomino-perineal excision in some cases. More recently, new criteria for endoscopic treatment of malignant lesions in the lower gastrointestinal tract (colon and rectum) have been well-defined (e.g., G1/G2, LO, invasion depth ≤1000 μm). The introduction of high definition flexible endoscopy and dedicated instruments specifically developed for performing more complex endoscopic resections, contributed to the increased interest of endoscopic community in underlying technical limitations and difficulties associated to the procedure[11]. Endoscopic submucosal dissection (ESD) is currently recognized as an effective treatment of larger (>15 mm) and poor lifting colonic lesions when en-bloc/R0 resection is required. Indeed, while standard endoscopic mucosal resection (EMR) is potentially associated with insufficient resection margin, ESD allows a correct judgment of either histological margin and stage. A recent metanalysis demonstrated that ESD rates of en-bloc curative resection were much higher but recurrence much lower (91.7%, 80.3%, and 0.9%, respectively) than those of EMR (46.7%, 42.3%, and 12.2%, respectively)[12]. However, ESD is considered a complex procedure due to some limitations such as risk of perforation, significantly higher than that associated with EMR (5.7 vs 1.4 per cent)[12]. One reported drawback of ESD is the lack of adequate tissue traction to allow a precise and effective dissection along the right plane as performed during surgery[13]. Another limitation is lack of triangulation compared to standard laparoscopy, so that endoluminal techniques remain difficult to accomplish due to limited view and available movements. That is, ESD procedure seems to be limited to few referral centers with great expertise in interventional endoscopy[14]. Several ESD techniques have been described to overcome these drawbacks with variable outcomes and reported complications and to date, an extensive use of ESD to address large lesions could have been implemented with a more simplified, standardized and repeatable procedure[15]. In fact, the reported length of ESD (range, 65–108 min) was about 3-fold longer when compared to standard EMR (range 29–30 min) (OR: 6.84; CI: 3.30–14.18)[13]. More recently, different devices (e.g., similar to laparoscopic Johannes) seemed to better guarantee an easier approach to resect lesions under tension and to remove tissue with overall improved oncological outcome. In this setting, the lack of reports determines the real necessity of further technical standardization.

## TECHNICAL NOTE

### Conventional flexible lateral dissection

Traditional technique of ESD includes six steps to be completed sequentially: a) lesion identification and mucosal marking, b) submucosal injection, c) precutting (the very first small incision into the mucosa), d) complete cutting of the circumferential incision, e) submucosal dissection, f) hemostasis. The lesion marking and

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
University of Campania Luigi Vanvitelli, Division of General, Mininvasive and Bariatric Surgery, Naples, Italy.
Corresponding author: Claudio Gambardella. E-mail: claudiog86@hotmail.it

the circumferential incision have been supported as a prudential approach reducing the incidence of uncompleted resection and perforation. This is related to the type of dissection; indeed, starting from the early introduction of needle-knife type devices (i.e., IT 1-2, Flex knives; Olympus America, Inc, Center Valley, Pa, USA. Hybrid knife; ERBE USA, Marietta, Ga. *Mucosectome*; *Pentax*, Tokyo, Japan) standard dissection is conducted by lateral movement of the endoscope with traction performed by the cap. This generates a simultaneous movement of dissection devices and the scope itself, along the surgical plane (FIGURE 1.A). A potential short-come of this technique may be represented by an intrinsic difficulty of maintaining a stable tissue traction as well as a steady clear image with an increased odd of achieving a wrong dissection line. This important limitation is more likely to occur when dealing with larger/occupying space lesion.

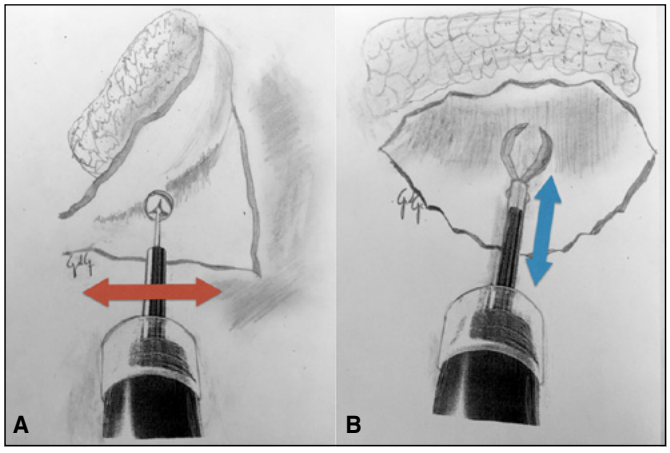



**FIGURE 1. A)** Endoscopic submucosal dissection performed by "flexible lateral dissection". Lateral movements of the endoscope tips allow to move the dissection and performing cut and suture. **B)** Endoscopic submucosal dissection accomplished by "fixed longitudinal dissection". The grasper grabs the tissue with subsequent energy delivery. The view remains stable being the scope maintained in a stationary position.

## Fixed longitudinal dissection

To overcome the above mentioned issues, we proposed an alternative technique for dissection based on the experience gained with single incision laparoscopic surgery. In this view, the main technical issue during ESD is to avoid tissue collapse, providing constant traction and optimal view during resection. The employment of a double channel endoscope enables operators to perform the ESD procedure by simultaneously introducing two instruments into the lumen. This allows to keep either an adequate traction even when cup cannot lift the tissue up or to achieve good haemostasis by combined use of mono- and bipolar energy graspers. Care should be taken to cauterize all small vessels before cutting, since bleeding can obscure surgical field and increase operative time. In case of active unexpected bleeding, grasper should grab and close the vessel whilst suction is carried out to confirm bleeding stops, so that diathermy energy can be finally applied with an available optimal view. The most important advantage of this "grasper assisted" dissection, lays on the opportunity of working along the longitudinal axis of the instruments (FIGURE 1.B). This allows during a large portion of dissection, to move forward into the deeper resection plane along the sub-mucosal layer with no need of moving the endoscopic tip, thereby enabling a less complicated and technically demanding procedure. Another advantage of grabbing the tissue into the grasper claws is to modulate the delivery of energy with a higher rather than lower power setting depending on the amount of tissue to address. Specifically, when a vessel is supposed to be included into the branches a longer preventive electrocoagulation to be applied is recommended. Another potential advantage of this technique is endoscopist may apply the required energy power after lifting back the grasper, assessing tissue consistency, and controlling the right plane of dissection. This potentially reduce the risk of diffusion of energy at deeper layer, thus the risk of serosal perforation.

With this in mind, we may assume this "fixed" technique of dissection reproduce more than the flexible one the principles derived from minimally invasive surgery. That is, it seems to facilitate and utterly improve safety of ESD. However, although it would seem more feasible, this newly described approach to ESD needs caution and should be initially reserved to smaller resection in selected patients with well exposed lesion. It is always necessary to inform the patient, an immediate surgical procedure (transanal excision or laparoscopic resection/repair) may be necessary in case of perforation or a second stage surgery may be necessary to extend the resection, according to final pathology. We are confident on that future progress in devices technology will improve feasibility of ESD and further facilitate widespread practice of such promising technique, according to all the required principles of surgical oncology.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the Division of General, Mini-invasive and Obesity Surgery- Master of Coloproctology and Master of Pelvi-Perineal Rehabilitation. University of Study of Campania "Luigi Vanvitelli" Naples, on reasonable request.

## Authors' contribution

Del Genio G, Lucido FS and Gambardella C: participated substantially in conception, design and execution of the study and in the analysis and interpretation of the data; also participated substantially in the drafting and editing of the manuscript. All authors contributed significantly to the present research, reviewed the entire manuscript and approved the final manuscript.

## Orcid

Gianmattia Del Genio: 0000-0001-5603-8970.
Francesco Saverio Lucido: 0000-0002-8778-4690.
Claudio Gambardella: 0000-0003-2277-2960.

Del Genio G, Lucido FS, Gambardella C. Aspectos técnicos da dissecação submucosal endoscópica. Da dissecção lateral à longitudinal: uma nova abordagem para tratar tumores de cólon. Arq Gastroenterol. 2021;58(4):566-8.
**Keywords** – Endoscopic submucosal dissection; transanal excision; NOTES.

## REFERENCES

1. Esposito D, Maione F, D'Alessandro A, Sarnelli G, De Palma GD. Endoscopic treatment of esophageal achalasia. World J Gastrointest Endosc. 2016;8:30-9. doi: 10.4253/wjge.v8.i2.30.
2. Del Genio G, Del Genio F, Schettino P, Limongelli P, Tolone S, Brusciano L, Avellino M, et al. Esophageal papilloma: Flexible endoscopic ablation by radiofrequency. World J Gastrointest Endosc. 2015;7:290-4. doi: 10.4253/wjge.v7.i3.290.
3. Veldkamp R, Kuhry E, Hop WC, Jeekel J, Kazemier G, Bonjer HJ, et al. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. Lancet Oncol. 2005;6:477-84. doi: 10.1016/S1470-2045(05)70221-7.
4. Yamamoto S, Inomata M, Katayama H, Mizusawa J, Etoh T, Konishi F, et al. Short-term surgical outcomes from a randomized controlled trial to evaluate laparoscopic and open D3 dissection for stage II/III colon cancer: Japan Clinical Oncology Group Study JCOG 0404. Ann Surg. 2014;260:23-30. doi:10.1097/SLA.0000000000000499.
5. Corleto VD, Pagnini C, Cattaruzza MS, Zykaj E, Di Giulio E, Margagnoni G, et al. Is proliferative colonic disease presentation changing? World J Gastroenterol. 2012;18:6614-9. doi: 10.3748/wjg.v18.i45.6614.
6. Galuppini F, Pennelli G, Loupakis F, Lanza C, Vianello L, Sacchi D, et al. BRAF p.V600E-specific immunohistochemical assessment in colorectal cancer endoscopy biopsies is consistent with the mutational profiling. Histopathology. 2017;71:1008-11. doi: 10.1111/his.13315.
7. Brusciano L, Gambardella C, Del Genio G, Tolone S, Lucido FS, Terracciano G, et al. Outlet obstructed constipation and fecal incontinence: is rehabilitation treatment the way? myth or reality. Arq Gastroenterol. 2020;5757:198. doi: 10.1590/s0004-2803.202000000-38.
8. Gambardella C, Brusciano L, Del Genio G, Tolone S, Terracciano G, Gualtieri G, et al. Predictive parameters to identify incontinent patients amenable for rehabilitation treatment: the muscular synergies evaluation. Arq Gastroenterol. 2019;56:452-53. doi: 10.1590/S0004-2803.201900000-76.
9. Hirao M, Masuda K, Asanuma T, Naka H, Noda K, Matsuura K, et al. Endoscopic resection of early gastric cancer and other tumors with local injection of hypertonic saline-epinephrine. Gastrointest Endosc. 1988;34:264-9. doi: 10.1016/s0016-5107(88)71327-9.
10. Hosokawa K, Yoshida S. Recent advances in endoscopic mucosal resection for early gastric cancer. Gan To Kagaku Ryoho. 1998;25:476-83.
11. Hsu WH, Sun MS, Lo HW, Tsai CY, Tsai YJ. Clinical practice of endoscopic submucosal dissection for early colorectal neoplasms by a colonoscopist with limited gastric experience. Gastroenterol Res Pract. 2013;2013:262171. doi: 10.1155/2013/262171.
12. Fujiya M, Tanaka K, Dokoshi T, Tominaga M, Ueno N, Inaba Y, et al. Efficacy and adverse events of EMR and endoscopic submucosal dissection for the treatment of colon neoplasms: a meta-analysis of studies comparing EMR and endoscopic submucosal dissection. Gastrointest Endosc. 2015;81:583-95. doi: 10.1016/j.gie.2014.07.034.
13. Kim GJ, Park SM, Kim JS, Ji JS, Kim BW, Choi H. Risk Factors for Additional Surgery after Iatrogenic Perforations due to Endoscopic Submucosal Dissection. Gastroenterol Res Pract. 2017;2017:6353456. doi: 10.1155/2017/6353456.
14. Yoshida N, Yagi N, Inada Y, Kugai M, Yanagisawa A, Naito Y. Prevention and management of complications of and training for colorectal endoscopic submucosal dissection. Gastroenterol Res Pract. 2013;2013:287173. doi: 10.1155/2013/287173.
15. Draganov PV, Gotoda T, Chavalitdhamrong D, Wallace MB. Techniques of endoscopic submucosal dissection: application for the Western endoscopist? Gastrointest Endosc. 2013;78:677-88. doi: 10.1016/j.gie.2013.07.033.

AG-2021-87
doi.org/10.1590/S0004-2803.202100000-100

# Indocyanine green and near-infrared fluorescence imaging in gastric cancer precision surgical approach

Erica **SAKAMOTO**, Andre Roncon **DIAS**, Marcus Fernando Kodama Pertille **RAMOS**, Adriana Vaz **SAFATLE-RIBEIRO**, Bruno **ZILBERSTEIN** and Ulysses **RIBEIRO JUNIOR**

Received: 27 April 2021
Accepted: 7 June 2021

Gastric cancer (GC) is the 4th most lethal cancer[1]. Radical gastrectomy is the main treatment for GC and D2 lymphadenectomy is recommended for advanced lesions, while limited lymph node dissection is adequate in early lesions, decreasing morbidity[2]. By tailoring the procedure according to the disease's extent, the best oncological results may be achieved while minimizing the patient's risk.

Currently, augmented reality is available and can be used for real-time assessment of the operative field and anatomy. One of the main modalities is the Indocyanine green (ICG) and Near-infrared (NIR) fluorescence imaging[3].

NIR light characteristics include low absorption, low scattering, and low autofluorescence, providing deeper tissue penetration than visible light. With fluorescent contrast agents, specific structures as lymphatic vessels, lymph nodes and blood vessels can be clearly visualized[3].

ICG is a sterile water-soluble tricarbocyanine dye, approved by the United States, Food and Drug Administration (FDA), with very rare reports of hypersensitivity reactions, besides being a nonionizing and nontoxic modality. It rapidly binds to plasma proteins, staying confined to the vascular compartment and, when excited by NIR light (700–900 nm), emits fluorescence at a wavelength of approximately 820 nm[3,4].

In GC, ICG and NIR fluorescence imaging may be used for sentinel lymph node biopsy and analysis, lymphadenectomy guidance and quality control, and localization of the tumor[4-6]. At present, a prospective, single-arm study is ongoing at our institution to evaluate the usefulness of ICG fluorescence imaging in GC (ClinicalTrials ID: NCT03021200). In this E-VIDEO*, we demonstrate ICG and NIR fluorescence applications in GC surgery.

First, a laparoscopic sentinel lymph node biopsy is performed. Intraoperative endoscopic injection of 0.2 mL of ICG is injected into the submucosal layer at four points around the lesion. The Sentinel Lymph node is identified (FIGURE 1) and removed for detailed analysis. Next, a robotic D2 gastrectomy in an obese patient is shown. ICG was endoscopically injected the day before and it allows for intraoperative identification of the lesion location and margin check (FIGURE 2), lymphadenectomy guidance and verification of its adequacy. In this particular case, a lymph node from station six was identified and rescued thanks to the NIR fluorescence (FIGURE 3). After the end of the surgery, the fluorescence system is activated, for final control of residual lymph nodes.

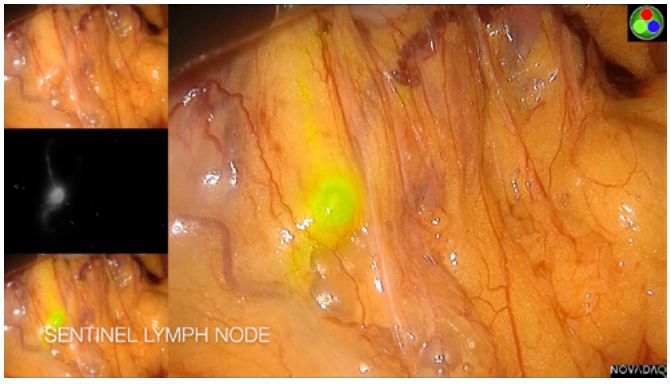



**FIGURE 1.** Sentinel Lymph node identification.

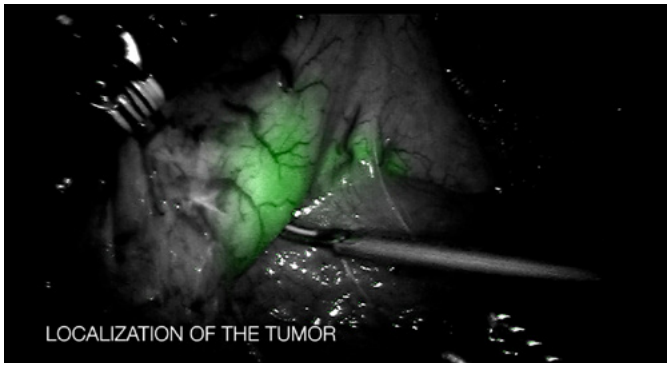



**FIGURE 2.** Localization of the tumor and determination of the surgical margins.

Declared conflict of interest of all authors: none
Disclosure of funding: the study is financed by PRONON (National Oncology Care Support Program by Brazilian Ministry of Health) and is registered online (Plataforma Brasil - CAAE: 56687616.5.0000.0065 - Clinical trial - NCT03021200).
Department of Gastroenterology, Instituto do Cancer, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR.
Corresponding author: Erica Sakamoto. E-mail: erica.sakamoto@hc.fm.usp.br
***E-VIDEO: https://youtu.be/Dvue8Uuhlks**

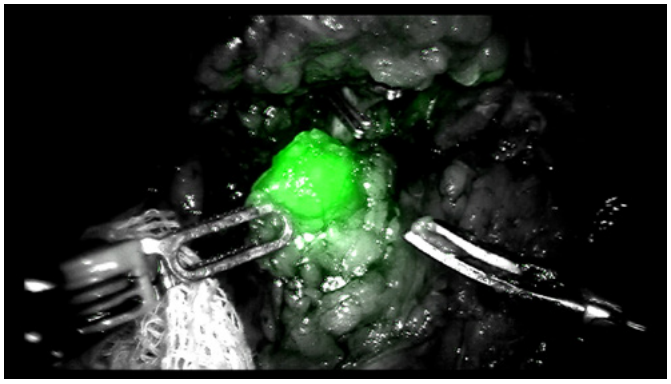


**FIGURE 3.** Station 6 lymph node.

## Authors' contribution

Dias AR carried out the operative procedure. Safatle-Ribeiro AV performed the endoscopic procedures. Sakamoto E edited the video. Dias AR, Sakamoto E, drafted the initial manuscript. Ramos MFKP, Safatle-Ribeiro AV, Zilberstein B and Junior UR supervised and commented on the manuscript. All authors discussed the results and contributed to the final manuscript.

## Orcid

Erica Sakamoto: 0000-0002-0845-0730.
Andre Roncon Dias: 0000-0003-3378-4916.
Marcus Fernando Kodama Pertille Ramos: 0000-0003-0200-7858.
Adriana Vaz Safatle-Ribeiro: 0000-0001-7686-8859.
Bruno Zilberstein: 0000-0002-1809-8558.
Ulysses Ribeiro-Junior: 0000-0003-1711-7347.

Sakamoto E, Dias AR, Ramos MFKP, Safatle-Ribeiro AV, Zilberstein B, Ribeiro Junior U. Uso da fluorescência a laser com infravermelho e indocianina verde no tratamento cirúrgico do câncer gástrico. Arq Gastroenterol. 2021;58(4):569-70.

## REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2021;71:209-49. doi.org/10.3322/caac.21660.
2. Kinami S, Nakamura N, Tomita Y, Miyata T, Fujita H, Ueda N, et al. Precision surgical approach with lymph-node dissection in early gastric cancer. World J Gastroenterol. 2019;25:1640-52. doi.org/10.3748/wjg.v25.i14.1640
3. Schaafsma BE, Mieog JSD, Hutteman M, van der Vorst JR, Kuppen PJK, Löwik CWGM, et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol. 2011;104:323-32. doi.org/10.1002/jso.21943.
4. Ushimaru Y, Omori T, Fujiwara Y, Yanagimoto Y, Sugimura K, Yamamoto K, et al. The Feasibility and Safety of Preoperative Fluorescence Marking with Indocyanine Green (ICG) in Laparoscopic Gastrectomy for Gastric Cancer. J Gastrointest Surg. 2019;23:468-76. doi.org/10.1007/s11605-018-3900-0.
5. Chen Q-Y, Xie J-W, Zhong Q, Wang J-B, Lin J-X, Lu J, et al. Safety and efficacy of indocyanine green tracer-guided lymph node dissection during laparoscopic radical gastrectomy in patients with gastric cancer: a randomized clinical trial. JAMA Surg. 2020;155:300-11. doi:10.1001/jamasurg.2019.6033.
6. Sakamoto E, Dias AR, Ramos MFKP, Zeide Charruf A, Ribeiro-Junior U, Zilberstein B, et al. Laparoscopic Completion Total Gastrectomy for Remnant Gastric Cancer. J Laparoendosc Adv Surg Tech A. 2020;31. doi.org/10.1089/lap.2020.0569.

AG-2021-90
doi.org/10.1590/S0004-2803.202100000-101

# Combined method for treating gastrocutaneous fistula after percutaneous endoscopic gastrostomy removal

Juliana Silveira Lima de **CASTRO**, Joao Guilherme Guerra de Andrade Lima **CABRAL**, Adriane Graicer **PELOSOF**, Alvaro Moura **SERAPHIM** and Claudia Sztokfisz **ZITRON**

Received: 26 April 2021
Accepted: 16 June 2021

A 56-year-old woman with food leaking by gastrocutaneous fistula (GCF), after removal of a 20 Fr percutaneous endoscopic gastrostomy (PEG), unresponsive to clinical treatment. She had PEG for 6 months, due to dysphagia and weight loss, related to squamous cell carcinoma of the esophagus and treatment with chemotherapy and radiotherapy.

We chose to close the ostomy with a simple hybrid technique, electrocoagulation associated with percutaneous suture guided by endoscopy. (E-VIDEO*). The procedure was performed with the patient under deep sedation and local anesthesia. Upper digestive endoscopy was performed identifying the gastric orifice of the GCF. (FIGURE 1). Initially electrocoagulation of the orifice, using coagulation current 40 Watts followed by percutaneous punctures on each corner of the GCF with a 14G peripheral intravenous catheter. (FIGURE 2). Subsequently a 3–0 nylon monofilament suture folded in half is passed through the catheter, forming the "loop" aspect. (FIGURE 3). The next step is to introduce a second 3–0 monofilament suture on the opposite corner catheter. This last

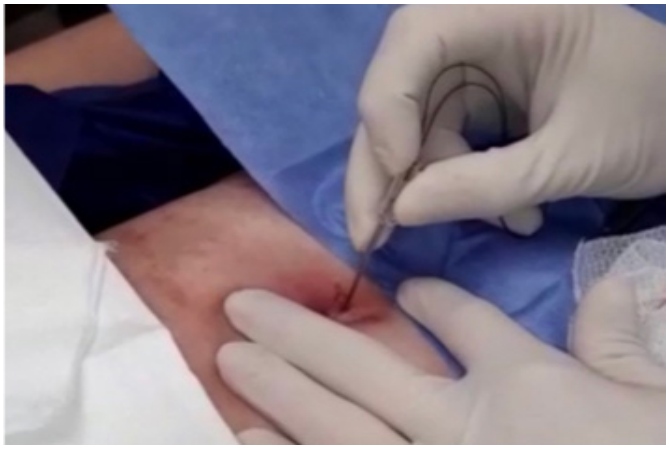


**FIGURE 2.** Percutaneous punctures on each corner of the gastrocutaneous fistula with a 14G peripheral intravenous catheter.

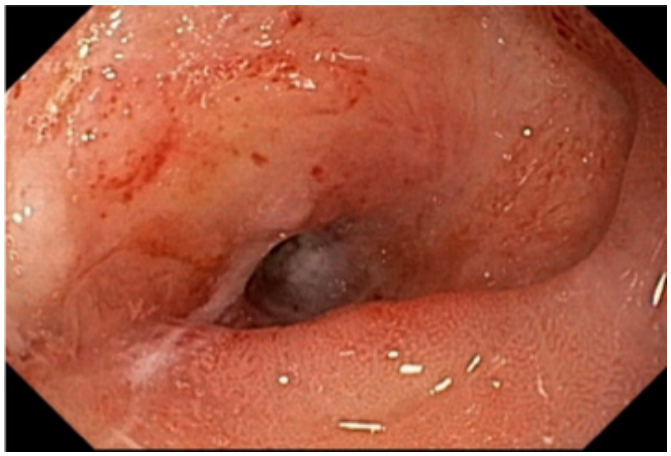


**FIGURE 1.** Upper digestive endoscopy was performed identifying the gastric orifice of the gastrocutaneous fistula.

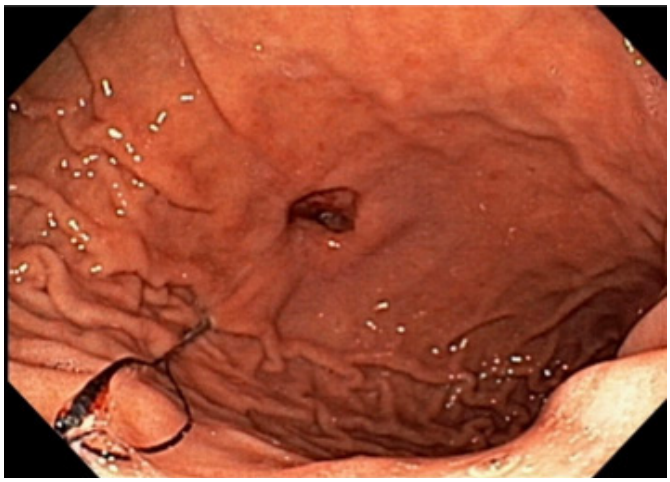


**FIGURE 3.** "Loop" aspect.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
A.C. Camargo Cancer Center, Unidade de Endoscopia, São Paulo, SP, Brasil.
Corresponding author: Juliana de Castro. E-mail: julianasilveira_@hotmail.com
***E-VIDEO: https://youtu.be/m5tyMpP8FgU**

suture should be guided by a biopsy forceps through the priorly formed suture "loop" under direct endoscopy view. (FIGURE 4). Once the first suture loop is pulled, the second suture will form a second loop on the gastric side with its booth extremities on the skin side, allowing the GCF to be closed by tying a surgical knot. (FIGURE 5).

In the follow up, patient returns after 15 days to remove the surgical knot with resolution of the GCF, without leakage of diet by GCF.

The opening of the wall after removal of PEG usually closes in about 1 to 3 days[1]. Persistent GCF after PEG removal is rare and uncommon in adults[2]. Surgical treatment has been largely replaced by endoscopy and several techniques have been described[3]. Electrocoagulation deepithelialize the tract and promote healing[4] and the suture causes mechanical closure[5]. The combined method for closing PEG with electrocoagulation associated with suture is simple, safe and has good results.

**Orcid**

Juliana Silveira Lima de Castro: 0000-0003-0280-8356.
Joao Guilherme Guerra de A. Lima Cabral: 0000-0001-7607-6982.
Adriane Graicer Pelosof: 0000-0001-9813-5832.
Alvaro Moura Seraphim: 0000-0002-3300-0467.
Claudia Sztokfisz Zitron: 0000-0001-9364-8460.

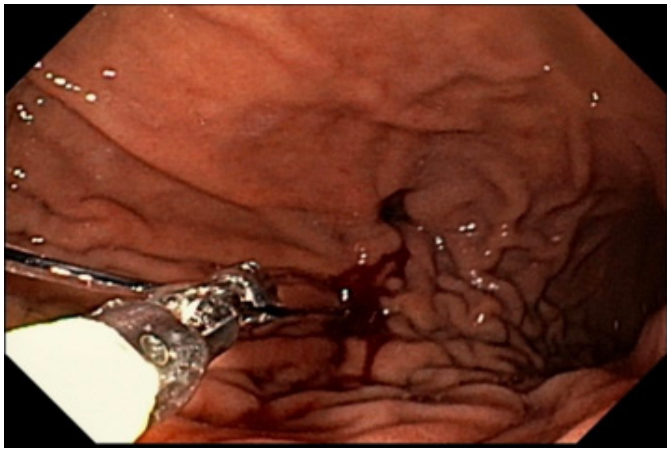


**FIGURE 4.** Last suture should be guided by a biopsy forceps through the priorly formed suture "loop" under direct endoscopy view.

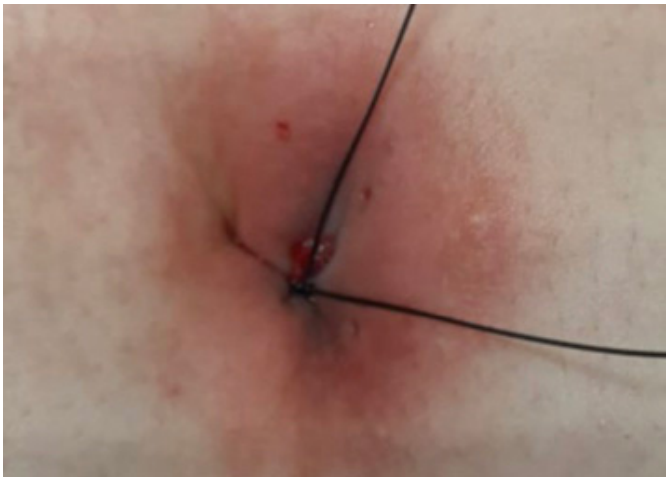


**FIGURE 5.** Surgical knot.

Castro JSL, Cabral JGGAL, Pelosof AG, Seraphim AM, Zitron CS. Método combinado para tratamento de fístula gastrocutânea após remoção de gastrostomia endoscópica. Arq Gastroenterol. 2021;58(4):571-2.

## REFERENCES

1. Hucl T, Spicak J. Complications of percutaneous endoscopic gastrostomy. Best Pract Res Clin Gastroenterol. 2016;30:769-81.
2. Omer J. Deen, Keely R. Parisian, Campbell Harris, Donald F Kirby. A Novel Procedure for Gastrocutaneous Fistula Closure. J Clin Gastroenterol. 2013;47:608-11.
3. Hameed H, Kalim S, Khan YI. Closure of a nonhealing gastrocutanous fistula using argon plasma coagulation and endoscopic hemoclips. Can J Gastroenterol Hepatol. 2009;23:217e9.
4. Duddempudi S, Ghevariya V, Singh M, Krishnaiah M, Anand S. Treatment of persistently leaking post PEG tube gastrocutaneous fistula in elderly patients with combined electrochemical cautery and endoscopic clip placement. South Med J. 2009;102:585-8.
5. Alberti-Flor JJ. Percutaneous-endoscopic suturing of gastrocutaneous fistula: report of two cases. Gastrointest Endosc. 2002;56:751-53.

PATROCÍNIO

IBEPEGE
Instituto Brasileiro de Estudos e Pesquisas de Gastroenterologia e Outras Especialidades

HOSPITAL IGESP