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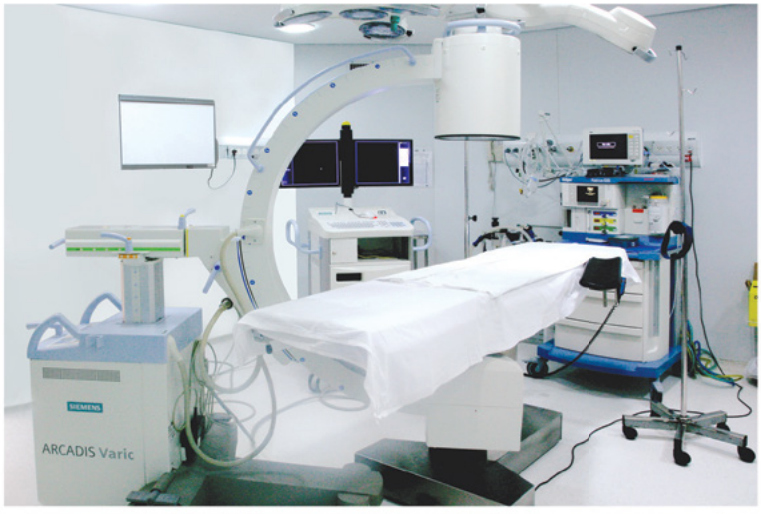

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Scope and policy

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

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

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10) All contributors who do not meet the criteria for authorship may be mentioned in Acknowledgments.
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New directions

Viebig RG. New directions. Arq Gastroenterol. 2021;58(1):1.

The **Archives of Gastroenterology** remains firm in its purposes: to promote and provide its content openly and free of charge and to mainly cater for Brazilian science a vehicle for publications in an appropriate and impersonal way. Gradually, by the merit and quality of its editors, reviewers, and authors, it has been awarded the rankings of national and international journals in the area of gastroenterology and related specialties.

One of the areas of gastroenterology that has evolved decisively is the inflammatory bowel disease (IBD) field where Brazilian specialists have been committed to publish their progress and stimulate the production of new lines of investigation, both in the epidemiological area and diagnosis and therapy. In 2004, a group of enthusiasts and interested researchers founded the Group of Studies of Inflammatory Disease of Brazil (GEDIIB), which has become Department of the Brazilian Federation of Gastroenterology that gives rise to intense academic activity and scientific production. Through the time, the departmental activities were highlighted not only by international publications but also in the Brazilian reality by IBD research and didactic activity through courses and congresses.

In this scenario, communication between general gastroenterologists and specialists has become even more important. This interactive platform provides better results for patients with IBD.

The **Archives of Gastroenterology** is very proud to have been chosen as a dissemination instrument in this area, and GEDIIB being another entity linked with the goals of our journal. This partnership is aligned with new structural and functional proposals of the journal that aim in the interest of the represented entities.

This restructuring does not hurt any of the precepts already practiced, but will impart more activities to each entity and decisive representation in the routine of the journal's office.

These new modes of action between GEDIIB and the **Archives of Gastroenterology**, considered exemplary, will serve as a model for other societies' performance.

Officially, GEDIIB now has an Associate Editor specific to the matter, who will determine and invite reviewers being responsible for the procedures required by the ScholarOne platform for the approval and rejection of submitted papers. The system will continue under the peer reviewer's policy and under the overview of the Editor-in-chief and Scientific Editor. Thus, GEDIIB brings an important contribution of modernity in the management of the representative body of the society and its relationship with the promotion of knowledge.

The **Archives of Gastroenterology** welcomes the entry of GEDIIB as a partner with great honor and hopes to have the company of other societies soon within the organizational chart. We thank Dr. Rogerio Saad Hossne for the initiative and for the strength he gave us. We congratulate Dr. Jose Luiz Parente and Dr. Genoile Oliveira Santana for their appointments as Associate Editor and Advisory Editor, respectively.

Let´s work.

Dr. Ricardo Guilherme VIEBIG*

Viebig RG. Novas direções. Arq Gastroenterol. 2021;58(1):1.

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Brazilian IBD Study Group position statement on SARS-CoV2 vaccination

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ABSTRACT – Mass vaccination offers the best strategy to fight against COVID-19 pandemic, and SARS-CoV2 vaccines are being approved in several countries for emergency use. In Brazil, vaccine approval is expected in the next few days, however potential concerns exist regarding vaccine recommendations for specific populations, such as patients with inflammatory bowel disease (IBD). To address these questions, the Brazilian IBD Study Group (GEDIIB) provides this practical advice with key recommendations about the COVID-19 vaccines in IBD population.

COVID-19 pandemic is a public health emergency of international concern and Brazil is currently one of the hardest hit regions, having one of the greatest COVID-19 death rates in the world[1]. Given that the introduction of SARS-Cov-2 vaccines represents the first chance to eliminate the virus on a long-term basis, many countries are already implementing mass vaccination programs. In Brazil, although no immunization program is planned so far, approval of vaccines is expected in the next few days. However, the availability of vaccines has raised some concerns in the media and public opinion regarding whether or not to vaccinate particular groups of patients, such as patients with inflammatory bowel diseases (IBD). Facing this scenario, this Brazilian IBD Study Group (GEDIIB) document aims to strongly recommend IBD patients to be given a COVID-19 vaccine once it is widely available. Moreover, this manuscript also may help both patients with IBD and physicians clarify specific safety issues that may arise, regarding COVID vaccination.

I. Should IBD patients be prioritized in vaccination allocation groups?

IBD patients often require treatment with immunosuppressant medications, which can increase their risk of infections. However, recent data suggest that they do not have an increased risk of infection of SARS-CoV-2 or the development of COVID-19 complications[2]. Therefore, it is unlikely that a patient with IBD will qualify for a priority allocation vaccination group based only on the diagnosis of IBD itself. Prioritization should be carried out on the basis of age and related comorbidities.

II. Which vaccines are currently available?

So far, no SARS-CoV-2 vaccine has been approved in Brazil. The first approved immunizations by the European and American regulatory agencies were the mRNA vaccines. In Brazil, it is very likely that our regulatory agency will issue emergence use authorization for Oxford/AstraZeneca and Sinovac SARS-CoV-2 vaccines. TABLE 1 provides an overview of SARS-CoV-2 vaccines.

III. Does immunosuppression affect the effectiveness of COVID-19 vaccine?

For other common vaccines, it has been demonstrated that immunosuppressant medications may result in some reduction in antibody formation and lower vaccine response. For instance, it has been shown that immune response to pneumococcal polysaccharide vaccination (PSV-23) is impaired in Crohn's disease (CD) patients on combination of TNF-blockers and immunomodulators[3] and that the serologic conversion rate to influenza vaccine is lower in immunossupressed IBD patients[4,5]. On the other hand, treatment with ustekinumab or vedolizumab does not appear to reduce responses to flu vaccine[6,7].

We do not know whether the available vaccines will be as effective in IBD patients, given that most vaccine trials excluded IBD patients. Taking into account the higher level of effectiveness of the available vaccines against symptomatic COVID-19, and severe COVID-19 (70–95%), much better than the flu vaccine (50–60%), it is anticipated that SARS-CoV-2 vaccine already protects at higher rate, even if treatment with immunosuppressant agents can make it slightly less effective.

Declared conflict of interest of all authors: Queiroz NSF reports receiving consulting and lecture fees from Janssen, Takeda, and Abbvie. Teixeira FV reports receiving consulting and lecture fees from Abbvie, Janssen, Pfizer and Takeda. Motta MP reports receiving lecture fees from Janssen and Takeda. Freire CCF reports receiving lecture fees from Abbvie, Janssen, Pfizer, Takeda and UCB. Chebli LA has served as a speaker for Janssen and Takeda. Saad Hossne R has received fees for serving as a speaker for Abbvie, Janssen, Pfizer and Takeda, advisory board member. Vasconcellos MAM has no conflict of interest.

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TABLE 1. Overview of approved SARS-CoV-2 vaccines.

Developer	Pfizer/ BioNTech	Moderna	Oxford/ AstraZeneca	Sinovac Biotech / Instituto Butantan
Name	BNT162b2	mRNA-1273	AZD1222	Coronavac
Doses	2 doses	2 doses	2 doses	2 doses
Time interval	3-12 weeks	4-12 weeks	4-12 weeks	2-3 weeks
Mechanism	mRNA encoding SARS-CoV2 spike protein	mRNA encoding SARS-CoV2 spike protein	Non-replicating adenovirus vector containing SARS-CoV2 spike protein gene	Inactivated SARS-CoV-2
Storage	-80ºC -60ºC	-20ºC	+2ºC a +8ºC	+2ºC a +8ºC
Efficacy	95%	94.1%	70%†	50.38% (overall) 78% (mild cases) 100% (moderate to severe)‡
Safety	No serious concerns. Twenty one cases of anaphylactoid reactions since approval*	No serious concerns	No serious concerns	No serious concerns

*Available at <https://www.cdc.gov/mmwr/volumes/70/wr/mm7002e1.htm>. Accessed on Jan. 11, 2020.

† Pooled data from two trials: 62% effective for volunteers given two full doses and 90% effective for a smaller subgroup who received a half dose followed by a full dose. ‡ Unpublished data disclosed by researchers.

IV. Is there any safety concern regarding SARS-Cov-2 vaccination in IBD patients?

There is no evidence to suggest an increased risk of the vaccine against SARS-CoV-2 in patients with IBD. Even though it is possible that other vaccines will soon become available, it is important to emphasize that, for those listed in TABLE 1, immunosuppression is not a contraindication. In addition, SARS-CoV2 vaccines have been tested in tens of thousands patients with safety profiles very similar to other vaccines commonly used in IBD patients, such as the flu vaccine. Also, IBD patients have been vaccinated with both influenza and pneumonia vaccines for many years with no indication of worsening IBD symptoms or flares following vaccination[4]. Thus, analogous to other vaccines used for many years, immunization appears very unlikely to affect IBD activity.

V. Should IBD patients postpone their biologic or hold their immunosuppression in order to get vaccinated?

We recommend that COVID-19 vaccine should be administered at any time regardless of last biologic infusion/subcutaneous dose received, as it is not a live vaccine. The one recommendation in this regard is that patients should avoid receiving their vaccine at the same day of an infusion/subcutaneous dose just in case the patient develop a reaction or adverse event it would be difficult to identify which one (vaccine or biologic) has caused it.

Therefore, in light of current evidence, we strongly support the recommendation to vaccinate all patients with IBD against SARS-CoV-2, regardless of their current treatment. For specific situations not discussed in this document, we recommend that the decision regarding whether to vaccinate or not should be taken individually, during shared decision making with patients.

CONCLUSION

This review represents an expert group opinion. It is in accordance with available evidence and positioning of main international IBD experts groups. Other approved vaccines and new data may be available in few weeks. Therefore, this position statement may change accordingly and will be reviewed frequently.

Authors' contribution

All authors were equally involved in study design, manuscript writing and review. All authors contributed to revision of the manuscript for important intellectual content, granted final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Queiroz NSF, Teixeira FV, Freire CCF, Motta MP, Vasconcellos MAM, Chebli LA, Saad Hossne R. Posicionamento do Grupo de Estudos da Doença Inflamatória Intestinal do Brasil sobre a vacinação para SARS-CoV2. Arq Gastroenterol. 2021;58(1):2-4.

RESUMO – A vacinação em massa oferece a melhor estratégia para enfrentamento da pandemia de COVID-19, e as vacinas contra SARS-CoV2 estão sendo aprovadas em vários países para uso emergencial. No Brasil, a aprovação da vacina é esperada em breve, no entanto, existem potenciais preocupações em relação às recomendações de vacinas para populações específicas, como pacientes com doença inflamatória intestinal (DII). Para responder essas questões, o Grupo Brasileiro de Estudos IBD (GEDIIB) fornece conselhos práticos com recomendações importantes sobre as vacinas para COVID-19 na população com DII.

REFERENCES

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020;20:533-4.
2. Brenner EJ, Ungaro RC, Gearry RB, Kaplan GG, Kissous-Hunt M, Lewis JD, et al. Corticosteroids, but not TNF Antagonists, are Associated with Adverse COVID-19 Outcomes in Patients with Inflammatory Bowel Diseases: Results from an International Registry. Gastroenterology. 2020;159:481-491.e3.
3. Melmed GY, Agarwal N, Frenck RW, Ippoliti AF, Ibanez P, Papadakis KA, et al. Immunosuppression impairs response to pneumococcal polysaccharide vaccination in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105:148-54.
4. Mamula P, Markowitz JE, Piccoli DA, Klimov A, Cohen L, Baldassano RN. Immune response to influenza vaccine in pediatric patients with inflammatory bowel disease. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2007;5:851-6.
5. Cullen G, Bader C, Korzenik JR, Sands BE. Serological response to the 2009 H1N1 influenza vaccination in patients with inflammatory bowel disease. Gut. 2012;61:385-91.
6. Caldera F, Hillman L, Saha S, Wald A, Grimes I, Zhang Y, et al. Immunogenicity of High Dose Influenza Vaccine for Patients with Inflammatory Bowel Disease on Anti-TNF Monotherapy: A Randomized Clinical Trial. Inflamm Bowel Dis. 2020;26:593-602.
7. Doornekamp L, Goetgebuer RL, Schmitz KS, Goeijenbier M, van der Woude CJ, Fouchier R, et al. High Immunogenicity to Influenza Vaccination in Crohn's Disease Patients Treated with Ustekinumab. Vaccines 2020;8(3).

ORIGINAL ARTICLE

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Impact of heartburn and regurgitation on individuals' well-being in the general population: a Brazilian national survey

Joaquim Prado P **MORAES-FILHO**[1], Gerson **DOMINGUES**[2], Decio **CHINZON**[1], Fabiana **ROVEDA**[3], Abner Augusto **LOBÃO NETO**[3] and Schlioma **ZATERKA**[1]

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ABSTRACT – **Background** – Heartburn and acid regurgitation are typical symptoms usually associated with gastroesophageal reflux disease (GERD). GERD is one of the gastrointestinal diagnosis with higher prevalence worldwide, significantly impairing patients' quality of life. **Objective** – The objective of this study was to analyze the impact of GERD-related symptoms in the Brazilian urban population. **Methods** – National telephone survey with community-dwelling Brazilian individuals. Self-reported prevalence and frequency of symptoms (heartburn / regurgitation) were assessed. Individuals rated the impact of symptoms in their general well-being using a numeric scale from 1 to 10 (1 = no impact; 10 = very intense, preventing the person to eat and perform daily routine activities). Descriptive and bivariate statistical analyses were performed. **Results** – The final sample was comprised of 1,773 subjects, 935 (52.7%) females, an average of 40 years old. The prevalence of heartburn and regurgitation in the past 6 months was 26.2% (n=466) and 11.0% (n=196), respectively. Women presented higher prevalence (heartburn n=266, 28.5% and regurgitation n=119, 12.7%) than men (n=200, 23.1% and n=78, 8.9%, respectively) (P<0.05). Heartburn in the past week was reported by 175 individuals (9.8%), while regurgitation episodes by 67 (3.8%). Absence of impact of the symptom in the overall well-being was observed for 82 subjects (17.6%) with heartburn and 18 individuals (9.2%) with regurgitation. Very intense impact was reported by 46 subjects (9.8%) with heartburn and 41 (20.9%) with regurgitation. Women's well-being was more affected than men's (mean score 5.45 vs 4.71, P<0.05). **Conclusion** – Heartburn and regurgitation were frequent symptoms, women with higher prevalence. These symptoms led to a substantial impact on individuals' well-being, women being more affected.

HEADINGS – Gastroesophageal reflux. Heartburn. Quality of life. Prevalence. Signs and symptoms. Surveys and questionnaires.

INTRODUCTION

Heartburn and regurgitation are typical symptoms associated with gastroesophageal reflux disease (GERD), although heartburn may also be observed in patients with functional heartburn[1]. GERD is one of the gastrointestinal diagnosis with higher prevalence worldwide, significantly impairing patients' acid related quality of life (HRQL)[2-4]. A national survey conducted in the United States with over 71,000 community-dwelling individuals observed that 61% reported ≥1 gastrointestinal symptom in the past week and heartburn and reflux were the most frequent self-reported symptoms (30.9%)[5].

In Brazil, a national survey interviewing 13,959 urban individuals identified an overall heartburn prevalence of 11.9%, at least once a week heartburn occurrence of 4.6% and GERD diagnosis in 7.3%[6]. Another Brazilian population-based survey observed that GERD symptoms were negatively associated with individuals' psychological well-being. After controlling for confounders, the odds of having GERD symptoms in the study was 2.14 times higher among those individuals self-assessing their psychological well-being as low as compared to those rating as high[7]. Thus, depending on the intensity and frequency of heartburn and regurgitation, the well-being and consequently the quality of life, may be significantly impaired.

The objective of the present study was to evaluate the impact of heartburn and regurgitation on the well-being of Brazilian individuals from a general urban population sample.

METHODS

A cross-sectional analysis of data was performed from a national telephone survey that aimed to determine the prevalence and distribution of gastrointestinal (GI) symptoms in community-dwelling Brazilian individuals.

The survey was conducted from August 6th to September 12th, 2018. A sample of 1800 adults, 18-69 years, was recruited from an already existing nationwide panel of individuals who take regular part in opinion and market research surveys. Sampling from the panel was conducted using quotas to represent the regions of the country. Sampled individuals were contacted by telephone and in-

Declared conflict of interest of all authors: Moraes-Filho JPP: speaker for Takeda. Domingues G: speaker for Takeda and Hypera. Chinzon D: advisory board member for Takeda and Hypera. Roveda F: medical manager at Takeda at the time of the study. Lobão Neto AA: medical director at Takeda. Zaterka S: advisory board member for Takeda and Ache; speaker for EMS and Takeda.

Disclosure of funding: The study was supported by Takeda Pharmaceuticals Brazil.

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vited to participate in the survey. After receiving information about study procedures and agreeing in participating, subjects were presented with questions about their demographic and socioeconomic status. Socioeconomic status was assessed using the 2015 Brazilian Criteria for Economic Classification[8], which categorizes into three socioeconomic groups: A (higher socioeconomic status, with higher estimated income and educational level); B (intermediate); and C (lower socioeconomic status)[8].

Symptoms prevalence was assessed using the following questions: (i) Have you had symptoms of heartburn and/or acid regurgitation in the last 6 months?; (ii) If yes, how many times did you have these symptoms in last 6 months?; (iii) When was the last time do you remember having these symptoms? Additionally, individuals were asked to rate the impact of the symptoms in their general well-being using a numeric scale from 1 to 10 (1 = no impact; 10 = very severe, preventing the person to eat or perform daily activities). Impact on well-being was assessed in individual levels from no impact (1) to very severe impact (10) and also in three impact levels. The following cut-offs were adopted to classify subjects according to the level of impact in general well-being:

- mild impact (1–3);
- moderate impact (4–7);
- severe impact (8–10).

This study was based on results of an opinion survey, thus no approval was required from a Research Ethics Committee. Likewise, it was not necessary to sign an Informed Consent Form. However, all procedures performed are governed by Ethical Standards of the Brazilian Association of Research Companies (ABEP) and of the European Society of Market Research (ESOMAR), in compliance with the International Standard for Quality on Market and Opinion Research – ISO 20252:2006 and the International Standard for Quality Management ISO 9001:2000.

Outcomes

The primary outcomes were the prevalence of heartburn and acid regurgitation (composite outcome) and the self-reported impact of these symptoms on individuals' general well-being. Among those who reported these GI symptoms, the average number of times they experienced symptoms in the past 6 months were also investigated as the proportion of respondents reporting these symptoms in the past week. The symptom severity in terms of well-being impairment were the secondary outcomes.

Statistical analyses

All statistical analyses were performed in SPSS (IBM SPSS Statistics for Windows Released 2015, Version 23.0. Armonk, NY: IBM Corp.). A two-tailed *P*-value of less than 0.05 was considered statistically significant. In bivariate analyses, categorical variables were compared using independent Z-test and continuous variables were explored using independent *t*-test.

RESULTS

The final sample was comprised of 1,773 subjects, 935 (52.7%) women, average of 40 years old. Socio-demographic sample characteristics are presented in TABLE 1.

TABLE 2 presents the findings on heartburn and regurgitation prevalence in the total sample and according to socio-demographic characteristics. Women had a significantly higher prevalence of heartburn (n=266, 28.5% vs n=200, 23.7%) and regurgitation

TABLE 1. Sample characteristics.

Characteristics		N	%
Age	18–24 years	292	16
	25–34 years	411	23
	35–44 years	395	22
	45–54 years	347	19
	55–69 years	355	20
	[Mean]	40.0	
Sex			
	Female	865	48
	Male	935	52
Geographic region			
	North	144	8
	Northeast	477	27
	Midwest	138	8
	Southeast	777	43
	South	265	15
Brazilian criteria for socio-economic classification			
	Class A	67	4
	Class B	512	28
	Class C	1220	68
Healthcare financing source			
	Brazilian Public Healthcare System	1083	60
	Private insurance or similar	505	28
	Out-of-pocket	194	11
	Other source	18	1

TABLE 2. Prevalence of heartburn and regurgitation in the past 6 months by socio-demographic characteristics.

Characteristics		Heartburn		Regurgitation	
		N	%	N	%
Total sample		466	26.2	196	11.0
Age					
	18–24 years (n=285)	69	24.2	29	10.2
	25–34 years (n=416)	106	25.4	38	9.1
	35–44 years (n=397)	110	27.7	43	10.8
	45–54 years (n=337)	89	26.4	39	11.6
	55–69 years (n=338)	93	27.5	47	13.9
Sex*					
	Female (n=931)	266	28.5	119	12.8
	Male (n=842)	200	23.7	78	9.3
Geographic region					
	North (n=136)	32	23.5	14	10.3
	Northeast (n=470)	119	25.3	54	11.5
	Midwest (n=136)	32	23.5	16	11.8
	Southeast (n=765)	224	29.3	83	10.8
	South (n=266)	59	22.2	29	10.9
Brazilian criteria for socio-economic classification					
	A (n=194)	57	29.4	26	13.4
	B (n=425)	114	26.8	52	12.2
	C (n=1154)	296	25.6	118	10.2

*Statistically significant differences for both symptoms frequency.

(n=119, 12.7% vs n=78, 8.9%). Other variables (age, geographic region, and socioeconomic status) were not significant (P>0.05).

Among those who reported each symptom, the average number of episodes that individuals had heartburn and regurgitation in the past 6 months was 7.74 and 9.62, respectively. Bivariate analysis did not indicate statistically significant differences for this outcome by age or sex.

Regarding the association of other variables (with average number of heartburn and regurgitation episodes in the previous 6 months) subjects classified in the C socio-economic category (lower income group)[8] had significantly higher mean number of regurgitation episodes (10.7 times/6 months) than subjects from the B category (7.43). The difference versus subjects in the A category (higher income group) did not reach statistical significance (9.10 times/6 months, P>0.05). The average number of heartburn episodes was not associated with socio-economic categories (P>0.05).

Heartburn in the past week was reported by 175 respondents (9.8% of the total sample), while regurgitation episodes occurred in the past week by 67 individuals (3.8%). (TABLE 3). Women had statistically significant higher prevalence of heartburn (n=115, 12.3% vs. n=60, 7.1%). Age and sex covariates were not associated with regurgitation frequency.

TABLE 3. Prevalence of heartburn and regurgitation in the past week by age group and sex (total sample).

Characteristics	Heartburn		Regurgitation	
	N	%	N	%
Any time in the past 6 months	466	26.2	196	11.0
In the past week	175	9.8	67	3.8
Age				
18–24 years (n=285)	30	10.6	12	4.2
25–34 years (n=416)	47	11.3	13	3.1
35–44 years (n=397)	41	10.3	16	4.0
45–54 years (n=337)	25	7.4	11	3.2
55–69 years (n=338)	33	9.8	15	4.4
Sex				
Female (n=931)	115	12.3*	46	4.9
Male (n=842)	60	7.1	21	2.5

*Statistically significant difference between men and women (heartburn only).

The self-reported impact of heartburn and regurgitation in subjects' well-being is presented in TABLE 4. Absence of impact of the symptom in the overall well-being was observed in 82 (17.6%) individuals with heartburn and 18 (9.2%) of individuals with regurgitation. Very severe impact was reported by 9.8% (n=46) of individuals with heartburn and 20.9% (n=41) with regurgitation. A similar pattern was observed for the predefined levels of impact (mild, moderate, and severe) with a higher impact associated more with regurgitation than with heartburn. Women had a lower proportion of "no impact" answers (n=39, 14.5% vs n=43, 21.7%, P<0.05) for heartburn and a higher proportion of very severe impact (impairing ability to eat or perform daily activities) for both heartburn (n=35, 13.2% vs n=11, 5.5%, P<0.05) and regurgitation (n=31, 26.1% vs n=9, 11.5%, P<0.05). Age groups comparison did not reach statistical significance for this outcome.

TABLE 4. Impact of heartburn and regurgitation in individuals' well-being.

	Heartburn (n=466)		Regurgitation (n=196)	
	N	%	N	%
No impact in individuals' well-being (score=1)	82	17.6	18	9.2
Very severe impact impairing ability to eat or perform daily activities (score=10)	46	9.8	41	20.9
Pre-defined levels of impact in individuals' well-being				
Mild (score=1-3)	147	31.5	43	21.9
Moderate (score=4-7)	192	42.5	72	36.7
Severe (score=8-10)	120	25.7	83	42.3
Average score of impact in well-being – mean (min-max)	5.18 (1–10)		6.28 (1–10)	

DISCUSSION

In the present study it was investigated a large sample of community-dwelling individuals to assess the prevalence and impact of heartburn and regurgitation symptoms in subject's general well-being. Although not representative of all Brazilian population, the results were representative of an important sector of the urban population represented by community-dwelling individuals, in terms of sex, age, geographic region and socio-economic status. The type of financing for healthcare services, with most individuals reported attending the National Public Healthcare System and being in the lower income group. The socio-economic criteria adopted in the study[8] also considered educational level, increasing the likelihood that individuals answering the survey were also similar to the general population regarding this particular variable. A questionnaire for evaluation of well-being not previously validated was used because of possible variables by telephone interviews.

The overall prevalence of heartburn and regurgitation was 26.2% and 11.0% respectively, with women having a higher frequency of both symptoms. In the previous Brazilian studies different methodologies were used as well as the outcome definitions, hindering our ability to compare findings[6,7]. A Brazilian study from 2005 observed a global prevalence of heartburn of 11.9%, pooling the prevalence of individuals reporting heartburn once a week (4.6%) and those with heartburn more than once a week (7.3%)[6]. Our findings indicate a self-reported frequency of heartburn in the past week of 9.8%, compatible to the mentioned study, but the outcome definition was different in terms of timing. Another study surveyed individuals using a GERD definition of heartburn at least once a week for the past 12 months and a psychological well-being assessment using a visual analogue scale[7]. Authors observed a prevalence of GERD in the past year of 31.3%, significantly associated with impaired psychological well-being. In the present sample, self-reported regurgitation symptoms have a higher impact in individuals' well-being than heartburn and this adverse effect is even more pronounced among women. These studies also observed that women were more affected than men by symptoms attributed to GERD, consistent with our findings and also with results from other contexts[6,7,9].

The main limitation of our study was the recall bias risk, since

prevalence of symptoms was self-reported and we adopted a 6 months recall period. Another limitation is related to its cross-sectional approach, limiting the ability to understand longitudinal patterns of symptoms occurrence. As GERD prevalence studies usually adopt weekly frequency of heartburn and regurgitation in the past year as diagnosis definition[10], our findings cannot be directly compared to observational data from previous studies. Taking into consideration the limitations of the present study in terms of observation of a specific populational sample, even so the findings unambiguously confirm the impairment on quality of life in individuals with heartburn/regurgitation.

Future studies may improve the understanding about GERD-related symptoms impact in individuals' quality of life using validated questionnaires and also other patient-reported outcomes such as productivity losses.

CONCLUSION

Heartburn and regurgitation were respectively present in 26.2% and 11.0% in the studied sample of the Brazilian population. Absence of impact of the symptoms in the overall well-being was observed in 17.6% and 9.2% of individuals with heartburn and regurgitation respectively.

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Authors' contribution

Moraes-Filho JPP: acquisition, analysis, or interpretation of data for the work; revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Moraes-Filho JPP, Domingues G, Chinzon D, Roveda F, Lobão Neto AA, Zaterka S. Impacto da pirose e regurgitação no bem-estar de indivíduos da população geral: um inquérito nacional brasileiro. Arq Gastroenterol. 2021;58(1):5-9.

RESUMO – **Contexto** – Pirose e regurgitação ácida são sintomas típicos usualmente relacionados à doença do refluxo gastroesofágico (DRGE). A DRGE é um dos diagnósticos gastrointestinais com maior prevalência mundial, afetando significativamente a qualidade de vida dos pacientes. **Objetivo** – Analisar o impacto de sintomas relacionados à DRGE em uma amostra da população brasileira urbana. **Métodos** – Inquérito nacional via telefone com indivíduos brasileiros em contexto comunitário. O inquérito foi conduzido entre 6 de agosto e 12 de setembro de 2018. A prevalência autorrelatada e a frequência dos sintomas foram avaliadas. Os respondentes classificaram o impacto dos sintomas no seu bem-estar geral utilizando uma escala numérica de 1 a 10 (1 = ausência de impacto; 10 = impacto muito grave, impedindo a pessoa de comer ou realizar atividades da vida diária). Análises estatísticas descritivas e bivariadas foram conduzidas. **Resultados** – A amostra final foi constituída por 1.773 indivíduos, 935 (52,7%) mulheres, com idade média de 40 anos. A prevalência de pirose e regurgitação nos últimos 6 meses foi de 26,2% (n=466) e 11,0% (n=196), respectivamente. Sexo feminino (pirose n=266, 28,5% e regurgitação n=119, 12,7%) apresentou prevalência mais alta do que o masculino (n=200, 23,1% e n=78, 8,9%, respectivamente) (P<0,05). Pirose na última semana foi relatada por 175 indivíduos (9,8%), enquanto episódios de regurgitação por 67 (3,8%). Ausência de impacto dos sintomas no bem-estar geral dos indivíduos foi observada para 82 respondentes (17,6%) com pirose e 18 (9,2%) daqueles com regurgitação. Impacto muito grave foi reportado por 46 (9,8%) indivíduos com pirose e 41 (20,9%) com regurgitação. Sexo feminino foi mais afetado pelos sintomas do que o masculino. **Conclusão** – Pirose e regurgitação foram bastante frequentes sendo o sexo feminino mais afetado. Tais sintomas levaram a impacto no bem-estar dos indivíduos, com maior prejuízo para mulheres.

DESCRITORES – Refluxo gastroesofágico. Azia. Qualidade de vida. Prevalência. Sinais e sintomas. Inquéritos e questionários.

REFERENCES

1. Hunt R, Chen M, Melo AC, Ford A, Lazebnik L, Lizarzabal M, et al. World Gastroenterology Organisation Global Guidelines: GERD Global Perspective on Gastroesophageal Reflux Disease. J Clin Gastroenterol. 2017;51:467-78.
2. Bruley Des Varannes S, Marek L, Humeau B, Lecasble M, Colin R. Gastro-esophageal reflux disease in primary care. Prevalence, epidemiology and Quality of Life of patients. Gastroenterol Clin Biol. 2006;30:364-70.
3. Eslick GD, Talley NJ. Gastroesophageal reflux disease (GERD): risk factors, and impact on quality of life-a population-based study. J Clin Gastroenterol. 2009;43:111-7.
4. Peery AF, Crockett SD, Barritt AS, Dellon ES, Eluri S, Gangarosa LM, et al. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States. Gastroenterology. 2015;149:1731-1741.e3.
5. Almario CV, Ballal ML, Chey WD, Nordstrom C, Khanna D, Spiegel BMR. Burden of Gastrointestinal Symptoms in the United States: Results of a Nationally Representative Survey of Over 71,000 Americans. Am J Gastroenterol. 2018;113:1701-10.
6. Moraes-Filho JPP, Chinzon D, Eisig JN, Hashimoto CL, Zaterka S. Prevalence of heartburn and gastroesophageal reflux disease in the urban Brazilian population. Arq Gastroenterol. 2005;42:122-7.
7. Oliveira SS, Santos IS, Silva JFP, Machado EC. [Gastroesophageal reflux disease: prevalence and associated factors]. [Article in Portuguese]. Arq Gastroenterol. 2005;42:116-21.
8. Associação Brasileira de Empresas de Pesquisa. Critério de Classificação Econômica Brasil 2015 [Internet]. ABEP. 2015 [cited 2019 Jun 18]. Available from: www.abep.org/Servicos/Download.aspx?id=09&p=cb
9. Soares RLS, Costa MC, Saad MAN, Salles MMS, Paes J, Menezes GM. Prevalence of Normal Endoscopic Findings in Women with Dyspeptic Symptoms in a Brazilian Community. Ann Clin Exp Metab. 2017;2:1024.
10. Eusebi LH, Ratnakumaran R, Yuan Y, Solaymani-Dodaran M, Bazzoli F, Ford AC. Global prevalence of, and risk factors for, gastro-oesophageal reflux symptoms: a meta-analysis. Gut. 2018;67:430-40.

ORIGINAL ARTICLE

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Good quality of life after more than a decade of living donor liver transplantation

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ABSTRACT – **Background** – Receptors of living donor liver transplantation (LDLT) have higher rate of postoperative biliary and vascular complications that may reduce posttransplant quality of life (QOL) due to the need of invasive and repetitive treatments. **Objective** – The purpose of our study is to assess the various aspects of QOL of receptors undergoing LDLT after 10 years of transplantation and to identify potential factors that might be associated with impaired QOL. **Methods** – Data of all patients with more than 10 years of LDLT were retrospectively evaluated. Patients were interviewed through a quality of life questionnaire (SF–36). **Results** – From a total of 440 LT performed in 17 years (from September 1991 through December 2008), 78 patients underwent LDLT, of which 27 were alive and 25 answered completely the questionnaire. There were 17 (68%) men and 8 (32%) women, with a mean age of 38.6±18.5 years at the time of transplantation and mean follow up time of 15.1±1.9 years. The average MELD was 16.4±4.9 and the main indication for LT was hepatic cirrhosis caused by hepatitis B virus (32%). When compared to the general population, LDLT patients had lower mental health score (66.4 vs 74.5, P=0.0093) and higher vitality score (87.8 vs 71.9, P<0.001), functional aspects (94.6 vs 75.5, P=0.002), social aspects (93 vs 83.9, P=0.005), physical aspects (92 vs 77.5, P=0.006), and emotional aspects (97.33 vs 81.7, P<0.001). General health status (73.28 vs 70.2, P=0.074) and pain (78.72 vs 76.7, P=0.672) scores were similar in both groups. **Conclusion** – It is concluded that the various aspects LDLT recipients' QOF are similar to those of the general population more than a decade after the transplant, except for the mental health domain which is lower.

HEADINGS – Liver transplantation. Living donors. Liver cirrhosis. Quality of life.

INTRODUCTION

With the enormous success of liver transplantation (LT), both in survival and in quality of life (QOL), the number of liver transplants increased remarkably all over the world in the last decades. However, the demand for LT increased more rapidly than the number of liver donors, causing elevated mortality of patients on a long waiting list. The growing disparity between the number of liver transplant candidates and the supply of deceased donor organs has motivated the development of living donor liver transplantation (LDLT).

The first LDLT was performed by Raia et al.[1] in Brazil in 1989 to overcome the shortage of cadaveric organs for pediatric recipients. LDLT in children has become accepted worldwide in a few years and helped to reduce the mortality of patients on the waiting list. Right lobectomy for adult-to-adult LDLT, a more complex and challenging procedure, was successfully performed by Yamaoka et al.[2] in Japan in 1994. Due to the risks of serious donor complications, including death, LDLT comprises a small percentage of total transplants in most countries. However, due to the limitation of cadaveric donation in some Asian countries, most LTs are performed with living donors. Although the results of LDLT are similar to those of deceased donor liver transplantation (DDLT), some complications are more common after LDLT, such as biliary stricture and fistula[3,4]. Post-transplant biliary complications may need prolonged endoscopic management, surgical treatment, and even retransplantation. This may reduce QOL after LT.

Several studies have evaluated the various aspects of QOL of donors of LDLT. However, only a few studies assessed the QOL of receptors of LDLT. Most of these studies are limited to patients undergoing LDLT in the short term. It has also not been determined whether the prolonged invasive treatment necessary to treat the higher rate of vascular and biliary complications following LDLT may decrease long-term QOL[3,4]. To the best of our knowledge there is no Brazilian study that evaluated the long-term QOL of patients who underwent LDLT. The objective of our study is to assess the various domains of QOL of receptors subjected to LDLT after 10 years and to identify potential factors that might be associated with impaired QOL.

METHODS

The present study was approved by the Ethical Committee of the Clinical Hospital of the Federal University of Paraná, Brazil (Protocol approval number CAAE 91362818.7.0000.0096). Data

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of all patients with more than 10 years of liver transplantation performed at the University Hospital of the Federal University of Paraná and *Nossa Senhora das Graças* Hospital, Curitiba, Brazil were retrospectively evaluated. All LT of the two hospitals were performed by the same transplantation team.

The following data were obtained from electronic medical records and study protocols: demographics, diagnosis, Child-Pugh classification, Model for End-Stage Liver Disease (MELD), peritransplant clinical factors, transplant technique, immunosuppressive regimen, graft function, and complications. Before May 2006, when MELD was officially introduced in Brazil, this score was retrospectively calculated based on the exams performed on the day of the transplantation. LT was performed using standard surgical techniques. After LT, patients were placed on standard immunosuppressive protocol consisting of calcineurin inhibitor-based immunosuppressive therapy (tacrolimus or cyclosporine), azathioprine or mycophenolate mofetil, and prednisone.

Patients' contact

All alive patients who underwent LT in our two hospitals before December 31, 2008 were contacted by telephone, e-mail or during medical consultation. The study was completely explained to the patients. Strict confidentiality was assured, and it was explained that the transplant members would not have access to answers from any individual patients. All questions were duly answered and doubts dispelled.

A letter with explanations, the SF-36 questionnaire, and the consent form to participate of this study were given personally or sent to the patients by e-mail. A few patients opted to answer the questionnaire by phone or completed the questionnaire during routine medical consultation.

Quality of Life Evaluation with the Short-Form 36 Questionnaire

Subjective QOL was assessed through Short-Form 36 (SF-36). The aim of this questionnaire is to assess differences of various domains of QOL (physical, functional, emotional and social aspects) between the general population and patients affected by a specific condition. This health survey is the most frequently employed QOL method to evaluate liver transplant recipients[5,6]. SF-36 is a generic, self-rated health survey designed to compare health status across diverse populations. This questionnaire is an internationally accepted general QOL survey validated by several authors, including for application in Brazilian population[7,8]. It offers broad-spectrum questions applicable to a variety of patient groups and enables comparison between different populations.

The questionnaire includes 36 questions divided into eight subscales or domains named general health, physical functioning, physical role functioning, body pain, mental health, emotional role functioning, social functioning, and vitality, as well as aggregate scores for physical and mental health. For each question, a score was attributed. Calculation was performed according to the SF-36 questionnaire protocol, composed by the data weighting phase and raw scale calculation phase 2, with scores ranging from 0 to 100. Higher scores indicate better health status. Another important advantage of the SF-36 questionnaire is the versatility of its application by self-filling, telephone, computer or personal interview for individuals above 14 years of age[6,9,10]. The time to answer all questions is usually 5 to 10 minutes[9,10].

In order to evaluate de QOL, the data obtained from the questionnaire answers of our patients was compared with those determined previously by Laguardia et al.[9] from the general Brazilian population and Cruz et al.[10] for the population of the south Brazilian region. The data from the study of these researchers was obtained from the general Brazilian population constituted of 12,423 randomly selected Brazilian men and women aged 18 years old or more from urban and rural areas of all five Brazilian regions who participated from the SF-36 questionnaire (Social Dimensions of Inequalities Study).

Statistical analysis

Values were expressed as mean ± SD (standard deviation) and median (minimum and maximum values). Qualitative variables were represented by absolute frequency (n) and relative frequency (%).

Nonparametric approaches were used since there was no normal distribution of the eight domains for any of the strata of the three grouping variables by the Kolmogorov-Smirnov test.

The Mann-Whitney nonparametric test was applied to evaluate the eight QOL domains provided by the SF-36 and their relation to the dichotomized MELD, dichotomized age (≤50 and >50 years), gender and presence or absence of acute complications. To evaluate the QOL with Child's classification, the Kruskal-Wallis nonparametric test was used. To analyze whether the mean scores of each of the eight SF-36 domains of the patients evaluated in the present study are different from those presented in previous studies of general population and population in the South of Brazil, the Student's *t*-test for one sample was used. The data were analyzed using the statistical software SPSS 22.0 considering a significance level of 5% (*P* value ≤0.05).

RESULTS

Of a total of 440 LT performed in the period of September of 1991 through December of 2008, there were 79 LDLT done in 78 patients. One of the LDLT was subjected to retransplantation. It was excluded from the study 342 patients (361 transplants, including 19 retransplants) who were subjected to deceased donor LT.

Of all patients who underwent LDLT, 27 (34.6%) were alive on December 31, 2018. Two of these patients had no follow-up in our hospital and were excluded from the study. The remaining 25 patients opted to participate of the research and responded all questions of the questionnaire of the QOL completely. These 25 patients were subjected to the study.

Clinical and epidemiological characteristics

Clinical and epidemiological characteristics of the 25 patients who have participated of the study are shown in TABLE 1. The mean follow-up time was 15.1±1.9 years (range, 12 to 19 years). There were 17 (68%) men and 8 (32%) women, with a mean age of 38.6±18.5 years (range, 0 to 60 years) at the time of transplantation and 53.6±17.5 years (range, 19 to 75 years) at the time of study analysis.

Almost all patients were class B (n=16; 64%) or class C (n=7; 28%) of the classification of Child-Pugh. Only 2 (8%) patients were class A.

The MELD varied from 4 to 27, with a mean of 16.4±4.9. Most patients (n=13; 52%) had MELD in the range of 10–17. For this purpose, additional points were not added to the MELD score of patients with associated hepatocellular carcinoma.

TABLE 1. Epidemiological, clinical characteristics, etiology of liver disease and SF-36 domains.

Characteristics	Patients
Number	25
Age at the time of transplantation (year)	
Mean ± SD	38.6±18.5
Median (min–max)	46 (0–60)
Age at the time of study analysis (year)	
Mean ± SD	53.6±17.5
Median (min–max)	61 (19–75)
Gender [n (%)]	
Male	17 (68)
Female	8 (32)
ICU time at the time of transplantation (days)*	
Mean ± SD	8.8±16.6
Median (min–max)	3 (1–80)
Hospital time at the time of transplantation (days)*	
Mean ± SD	23.2±21.6
Median (min–max)	16.5 (9–110)
Follow-up time (year)	
Mean ± SD	15.1±1.9
Median (min–max)	15 (12–19)
Child-Pugh Classification [n (%)]	
A	2 (8)
B	16 (64)
C	7 (28)
MELD Score [n (%)]	
<10	2 (8)
10–17	15 (60)
18–22	4 (16)
>23	4 (16)
Etiology [n (%)]	
Hepatitis B virus	8 (32)
Alcoholic cirrhosis	4 (16)
Autoimmune hepatitis	3 (12)
Primary sclerosis sholangitis	3 (12)
Hepatitis C virus	3 (12)
Biliary atresia	2 (8)
Type 1 glycogenesis	1 (4)
Cryptogenic cirrhosis	1 (4)

SD: standard deviation; MELD: Model for End-Stage Liver Disease. *Total n=24.

The main indications for LT were hepatic cirrhosis caused by hepatitis B virus (HBV) (n=8), alcoholic cirrhosis (n=4), autoimmune hepatitis (n=3), primary sclerosis cholangitis (n=3) and hepatitis C virus (HCV) (n=3). All patients with alcoholic cirrhosis had at least 6 months of alcohol abstinence before the LT.

Of the 25 patients analyzed, 18 (72%) of them had one or more complications of cirrhosis. The main liver complications recorded before the LT were ascites in 13 (52%) patients, hepatic encephalopathy in 7 (28%) patients and upper gastrointestinal hemorrhage in 7 (28%) patients. Other pre-LT complications were jaundice, spontaneous bacterial peritonitis, hepatorenal syndrome, and osteoporosis, one of each.

Postoperative complications

Fourteen patients (54%) had early postoperative complications. Early postoperative complication was defined as any clinical and surgical complication that occurred within 30 days after LT. The main complication was biliary fistula, which occurred in 6 (24%) patients. Other complications are shown in TABLE 2. Four patients were subjected to abdominal reoperation within 30 days after LT, two for abdominal lavage and biliary drainage due to biliary fistula and two for deceased liver retransplantation due to hepatic artery thrombosis.

TABLE 2. Early and late postoperative complications.

Characteristics	Patients
Early postoperative complications [n (%)]*	
Biliary fistula	6 (24)
Hepatic artery thrombosis	2 (8)
Septic shock	1 (4)
Pleural effusion	1 (4)
Parenchymal ischemia	1 (4)
Pyelonephritis in kidney graft	1 (4)
CMV infection	1 (4)
Resistant gastroenteritis	1 (4)
Late postoperative complications [n (%)]*	
Biliary stricture	9 (36)
Recurrent autoimmune hepatitis	4 (16)
Hepatitis B virus reactivation	3 (12)
Hepatitis C virus reactivation	2 (8)
Alcohol relapse	1 (4)
CMV infection	1 (4)
Perfusional graft disorder	1 (4)

*N=25, a single patient may have had more than one complication. CMV: cytomegalovirus.

After 30 days of LT to date, 10 (40%) patients did not present any chronic complications. Among the others 15 (60%) patients, the main complication was biliary stricture which occurred in 9 (36%) patients. Other complications are shown in TABLE 2.

Quality of life evaluation

The QOL data obtained from the questionnaire answers by our patients is shown in TABLE 3. The domain scores of LDLT patients varied from 66.4 (mental health) to 97.33 (emotional aspect limitations). These data were expressed as average with 95% CI.

LDLT patients had lower mental health (MH) score than the general Brazilian population [66.4 (60.5–72.3, 95% CI) vs 74.5, P=0.009] (TABLE 3). On the contrary, vitality (V) [87.8 (81.9–93.7, 95% CI) vs 71.9, P<0.001], functional aspects (FA) [94.6 (91.8–97.4, 95% CI) vs 75.5, P=0.002], social aspects (SA) [93 (86.8–99.2, 95% CI) *vs* 83.9, P=0.005], physical aspects (PA) [92 (82.3–100, 95% CI) vs 77.5, P=0.006], emotional aspects (EA) [97.33 (93.5–100, 95% CI) vs 81.7, P<0.001] scores were higher in LDLT patients than in the general population. General health status (GH) [73.28 (69.9–76.7, 95% CI) vs 70.2, P=0.074] and pain (P) [78.72 (69–88.4, 95% CI) vs 76.7, P=0.672] scores were similar in the two groups.

When compared to the population of Southern Brazil, LDLT patients had also lower mental health score [66.4 (60.5–72.3, 95% CI) vs 73.82, P=0.016] (TABLE 3). However, LDLT recipients had better quality of life in almost all the domains, except for the general health status [73.28 (69.9–76.7, 95% CI) vs 71.1, P=0.199], which there was no difference between the two groups.

TABLE 3. Comparation of mean scores of QOL between LDLT Patients vs Brazil´s population vs South Brazil´s population.

QOL	LDLT (n=25) mean	Brazil's population (n=12,423) mean	*P* value Student t test*	South Brazil population (n=755) mean	*P* value Student *t* test*
Vitality	87.8	71.9	<0.001	66.85	<0.001
Mental health	66.4	74.5	0.009	73.82	0.016
General health status	73.28	70.2	0.074	71.1	0.199
Pain	78.72	76.7	0.672	67.53	0.026
Functional aspects	94.6	75.5	0.002	82.45	0.005
Social aspects	93	83.9	0.005	78.3	<0.001
Physical aspects	92	77.5	0.006	74.73	0.001
Emotional aspects	97.33	81.7	<0.001	70.02	<0.001

QOL: quality of life; LDLT: liver donor liver transplantation. Numbers were expressed in mean. *Statistically significant ($P<0.05$).

TABLE 4 illustrated that there was no difference between patients with MELD ≤15 and those with MELD >15 in any of eight domains of QOL assessed. There was also no difference when patients were divided according to the Child classification, gender or age (TABLE 4). Mental health aspects score was lower for patient with acute complications (58.91±18.51 vs 72.29±5.54, P=0.033). There was no other difference between the groups.

DISCUSSION

Advances in intensive care medicine, immunosuppression, perioperative management, and surgical techniques allowed impressive improvement in patient survival after LT in the last decades. Presently, LT 5- and 10-year survival rate is about 75% and 70%, respectively. Several studies have also documented excellent QOL after LT, with most patients returning to normal life[11-14]. However, there are only a few studies that assess QOL after LDLT and almost all these studies are limited to the QOL of the donor[15-18]. Receptors of LDLT have some important different aspects from those of DDLT that justify a distinct evaluation[19].

Receptors of LDLT have higher rate of postoperative biliary and vascular complications[20,21]. Biliary technical complications are common following reconstitution of one to three tiny bile ducts from partial liver grafts obtained from living donors[22]. The rate of vascular complications, mainly hepatic artery thrombosis, is also higher in receptors of LDLT due to small hepatic artery of a hepatic lobe rather than a large artery from the entire liver. In addition to the higher rate of these complications, their complexity is expressive. They are associated with increase hospital stay, cost, morbidity and mortality. Quality of life is reduced due to need of repeated admissions and invasive treatments, such as percutaneous, endoscopic and surgical procedures to treat biliary and vascular complications. Retransplantation rate is also higher in these receptors[20].

Our study evaluated the QOL of recipients of LDLT with more than 10 years of successful transplantation. Our mean follow-up time was 15 years. The survival rate of 34,6% observed in our study is lower than that of most American, European, and Japanese hospitals. Presently, 10-year survival rate of patients subjected to LDLT in the United States and Europe is about 50 to 60%. In Japan, this rate is even higher, about 70%[23]. Our lower patient survival may be possibly due to several medical limitations of developing countries, like Brazil, mainly scarcity of appropriate hospital resources and patients´ economic and cultural differences.

The goal of LT is not only to obtain long survival rate, but also to achieve an excellent QOL in all aspects, including physical, psychological, and social domains. With the significant improvement in survival of patients undergoing LT in the last decades, several transplantation centers and government and insurance health organizations have begun to focus attention on QOL after LT[5,24,25]. The evaluation and applications of QOL after LT have expanded dramatically in the last years[11,12]. Studies with different follow-up and questionnaires have been used to assess QOL following LT[6,24,25]. QOL assessment should consider both objective data obtained by the medical team and patient´s opinions of their health, reflecting how they really feel, and how much their disease affects their way of life.

The World Health Organization Committee for QOL defined that QOL is a "multidimensional construct that is affected by physical health, psychological health, functional status, social relationships, personal beliefs"[26]. The method of QOL evaluation used in our study was the SF-36, which has been validated in several countries. It is the most frequently method employed to evaluate QOL of liver transplant recipients. This questionnaire assesses eight domains on general health, functional capacity, physical aspects, bodily pain, mental health, emotional role functioning, social functioning, and vitality.

The few publications on QOL after LDLT are limited either to one or few QOL domains assessed or to patients with follow-up less than 5 years after LT[5,24,25]. Our study assessed all eight QOL domains of LDLT recipients with more than 10 years of successful transplantation.

Our study showed that recipients of LDLT with more than 10 years of successful transplantation had good QOL rates in all domains. When compared with the QOL of the general Brazilian population and the population of the Southern region of Brazil, LDLT recipients had better or similar QOL in all domains, except for mental health. Mental health domain was lower in transplanted patients than in the general Brazilian population or in the population of the Southern region of the country.

Several studies demonstrated that socioeconomic status, such as marital status, education level, social support, income, profession and employment are crucial factors that influence QOL after LT[6,27-30]. Since Brazil is a large country with enormous socioeconomic and cultural differences between the Brazilian regions, we considered that was is important to compare QOL of LDLT patients not only with that of the general population, but also with that of the South Brazilian region, where our hospitals are located.

TABLE 4. QOL after 10 years of LDLT According to MELD (≤ 15 and >15), Child (A, B or C), Age (≤ 50 and >50 years), Gender and Occurrence of Acute Complications.

QOL		EA	PA	SA	FA	P	GH	MH	VT
MELD									
≤ 15 (n=11)	Mean ± SD	96.96±10.07	100±0	89.77±16.6	95.91±4.37	76.55±21.77	70.18±7.03	65.82±16.53	87.27±12.92
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (50–100)	95 (90–100)	72 (40–100)	67 (60–80)	72 (20–76)	90 (55–100)
>15 (n=14)	Mean ± SD	97.61±893	85.71±30.56	95.54±13.52	93.57±8.19	80.43±25.53	75.71±8.56	66.86±12.98	88.21±15.64
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (50–100)	95 (75–100)	92 (30–100)	80 (57–87)	72 (32–76)	92.5 (45–100)
	P Mann-Whitney test*	0.936	0.373	0.373	0.727	0.536	0.075	0.979	0.572
Child									
A (n=2)	Mean ± SD	100±0	100±0	100±0	95±7.07	92±11.31	63.5±4.95	64±5.66	87.5±10.61
	Median (min–max)	100 (100–100)	100 (100–100)	100 (100–100)	95 (90–100)	92 (84–100)	63.5 (60–67)	64 (60–68)	87.5 (80–95)
B (n=16)	Mean ± SD	91.91±8.37	90.63±27.2	89.84±17.81	94.06±6.88	74.75±23.57	74±6.79	65±16.94	86.88±16.21
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (50–100)	95 (75–100)	72 (40–100)	75 (62–85)	72 (20–76)	92.5 (45–100)
C (n=7)	Mean ± SD	95.23±12.62	92.86±18.9	98.21±4.72	95.71±7.32	84±25.95	74.43±10.88	70.29±8.6	90±11.18
	Median (min–max)	100 (66.6–100)	100 (50–100)	100 (87.5–100)	100 (80–100)	100 (30–100)	80 (57–87)	76 (56–76)	90 (70–100)
	P Kruskal-Wallis test*	0.744	0.866	0.433	0.703	0.445	0.230	0.496	0.806
Sex									
Men (n=17)	Mean ± SD	98.04±8.10	91.18±26.43	95.59±12.45	94.12±5.93	80.59±24.14	72.59±8.6	68.71±11.68	87.94±13.81
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (50–100)	95 (80–100)	84 (30–100)	75 (57–87)	76 (32–76)	90 (45–100)
Women (n=8)	Mean ± SD	95.83±11.81	93.75±17.68	87.5±18.9	95.63±8.63	74.75±23.28	74.75±7.8	61.5±18.75	87.5±16.04
	Median (min–max)	100 (66.6–100)	100 (50–100)	100 (50–100)	100 (75–100)	72 (41–100)	76 (62–85)	68 (20–76)	92.5 (55–100)
	P Mann-Whitney test*	0.798	1.0	0.374	0.288	0.549	0.549	0.238	0.887
Age at the time of transplantation									
≤50 (n=17)	Mean ± SD	98.04±8.10	91.18±26.43	90.44±17.42	95.88±6.9	77.59±23.36	74.53±8.02	65.88±16.62	88.53±16.28
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (50–100)	100 (75–100)	84 (40–100)	75 (60–87)	72 (20–76)	95 (45–100)
>50 (n=8)	Mean ± SD	95.83±11.81	93.75±17.68	98.44±4.42	91.88±5.94	81.13±25.36	70.63±8.63	67.5±8.4	86.25±9.16
	Median (min–max)	100 (66.6–100)	100 (50–100)	100 (87.5–100)	92.5 (80–100)	92 (30–100)	71 (57–80)	68 (56–76)	85 (70–100)
	P Mann-Whitney test*	0.798	1.0	0.440	0.057	0.711	0.315	0.711	0.262
Acute complications									
Yes (n=11)	Mean ± SD	96.96±10.07	95.45±15.06	89.77±20.01	95±7.07	83.73±22.86	70.91±9.6	58.91±18.51	84.09±19.60
	Median (min–max)	100 (66.6–100)	100 (50–100)	100 (50–100)	100 (80–100)	78 (40–100)	75 (57–85)	60 (20–76)	95 (45–100)
No (n=14)	Mean ± SD	96.61±8.93	89.29±28.95	95.54±9.31	94.29±6.75	74.79±24.16	75.14±6.81	72.29±5.54	90.71±7.56
	Median (min–max)	100 (66.6–100)	100 (0–100)	100 (75–100)	100 (75–100)	78 (30–100)	77.5 (65–87)	76 (60–76)	90 (80–100)
	P Mann-Whitney test*	0.936	0.809	0.727	0.609	0.373	0.244	0.033	0.767

VT: vitality; MH: mental health; GH: general health status; P: pain; FA: functional aspects; SA: social aspects; PA: physical aspects; EA: emotional aspects. Numbers were expressed in mean ±SD, median (min–max). *Statistically significant ($P<0.05$).

Similar to our findings, some other studies have documented a significant QOL improvement of recipients of LDLT. El-Meteini et al. have shown improvement in all eight domains evaluated six months after LT by the Short-Form 36 of all 35 patients subjected to LDLT in Egypt[5]. Kawagishi et al. have demonstrated that most children who underwent LDLT had normal school achievements and physical development more than 10 years after LT. In their experience, four of six recipients who showed growth retardation with low body weights reached average heights and body weights for their ages after LDLT. Biliary stenosis was the most significant prognostic factor in terms of QOL because of the need of frequent hospital readmissions[25].

Noma et al.[24] evaluated the psychosocial state of 40 recipients three to 5 years after LDLT. They indicated that the recipients had a decline of psychosocial QOL due to the incapacity to return to full time work after the transplant and because of the guilty feelings to the donors after the transplantation. The authors concluded that the main predictor of psychosocial states of the recipients was the length of wait for LDLT[24].

The reasons for the lower mental health rate in transplanted patients observed in our report as well as in other studies may be multifold. Presence of comorbidities and complications, immunosuppressors' side-effects, and recurrence of liver disease may play an important role[31]. In our study, the presence of acute post-transplant complications was one of the factors that contributed to this decrease. Some recipients of living donors report distress because living organs were donated by healthy donors. In a systematic review, Thys et al.[32] reported that although pediatric recipients of LDLT had improved coping skills and satisfactory peer relationships, they also had anxiety, depressive symptoms, and negative body image, and were concerned about the future. Psychological problems were sometimes induced by feelings of guilt and indebtedness toward the donor[33].

Our finding of lower mental health in patients with more than a decade of LDLT has important clinical application. Transplanted patients should have routine psychological evaluation and therapeutic measures instituted when mental health changes are recognized. A multidisciplinary approach with psychological treatment and social intervention may be helpful for rehabilitating these patients[34-38]. The major strength of our study is that the study was limited to recipients with more than 10 years of survival after LDLT. In addition, almost all living patients participated of the study.

The major limitation of our study is the retrospective evaluation of the data and the small number of patients. This is minimized because all medical and surgical procedures were coordinated and supervised by the same transplant team and the data were retrieved from electronic medical records and study protocols. The drastic reduction of LDLT worldwide in the last decades limits the possibility to perform studies with large number of LDLT in a single institution. Multicenter studies may overcome this limitation. Prospective study and QOL comparison between deceased liver transplant and LDLT may also provide valuable contribution to this important subject.

It is concluded that long-term QOL in recipients of LDLT is similar that of general population, except for mental health domain which is reduced.

Authors' contribution

Nogueira IR: protocol development; data collection; writing of the manuscript. Coelho JCU: protocol development; interpretation of data; writing of the manuscript. Domingos MF: data collection; approval of the final version of the manuscript. Parolin MB: data collection; revision of the manuscript. Matias JEF, Freitas ACT: data interpretation; approval of the final version of the manuscript. Martins EL: analysis of data; drafting of the manuscript. Costa MAR: analysis and interpretation of data.

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Nogueira IR, Coelho JCU, Domingos MF, Parolin MB, Matias JEF, Freitas ACT, Martins EL, Costa MAR. Boa qualidade de vida após mais de uma década de transplante hepático inter-vivos. Arq Gastroenterol. 2021;58(1):10-6.

RESUMO – **Contexto** – Receptores de transplante hepático inter-vivo (THIV) apresentam elevada taxa de complicações biliares e vasculares pós-operatórias que podem reduzir a qualidade de vida (QV) devido à necessidade de tratamentos invasivos e repetitivos. **Objetivo** – O objetivo deste estudo é avaliar os vários aspectos da qualidade de vida dos pacientes submetidos a THIV após 10 anos de transplante e identificar possíveis fatores que possam estar associados à diminuição da QV. **Métodos** – Os dados de todos os pacientes com mais de 10 anos de THIV foram avaliados retrospectivamente. Os pacientes foram entrevistados por meio de um questionário de qualidade de vida (SF-36). **Resultados** – Do total de 440 transplantes hepáticos realizados em 17 anos (setembro de 1991 a dezembro de 2008), 78 pacientes foram submetidos a THIV, dos quais 27 estavam vivos e 25 responderam completamente ao questionário. Destes, 17 (68%) homens e 8 (32%) mulheres, com idade média de 38,6±18,5 anos no momento do transplante e tempo médio de acompanhamento de 15,1±1,9 anos. O MELD médio foi de 16,4±4,9 e a principal indicação para o transplante hepático foi cirrose hepática causada pelo vírus da hepatite B, 32%. Quando comparado com a população geral, os pacientes submetidos a THIV apresentaram menor escore de saúde mental (66,4 vs 74,5; *P*=0,0093) e escores mais altos de vitalidade (87,8 vs 71,9; *P*<0,001), aspectos funcionais (94,6 vs 75,5; *P*=0,002), aspectos sociais (93 vs 83,9; *P*=0,005), aspectos físicos (92 vs 77,5; *P*=0,006), e aspectos emocionais (97,33 vs 81,7; *P*<0,001). Os escores do estado geral de saúde (73,28 vs 70,2; *P*=0,074) e de dor (78,72 vs 76,7; *P*=0,672) eram similares nos dois grupos. **Conclusão** – Conclui-se que os vários aspectos da QV dos receptores de transplante hepático inter-vivo são semelhantes aos da população geral mais de uma década após o transplante, exceto o domínio da saúde mental que é menor.

DESCRITORES – Transplante de fígado. Doadores vivos. Cirrose hepática. Qualidade de vida.

REFERENCES

1. Raia S, Nery JR, Mies S. Liver transplantation from liver donors. Lancet. 1989;2:497.
2. Yamaoka Y, Washida M, Honda K, Tanaka K, Mori K, Shimahara Y, et al. Liver transplantation using a right lobe graft from a living related donor. Transplantation. 1994;57:1127-30.
3. Ogiso S, Kamei H, Onishi Y, Kurata N, Jobara K, Kawashima H, et al. Decreased long-term graft survival in persistent biliary complications after right-lobe living-donor liver transplantation. Clin Transplant. 2020;34:e13771.
4. Coelho JCU, Leite LO, Molena A, Freitas ACT, Matias JEF. Biliary complications after liver transplantation. Arq Bras Cir Dig. 2017;30:127-31.
5. El-Meteini M, Montasser IF, El Gendy E, Dabbous H, Hashem RE, William P, et al. Assessment of health-related quality of life in Egyptian HCV-infected recipients after living donor liver transplantation. J Dig Dis. 2015;16:675-82.
6. Onghena L, Develtere W, Poppe C, Geerts A, Troisi R, Vanlander A, et al. Quality of life after liver transplantation: State of the art. World J Hepatol. 2016;8: 749-56.
7. Ciconelli RM, Ferraz MB, Santos W, Meinão I, Quaresma MR. Tradução para a língua portuguesa e validação do questionário genérico de avaliação de qualidade de vida SF-36 (Brasil SF-36). Rev Bras Reumatol. 1999;39:143-50.
8. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30:473-83.
9. Laguardia J, Campos MR, Travassos C, Najar AL, Anjos LA, Vasconcellos MM. Dados normativos brasileiros do questionário Short Form-36 versão 2. Rev Bras Epidemiol. 2013;16:889-97.
10. Cruz LN, Fleck MPA, Oliveira MR, Camey SA, Hoffmann JF, Bagattini AM, et al. Health-related quality of life in Brazil: normative data for the SF-36 in a general population sample in the south of the country. Cien Saude Colet. 2013;18: 1911-21.
11. Desai R, Jamieson NV, Gimson AE, Watson CJ, Gibbs P, Bradley JA, et al. Quality of life up to 30 years following liver transplantation. Liver Transpl. 2008;14:1473-9.
12. Sullivan KM, Radosevich DM, Lake JR. Health-related quality of life: Two decades after liver transplantation. Liver Transpl. 2014;20:649-54.
13. Belle SH, Porayko MK, Hoofnagle JH, Lake JR, Zetterman RK. Changes in quality of life after liver transplantation among adults. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Liver Transplantation Database (LTD). Liver Transpl Surg. 1997;3:93-104.
14. Duffy JP, Kao K, Ko CY, Farmer DG, McDiarmid SV, Hong JC, et al. Long-term patient outcome and quality of life after liver transplantation: analysis of 20-year survivors. Ann Surg. 2010;252:652-61.
15. Coelho JC, Parolin MB, Baretta GAP, Pimentel SK, Freitas ACT, Colman D. Donor quality of life after living donor liver transplantation. Arq Gastroenterol. 2005;42:83-8.
16. Benzing C, Schmelzle M, Oellinger R, Gruettner K, Muehlisch AK, Raschzok N, et al. Living-Donor Liver Transplant: An Analysis of Postoperative Outcome and Health-Related Quality of Life in Liver Donors. Exp Clin Transplant. 2018;16:568-74.
17. Coelho JCU, Freitas ACT, Matias JEF, Godoy JL, Zeni Neto C, Parolin MB, et al. Donor complications including the report of one death in right-lobe living donor liver transplantation. Dig Surg. 2007;24:191-6.
18. Kikuchi R, Mizuta K, Urahashi T, Sanada Y, Yamada N, Onuma E, et al. Quality of life after living donor liver transplant for biliary atresia in Japan. Pediatr Int. 2018;60:183-90.
19. Miller CM, Quintini C, Dhawan A, Durand F, Heimbach JK, Kim-Schluger HL, et al. The International Liver Transplantation Society Living Donor Liver Transplant Recipient Guideline. Transplantation. 2017;101:938-44.
20. Abu-Gazala S, Olthoff KM. Status of adult living donor liver transplantation in the United States: Results from the adult-to-adult living donor liver transplantation cohort study. Gastroenterol Clin North Am. 2018;47:297-311.
21. Samstein B, Smith AR, Freise CE, Zimmerman MA, Baker T, Olthoff KM, et al. Complications and their resolution in recipients of deceased and living donor liver transplants: findings from the A2ALL Cohort Study. Am J Transplant. 2016;16:594-602.
22. Miyagi S, Kakizaki Y, Shimizu K, Miyazawa K, Nakanishi W, Hara Y, et al. Arterial and biliary complications after living donor liver transplantation: a single-center retrospective study and literature review. Surg Today. 2018;48:131-9.
23. Umeshita K, Inomata Y, Furukawa H, Kasahara M, Kawasaki S, Kobayashi E, at al. Liver transplantation in Japan: Registry by the Japanese Liver Transplantation Society. Hepatol Res. 2016;46:1171-86.
24. Noma S, Hayashi A, Uehara M, Uemoto S, Murai T. Comparison between psychosocial long-term outcomes of recipients and donors after adult-to adult living donor liver transplantation. Clin Transplant. 2011;25:714-20.
25. Kawagishi N, Takeda I, Miyagi S, Satoh K, Akamatsu Y, Sekiguchi S, et al. Quality of life and problems affecting recipients more than 10 years after living donor liver transplantation. Transplant Proc. 2009;41:236-7.
26. The World Health Organisation quality of life assessment (WHOQOL) Position paper from the World Health Organization. Soc Sci Med. 1995;41:1403-9.
27. Yang LS, Shan LL, Saxena A, Morris DL. Liver transplantation: A systematic review of long-term quality of life. Liver Int. 2014;34:1298-313.
28. Bownik H, Saab S. Health-Related Quality of Life After Liver Transplantation for Adult Recipients. Liver Transpl. 2009;15:S42–S9.
29. Coelho JCU, Freitas ACD, Matias JEF, Pissaia Jr A, Godoy JL, Zeni JOV. Sexual dysfunction in males with end-stage liver disease: partial recovery after liver transplantation. J Liver: Dis Transplant. 2014;3:1-4.
30. Parolin MB, Rabinovitch I, Urbanetz AA, Scheidemantel C, Cat ML, Coelho JCU. Impact of successful liver transplantation on reproductive function and sexuality in women with advanced liver disease. Transplant Proc. 2004;36:943-4.
31. Pérez-San-Gregorio MA, Martín-Rodríguez A, Domínguez-Cabello E, Férnandez-Jiménez E, Borda-Más M, Bernardos-Rodríguez A. Mental health and quality of life in liver transplant and cirrhotic patients with various etiologies. Int J Clin Health Psychol. 2012;12:203-18.
32. Thys K, Schwering KL, Siebelink M, Dobbels F, Borry P, Schotsmans P, et al. Psychosocial impact of pediatric living-donor kidney and liver transplantation on recipients, donors, and the family: a systematic review. Transpl Int. 2015;28:270-80.
33. Shizuku M, Kamei H, Kimura H, Kurata N, Jobara K, Yoshizawa A, et al. Clinical features and long-term outcomes of living donors of liver transplantation who developed psychiatric disorders. Ann Transplant. 2020;25:e918500.
34. Miller LR, Paulson D, Eshelman A, Bugenski M, Brown KA, Moonka D, et al. Mental health affects the quality of life and recovery after liver transplantation. Liver Transpl. 2013;19:1272-8.
35. Grover S, Sarkar S. Liver transplant-psychiatric and psychosocial aspects. J Clin Exp Hepatol. 2012;2:382-92.
36. Pérez-San-Gregorio MÁ, Martín-Rodríguez A, Borda-Mas M, Avargues-Navarro ML, Pérez-Bernal J, Conrad E, et al. Post-traumatic growth and its relationship to quality of life up to 9 years after liver transplantation: a cross-sectional study in Spain. BMJ Open. 2017;7:e017455.
37. Dąbrowska-Bender M, Kozaczuk A, Pączek L, Milkiewicz P, Słoniewski R, Staniszewska A. Patient quality of life after liver transplantation in terms of emotional problems and the impact of sociodemographic factors. Transplant Proc. 2018;50:2031-8.
38. Dunn MA, Rogal SS, Duarte-Rojo A, Lai JC. Physical function, physical activity, and quality of life after liver transplantation. Liver Transpl. 2010;26:702-8.

ORIGINAL ARTICLE

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The acceptance of changes in the management of patients with acute pancreatitis after the revised Atlanta Classification

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ABSTRACT – **Background** – New recommendations for the management of patients with acute pancreatitis were set after the Atlanta Classification was revised in 2012. **Objective** – The aim of the present systematic review is to assess whether these recommendations have already been accepted and implemented in daily medical practices. **Methods** – A systematic literature review was carried out in studies conducted with humans and published in English and Portuguese language from 10/25/2012 to 11/30/2018. The search was conducted in databases such as PubMed/Medline, Cochrane and SciELO, based on the following descriptors/*Boolean* operator: "Acute pancreatitis" AND "Atlanta". Only Randomized Clinical Trials comprising some recommendations released after the revised Atlanta Classification in 2012 were included in the study. **Results** – Eighty-nine studies were selected and considered valid after inclusion, exclusion and qualitative evaluation criteria application. These studies were stratified as to whether, or not, they applied the recommendations suggested after the Atlanta Classification revision. Based on the results, 68.5% of the studies applied the recommendations, with emphasis on the application of severity classification (mild, moderately severe, severe); 16.4% of them were North-American and 14.7% were Chinese. The remaining 31.5% just focused on comparing or validating the severity classification. **Conclusion** – Few studies have disclosed any form of acceptance or practice of these recommendations, despite the US and Chinese efforts. The lack of incorporation of these recommendations didn't enable harnessing the benefits of their application in the clinical practice (particularly the improvement of the communication among health professionals and directly association with the worst prognoses); thus, it is necessary mobilizing the international medical community in order to change this scenario.

HEADINGS – Pancreatitis. Classification. Severity of illness index. Prognosis.

INTRODUCTION

Pancreatitis consists in the inflammatory process affecting the pancreatic tissue and adjacent areas; the disease can present acute or chronic evolution and records significant incidence in its acute form (from 13 to 45 patients per 100,000 inhabitants per year, previously demonstrated from a nationwide survey in Japan)[1,2]. Its diagnosis is based on the identification of at least two of the following symptoms: pain in the upper abdomen; amylase and/or lipase values higher than three times the normal reference values; evidence of inflammation in the pancreatic and/or peripancreatic tissue based on complementary imaging examination[3].

Propositions focused on changing the concepts and treatment of acute pancreatitis, mainly on the best way to determine the severity of patients, from the beginning of symptoms (abdominal pain), emerged after the publication of the Revised Atlanta Classification on 10/25/2012[4]. Currently, based on the new recommendations, systemic or local signs of inflammation, the presence or absence of temporary or persistent organ failure (determined based on the modified Marshall classification), and the incidence of local complications (i.e., the incidence of acute liquid collections and of sterile or infected necrosis) are criteria adopted to determine the severity of patients with acute pancreatitis[3-5]. Thus, patients with acute pancreatitis were classified as MILD (absence of organ failure and local or systemic complications), MODERATELY SEVERE (absence of organ failure or, when it happens, it is transient – i.e., it disappears within 48 hours – and can be associated, or not, with local or systemic complications), SEVERE (persistent organ failure – i.e., it remains for more than 48 hours and, when associated with infected pancreatic necrosis, features the most severe conditions, which are associated with the highest mortality rates)[2-5].

Another recent attempt to enhance the severity classification of the patients with acute pancreatitis was the Determinant-Based Classification (DBC). This classification was also developed by several experts worldwide and published simultaneously a the revised Atlanta Classification by the end of 2012, consolidating that the presence of local determinants (sterile or infected pancreatic and/or peripancreatic necrosis) and systemic determinants (transient or persistent organ failure) would be the most appropriated criteria to classify the patients into four categories: mild, moderate, severe and critic, related to their severity[5,6-11]. However, the DBC was

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more minded to the establishment of the severity classification, meanwhile, the revised Atlanta Classification of 2012 presents wider recommendations related to the clinical management of the patients with acute pancreatitis[6-11].

Thus, the use of multifactor scoring methods such as Ranson, APACHE II, Glasgow, SAPS II, among others, to determine the severity of patients with acute pancreatitis is no longer recommended[12,13]. The delay in establishing patients' severity level is one of the main disadvantages associated with the use of scoring methods such as the Ranson criteria (the most used system in past clinical practices), which requires 48 hours to determine the severity of acute pancreatitis[14]. Moreover, this 48-hour period is not based on the onset of the clinical picture, but on the hospitalization date, when the first complementary serum exams are performed[14]. Although APACHE II (another among the most used criteria) can be calculated in the first 24 hospitalization hours, it was originally developed to be applied in Intensive Care Units and requires the analysis of 12 parameters in order to estimate a possible organ failure. Therefore, this complex method comprises many criteria that are not directly correlated to the prognosis of patients with pancreatitis[15].

In addition, other possible serum markers have been evaluated to help improving the implementation of criteria capable of determining the severity of patients with acute pancreatitis, based on laboratory tests such as hematocrit, urea, C-reactive protein and other inflammatory cytokines[16]. Some prognostic risk factors focused on predicting the most severe forms of acute pancreatitis have also been investigated. So far, only overweight (body mass index >25 kg/m^2) and hyperglycemia (blood glucose level higher than 11.1 mmol/L or 200 mg/dL) have been identified and established as eligible factors[13,17].

The best detailing of local complications such as acute peripancreatic collections, sterile or infected pancreatic and peripancreatic necrosis, pseudocysts, and sterile or infected delimited pancreatic necrosis stands out among concepts that changed after the Atlanta Classification revision[4,18,19]. Acute peripancreatic collections can develop near the pancreas in the early stage of the disease (often within 48 hours after clinical picture onset) and do not present internal solid component[4,18,19]. Pseudocysts are amylase-rich liquid collections (without solid components) whose external area simulates a pseudocapsule in the pancreatic and/or peripancreatic region; they emerge after four weeks of disease evolution[4,18,19]. On the other hand, acute necrotic collections are liquid collections associated with necrotic tissues in the pancreas and/or in the peripancreatic region; in most cases, they can maintain communication with the pancreatic duct or with its branches[4]. Delimited pancreatic necrosis is a necrotic collection found within a fibrotic capsule; it often becomes fibrotic 4 weeks after the onset of acute necrotizing pancreatitis[4,18-20].

Finally, based on the best scientific evidences, the application of the new concepts and recommendations published after the revision of the Atlanta Classification in the clinical practice has several advantages and benefits such as: 1. Improving the therapeutic conduct and dialogue among different health professionals involved in the management of patients with acute pancreatitis through the establishment of new criteria, as well as of more uniform and precise terminologies, to diagnose and identify, mainly, the forms of local complications[21-24]; 2. Improving the severity stratification of patients with acute pancreatitis, based on the importance given to the incidence of organ failure in the classification of (mild, moderately severe and severe) acute pancreatitis[4,21]; 3. Facilitating and improving the management and monitoring of the therapeutic success of patients with acute pancreatitis through the inclusion of new imaging criteria to classify the tomographic findings in the evaluation of these patients[22]; 4. Helping the medical community to plan clinical studies based on standardized parameters, which will have impacts on the recommendations for the establishment of future interventions and specific treatments for patients with acute pancreatitis[18,21,24]; 5. Identifying the role played by infected necrosis as determinant factor of high mortality rates associated with the prognosis of acute pancreatitis[21,22].

Unfortunately, despite the advantages and benefits mentioned above, it seems that the world has not fully adhered to the new recommendations issued after the Revised Atlanta Classification for acute pancreatitis[4]. Thus, the aims of the current systematic review were to evaluate this scenario and to investigate whether these recommendations have already been accepted and implemented in current medical practices[24,25].

METHODS

A systematic review of the medical literature, based on recommendations of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes) protocol, was carried out in studies conducted with humans, which were published in English and Portuguese languages, from 10/25/2012 to 11/30/2018. The search was performed in electronic databases such as PubMed/Medline, SciELO and Cochrane, based on the following descriptors/*Boolean* operator: "Acute pancreatitis" AND "Atlanta"[26,27]. Inclusion and exclusion criteria were applied and a qualitative analysis of the studies was performed to select valid and eligible articles for future inferences disclosed in the current review.

Inclusion criteria

Only articles meeting the following characteristics were included in the study: 1. Randomized Clinical Trials-type studies; 2. Studies comparing the efficiency of prognostic markers (chemical/biological/clinical parameter) to established concepts, after the Atlanta Classification revision, in order to determine the severity of patients with acute pancreatitis; 3. Studies focused on investigating parameters and/or prognostic markers for acute pancreatitis, based on criteria established after the Atlanta Classification revision – even if only to divide the groups evaluated in the study sample; 4. Studies comparing rating scores and prognosis predictors for the classification and determination of acute pancreatitis severity cases (among them, the criteria established after the Atlanta Classification revision); 5. Studies focused on presenting the clinical evolution of acute pancreatitis with, or without, therapeutic intervention and on determining the severity of patients based on recommendations established after the Atlanta Classification revision; 6. Studies aimed at evaluating or validating the recommendations established after the Atlanta Classification revision.

Exclusion criteria

We excluded studies that did not meet the inclusion criteria, as well as those that did not mention the recommendations established after the Atlanta Classification revision (i.e., studies published after 2012, whose implementation and/or data collection were performed before this year, when the Atlanta Classification revision had not yet been published and, consequently, released for consultation).

Data extraction and qualitative analysis

The search for studies was based on pre-established qualitative criteria, as described in the methodology of the current review. The information was collected and recorded in a standardized Excel data sheet (Microsoft Corp., Redmond, WA). Discrepancies identified during data sorting or throughout the extraction process were resolved through consensus among the authors. The initial qualitative selection of the studies was based on ABSTRACT reading in order to analyze the aims and outcomes, according to pre-established inclusion and exclusion criteria. Subsequently, the selected studies were fully read to enable[6-9,11,16,28-110].

Exceptionally, in order to clarify some discussion points, the search in the databases was extended to studies presenting design different from the randomized clinical trial, a fact that enabled using more than one study[111]. However, according to the inclusion and exclusion criteria of this review, the selected study, which presented different design from the clinical trial, was not taken into consideration in the results, nor was it added to the tables, in order to respect the methodological proposal of this systematic review[111].

RESULTS

One hundred and seventy-four (174) studies were initially identified. Next, they were subjected to the inclusion and exclusion criteria and to qualitative analysis (according to the PRISMA protocol), which made it possible selecting 89 valid studies, as shown in FIGURE 1[26,27].

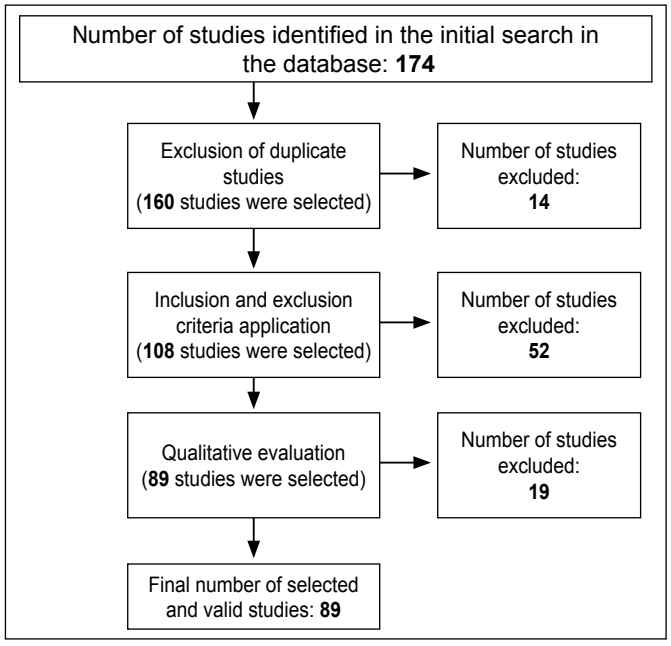

FIGURE 1. Outlining the selection of valid studies based on PRISMA Protocol.

The herein selected 89 valid studies were recorded and stratified as to whether, or not, they applied the recommendations and/or concepts proposed and disclosed after the Atlanta Classification revision. The studies applying these recommendations were those that, based on these principles, established and determined the severity of the investigated patients (mild, moderately severe, severe) in order to evaluate their clinical evolution (TABLE 1)[16,28-87].

Other studies that did not apply these concepts and/or recommendations to categorize patients' severity were subdivided as follows: studies that just compared the efficiency of prognostic markers (chemical / biological / clinical parameter) and of old rating scores to the new concepts used to determine the severity of patients, which were disclosed after the Atlanta classification revision (TABLE 2)[6-9,88-100]; and studies that just evaluated and validated the recommendations issued after the Atlanta Classification revision (TABLE 3)[11,101-110].

Based on TABLES 1, 2 and 3, 68.5% (61/89) of the studies applied the recommendations disclosed after the Atlanta Classification revision. Most studies that have applied the recommendations after the Atlanta Classification revision were North-American (16.4% = 10/61) and Chinese (14.7% = 9/61) (FIGURE 2). On the other hand, most studies that have compared prognostic factors to rating scores in order to evaluate the new recommendations were Chinese (41.2% = 7/17), and they were followed by North-American studies (11.8% = 2/17).

DISCUSSION

Most studies (68.5% = 61/89) adhered to and applied the new recommendations published after the Atlanta Classification revision to their sampling. The main aim of more than half of these studies (55.7% = 34/61) was to evaluate the clinical evolution of patients based on the application of the new severity classification proposal (mild, moderately severe and severe). The remaining studies (44.2% = 27/61) used these recommendations to divide their sample; they took the severity classification as standard and, later, they investigated other factors capable of determining the prognosis of patients. In fact, these studies stood out, mainly because they presented and reinforced the reliability of the severity classification published after the Atlanta revision. Publications from countries located in continents such as Africa and Oceania were not identified. Latin America also presented few studies; Brazil (3.3% = 2/61) and Mexico (4.9% = 3/61) were the only Latin American countries presenting studies about this topic. In addition, although no European country has individually played a significant role in the number of publications, the analysis of the whole set of publications enabled seeing that Poland (6.6% = 4/61), Croatia (4.9% = 3/61) and Finland (4.9% = 3/61) have made considerable contributions to understand the natural clinical evolution of patients which severity was determined by the revised Atlanta classification. Despite the Chinese and American leadership in the number of publications, one should take into consideration a possible numerical overestimation bias due to their great economic and demographic power[112]. (FIGURE 2)

China (41.2% = 7/17) and the United States (11.8% = 2/17) also recorded the largest number of publications among studies that just compared the efficiency of prognostic markers (C-reactive protein, hematocrit, red cell distribution width – RDW, serum calcium, thrombin-antithrombin III complex, brain natriuretic peptide – BNP, procalcitonin, apolipoprotein B, pentraxin 3 -PTX3, growth differentiation factor 15 – GDF-15, urea and body mass index) and of old rating scores (Ranson, Apache II, BISAP, PANC 3, DBC) to the new concepts aimed at determining the severity of patients with acute pancreatitis, which were released after the Atlanta classification revision (TABLE 2)[16,38,41,60,67,87,99]. However, European countries such as Spain (11.8% = 2/17) and the United Kingdom (11.8% = 2/17)

TABLE 1. List of studies that applied the recommendations and concepts proposed after the Atlanta Classification revision, establishing the severity of the investigated patients in mild, moderately severe or severe, in order to evaluate their clinical evolution.

Author	Publication year	Number of participants	Adopted Atlanta Revision Criteria	Nationality
Weitz G[28]	2014	391	Severity Classification	Germany
Karabulut U[29]	2014	98	Severity Classification	Brazil
Murilo GB[30]	2016	58	Severity Classification	Brazil
Sun Y[31]	2015	43	Severity Classification	China
Zhu Y[32]	2017	3260	Severity Classification	China
Zeng Y[33]	2014	90	Severity Classification	China
Li G[34]	2016	35	Diagnosis	China
Deng LH[16]	2017	70	Severity Classification	China
Lin S[35]	2017	671	Severity Classification	China
Qi X[36]	2017	204	Severity Classification	China
Jia R[37]	2015	85	Severity Classification	China
Xiao S[38]	2015	153	Diagnosis	China
Joon HC[39]	2015	153	Severity Classification	South Korea
Huh JH[40]	2018	191	Severity Classification	South Korea
Kim BG[41]	2013	50	Severity Classification / Diagnosis	South Korea
Cho JH[42]	2018	60	Severity Classification	South Korea
Mikolasevic[43]	2016	198	Severity Classification	Croatia
Mikolasevic[44]	2016	609	Severity Classification / Diagnosis	Croatia
Trgo G[45]	2016	40	Severity Classification	Croatia
Vujasinovic[46]	2014	100	Severity Classification	Slovenia
María CP[47]	2016	56	Severity Classification	Spain
Bozhychko[48]	2017	233	Severity Classification	Spain
Ellery KM[49]	2017	122	Severity Classification / Diagnosis	USA
Sugimoto M[50]	2015	663	Severity Classification	USA
Gougol A[51]	2017	500	Severity Classification	USA
Vipperla K[52]	2017	121	Severity Classification	USA
Vlada AC[53]	2013	67	Severity Classification	USA
Buxbaum J[54]	2014	25	Severity Classification	USA
Buxbaum J[55]	2016	60	Severity Classification	USA
Dimagno M[56]	2014	223	Diagnosis	USA
Bishu S[57]	2018	357	Severity Classification	USA
Bem MD[58]	2016	175	Severity Classification	USA
Nieminen A[59]	2014	25	Diagnosis	Finland
Nikkola A[60]	2017	35	Severity Classification	Finland
Nukarinen E[61]	2016	176	Severity Classification	Finland
Bakker OJ[62]	2013	639	Severity Classification	Netherlands
Párniczky A[63]	2015	-*	Severity Classification	Hungary
Poropat G[64]	2012	162	Severity Classification /Complications	India
John BJ[65]	2017	134	Severity Classification	India
Stirling AD[66]	2017	337	Severity Classification	Ireland
Losurdo G[67]	2016	90	Severity Classification	Italy
Sugawara S[68]	2017	23	Severity Classification	Japan
Andrius K[69]	2016	142	Severity Classification / Diagnosis	Lithuania
Chacó MA[70]	2017	27	Severity Classification	Mexico
Riquelme F[71]	2016	137	Severity Classification / Diagnosis	Mexico
Jesus E[72]	2017	198	Severity Classification	Mexico
Głuszek S[73]	2015	10	Severity Classification	Poland
Michal L[74]	2015	103	Severity Classification / Diagnosis	Poland
Kuśnierz C[75]	2017	66	Diagnosis	Poland
Sporek M[76]	2016	65	Diagnosis	Poland
Huggett MT[77]	2015	19	Severity Classification / Pancreatic Necrosis	United Kingdom
Suppiah A[78]	2013	146	Severity Classification	United Kingdom
Haffar S[79]	2017	54	Severity Classification / Diagnosis	Syria
Bertilsson S[80]	2015	1457	Severity Classification	Sweden
Ragnarsson[81]	2016	254	Severity Classification	Sweden
Shen HN[82]	2012	1.131.927	Severity Classification	Taiwan
Ince AT[83]	2014	84	Severity Classification	Turkey
Madaria E[84]	2016	40	Pancreatic Necrosis / Complications	Turkey
Senturk H[85]	2015	68	Diagnosis	Turkey
Fidan S[86]	2018	76	Severity Classification	Turkey
Türkoğlu A[87]	2014	92	Severity Classification	Turkey

* Multicenter study in progress, expected sample larger than 1,200 patients.

TABLE 2. List of studies that compared the efficiency of prognostic markers (chemical / biological / clinical parameter) and of old rating scores to the new concepts used to determine the severity of patients, which were disclosed after the Atlanta classification revision.

Author	Publication year	Number of participants	Adopted Atlanta Revision Criteria	Nationality
Koziel D[88]	2015	822	Severity Classification	Canada
Liu J[89]	2016	214	Severity Classification	China
Guo Q[6]	2015	973	Severity Classification	China
Chen Y[7]	2015	395	Severity Classification	China
He WH[90]	2017	708	Severity Classification	China
Zhang J[91]	2014	155	Severity Classification	China
Xiao Don[92]	2015	573	Severity Classification / Pancreatic Necrosis	China
Yang Z[93]	2015	1308	Severity Classification	China
Lee KJ[94]	2016	146	Severity Classification	South Korea
Zubia OF[95]	2016	374	Severity Classification	Spain
Acevedo N[96]	2014	459	Severity Classification	Spain
Kadiyala V[8]	2016	338	Severity Classification	USA
Nawaz H[97]	2013	256	Severity Classification	USA
Jones MJ[98]	2017	629	Severity Classification	United Kingdom
Bansal SS[9]	2016	228	Severity Classification	United Kingdom
Ikeura T[99]	2016	1159	Severity Classification	Japan
Gravito S[100]	2018	312	Severity Classification / Diagnosis	Portugal

TABLE 3. Studies that just evaluated and validated the recommendations and concepts presented after the Atlanta Classification revision.

Author	Publication year	Number of participants	Adopted Atlanta Revision Criteria	Nationality
Chen C[101]	2017	208	Imaging examinations / Severity Classification	China
Huang J[102]	2016	3212	Severity Classification	China
Choi JH[103]	2014	553	Severity Classification / Pancreatic Necrosis	South Korea
Kim EJ[104]	2017	258	Severity Classification	South Korea
Bouwense SA[105]	2017	55	Imaging examinations / Severity Classification	Netherlands
Talukdar R[106]	2014	163	Severity Classification	India
Thandassery RB[107]	2013	151	Severity Classification	India
Povilas I[108]	2017	103	Severity Classification	Lithuania
Lakhey PJ[109]	2013	172	Severity Classification	Nepal
Gluszek S[110]	2012	1044	Severity Classification	Poland
Fernandes SR[11]	2016	525	Severity Classification	Portugal

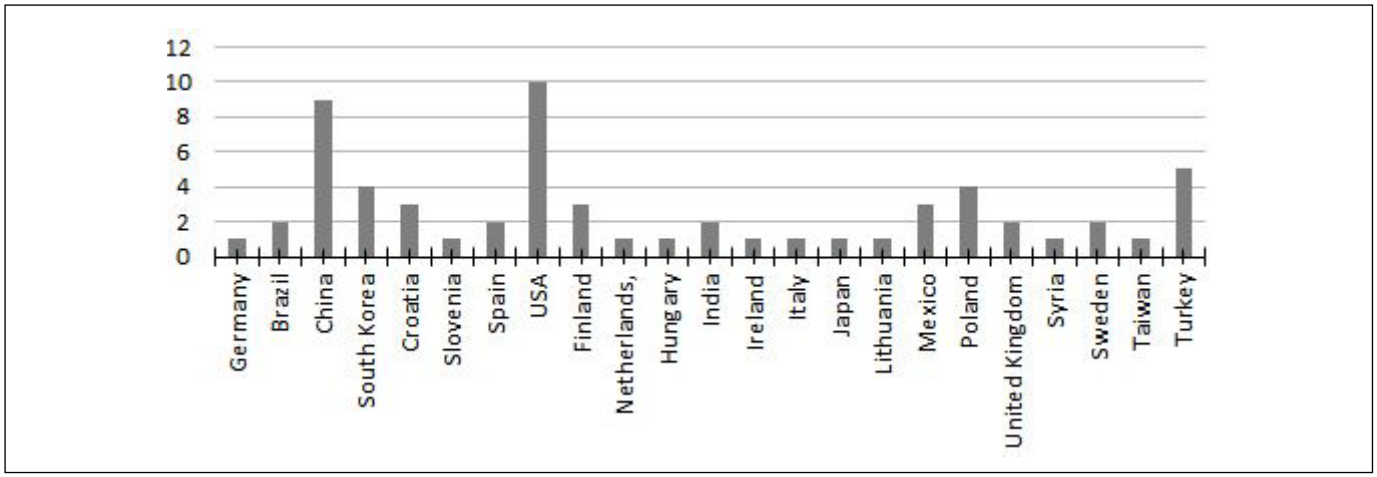

FIGURE 2. Studies that applied the recommendations of the Classification after the Atlanta revision, according to publication country.

recorded the most significant contributions in this group of studies, helping to enhance the knowledge about different prognostic markers and rating scores (especially the Determinant-Based Classification). Nevertheless, they did not classify the investigated patients to assess their prognosis based on the recommendations published after the Atlanta Classification revision, fact that enabled seeing a misalignment in the application of these recommendations in clinical practices.

Interestingly, the evaluation of studies aimed at just validating the recommendations issued after the Atlanta classification revision did not show any US publication, which may suggest that this country may have already incorporated the new recommendations in clinical practices associated with the management of acute pancreatitis[58,59].

In addition, the intention to only classify the severity of patients (90.2% = 55/61) and, to a lesser extent, to use other concepts such as the diagnostic criteria (19.7% = 12/61) and the new definition of pancreatic necrosis (4.9% = 3/61) has prevailed even among publications that had already acknowledged the new recommendations published after the Atlanta Classification revision. However, it is worth taking into consideration and clarifying that the truth about the real application of the new concepts and recommendations released after the Atlanta Classification revision in clinical practices deserves further investigation.

Furthermore, besides the similarity methods used to classify the severity of their patients, the greatest number of studies that the main objective was supposed to compare the Determinant-Based Classification with Atlanta revised Classification still devalue the persistent organ failure as the main criteria to considerate the worsts prognosis of the patients with acute pancreatitis[5-11]. Because, even those with infected necrosis, when weren't simultaneously affected for some persistent organ failure, had more expectation to survive, a fact which proves that infected necrosis alone isn't formal indication for open necrosectomy. So, it's necessary a more systematic approach, in a proper time and initiated by minimally invasive procedures as imaging guided percutaneous drainage and endoscopic techniques, until be necessary more invasive measures (video laparoscopy or even laparotomy), mainly if a more significant number of organs has failure and there isn't clinical improvement signals[92]. In fact, the revised Atlanta Classification consider the exacerbation of comorbidities and the presence of other different local complications than pancreatic and/or peripancreatic necrosis (as acute liquid collection and pseudocysts) significant criteria to classify patients as moderately severe (instead of only mild, as it would be in the Determinant-Based Criteria if the patient wasn't with organ failure). Moreover, the Atlanta Classification identifies organ failure in a simple form (based on the modified Marshall Classification), establishing a more accurate classification of the patients with acute pancreatitis than the DBC, which leads to a more suitable method to select patients, specific for those who are included in medical researchs[5-11].

It is essential mentioning that few published studies, which adopted a different design from the randomized clinical trial, addressed the application of recommendations published after the Atlanta Classification revision in clinical practices. Among them, it is necessary highlighting the study by Staubli et al., who interviewed 233 physicians, who were heads of surgical or internal departments of 85 hospitals (public and private) in Switzerland, based on an online questionnaire, or on telephone conversations, about the management of patients with acute pancreatitis[111]. The aforementioned study has shown that most physicians assessed the severity of patients with acute pancreatitis based on Ranson (87%) or APACHE II (23%) scores; few of them used the classification established and disclosed after the Atlanta revision (12%)[111]. Assumingly, the scenario presented in the study published in 2017 by Staubli et al. reinforces the current lack of theoretical knowledge by medical professionals on the subject, as well as a possible outdated teaching process currently in progress in medical schools[111].

CONCLUSION

Unfortunately, changes in the management of patients with acute pancreatitis may need to be taken into consideration, from the teaching process of future physicians to the updating the professionals who are currently dealing with these patients. This assumption is reinforced by the limited number of countries that reported to have started to incorporate the recommendations released after the Atlanta Classification revision in their clinical practice. This is a worrisome situation, since the incorporation of these recommendations, mainly of those associated with the new proposal to classify the severity of patients with acute pancreatitis could considerably facilitate the communication between health professionals, as well as have a directly association with the hospitalization time, mortality rates, ICU admission, need of interventions, nutritional support and longer hospital stay, mainly of patients facing the most severe conditions[11]. Finally, the disclosure of the current systematic review could encourage the outspread of the new recommendations in order to enable a larger number of nations to perceive, as soon as possible, the importance of updating and changing the herein presented scenario.

Authors' contribution

Alves JR: study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; study supervision. Ferrazza GH: acquisition of data; analysis and interpretation of data; drafting of the manuscript. Nunes Junior IN: acquisition of data; analysis and interpretation of data. Teive MB: critical revision of the manuscript for important intellectual content.

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RESUMO – **Contexto** – Após a revisão da Classificação de Atlanta, em 2012, foram estabelecidas novas recomendações no manejo dos pacientes com pancreatite aguda. **Objetivo** – Objetiva-se avaliar o grau de aceitação e implementação dessas recomendações na prática clínica. **Métodos** – Foi realizada revisão sistemática da literatura com auxílio das bases: PubMed/Medline, Cochrane e SciELO, por meio de busca de estudos em humanos, publicados em inglês e português, no período de 25/10/2012 até 30/11/2018, utilizando os descritores e operador *booleano*: "Acute pancreatitis" E "Atlanta". Foram incluídos apenas estudos do tipo Ensaios Clínicos Randomizados que avaliaram alguma recomendação relacionada a revisão da Classificação de Atlanta após 2012. **Resultados** – Foram selecionados 89 estudos após aplicação dos critérios de inclusão, exclusão e avaliação qualitativa. Esses foram estratificados quanto à aplicação ou não das recomendações propostas após a revisão da Classificação de Atlanta. Verificou-se que 68,5% dos estudos aplicaram essas recomendações, principalmente, na classificação da gravidade dos pacientes (leve, moderadamente grave, grave). Desses 16,4% eram estudos de origem norte-americana e 14,7% chineses. Os outros 31,5% limitaram-se a comparar ou apenas validar essa classificação de gravidade. **Conclusão** – Poucos estudos divulgaram alguma forma de implementação das novas recomendações, apesar dos esforços norte-americanos e chineses. A falta da aparente incorporação dessas recomendações na prática clínica, não permitiu o aproveitamento de suas vantagens (principalmente a melhora da comunicação entre os profissionais e estabelecimento da classificação e identificação precoce dos pacientes mais graves), sendo necessário toda a comunidade médica internacional se mobilizar de alguma forma para mudar esse cenário.

DESCRITORES – Pancreatite. Classificação. Índice de gravidade de doença. Prognóstico.

REFERENCES

1. Forsmark, Chris E, Timothy B. Gardner, eds. Prediction and management of severe acute pancreatitis. Springer New York, 2015.
2. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144:1252-61.
3. Tenner S, Baillie J, DeWitt J, Vege SS. American College of Gastroenterology Guideline: Management of Acute Pancreatitis. Am J Gastroenterol. 2013;108:1400-15.
4. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62:102-11.
5. Yang CJ, Chen J, Phillips ARJ, Windsor JA, Petrov MS. Predictors of severe and critical acute pancreatitis: A systematic review. Dig Liver Dis. 2014;46:446-51.
6. Guo Q, Li M, Chen Y, Hu W. Pancreatology Determinant-based classification and revision of the Atlanta classification, which one should we choose to categorize acute pancreatitis? Pancreatology. 2015;1-6.
7. Chen Y, Ke L, Tong Z, Li W, Li J. Association between severity and the determinant-based classification, Atlanta 2012 and Atlanta 1992, in acute pancreatitis: a clinical retrospective study. Medicine (Baltimore). 2015;94(13):e638.
8. Kadiyala V, Suleiman SL, McNabb-Baltar J, Wu BU, Banks PA, Singh VK. The Atlanta Classification, Revised Atlanta Classification, and Determinant-Based Classification of Acute Pancreatitis: Which Is Best at Stratifying Outcomes? Pancreas. 2016;45:510-5.
9. Bansal SS, Hodson J, Sutcliffe RS, Sutcliffe RS, Marudanayagam R, Muiesan P, et al. Performance of the revised Atlanta and determinant-based classifications for severity in acute pancreatitis. Br J Surg. 2016;103:427-33.
10. Dellinger EP, Forsmark CE, Layer P, Layer P, Levy P, Maravi-Poma E, et al. Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. Ann Surg. 2012;256:875-80.
11. Fernandes SR, Carvalho J, Santos P, Moura CM, Antunes T, Velosa J. Atlanta, revised Atlanta, and Determinant-based classification--application in a cohort of Portuguese patients with acute pancreatitis. Eur J Gastroenterol Hepatol. 2016;28:20-4.
12. Mounzer R, Langmead CJ, Wu BU, Evans AC, Bishehsari F, Muddana V, et al. Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. Gastroenterology. 2012;142:1476-82.
13. Ferreira Ade F, Bartelega JA, Urbano HC, de Souza IK. Acute pancreatitis gravity predictive factors: which and when to use them? ABCD, Arq Bras Cir Dig. 2015;28:207-11.
14. Amálio SMRA, Macedo MATV, Carvalho SMMA, Moreno RP. Avaliação da mortalidade na pancreatite aguda grave: estudo comparativo entre índices de gravidade específicos e gerais. Rev Bras Ter Intensiva. 2012;24:246-51.
15. Wu BU, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. Gut. 2008;57:1698-703.
16. Deng LH, Hu C, Cai WH, Chen WW, Zhang XX, Shi N, et al. Plasma cytokines can help to identify the development of severe acute pancreatitis on admission. Medicine (Baltimore). 2017;96:e7312.
17. Jin Z, Xu L, Wang X, Yang D. Risk Factors for Worsening of Acute Pancreatitis in Patients Admitted with Mild Acute Pancreatitis. Med Sci Monit. 2017;23:1026-32.
18. Souza GD, et al. Artigo de Revisão Entendendo o Consenso Internacional Para as Pancreatites Agudas: classificação de Atlanta 2012. ABCD Arq Bras Cir Dig. 2016;29:206-10.
19. Baker ME, Nelson RC, Rosen MP, Blake MA, Cash BD, Hindman NM, et al. ACR Appropriateness Criteria® acute pancreatitis. Ultrasound Q.2014;30:267-73.
20. Ashley SW, Perez A, Pierce EA, Brooks DC, Moore FD, Whang EE, Banks PA, Zinner MJ. Necrotizing pancreatitis: Contemporary Analysis of 99 Consecutive Cases. Ann Surg. 2001;234:572-9.
21. Lankisch PG, Apte M, Banks PA. Acute pancreatitis. Lancet. 2015;6736:1-12.
22. Cunha EFC, Rocha MS, Pereira FP, Blasbalg R, Baroni RH. Necrose pancreática delimitada e outros conceitos atuais na avaliação radiológica da pancreatite aguda. Radiol Bras. 2014;47:165-75.
23. Sheu Y, Furlan A, Almusa O, Papachristou G, Bae KT. The revised Atlanta classification for acute pancreatitis: a CT imaging guide for radiologists. Emerg Radiol. 2012;19:237-43.
24. Ribeiro G, Silva G, Martins M, Bogea E, Cantanhede J, Abreu J. Etiologia E Mortalidade Por Pancreatite Aguda: Uma Revisão Sistemática. Arquivos Catarinenses de Medicina. 2017;46:168-81.
25. Tércio DC, José P, Edivaldo U, Samir R. A Brazilian survey regarding the management of acute pancreatitis. Revista do Colégio Brasileiro de Cirurgiões, 2008. p. 304-10.
26. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009;62:1006-12.
27. Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011.
28. Schmidt K, Büning J, Weitz G, Woitalla J, Wellh P, Fellermann K. Detrimental effect of high volume fluid administration in acute pancreatitis e A retrospective analysis of 391 patients. Pancreatology. 2014;14:478-83.
29. Karabulut U, Koyuncu MB, Sezgin O, Ucbilek E, Aydin MK, Altintas E. Mo1327 Early Oral Feeding and Selection of Initial Diet in Mild Acute Pancreatitis. Gastroenterology. 146(5):S-621.

30. Beduschi MG, Mello AL, VON-Mühlen B, Franzon O. The panc 3 score predicting severity of acute pancreatitis. Arq Bras Cir Dig. 2016;29:5-8.
31. Sun Y, Lu Z, Zhang X, Geng X, Cao L, Yin L. Pancreatology. The effects of fluid resuscitation according to PiCCO on the early stage of severe acute pancreatitis. Pancreatology. 2015.
32. Zhu Y, Pan X, Zeng H, Xia L, Liu P, Lv N. A Study on the Etiology, Severity, and Mortality of 3260 Patients With Acute Pancreatitis According to the Revised Atlanta Classification in Jiangxi, China Over an 8-Year Period. Pancreas, 2017;46:504-9.
33. Zeng Y, Zhang W, Lu Y, Huang C, Wang X. Impact of hypertriglyceridemia on the outcome of acute biliary pancreatitis. Am J Med Sci. 2014;348:399-402.
34. Li G, Wang X, Shen X, Ke L, Li W, Tong Z, Zhou J. Positive end-expiratory pressure setting guided by esophageal pressure in patients with intra-abdominal hypertension: a prosp ective, randomized, open-label trial; Pancreatology, 2016;16:S25.
35. Lin S, Hong W, Basharat Z, Wang Q, Pan J, Zhou M. Blood Urea Nitrogen as a Predictor of Severe Acute Pancreatitis Based on the Revised Atlanta Criteria: Timing of Measurement and Cutoff Points. 2017;2017.
36. Qi X, Yang F, Huang H, Du Y, Chen Y, Wang M, et al. OPEN A reduced lymphocyte ratio as an early marker for predicting acute pancreatitis. Nat Publ Gr. 2017;(415):1–6.
37. Jia R, Tang M, Qiu L, Sun R, Cheng L, Ma X, et al. Increased interleukin-23/17 axis and C-reactive protein are associated with severity of acute pancreatitis in patients. Pancreas. 2015;44:321-5.
38. Shen X, Sun J, Ke L, Zou L, Li B, Tong Z, Li W, Li N, Li J. Reduced lymphocyte count as an early marker for predicting infected pancreatic necrosis. BMC Gastroenterol. 2015;15:147.
39. Kim TN, Kim SB, Cho JH, Kim KH. Comparison of clinical course and outcome of acute pancreatitis according to two main etiologies: alcohol vs. gallstone. Pancreatology. 2009;14(3):S62.
40. Huh JH, Jung S, Cho SK, Lee KJ, Kim JW. Predictive value of apolipoprotein B and A-I ratio in severe acute pancreatitis. J Gastroenterol Hepatol. 2018;33: 548-53.
41. Kim BG, Noh MH, Ryu CH, Nam HS, Woo SM, Ryu SH, et al. A comparison of the BISAP score and serum procalcitonin for predicting the severity of acute pancreatitis. Korean J Intern Med. 2013;28:322-9.
42. Kim J, Cho JH, Kim EJ, Kim Y, Kim HM, Kim Y. Mo1253 - Risk Factors of Recurrent Acute Pancreatitis and Progression to Chronic Pancreatitis after the first Attack of Acute Pancreatitis. Gastroenterology. 2018;154:S–721–S–722.
43. Mikolasevic I, Orlic L, Poropat G, Jakopcic I, Stimac D, Klanac A, et al. European Journal of Internal Medicine Nonalcoholic fatty liver and the severity of acute pancreatitis. Eur J Intern Med. 2017 Mar;38:73-78.
44. Mikolasevic I, Milic S, Orlic L, Poropat G, Jakopcic I, Franjic N, et al. Metabolic syndrome and acute pancreatitis. Eur J Intern Med. 2016
45. Trgo G, Zaja I, Bogut A, Kovacic V, Meter I, Vucic LM, et al. Association of Asymmetric Dimethylarginine With Acute Pancreatitis-Induced Hyperglycemia. Pancreas. 2016;45:694-99.
46. Vujasinovic M, Tepes B, Makuc J, Rudolf S, Zaletel J, Vidmar T, et al. Pancreatic exocrine insufficiency, diabetes mellitus and serum nutritional markers after acute pancreatitis. World J Gastroenterol. 2014;20(48):18432-8.
47. Pintado M, Trascasa M, Arenillas C, Ortiz Y, Zárate D, Pardo A, et al. European Journal of Internal Medicine. New Atlanta Classification of acute pancreatitis in intensive care unit: Complications and prognosis. Eur J Intern Med. 2016; 30:82-87.
48. Bozhychko M, Cárdenas-Jaén K, Ruiz-Rebollo ML, de Madaria E. Use of statins and nonsteroidal anti-inflammatory drugs and severity of acute pancreatitis. Pancreatology, 2017. 17(5), S12.
49. Ellery KM, Ms DO, Kumar S, Mmm WC, Gariepy C. The Benefits of Early Oral Nutrition in Mild Acute Pancreatitis. J Pediatr. 2017;1-6.
50. Sugimoto M, Sonntag DP, Flint GS, Boyce CJ, Kirkham JC, Harris TJ, et al. A percutaneous drainage protocol for severe and moderately severe acute pancreatitis. Surg Endosc. 2015;29:3282-91.
51. Gougol A, Dugum M, Dudekula A, Greer P, Slivka A, Whitcomb DC, et al. Clinical outcomes of isolated renal failure compared to other forms of organ failure in patients with severe acute pancreatitis. World J Gastroenterol. 2017;23:5431-7.
52. Vipperla K, Somerville C, Furlan A, Koutroumpakis E, Saul M, Chennat J, et al. Clinical Profile and Natural Course in a Large Cohort of Patients With Hypertriglyceridemia and Pancreatitis. J Clin Gastroenterol. 2017;51:77-85.
53. Vlada AC, Schmit B, Perry A, Trevino JG, Behrns KE, Hughes SJ. Failure to follow evidence-based best practice guidelines in the treatment of severe acute pancreatitis. HPB (Oxford). 2013;822-7.
54. Buxbaum JL, Quezada M, Da B, Jani N, Lane C, Mwengela D, et al. Early Aggressive Hydration Hastens Clinical Improvement in Mild Acute Pancreatitis. Am J Gastroenterol. 2017;112:797-803.
55. Buxbaum JL, Da B, Quezada M, Jani N, Mwengela D, Thomas E, et al. S-64 AGA Abstracts. Gastroenterology. 2016;150:S64-5.
56. Dimagno MJ, Wamsteker E, Rizk RS, Spaete JP, Gupta S, Sahay T, et al. A Combined Paging Alert and Web-Based Instrument Alters Clinician Behavior and Pancreatitis. Am J Gastroenterol. 2014;109:306-15.
57. Bishu S, Koutroumpakis E, Mounzer R, Stello K, Pollock N, Evans A. Papachristou G. The-251 A/T Polymorphism in the IL8 Promoter is a Risk Factor for Acute Pancreatitis. Pancreas. 2018;47:87-91.
58. Da B, Quezada M, Shulman I, Sheibani S, Buxbaum JL. Severe Obesity Predicts Adverse Outcomes in Acute Pancreatitis. American Journal of Gastroenterology. 2015;110:S14. https://doi.org/10.1038/ajg.2015.269.
59. Nieminen A, Maksimow M, Mentula P, Kyhälä L, Kylänpää L, Puolakkainen P, et al. Circulating cytokines in predicting development of severe acute pancreatitis. Crit Care. 2014;18:R104.
60. Nikkola A, Aittoniemi J, Huttunen R, Rajala L, Nordback I, Sand J, et al. Plasma Level of Soluble Urokinase-type Plasminogen Activator Receptor Predicts the Severity of Acute Alcohol Pancreatitis. Pancreas. 2017;46:77-82.
61. Nukarinen E, Lindström O, Kuuliala K, Kylänpää L, Pettilä V, Puolakkainen P, et al. Association of Matrix Metalloproteinases -7, -8 and -9 and TIMP -1 with Disease Severity in Acute Pancreatitis. A Cohort Study. PLoS One. 2016;11:e0161480.
62. Bakker OJ, van Santvoort H, Besselink MG, Boermeester MA, van Eijck C, Dejong K, et al. Extrapancreatic necrosis without pancreatic parenchymal necrosis: a separate entity in necrotising pancreatitis? Gut. 2013;62:1475-80.
63. Párniczky A, Mosztbacher D, Zsoldos F, Tóth A, Lásztity N, Hegyi P, et al. Analysis of Pediatric Pancreatitis (APPLE Trial): Pre-Study Protocol of a Multinational Prospective Clinical Trial. Digestion. 2016;93:105-10.
64. Doley RP, Yadav TD, Wig JD, Kochhar R, Singh G, Bharathy KG, et al. Enteral nutrition in severe acute pancreatitis. JOP. 2009;10:157-62.
65. John BJ, Sambandam S, Garg P, Singh G, Kaur M, Baskaran R, et al. Persistent Systemic Inflammatory Response Syndrome predicts the need for tertiary care in Acute Pancreatitis. Acta Gastroenterol Belg. 2017;80:377-80.
66. Stirling AD, Moran NR, Kelly ME, Ridgway PF, Conlon KC. The predictive value of C-reactive protein (CRP) in acute pancreatitis – is interval change in CRP an additional indicator of severity? HPB (Oxford). 2017;19:874-80.
67. Losurdo G, Iannone A, Principi M, Barone M, Ranaldo N, Ierardi E, et al. European Journal of Internal Medicine Acute pancreatitis in elderly patients: A retrospective evaluation at hospital admission. Eur J Intern Med. 2016; 30:88-93.
68. Sugawara S, Arai Y, Sone M, Katai H. Frequency, severity, and risk factors for acute pancreatitis after percutaneous transhepatic biliary stent placement across the papilla of vater. Cardiovasc Intervent Radiol. 2017;40:1904-10.
69. Karpavicius A, Dambrauskas Z, Gradauskas A, Samuilis A, Zviniene K, Kupcinskas J, et al. The clinical value of adipokines in predicting the severity and outcome of acute pancreatitis. BMC Gastroenterol. 2016;16:99.
70. Chacón-portillo MA, Payró-ramírez G, Peláez-luna MC, Uscanga-domínguez LF, Vasquéz-ortiz Z, Orihuela C, et al. Abnormal Cardiovascular Findings in Acute Pancreatitis: Are They Associated with Disease Severity ? Rev Inves Clin. 2017;314-8.
71. Riquelme F, Marinkovic B, Salazar M, Martínez W, Catan F, Uribe-Echevarría S, et al. Early laparoscopic cholecystectomy reduces hospital stay in mild gallstone pancreatitis. A randomized controlled trial. HPB (Oxford). 2020;22:26-33.
72. Cuellar-Monterrubio JE, Robles RM, Gonzalez-Moreno EI, Borjas-Almaguer OD, Garcia-Compean D, Maldonado Garza HJ, González-González JA. Guidelines Versus Directed IV Fluid Therapy in Acute Pancreatitis of More than 24 Hours of Clinical Evolution: A Prospective Randomized Clinical Trial Gastroenterology. Gastroenterology 152(5):S281. DOI: 10.1016/S0016-5085(17) 31235-0.
73. Głuszek S, Nawacki Ł, Matykiewicz J, Kot M, Kuchinka J. Severe Vascular Complications Of Acute Pancreatitis. Pol Przegl Chir. 2015;87:485-90.
74. Madaria E, Herrera-Marante I, Gonzalez-Camacho V, Bonjoch L, Quesada-Vazquez N, Almenta-Saavedra I, et al. Fluid resuscitation with lactated Ringer's solution vs normal saline in acute pancreatitis: A triple-blind, randomized, controlled trial. HPB (Oxford). 2018;6:63-72.
75. Kuśnierz-Cabala B, Gala-Błądzińska A, Mazur-Laskowska M, Dumnicka P, Sporek M, Matuszyk A, et al. Serum Uromodulin Levels in Prediction of Acute Kidney Injury in the Early Phase of Acute Pancreatitis. Molecules. 2017; 22:988.
76. Sporek M, Dumnicka P, Gala-Błądzińska A, Małgorzata Mazur-Laskowska, Walocha J, Ceranowicz P, et al. Determination of serum neutrophil gelatinase-associated lipocalin at the early stage of acute pancreatitis. Folia Med Cracov. 2016;56:5-16.
77. Huggett MT, Oppong KW, Pereira SP, Keane MG, Mitra V, Charnley RM, Nayar MK. Endoscopic drainage of walled-off pancreatic necrosis using a novel self-expanding metal stent. Endoscopy. 2015;47:929-32.

78. Suppiah A, Malde D, Arab T, Hamed M, Allgar V, Smith AM, Morris-Stiff G. The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg. 2013;17:675-81.
79. Haffar S, Bazerbachi F, Prokop L, Watt KD, Murad MH, Chari ST. Frequency and prognosis of acute pancreatitis associated with fulminant or non-fulminant acute hepatitis A: A systematic review. Pancreatology. 2017;17:166-75.
80. Bertilsson S, Swärd P, Kalaitzakis E. Factors That Affect Disease Progression After First Attack of Acute Pancreatitis. Clin Gastroenterol Hepatol. 2015;13:1662-9.e3.
81. Ragnarsson T, Andersson R, Ansari D, Persson U, Andersson B. Acute biliary pancreatitis: focus on recurrence rate and costs when current guidelines are not complied. Scand J Gastroenterol. 2017;52:264-9.
82. Shen HN, Chang YH, Chen HF, Lu CL, Li CY. Increased risk of severe acute pancreatitis in patients with diabetes. Diabet Med. 2012;29:1419-24.
83. İnce AT, Senturk, H, Singh VK, Yildiz, K, Danalioğlu A, Çinar A, et al. A randomized controlled trial of home monitoring versus hospitalization for mild non-alcoholic acute interstitial pancreatitis: A pilot study. Pancreatology. 2014;14:174-8.
84. de-Madaria E, Herrera-Marante I, González-Camacho V, Bonjoch L, Quesada-Vázquez N, Almenta-Saavedra I, et al. Fluid resuscitation with lactated Ringer's solution vs normal saline in acute pancreatitis: A triple-blind, randomized, controlled trial. United European Gastroenterol J. 2018;6:63-72.
85. Tozlu M, Kayar Y, İnce AT, Baysal B, Şentürk H. Low molecular weight heparin treatment of acute moderate and severe pancreatitis: A randomized, controlled open-label study. Turk J Gastroenterol. 2019;30:81-7.
86. Fidan S, Erkut M, Cosar AM, Yogun Y, Örem A, Sönmez M, Arslan M. Higher Thrombin-Antithrombin III Complex Levels May Indicate Severe Acute Pancreatitis. Dig Dis. 2018;36:244-51.
87. Abdullah B, Dusak A, Kaplan I, Gümüs M. The potential role of BMI, plasma leptin, nesfatin-1 and ghrelin levels in the early detection of pancreatic necrosis and severe acute pancreatitis: A prospective cohort study. Int J Surg. 2014;12: 1310-3.
88. Koziel D, Gluszek S, Matykiewicz J, Lewitowicz P, Drozdzak Z. Comparative analysis of selected scales to assess prognosis in acute pancreatitis. Can J Gastroenterol Hepatol. 2015;29:299-303.
89. Liu J, Cao F, Dong XM, Yu Li P, Li HC, Qi BJ, Li F. Early prediction of organ failure under the revised Atlanta classification. Turk J Gastroenterol. 2017;28:46-52.
90. He WH, Zhu Y, Zhu Y, Jin Q, Xu HR, Xion ZJ, et al. Comparison of multifactor scoring systems and single serum markers for the early prediction of the severity of acute pancreatitis. J Gastroenterol Hepatol. 2017;32:1895-901.
91. Zhang J, Shahbaz M, Fang R, Liang B, Gao C, Gao H, et al. Comparison of the BISAP scores for predicting the severity of acute pancreatitis in Chinese patients according to the latest Atlanta classification. J Hepatobiliary Pancreat Sci. 2014;21:689-94.
92. Xu XD, Wang ZY, Zhang LY, Ni R, Wei FX, Han W, et al. Acute Pancreatitis Classifications: Basis and Key Goals. Medicine (Baltimore). 2015;94:e2182.
93. Yang Z, Dong L, Zhang Y, Yang C, Gou S, Li Y, et al. Prediction of Severe Acute Pancreatitis Using a Decision Tree Model Based on the Revised Atlanta Classification of Acute Pancreatitis. PLoS One. 2015;10:e0143486.
94. Lee KJ, Kim HM, Choi JS, Kim YJ, Kim YS, Cho JH. Comparison of Predictive Systems in Severe Acute Pancreatitis According to the Revised Atlanta Classification. Pancreas. 2016;45:46-50.
95. Zubia-Olaskoaga F, Maraví-Poma E, Urreta-Barallobre I, Ramírez-Puerta MR, Mourelo-Fariña M, Marcos-Neira MP, et al. Comparison Between Revised Atlanta Classification and Determinant-Based Classification for Acute Pancreatitis in Intensive Care Medicine. Why Do Not Use a Modified Determinant-Based Classification? Crit Care Med. 2016;44:910-7.
96. Acevedo-Piedra NG, Moya-Hoyo N, Rey-Riveiro M, Gil S, Laura S, Juan M, et al. Validation of the determinant-based classification and revision of the Atlanta classification systems for acute pancreatitis. Clin Gastroenterol Hepatol. 2014;12:311-6.
97. Nawaz H, Mounzer R, Yadav D, Yabes JG, Slivka A. Revised Atlanta and Determinant-Based Classifi cation : Application in a Prospective Cohort of Acute Pancreatitis Patients. Am J Gastroenterol [Internet]. 2013;108:1911-7.
98. Jones MJ, Neal CP, Ngu WS, Dennison AR, Garcea G. Early warning score independently predicts adverse outcome and mortality in patients with acute pancreatitis. Langenbecks Arch Surg. 2017;402:811-9.
99. Ikeura T, Horibe M, Sanui M, Sasaki M, Kuwagata Y, Nishi K, Kariya S, et al. Validation of the efficacy of the prognostic factor score in the Japanese severity criteria for severe acute pancreatitis: A large multicenter study. United European Gastroenterol J. 2017;5:389-97.
100. Gravito-Soares M, Gravito-Soares E, Gomes D, Almeida N, Tomé L. Red cell distribution width and red cell distribution width to total serum calcium ratio as major predictors of severity and mortality in acute pancreatitis. BMC Gastroenterol. 2018;18:108.
101. Chen C, Huang Z, Li H, Song B, Yuan F. Evaluation of extrapancreatic inflammation on abdominal computed tomography as an early predictor of organ failure in acute pancreatitis as defined by the revised Atlanta classification. Medicine (Baltimore). 2017;96(15).
102. Huang J, Qu H, Zheng Y, Song X, Li L, Xu Z. The revised Atlanta criteria 2012 altered the classification, severity assessment and management of acute pancreatitis. Hepatobiliary Pancreat Dis Int.. 2016;15:310-5.
103. Choi J, Kim M, Oh D, Hyun W, Hyun D, Soo S, et al. Clinical relevance of the revised Atlanta classification focusing on severity stratification system. Pancreatology. 2014;14:324-9.
104. Kim EJ, Cho JH, Oh KY, Kim SY, Kim YS. The Risk Factors for Moderately Severe and Severe Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis According to the Revised Atlanta Classification. Pancreas. 2017;46:1208-13.
105. Bouwense SA, van Brunschot S, van Santvoort HC, Besselink MG, Bollen TL, Bakker OJ, et al. Describing Peripancreatic Collections According to the Revised Atlanta Classification of Acute Pancreatitis: An International Interobserver Agreement Study. Pancreas. 2017;46:850-7.
106. Talukdar R, Bhattacharrya A, Rao B, Sharma M, Reddy DN. Pancreatology Clinical utility of the Revised Atlanta Classification of acute pancreatitis in a prospective cohort: Have all loose ends been tied? Pancreatology. 2014;14:257-62.
107. Thandassery RB, Yadav TD, Dutta U, Appasani S, Singh K, Kochhar R. Prospective validation of 4-category classification of acute pancreatitis severity. Pancreas. 2013;42:392-6.
108. Ignatavicius P, Gulla A, Cernauskis K, Barauskas G, Dambrauskas Z. How severe is moderately severe acute pancreatitis? Clinical validation of revised 2012 Atlanta Classification. World J Gastroenterol. 2017;23(43):7785-90.
109. Lakhey PJ, Bhandari RS, Kafle B, Singh KP, Khakurel M. Validation of 'Moderately Severe Acute Pancreatitis' in patients with Acute Pancreatitis. JNMA J Nepal Med Assoc. 2013;52:580-5.
110. Gluszek S, Kozie D. Prevalence and progression of acute pancreatitis in the świętokrzyskie voivodeship population. Pol Przegl Chir. 2012;84:618-25.
111. Staubli SM, Oertli D, Nebiker CA. Assessing the severity of acute pancreatitis (ASAP) in Switzerland: a nationwide survey on severity assessment in daily clinical practice. Pancreatology. 2017;7:356-63.
112. United Nations Development Programme – UNDP. 2018. 2018 Statistical Update: Human Development Indices and Indicators. New York. Available from: http:// http://hdr.undp.org/en/content/human-development-indices-indicators-2018-statistical-update.

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Noninvasive breath tests for diagnosis of SIBO and lactose intolerance in patients on chemotherapy treatment for colorectal and gastric cancer

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ABSTRACT – **Background** – Worldwide, colorectal cancer (CRC) and gastric cancer (GC) are the third and the fifth most prevalent, respectively. Diarrhea is a common symptom in patients on chemotherapy or radiotherapy treatment and can reduce treatment tolerance. Surgical resections and chemotherapy change the intestinal microbiota that can lead to lactose intolerance, small intestinal bacterial overgrowth (SIBO). **Objective** – The aim of the study was to evaluate the frequency of diarrhea in patients with CRC and GC on chemotherapy with SIBO or intolerance of lactose. **Methods** – This is a descriptive and observational study with patients of both sexes, over 18 years old, in treatment in the Gastro-Oncology outpatient clinic of the Federal University of São Paulo. Patients with a confirmed diagnosis of CRC or GC during chemotherapy treatment were included. To detect bacterial overgrowth and lactose intolerance, breath hydrogen test with lactulose and lactose was done. Number and aspects of the evacuations and toxicity degree were collected. For the nutritional assessment, weight and height were performed to calculate the BMI. and the Patient Generated Subjective Global Assessment (PG-SGA). **Results** – A total of 33 patients were included, 29 with CRC and 3 with GC. Most of them were male (57.57%), mean age of 60.03±10.01 years and in chemotherapy with fluoropyrimidine and oxaliplatin (54.5%). Diarrhea was present in 57.6% and 30.3% had toxicity grade 2. According to the BMI, 78.9% were eutrophics, obese or overweight, but according to PG-SGA, 84.9% had moderate or severe nutritional risk grade. Between patients, 45% had lactose intolerance and 9% SIBO. Diarrhea grade 2-3 was observed in 66.6% of patients with SIBO and 66.7% of that with lactose intolerance. No statistical difference was observed between patients with SIBO or lactose intolerance and grade of diarrhea. **Conclusion** – Diarrhea was a frequent symptom in chemotherapy patients with gastric or colorectal cancer independent of the presence of SIBO or lactose intolerance. Surgery and chemotherapy treatment impacted in the intestinal habit of patients. Diagnosis of other causes of diarrhea may contribute to a better tolerance to treatment and quality of life.

HEADINGS – Colorectal neoplasms, drug therapy. Stomach neoplasms, drug therapy. Diarrhea. Bacteria, growth & development. Lactose intolerance.

INTRODUCTION

According to Globocan, in 2018, there were more than 1.8 million new cases of colorectal cancer (CRC) with 881 thousand deaths and 1.0 million new cases of gastric cancer (GC) with 783 thousand deaths, worldwide. In Brazil, 41.000 new cases of CRC and 21.000 new cases of GC had been estimated each year for the 2020–2022 period[1,2].

The prognosis depends mainly on the pathological stage. The treatment (surgical resection, adjuvant or neoadjuvant chemotherapy and radiotherapy) have an important influence on prognosis but also may cause pain, constipation or diarrhea, nausea, vomiting, lack of appetite, contributing to the reduction of food intake and consequent malnutrition[3,4].

Diarrhea is a common symptom in patients on chemotherapy or radiotherapy treatment[5]. Treatment-related diarrhea is a very common toxicity, which can reduce treatment tolerance[6]. Long-term changes in bowel function after cancer treatment are common and can decrease the patient's quality of life[7]. Between 15% to 66% of cancer patients treated with surgery, radiation and chemotherapy suffer from chronic gastrointestinal symptoms[8].

Despite these kinds of treatments, diarrhea may be caused by others factors as intestinal bacterial disorders, known as small intestinal bacterial overgrowth (SIBO) or intolerance to lactose.

Surgical resection of the gastrointestinal tract and chemotherapy can lead to malnutrition that is correlated with poor prognosis and intolerance to chemotherapy treatment. Body mass index (BMI) is an easy, lower cost and fast method to diagnose malnutrition, however it does not evaluate loss of weight during the last months[9]. The Patient-Generated Subjective Global Assessment (PG-SGA), is a subjective, noninvasive method that can detect individuals at nutritional risk, with sensibility and specificity of the 98% and 82%, respectively. This tool can identify early patients with risk of malnutrition who will benefit from specialized nutritional intervention[9,10].

SIBO is defined by the abnormal and excessive presence of the number of bacteria in the small intestine and is associated with a group of clinical symptoms, such as abdominal distention, flatulence, abdominal pain, nausea, dyspepsia, fatigue, diarrhea and constipation[11]. Alterations of the defense mechanisms of the gastrointestinal tract, such as gastric acid secretion, motility and integrity of the intestinal mucosa may cause the disease. More than 40% of patients undergoing subtotal colectomy with resection of

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the ileocecal valve report persistent gastrointestinal symptoms, suggesting that it may predispose to SIBO[12]. This disease may occur in patients during radiotherapy or chemotherapy[13].

Clinical studies suggest that bacterial overgrowth of the small intestine, malabsorption of bile acids and pancreatic insufficiency are factors that can increase chemotherapy-induced symptoms in gastrointestinal tract.

Lactose intolerance is a pathological condition characterized by abdominal symptoms caused by lactase deficiency enzyme caused by polymorphisms in the MCM6 gene. These polymorphisms can be different according to the region[14]. Humans born with initially elevated levels of lactase in intestinal enterocytes that decrease in adults[14]. The expression of lactase is decreased in approximately 2/3 of the world population, leading to lactose intolerance.

During chemotherapy, there is an increase in transit and changes in the intestinal flora, which can also lead to lactose intolerance and malabsorption of bile acids[15,16].

The aim of the study was to evaluate the frequency of diarrhea among patients with CRC and GC on chemotherapy and SIBO or intolerance of lactose.

METHODS

This is a descriptive and observational study with patients of both sexes, over 18 years old, in treatment in the Gastro-Oncology and Clinical Oncology outpatient clinic of the Federal University of São Paulo. The study was submitted to the Local Ethics Committee and was approved under CAAE n. 81597517.0.0000.5505. All the patients were informed and signed the informed consent form.

Patients with a confirmed diagnosis of colorectal or gastric cancer during chemotherapy were included. Subjects with other types of neoplasia were excluded.

For the construction of an excel spreadsheet, age, sex, histological type of the tumor and its stage, presence of diarrhea, number of bowel movements, degree of toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE)[17] and aspect of them according to the Bristol[18] scale were collected.

To assess the degree of toxicity of diarrhea (Grade 1–5), the CTCAE[17] was used. In addition to the CTCAE, the Bristol Scale[18], proposed by Lewis and Heaton, was used, which is a medical scale designed to categorize shape of human stools.

Breath noninvasive test

Small intestinal bacterial overgrowth and lactose intolerance were done in the laboratory of breath tests of the *Hospital São Paulo*, in fasting patients in chemotherapy treatment. During the last 24 hours a special diet without fibers or food with fermentative effect was done. They were instructed to do a good oral hygiene and not smoke for 4 hours before the test. Using a disposable gas mouth blowpipe, the patient investigated subject exhaled slowly for as long as possible. The first exhalation was done at the beginning and after an ingestion of a liquid sweetened with lactulose or lactose for the test of SIBO or lactose intolerance respectively at repeated intervals of 20 minutes for a total of 150 minutes. Up to 90 minutes values ≥20 PPM were considered positive for bacterial overgrowth. Some patients had a second elevation after 90 min that had also measured. The breath exams were done in different days[19]. Lactose intolerance is an elevation ≥20 PPM at any time during the test (values of ≥20 PPM and <70 PPM is considered low grade intolerance and values >70 PPM are considered high grade intolerance).

Nutritional assessment and nutrition risk

For the nutritional assessment, weight and height were collected to calculate the BMI. Its classification was carried out according to the World Health Organization for adults[20] and Lipschitz[21] for elderly patients. Before the expired hydrogen test, all patients answered the PG-SGA.

Statistical analysis

Exploratory data analysis included mean, median, standard deviation and variation for continuous variables and number and proportion for categorical variables. Comparison of the mean age between the two groups was made using the *t*-Student. Comparison of categorical variables between the two groups was performed using Fischer's exact test. Statistical analysis was performed using the software IBM-SPSS Statistics version 24 (IBM Corporation, NY, USA). Two tailed testes were done and values of $P<0.05$ were considered significant.

RESULTS

Fifty-four patients were selected, but only 33 patients were included. The others were excluded because they had already finished the chemotherapy or dropped out of the study. The majority were male (57.6%) and the mean age was 60.03 (±10.1) years. Thirty patients had CRC and 3 GC. Thirty-two patients performed breath test with lactulose and 20 with lactose.

According to the BMI, 26 (78.9%) had eutrophic, obese or overweight, and only seven had malnutrition. However, the PG-SGA showed that 28 (84.9%) had nutritional moderate risk (grade B) or severe risk (grade C) (TABLE 1). Most of the patients had adenocarcinoma (88%), three had neuroendocrine tumor and one gastrointestinal stromal tumor.

SIBO was positive in 3/32 (9.1%) patients and lactose intolerance in 9/20 (45%) patients, all with low grade (TABLE 2). None of them had SIBO and lactose intolerance. Nineteen (57.6%) had diarrhea, being grade 2/3 in 13 (39.4%). Most of the patients were on chemotherapy with 5-fluorouracil/capecitabine and oxaliplatin (54.5%) or 5 fluorouracil / capecitabine (33.2%). 5Fluorouracil (5FU) and capecitabine are fluoropyrimidines used intravenous and oral, respectively.

In the comparative analysis of patients with or without SIBO, no significant differences were detected in terms of gender or age (TABLE 3). Diarrhea toxicity according to CTCAE among patients with and without SIBO was analyzed; the grades 2/3 were observed in 2/3 (66.6%) of the patients with SIBO. According to Bristol's scale, 66.6% of the patients presented grade 5–7.

Lactose intolerance also had no significant difference between genders or age (TABLE 4). The proportions were similar for elderly between groups. No difference was observed between the chemotherapy treatments and lactose intolerance. According to Bristol`s scale 77.8% of the patients with lactose intolerance presented grade 5–7. Two patients had high grade intolerance of lactose with values >70 PPM and Bristol scale 6 and 7. Although the difference between the grade of toxicity of diarrhea or the Bristol scale was not statistically significant, patients with SIBO or lactose intolerance had a higher percentual of patients with diarrhea grade 2/3 and Bristol scale of 5–7.

TABLE 1. Sociodemographic and clinical data of patients included in the study.

Variable	N=33
Age, years	60.03±10.1
Age range, n (%)	
Adults (19–59 years)	13 (39.4)
Elderly (≥ 60 years)	20 (60.6)
Gender, n (%)	
Male	19 (57.6)
Female	14 (42.4)
Tumor Location, n (%)	
Rectum	16 (48.5)
Right Colon	7 (21.1)
Left Colon	7 (21.1)
Stomach	3 (9.1)
Nutritional status (BMI) n (%)	
Malnutrition	7 (21.1)
Eutrophy	15 (45.5)
Overweight	5 (15.2)
Obesity I	6 (18.2)
Nutritional risk (PG-SGA) (n %)	
A	5 (15.2)
B	15 (45.5)
C	13 (39.4)

Continuous variables are described as mean ± standard-deviation; categorical variables are described in number (percentage). BMI: body mass index; PG-SGA: Patient Generated-Subjective Global Assessment.

TABLE 2. Distribution of the patients according to diarrhea, chemotherapy, or target drugs treatment, SIBO and lactose intolerance.

Characteristic	N=33 N (%)
SIBO, n (%)	
Yes	3 (9.1)
No	29 (87.9)
ND	1 (3.0)
Lactose intolerance, n (%)	
Yes	9 (27.3)
No	11 (33.3)
ND	13 (39.4)
Diarrhea* n (%)	
Grade 0	14 (42.4)
Grade 1-3	19 (57.6)
Chemotherapy/target drugs n (%)	
Octreotide/everolimus	3 (9.1)
5FU/oxaliplatine	18 (54.5)
5FU/Capecitabina	7 (21.1)
5FU/Capecitabina + RT	4 (12.1)
Imatinib	1 (3.0)

SIBO: small intestinal bacterial overgrowth; RT: radiotherapy.

TABLE 3. Descriptive and comparative analysis of SIBO, according to demographic and clinical data of patients.

	SIBO		I
	Negative (n=29)	Positive (n=3)	*P* value
Gender			
Male	18 (62.1)	0 (0)	0.07
Female	11 (37.9)	3 (100)	
Age, years	63.07±10.2	65.67±11.0	0.68
Age range			
Adults (19–59 years)	11 (37.9)	1 (33.3)	1.000
Elderly (≥60 years)	18 (62.1)	2 (66.7)	
Tumor location, n (%)			
Colorectal cancer	26 (89.7)	3 (100)	–
Gastric cancer	3 (10.3)	0 (0)	
Histological type, n (%)			
Adenocarcinoma	25 (86.2)	3 (100)	1.00
NET/GIST	4 (13.7)	0	
Diarrhea toxicity (CTCAE), n (%)			
Grade 0-1	13 (44.8)	1 (33.3)	1.00
Grade 2-3	16 (55.2)	2 (66.6)	
Bristol's scale			
1-3	3 (10.3)	0	
4-7	26 (89.6)	3 (100)	1.00
Surgical procedure, n (%)			
Intestinal surgery	22 (75.8)	1 (33.3)	
Gastric surgery	2 (6.9)	0	–
No surgery	5 (17.2)	2 (66.7)	
Chemotherapy with 5FU/oxaliplatin, n (%)			
Yes	17 (58.62)	1 (33.3)	0.57
No	12 (41.38)	2 (66.7)	
*Chemotherapy with capecitabine/5FU, n (%)			
Yes	9 (31.03)	2 (66.7)	0.27
No	20 (68.96)	1 (33.3)	
Nutritional status (BMI), n (%)			
Malnutrition	6 (20.7)	1 (33.3)	0.53
Eutrophy/overweight/ obesityI	23 (79.3)	2 (66.7)	
Nutritional risk (PG-SGA), n (%)			
A/B	18 (62.1)	2 (66.6)	1.00
C	11 (37.9)	1 (33.3)	

Continuous variables are described as mean ± standard-deviation; categorical variables are described in number (percentage). CTCAE: Common Terminology Criteria for Adverse Events; 5FU:5-Fluorouracil; SIBO: Small Intestinal Bacterial Overgrowth; NET: neuroendocrine tumor; GIST: gastrointestinal stromal tumor; BMI: body mass index; PG-SGA: Patient Generated-Subjective Global Assessment. *With or without radiotherapy.

TABLE 4. Descriptive and comparative analysis of lactose intolerance, according to patients' demographic and clinical data.

	Lactose intolerance		
	Negative (n=11)	**Positive (n=9)**	***P* value**
Gender, n (%)			
Male	6 (54.5)	7 (77.8)	0.37
Female	5 (45.4)	2 (22.2)	
Age, years	64.3±12.9	61.9±8.5	0.64
Age range			
Adults (19–59 years)	4 (36.3)	3 (33.3)	1.00
Elderly (≥60 years)	7 (63.6)	6 (66.7)	
Tumor location, n (%)			
Colorectal cancer	10 (90.9)	7 (77.8)	–
Gastric cancer	1 (9.1)	2 (2.2)	
Histological type, n (%)			
Adenocarcinoma	10 (90.9)	8 (89)	1.00
NET/GIST	1 (9.1)	1 (11.1)	
Diarrhea toxicity (CTCAE), n (%)			
Gade 0-1	6 (54.6)	3 (33.3)	0.66
Grade 2-3	5 (45.4)	6 (66.7)	
Bristol's scale n (%)			
1-3	1 (9.1)	1 (11)	1.00
4-7	10 (90.9)	8 (89)	
Surgical procedure, n (%)			
Intestinal surgery	9 (81.2)	5 (55.5)	–
Gastric surgery	1 (9.1)	2 (22.2)	
No surgery	1 (9.1)	2 (22.2)	
Chemotherapy with 5FU/oxaliplatin, n (%)			
Yes	8 (72.7)	2 (22.2)	0.17
No	3 (27.3)	7 (77.8)	
*Chemotherapy with capecitabine/5FU, n (%)			
Yes	1 (9.1)	6 (66.7)	1.00
No	10 (90.9)	3 (33.3)	
Nutritional status (BMI), n (%)			
Malnutrition	3 (27.3)	2 (22.2)	1.00
Eutrophy/overweight/obesity I	8 (72.7)	7 (77.8)	
Nutritional risk (PG-SGA), n (%)			
A/B	6 (54.5)	7 (77.8)	0.37
C	5 (45.5)	2 (22.2)	

Continuous variables are described as mean ± standard-deviation; Categorical variables are described in number (percentage). CTCAE: Common Terminology Criteria for Adverse Events; 5FU: 5-Fluorouracil; NET: neuroendocrine tumor; GIST: gastrointestinal stromal tumor; BMI: body mass index; PG-SGA: Patient Generated-Subjective Global Assessment. *With or without radiotherapy.

DISCUSSION

Diarrhea is a common side effect during chemotherapy and is usually attributed to the toxicity of the drugs. In our study, 57.6% of the patients presented diarrhea.

Schmoll et al.[22], published in a large study that patients with CRC stage III treated with adjuvant chemotherapy (capecitabine and oxaliplatin or 5FU) had diarrhea in 60–68% being severe in 20% (grade 3 or 4). When associated to radiotherapy, this risk increases 21%[23]. In our study, 66.6% of the patients with SIBO and 77.8% with lactose intolerance had diarrhea.

Decrease concentration of acid by surgical resection of the stomach or chronic use of inhibitor of protonic pump, immunodeficiency, surgery with transit deviation and resection of the ileum cecal valve with reflux of the bacteria from the colon and decrease of the motility of the intestine can induce SIBO. Bacterial overgrowth causes diarrhea because the enzymes of the bacteria compete with the enzymes of the digestive wall.

SIBO can be detected by noninvasive breath tests with glucose or lactulose. However, there is no consensus about which one is better glucose or lactose. Lactulose is an osmotic sugar that can accelerate the intestinal transit and the diagnosis can reproduce colonic bacteria increasing the number of false positive. In the other hand glucose can be absorbed fast and did not show bacteria on the ileum (increasing the number of false negative).

Losurdo et al.[18], described in a metanalysis that glucose had a sensitivity of 54.5% and a specificity of 83.2% and lactulose had a sensitivity of 42% and a specificity of 70.6%.

In our study among the 32 patients, two patients with SIBO had not performed surgery and one performed intestinal resection. Rao et al reported that prevalence of SIBO in patients with unexplained abdominal pain, gas, bloating and diarrhea was twice (62%) in patients that performed colectomy compared to the control group without colectomy (32%). They described that the intestinal flora was different between the groups, with predominance of aerobic bacteria and less anaerobic microorganisms in patients with SIBO post-colectomy[11].

Brägelmann et al., described that in 80 patients with GC submitted to gastrectomy, 63% had SIBO with abdominal distension in 71%, without significant changes in other characteristics, including nutritional status according to BMI[24]. Newberry et al., also did not correlate SIBO with malnutrition by BMI[25]. In our study, we did not find significant difference between nutritional status in patients with SIBO.

Saffouri et al., compared the presence of SIBO by quantitative duodenal aspirate to the presence of diarrhea, abdominal pain and bloating and did not find a positive correlation between symptoms and SIBO[26]. In our study, between the three patients with SIBO in chemotherapy, two had diarrhea grade 2/3 and Bristol scale 5–7.

The study of Liang evaluated 112 patients with GC and 88 with CRC and observed that 73 (65.2%) patients with GC and 53 (60.2%) with CRC presented SIBO studied by glucose-H2-breath test (GHBT)[27]. In our study, we evaluated 30 patients with CRC and three with GC, but only three patients with CRC had SIBO by lactulose H2 breath test.

Osterlund et al., performed a study with 150 patients with CRC, treated with 5-FU and leucovorin, 24% presented hypolactasia before chemotherapy and 35% during the treatment. In general, 94% of the patients presented symptoms compatible with lactose intolerance. Seventy-six per cent of them had diarrhea grade 0–2 and 24% grade 3–4[28]. In our study, lactose intolerance was diagnosed in 45% of the patients, 66.7% of them had diarrhea, being Bristol's scale 5–7 in 77.8%.

Regarding to the nutritional status, Osterlund et al.[28], described that 71% were well nourished, according to SGA. In our study, most of the patients had not malnutrition according to the BMI. Considering the PG-SGA, two patients with SIBO had moderate or severe nutrition risk. Regarding the patients with lactose intolerance, 22.2% presented severe risk of nutrition.

The small number of patients included was a limitation of the study. Many patients did not accept to do both breath tests

during the chemotherapy treatment. We believe that this difficulty was a consequence of the patients' extensive routine procedures as medical consultations, chemotherapy days, routine blood, and imaging exams, beyond the treatment side toxicity such as fatigue, nausea, and diarrhea. Another limitation was the choice of a breath test in the diagnosis of SIBO. The gold standard for the diagnosis of this disease is the culture of jejunal aspiration, that is an invasive and expensive exam, not routinely done in our country. However, it was interesting to demonstrate that these patients had others causes of diarrhea that can explain or intensify the diarrhea of patients on chemotherapy and gastrointestinal or colorectal cancer.

In conclusion more than half of the patients on chemotherapy with gastrointestinal cancer had diarrhea. Nine per cent of the patients had SIBO and 45% had lactose intolerance. Regarding the nutritional status, most of the patients had eutrophic, overweight or obesity by the BMI independent of the presence of SIBO or intolerance of lactose. According to PG-SGA, moderate or severe nutritional risk was present in a high percent of the patients. Although we did not find correlation between SIBO or lactose intolerance and intensity or characteristic of the stools, the diagnosis of other causes of diarrhea can contribute to a specific treatment with better tolerance to chemotherapy and better quality of life.

Authors' contribution

Gonçalves AR did the inclusion of the patients, performance of the breath tests with the laboratory technicians, statistical analysis and writing the article. Ambrogini Jr O. contributed to the interpretation of the breath tests and the concept of the study. Forones NM contributed to the concept, design and the finalization of the manuscript.

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Gonçalves AR, Ambrogini Jr O, Forones NM. Testes respiratórios não invasivos para o diagnóstico de SBID e intolerância à lactose em pacientes com câncer colorretal e gástrico em tratamento quimioterápico. Arq Gastroenterol. 2021;58(1):26-31.

RESUMO – **Contexto** – Mundialmente, o câncer colorretal (CCR) e gástrico (CG) são a terceira e a quinta causa de câncer mais prevalente, respectivamente. A diarreia é um sintoma comum entre os pacientes em quimioterapia ou radioterapia e pode reduzir a tolerância ao tratamento. Quimioterapia e ressecções cirúrgicas causam alterações da microbiota intestinal que podem levar a intolerância à lactose e ao supercrescimento bacteriano do intestino delgado (SBID). **Objetivo** – Avaliar a presença de diarreia nos pacientes com câncer colorretal e gástrico em quimioterapia e a presença de SBID ou intolerância à lactose. **Métodos** – Foi realizado um estudo descritivo, observacional com pacientes ambulatoriais de ambos os sexos, maiores de 18 anos, em tratamento no ambulatório de gastro-oncologia da Universidade Federal de São Paulo. Foram incluídos pacientes com diagnóstico confirmado de CCR ou CG durante tratamento quimioterápico. Para detectar supercrescimento bacteriano e intolerância à lactose, foram realizados testes respiratórios com lactulose e lactose respectivamente. Número, aspecto das evacuações e grau de toxicidade foram coletados. Para a avaliação nutricional foram aferidos peso e altura para cálculo do IMC e para avaliação do risco nutricional foi realizada a avaliação subjetiva global produzida pelo próprio paciente (ASG-PPP). **Resultados** – Foram incluídos 33 pacientes, 29 com CCR e 3 com CG. A maioria era do sexo masculino (57,5%) com média de idade 60,03±10,01 anos e em tratamento quimioterápico com fluoropirimidina e oxaliplatina (54,5%). Diarreia foi relatada por 57,6% dos pacientes sendo em 30% grau 2. Pelo IMC, 78,9% apresentavam eutrofia, sobrepeso ou obesidade grau 1, mas pela ASG-PPP 84,9 apresentavam risco nutricional moderado ou severo. Entre os pacientes 9% apresentavam SBID e 45% intolerância à lactose. Diarreia grau 2-3 foi observada em 66,6% daqueles pacientes com SBID e 66,7% dos com intolerância à lactose. Não encontramos diferenças estatísticas entre os pacientes com SBID ou intolerância à lactose e intensidade de diarreia. **Conclusão** – Diarreia foi um sintoma frequente entre os pacientes com câncer gástrico ou colorretal em quimioterapia independente da presença de SBID ou intolerância à lactose. Cirurgia e quimioterapia impactaram no hábito intestinal dos pacientes. O diagnóstico de outras causas de diarreia pode contribuir para a melhor tolerância do tratamento e qualidade de vida.

DESCRITORES – Neoplasias colorretais, tratamento farmacológico. Neoplasias gástricas, tratamento farmacológico. Diarreia. Bactéria, crescimento & desenvolvimento. Intolerância à lactose.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: Cancer J Clin. 2018;68:394-424.
2. Instituto Nacional do Câncer. Incidência de Câncer no Brasil Estimativa 2020. [Internet]. [Acessed 2020 March 25]. Available from: https://www.inca.gov.br/publicacoes/livros/estimativa-2020-incidencia-de-cancer-no-brasil.
3. Holmes AC, Riis AH, Erichsen R, Fedirko V, Ostenfeld EB, Vyberg M et al. Descriptive Characteristics of Colon and Rectal Cancer Recurrence in Danish Population Based-Study. Acta Oncologica. 2017;56:1111-9.
4. Carlotto A, Hogsett VL, Maiorini EM, Razulis JG, Sonis ST. The economic burden of toxicities associated with cancer treatment: review of the literature and analysis of nausea and vomiting, diarrhea, oral mucositis and fatigue. Pharmacoeconomics. 2013;31:753-66.
5. Andreyev J, Ross P, Donnellan C, Lennan E, Leonard P, Waters C, Wedlake L et al., Guidance on the management of diarrhoea during cancer chemotherapy. Lancet Oncol. 2014;15:e447-60. doi: 10.1016/S1470-2045(14)70006-3.
6. Benson AB, Ajani JA, Catalano RB, Engelking C, Kornblau SM, Marteson JA Jr, et al., Recommended Guidelines for the Treatment of Cancer Treatment-Induced Diarrhea. J Clin Oncol. 2004;22:2918-26.
7. Muls AC, Lalji A, Marshall C, Butler L, Shaw C, Vyoral S, et al. The Holistic Management of Consequences of Cancer Treatment by a Gastrointestinal and Nutrition Team: A Financially Viable Approach to an Enormous Problem? Clin Med. 2016;16:240-6.
8. Larsen HM; Borre M; Christensen P; Drewes AM, Laurberg S, Krogh K, et al. Clinical Evaluation and Treatment of Chronic Bowel Symptoms Following Cancer in Colon and Pelvic Organs. Acta Oncologica. 2019;58:776-81.

9. Ozorio GA, Barão K, Forones NM. Cachexia Stage, Patient-Generated Subjective Global Assessment, Phase Angle, and Handgrip Strength in Patients with Gastrointestinal Cancer. Nutr Cancer. 2017;19:1-8.
10. Faramarzi E, Mahdavi R, Mohammad-Zadeh M, Nasirimotlagh B. Validation of Nutritional Risk Index Method Against Patient Generated Subjective Global Assessment in Screening Malnutrition in Colorectal Cancer Patients. Chin. F. Cancer Res. 2013;25:544-8.
11. Rezaie A, Pimentel M, Rao SS. How to Test and Treat Small Intestinal Bacterial Overgrowth: An Evidence-Based Approach. Curr Gastroenterol Rep. 2016; 18:1-11.
12. Rao SS, Tan G, Abdulla D, Yu S, Larion S Leelasinjaroen P. Does Colectomy Predispose to Small Intestinal Bacterial (SIBO) and Fungal Overgrowth (SIFO)? Clin Transl Gastroenterol. 2018;9:146. doi: 10.1038/s41424-018-0011-x.
13. Muls AC. Gastrointestinal consequences of cancer treatment and the wider context: A bad gut feeling. Acta Oncol. 2014;53:297-306.
14. Friedrich DC, Santos EB, Ribeiro-dos-Santos AKC, Hutz MH. Several Different Lactase Persistence Associated Alleles and High Diversity of the Lactase Gene in the Admixed Brazilian Population. PLoS One. 2012;7:e46520.
15. Ji J, Sundquist J, Sundquist K. Lactose Intolerance and Risk of Lung, Breast and Ovarian Cancers: Aetiological Clues from a Population-Based Study in Sweden. Br J Cancer 2015;112:149-52.
16. Kim JW. Lactose Intolerance and Colorectal Cancer. Ann Coloproctol. 2017;33:157-8.
17. Common Terminology Criteria for Adverse Events (CTCAE) Version 5,0. U.S Department of Health and Human Services. 2017. Available from: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf
18. Lewis SJ, Heaton KW. Stool Form Scale as a Useful Guide to Intestinal Transit Time. Scand J Gastroenterol 1997;32:920-4.
19. Losurdo G, Leandro G, Ierardi E, Perri F, Barone M, Principi M, et al., Breath Tests for the Non-invasive Diagnosis of Small Intestinal Bacterial Overgrowth: A Systematic Review With Meta-analysis. J Neurogastroenterol Motil. 2020;26:16-28.
20. World Health Organization. Obesity: Preventing and Managing the Global Epidemic: Report of WHO Consultation on Obesity. Geneva. WHO. 1997, 178p.
21. Lipschitz DA. Screening for Nutrition Status in Elderly. Prime Care. 1994; 21:55-67.
22. Schmoll HJ, Cartwright T, Tabernero J, Nowacki MP, Figer A, Maroun J et al., Phase III Trial of Capecitaine plus Oxaliplatine as adjuvante therapy for Stage III Colon Cancer: A Planned Safety Analysis in 1864 Patients. J Clin Oncol. 2007;25:102-9.
23. Glynne-Jones R, Dunst J, Sebag-Montefiore D. The integration of oral capecitabine into chemoradiation regimens for locally advanced rectal cancer: how successful have we been? Ann Oncol. 2006;17:361-71.
24. Brägelmann R, Armbrecht U, Rosemeyer D, Schineider B, Zilly W, Stockbrügger W. Small Bowel Bacterial Overgrowth in Patients After Total Gastrectomy. Eur J Clin Invest. 1997;27:409-16.
25. Newberry C, Tierney A, Pickett-Blakely O. Lactulose Hidrogen Breath Test Result is Associated with Age and Gender. Biomed Res Int. 2016;2016:1064029.
26. Saffouri GB, Shields-Cutler RR, Chen J, Yang Y, Lekatz HR, Hale VL, et al. Small Intestinal Microbial Dysbiosis Underlies Symptoms Associated with Functional Gatrointestinal Disorders. Nat Commun. 2019;10:1-11. https://doi.org/10.1038/s41467-019-09964-7.
27. Liang S, Xu L, Zhang D, Wu Z. Effect of Probiotics on Small Intestinal Bacterial Overgrowth in Patients with Gastric and Colorectal Cancer. Turk J Gastroenterol. 2016;27:227-32.
28. Osterlund P, Ruotsalainen T, Peuhkuri K, Korpela R, Ollus R, Ikonen M, et al. Lactose Intolerance Associated with Adjuvant S-Fluourouracil-Based Chemotherapy for Colorectal Cancer. Clin Gastroenterol Hepatol 2004;2:696-703.

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Inappropriate usage of intravenous proton pump inhibitors and associated factors in a high complexity hospital in Brazil

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ABSTRACT – **Background** – Intravenous (IV) use of proton pump inhibitors (PPIs) is advised only in cases of suspected upper gastrointestinal bleeding (UGIB) or impossibility of receiving oral medication, although there has been a persistent practice of their inappropriate use in health institutions. **Objective** – The purpose of our study was to measure the inappropriate use of IV PPIs in a high complexity hospital in Brazil and to estimate its costs. **Methods** – Retrospective study of 333 patients who received IV omeprazole between July and December of 2018 in a high complexity hospital in Brazil. **Results** – IV omeprazole was found to be appropriately prescribed in only 23.4% patient reports. This medication was administered mainly in cases of suspected UGIB (19.1%) and stress ulcer prophylaxis in patients with high risk of UGIB unable to receive medication orally (18.7%). It was observed a statistically significant association between adequate prescription and stress ulcer prophylaxis in patients with high risk of UGIB unable to receive medication orally; patient *nil per os* with valid indication for PPIs usage; prescription by intensive care unit doctors; prescription by emergency room doctors; intensive care unit admission; evolution to death; sepsis; and traumatic brain injury ($P<0.05$). On the other hand, inadequate prescription had a statistically significant association with surgical ward prescription and non-evolution to death ($P<0.05$). The estimated cost of the vials prescribed inadequately was US$1696. **Conclusion** – There was a high number of inappropriate IV omeprazole prescriptions in the studied hospital, entailing greater costs to the institution and unnecessary risks.

HEADINGS – Proton pump inhibitors. Peptic ulcer. Gastrointestinal hemorrhage. Inappropriate prescribing. Off-label use. Endoscopy. Medical overuse.

INTRODUCTION

The most efficient medications for gastric acid suppression nowadays are proton pump inhibitors (PPIs), available in the Market since 1989 with the launch of omeprazole[1]. These pharmaceutical drugs inhibit H+, K+-ATPase of gastric parietal cells, causing an increase in stomach pH[2].

The main clinical indications approved by the Food and Drug Administration for the administration of this class of medication are treatment for erosive esophagitis and its maintenance; treatment of gastro esophageal reflux; reduction of risk of gastric ulcer associated to non-steroid anti-inflammatory drug (NSAID); *Helicobacter pylori* eradication; hyper secretive pathological conditions, such as Zollinger-Ellison syndrome; and duodenal ulcer treatment and its maintenance[3].

However, the intravenous (IV) preparations, according to recent studies, are reserved for cases of patients with: gastric hyper secretion associated with neoplastic conditions and Zollinger-Ellison unable to receive medication orally; severe cases of non-variceal upper gastrointestinal bleeding (UGIB); gastrointestinal hemorrhage with risk of recurrent continuous bleeding; and in stress peptic ulcer prophylaxis in high risk patients on the intensive care unit (ICU) without access to enteral feeding or unable to receive orally, nil per os (NPO)[4,5]. According to the Brazilian National Health Surveillance Agency (ANVISA), intravenous sodium omeprazole is indicated when the usage of the pharmaceutical form of pills is not possible. Sodium omeprazole is to be used to treat: gastric or duodenal peptic ulcer; reflux esophagitis; Zollinger-Ellison syndrome; and prophylaxis for aspiration of gastric content during general anesthesia in high risk patients[6].

The adequate use of IV PPIs in patients with suspected non-variceal UGIB, according to current international guidelines, consists on the implementation of an initial bolus of 80 mg and, subsequently, an infusion of 8 mg/h for 72 hours, executing a new treatment for 72 hours if recurrence of bleeding[7,8]. However, in a systematic review and meta-analysis conducted by Sachar, Vaidya and Laine, it was observed that the utilization of PPIs intermittently, with an initial bolus of 80 mg followed by 40 mg every 12 hours presented similar outcomes to the continuous infusion of 8 mg/h[9]. In patients unable to receive oral drugs, the IV dosage should be individualized in accordance to the specific indication[10,11].

The inappropriate use of IV PPIs has been observed in various studies, especially in cases without the suspicion of UGIB[4,11-14]. There are, however, studies demonstrating that oral preparations of PPIs have similar efficacy to IV preparations in cases of bleeding ulcers suggesting that it seems to be no need for such an excessive IV administration in hospitals resulting undoubtedly to higher institutional costs[15-17].

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There is little data regarding the usage of IV PPIs in Brazil and Latin America. This present study had, therefore, the objective of evaluating the indications, posologies, duration of treatment and cost of IV omeprazole prescriptions, the only IV PPI available in the studied institution.

METHODS

This present study was approved by the Ethics and Research with Human Beings Committee from the *Universidade do Extremo Sul Catarinense* (3.084.465) and from the Hospital São José Criciúma (3.145.505).

This study was held in a high complexity hospital in the Southern region of the state of Santa Catarina, Brazil, estimating that in six months about a thousand patients receive IV omeprazole in this institution and the minimum sample calculation was of 291 patient records. Three hundred thirty three patient records of patients who received IV omeprazole were assessed retrospectively in the period of July to December of 2018. Patients with age inferior to 18 years were excluded.

An instrument for data collection was used, evaluating sex, age, race and comorbities of the patient; which was the indication for initiating treatment with IV omeprazole; what were the reasons for its use when there was no suspected UGIB; if the patient was subjected to upper digestive endoscopic exam (UDE) or surgical procedure in case of suspected upper gastrointestinal bleeding and reasons for non-subjection of the patient; UDE findings, if performed; surgical procedure findings, if performed; IV omeprazole's posology; duration of treatment; service responsible for prescribing the medication; admission in the intensive care unit during hospital stay; prescription by emergency room doctor; if there was evolution to death during hospital stay; if there was IV omeprazole suspension in 48 hours when initiated by inadequate reason; total number of vials of this pharmaceutical drug utilized during hospital stay; number of vials utilized inadequately; cost involved with the use of this medication; if the indication of use was adequate; if the dosage was adequate; if the duration of treatment was adequate and if the prescription of IV omeprazole was entirely correct.

The indications considered as adequate for the administration of this IV drug were upper gastrointestinal bleeding or its suspicion before a confirmatory procedure, like endoscopy or surgery; stress ulcer prophylaxis in patients with high risk of upper gastrointestinal bleeding and unable to receive medication orally; and patient in NPO with valid indication for oral PPI. Of the patients with suspected non-variceal UGIB, were considered with appropriate indication those presenting peptic ulcer disease with stigmata of high risk of rebleeding in the endoscopic or surgical report according to Forrest classification: Forrest IA (spurting hemorrhage), IB (oozing hemorrhage), IIA (visible vessel) or IIB (adherent clot), in addition to erosive disease of the esophagogastric mucous membrane with active bleeding, tumoral lesion with active bleeding, or while awaiting for confirmatory procedure. If the patients did not match the formerly cited situations, it was also considered an adequate indication those patients who had the medication suspended in up to 48h after the result of the endoscopy. Of the patients unable to receive enteral diet, those in NPO or intolerant to oral medication with valid indication for PPIs were considered with adequate indication, or those that were receiving prophylaxis for stress ulcer because of high risk of upper gastrointestinal bleeding.

For stress ulcer prophylaxis, we considered as critically ill patients with high risk for upper gastrointestinal bleeding those with at least one of the following criteria: hemorrhagic diathesis – platelet count <50.000 by mm^3, an International Normalized Ratio (INR) >1.5 or an activated partial thromboplastin time (aPTT) >2 the control value; mechanical ventilation >48 hours; history of gastrointestinal ulcer or gastrointestinal bleeding in the last year; traumatic brain injury; traumatic spinal cord injury; burn; or at least two of the following findings: sepsis; period of intensive care unit stay superior to one week; gastroinstestinal bleeding for six or more days; glucocorticoid therapy (superior to 250 mg of hydrocortisone or equivalent); or use of NSAID or antiplatelet agents[5].

Posology was considered adequate when, in the case the patient had UGIB suspicion, a bolus of 80 mg followed by continuous infusion of 8 mg/h for 72 hours or bolus of 80 mg followed by 40 mg every 12 hours for 72 hours was instituted. If the usage was due to stress ulcer prophylaxis in patients with high risk of UGIB unable to receive it orally or in patients in NPO with valid indication for oral PPI, it was considered adequate the use of 40 mg of intravenous omeprazole once a day.

Regarding the duration of treatment, all the patients who did not present any indication for intravenous omeprazole use and those with UGIB suspicion who did not suspend the medication in up to 48 hours after the absence of valid indication or absence of stigmata of peptic ulcer with high risk of rebleeding in the diagnostic procedure results were considered inadequate.

In cases where the indication was considered inadequate, dosage and duration of treatment were also considered inadequate. Only when indication, dosage and duration of treatment were adequate, the prescription was considered entirely adequate.

The collected data was analyzed with the help of the software IBM Statistical Package for the Social Sciences (SPSS) version 21.0. The quantitative variables were expressed by median and interquartile amplitude or minimum and maximum value when these did not present normal distribution and by mean and standard deviation when these did present with normal distribution. Qualitative variables were expressed by frequency and percentage.

For the calculation of the number of inadequately prescribed vials and the cost of these to the hospital, we multiplied the number of days of inadequate prescription by the number of vials used in the period. The hospital onus was calculated by multiplying the total number of inadequate vials by the cost of each vial in the time of the study.

The statistical tests were made with a significance level $\alpha = 0.05$ and, therefore, 95% confidence interval. The data distribution in relation to normality was evaluated by application of the Kolmogorov-Smirnov test. The investigation of association between qualitative variables was made by application of the Pearson's chi-square test, Fisher's exact test and the likelihood ratio test, followed by residual analysis when statistical significance was observed.

RESULTS

Of the 333 patients analyzed, 13 were excluded due to age inferior to 18 years, with a subsequent sample of 320 patients. The studied population was composed mainly by men (54.4%), whites (93.4%) with a mean age of approximately 60 years (±16.29). The comorbities most commonly found in these patients are described in TABLE 1.

TABLE 1. Demographic data and comorbities of patients using intravenous omeprazole.

	Mean ±SD, n (%) n=320
Age (years)	59.92±16.29
Sex	
Male	174 (54.4)
Female	146 (45.6)
Race	
White	298 (93.4)
Black	11 (3.4)
Brown	9 (2.8)
Indigenous	1 (0.3)
Not specified	1
Comorbities	
Systemic arterial hypertension	125 (39.1)
Pneumonia	81 (25.3)
Diabetes Mellitus	76 (23.8)
Malignant neoplasia	62 (19.4)
Acute coronary syndrome	45 (14.1)
Sepsis	44 (13.8)
Cerebrovascular accident	28 (8.8)
COPD	20 (6.3)
Congestive heart failure	15 (4.7)
Chronic hepatopathy	15 (4.7)
Traumatic brain injury	12 (3.8)
UTI	10 (3.1)
Gallstones	9 (2.8)
Chronic kidney disease	9 (2.8)
Other	95 (29.7)

SD: standard deviation; COPD: chronic obstructive pulmonar disease; UTI: urinary tract infection.

The suspicion of presence of UGIB was the most frequent indication for initiating the use of IV omeprazole, occurring in 19.1% of the cases. The median of duration of treatment was of 4.00 days, with an interquartile amplitude of 2.00–8.00, and periods of duration varying from 1 to 61 days were found (TABLE 2).

The largest part of the prescriptions was made by doctors of the ICU (39.7%) and the prescription was initiated by emergency room doctors in only 10.3% of the cases. Half of the patients were admitted to the ICU during hospital stay and 69 (21.6%) patients evolved to death (TABLE 3).

Regarding the group of patients with UGIB suspicion, 34.4% did not undergo any procedure for confirmation of presence of upper gastrointestinal bleeding. The endoscopic report of patients who did undergo UDE showed normal or unspecific findings in most cases (31.6%). Regarding the 28 (73.6%) patients who did not have confirmed high risk of bleeding, 9 (32.2%) remained utilizing the IV omeprazole for more than 48 hours after receiving the UDE report (TABLE 4).

The indication for use of IV omeprazole was considered adequate in only 41.6% of cases. Regarding posology, the following findings were observed in reference to the group with UGIB suspicion: 15 (24.6%) in use of initial bolus of 80 mg followed by

TABLE 2. Indications for initiating intravenous omeprazole usage and duration of treatment.

	n (%), Median (minimum – maximum) n=320
Adequate indications	
Upper gastrointestinal bleeding suspicion	61 (19.1)
Stress ulcer prophylaxis in patient with high risk of UGIB unable to receive orally	60 (18.7)
Patient in NPO with valid PPI indication	12 (3.7)
Inadequate indications	
PUD prophylaxis after surgical procedure	58 (18.1)
Stress ulcer prophylaxis in patients with low risk of UGIB	40 (12.5)
Abdominal pain	23 (7.2)
Absence of clear indication	19 (5.9)
Stress ulcer prophylaxis and high risk of UGIB with possibility of oral administration	12 (3.7)
Anticoagulants/ platelet antiaggregant/ anti-inflammatories usage	11 (3.4)
Abdominal pain associated with vomiting	8 (2.5)
Other	16 (5.0)
Duration of treatment (days)	4.00 (1–61)

UGIB: upper gastrointestinal bleeding; PUD: peptic ulcer disease; NPO: nil per os; PPI: proton pump inhibitor.

TABLE 3. Sectors involved in prescribing intravenous omeprazole and evolution to death.

	n (%) n=320
Prescribing sector	
ICU	127 (39.7)
General medicine	81 (25.3)
Surgery	56 (17.5)
Oncology	18 (5.6)
Gastroenterology	16 (5.0)
Cardiology	8 (2.5)
Pneumology	5 (1.6)
Other	9 (2.8)
Prescription by ER doctor	
Yes	33 (10.3)
No	287 (89.7)
Admission in the ICU during hospital stay	
Yes	160 (50.0)
No	160 (50.0)
Evolution to death	
Yes	69 (21.6)
No	251 (78.4)

ICU: intensive care unit; ER: emergency room.

TABLE 4. Performance of procedures for confirmation of bleeding in patients using intravenous omeprazole with suspicion of upper gastrointestinal bleeding.

	n (%) n=61
Procedure	
UDE	38 (62.3)
Surgery	2 (3.3)
Gastric or duodenal perforated ulcer	2 (100.0)
Not performed	21 (34.4)
Reason for not performing any procedure (n=21)	
Critically ill patient	6 (28.6)
Recent UDE	4 (19.0)
Unnecessary UDE or patient too well for procedure	4 (19.0)
Not informed	7 (33.4)
UDE results (n=38)	
Normal or unspecific findings	12 (31.6)
Peptic ulcer disease with stigmata of low risk of rebleeding (Forrest IIC or III)	10 (26.3)
Peptic ulcer disease with stigmata of high risk of rebleeding (Forrest IA, IB, IIA or IIB)	8 (21.1)
Gastro-esophageal varicose veins	3 (7.9)
Esophagogastric mucous membrane erosive disease without active bleeding	2 (5.3)
Esophagogastric mucous membrane erosive disease with active bleeding	1 (2.6)
Tumoral lesion with active bleeding	1 (2.6)
Gastric angiodysplasia without signs of recent bleeding	1 (2.6)
Medication suspended in up to 48h in case of absence of indication for maintenance of intravenous omeprazole (n=28)	
Yes	19 (67.8)
No	9 (32.2)

UDE: upper digestive endoscopy.

40 mg every 12 hours and 8 (13.1%) in use of bolus of 80 mg on IV omeprazole followed by continuous infusion of 8 mg/h for 72 hours. In regard to the group of patients with stress ulcer prophylaxis and high risk of gastrointestinal bleeding unable to receive medication orally or in NPO with valid indications for PPI usage, 70 (97.2%) used 40 mg of IV omeprazole once a day. In any other case, the posology was considered inadequate.

Considering that when the indication was considered inadequate, dosage and duration were also considered inadequate, only 93 (29.1%) patients were in use of correct doses and only 101 (31.6%) patients presented adequate duration of treatment. Prescription was entirely adequate in only 23.4% of analyzed prescriptions (TABLE 5). Comparing the group with UGIB suspicion and the one that initiated IV omeprazole for other reasons, prescription was entirely adequate in 31.1% and 21.6% of cases, respectively, thus having no statistically significant difference (P=0.114) (TABLE 6).

The median of the number of vials utilized per patient was 6.00, with variations from 1–74 phials per patient and interquartile amplitude of 2.50–11.00. The median for the number of inadequate vials per patient was 3.00, with variations from 0–60 inadequate vials per patient and interquartile amplitude of 0.00–7.00. It was

TABLE 5. Evaluation of prescriptions of patients who used intravenous omeprazole.

	n (%) n=320
Adequate indication	
Yes	133 (41.6)
No	187 (58.4)
Adequate dosage*	
Yes	93 (29.1)
No	227 (70.9)
Adequate duration*	
Yes	101 (31.6)
No	219 (68.4)
Entirely adequate prescription	
Yes	75 (23.4)
No	245 (76.6)

*When the indication was considered inadequate, dosage and duration were also considered inadequate. Source: Research data, 2019.

TABLE 6. Factors associated with adequate and inadequate prescription of intravenous omeprazole.

	n (%)		
	Adequate prescription	Inadequate prescription	*P*-value
Adequate indications	n=74	n=180	
Suspected upper gastrointestinal bleeding	19 (31.1)	42 (68.9)	<0.001†
Stress ulcer prophylaxis in patient with high risk of UGIB unable to receive orally	44 (73.3)[a]	16 (26.7)	
Patient in NPO and valid indication for PPI usage	11 (91.7)[a]	1 (8.3)	
Prescribing sector	n=75	n=223	
ICU	44 (34.6)[a]	83 (65.4)	0.010†
General medicine	18 (22.2)	63 (77.8)	
Surgery	7 (12.5)	49 (87.5)[a]	
Prescription by ER doctor	n=75	n=245	
Yes	15 (45.5)[a]	18 (54.5)	0.002‡
No	60 (20.9)	227 (79.1)[a]	
ICU admission	n=75	n=245	
Yes	50 (31.3)[a]	110 (68.8)	0.001‡
No	25 (15.6)	135 (34.4)[a]	
Evolution to death	n=75	n=245	
Yes	31 (44.9)[a]	38 (55.1)	<0.001‡
No	44 (17.5)	207 (82.5)[a]	
Sepsis			
Yes	23 (54.5)[a]	20 (45.5)	<0.001‡
No	51 (18.5)	225 (81.5)[a]	
Traumatic brain injury			
Yes	8 (66.7)[a]	4 (33.3)	0.001†
No	67 (21.8)	241 (78.2)[a]	

UGIB: upper gastrointestinal bleeding; NPO: nil per os; PPI: proton pump inhibitor; ICU: intensive care unit; ER: emergency room. †Value obtained after applying the Likelihood Ratio test; ‡Value obtained after applying the Pearson qui-square test. [a]Statistically significant value after residual analysis.

utilized a total of 2853 vials in these 320 patients and, of these, 1696 were considered inadequate. Considering the approximate value of each vial is US$1.00, it is estimated a cost of US$1696.00 spent with inadequate use of IV omeprazole prescribed for these patients, and a total of US$2853.00 with the total of utilized vials evaluated in this study. As it was collected about a third of the number of patient reports of those who used IV omeprazole in six months in the studied hospital, it is estimated a cost of about US$5088.00 with the administration of inadequate vials and a cost of US$8559.00 with the total of vials of IV omeprazole administrated in a period of one semester.

It was observed a statistically significant association between adequate prescription and the following factors: stress ulcer prophylaxis in patients with high risk of UGIB unable to receive medication orally; patient in NPO with valid indication for PPI usage; ICU doctor prescription; evolution to death; sepsis; and traumatic brain injury (P<0.05). Inadequate prescription, however, had a statistically significant association with prescription by the surgical ward and non-evolution to death (P<0.05) (TABLE 6).

DISCUSSION

This study was conducted in a high complexity hospital evaluating the usage of IV omeprazole during a period of six months. It was verified that the prescription was inadequate in 76.6% of cases, a higher rate than those observed in studies conducted in other countries[4,11-14].

In the study by Lai et al., in 76.4% of the cases an unexplained abdominal pain was the reason for initiating the use of IV PPIs and, in these cases, 68.9% had UGIB suspicion[14]. In this present study, the most frequent reason for initiating the use of IV omeprazole was UGIB suspicion, however this occurred in only 19.1% of cases. Other studies demonstrated that most patients receiving IV PPIs, did it for stress ulcer prophylaxis, data that is compatible with this study, in which it was administered IV omeprazole in 53% of the patients for prophylaxis of peptic ulcer disease, if we add the patients in high and low risk[4,13].

Former studies observed that most of the doctors responsible for IV PPI prescription were part of the surgical ward[11,14], a data that differs from this present study, in which most of the prescribing doctors were from the ICU (39.7%) and from the clinical ward (25.3%). In this study, it was also observed that half of the patients had an ICU stay. It is possible that patients who were evaluated at the studied institution had a larger admission in this sector in light of being a tertiary hospital, with cases of higher gravity, which could be better evaluated in posterior studies with a larger number of patients.

On a study made in England, IV PPI prescriptions were found to be inadequate in 75.4% of cases, and most of these patients receiving this medication inadequately had no suspicion of UGIB[11]. In this present study there was a larger percentage of patients in use of IV omeprazole inadequately in the group without UGIB suspicion. There was no statistically significant difference, however, between the group with UGIB suspicion and the group that initiated IV omeprazole for other reasons.

In a Canadian study that evaluated IV PPI prescriptions in cases of UGIB suspicion, it was found that 68% of the patients underwent an UDE. Of those that underwent endoscopic procedure, 86.2% had no stigmata for high risk of rebleeding and, of these, 56.9% remained in use of the medication despite the low risk of rebleeding[18]. In regard to the present study, 73.6% of the patients who underwent UDE did not have high risk of rebleeding, a similar finding to the one observed in the Canadian study, but only 32.3% of these remained utilizing IV omeprazole 48 hours after the procedure.

In this study, the adequate prescription was associated with stress ulcer prophylaxis in patients with high risk of UGIB unable to receive oral medication; patient in NPO with valid indication for PPI usage; prescription by ICU doctor; prescription by emergency room doctor; ICU admission; evolution to death; sepsis; and traumatic brain injury. The inadequate prescription was associated to surgical ward prescription and non-evolution to death. It is important to highlight that traumatic brain injury and sepsis combined to long stay in ICU were considered adequate indications for stress ulcer prophylaxis in patients with high risk of UGIB unable to receive oral medication. Since all these factors are associated with greater severity and were more prevalent in patients with adequate prescription, we believe they contributed to the association between adequate prescription and evolution to death. The factors associated with adequate and inadequate prescriptions are relevant in facilitating the identification and correction of inadequate practices in health institutions.

This study presented limitations regarding sample. Although the minimum sample was matched, the total number of patient records in use of IV omeprazole in the studied period was not analyzed due to little time available for data collection, and also this being a retrospective study. It is important to point out, however, that there is not sufficient data in literature about how the administration of IV PPIs is carried out in Brazil and Latin America. Thus, this article is still relevant so that greater information regarding the hospital practices in the studied region is obtained.

Knowing that IV PPIs entail a high cost to institutions, a few models were already proposed, suggesting that the administration of these IV medications in every case of suspected UGIB probably is not a cost-effective approach, and also most of the patients treated for UGIB are not hemodynamically unstable[11,19]. Although UGIB suspicion still being a formal indication for the use of IV PPIs, a few studies already demonstrated that patients who tolerate oral medication can receive this class of medication this way, which is effective even in cases of bleeding peptic ulcers, entailing a smaller cost that the usage of IV vials[15-17].

Some therapeutic strategies aiming to minimize IV PPIs prescription errors have been shown in literature. In a study conducted by Kaplan et al., it was observed that, initially, only 50% of the patients receiving IV pantoprazole had adequate indication. A multidisciplinary intervention involving medical education; computerized dosage model; pharmaceutical intervention when a patient without suspected UGIB and with tolerance for oral medication received IV PPI and recommendation of a consult with a gastroenterologist when a continuous infusion of these medications was applied. After this intervention, there was a significant reduction of inadequate prescriptions in the groups with UGIB suspicion (26%) and without UGIB suspicion (41%)[12].

In the study conducted by Lai et al., every fourth IV PPI prescription received by the pharmaceutical sector was traced against the guidelines from that hospital. PPIs were incorrectly prescribed in 52.8% of cases and interventions were more effective when made by senior doctors (100%), followed by clinical pharmacists (50%)[14].

Despite PPIs being considered one of the safest pharmaceuti-

cal classes, there are potential side effects with chronic use of these medications and gastric suppression in the long term[20]. These drugs present few interactions with other drugs. The most notable would be caused by the inhibition of the P450 2C19 cytochrome, especially by omeprazole, leading to a reduction of clopidogrel conversion in its active metabolite, enlarging the risk of cardiovascular diseases in patients who use both medications simultaneously[21]. Other drugs which may also be affected by inhibiting the P450 2C19 cytochrome are diazepam, phenytoin and warfarin, while dexlansoprazole and lansoprazole may induce theophylline metabolism by the P450 1A2 cytochrome[22].

The most important side effects related to the usage of these drugs are rebound gastric hypersecretion; hypergastrinemia; hypomagnesemia; reduction of calcium, iron and vitamin B12 absorption; greater risk of bone fracture; enteric infections, with diarrhea by Clostridium difficile the most significant within those; the possibility of spontaneous bacterial peritonitis in cirrhotic patients; community acquired pneumonia; nephrotoxicity and possible augmented risk of dementia and myopathies. There is an inverse relation between gastric acidity and plasmatic levels of gastrin, justifying the hypergastrinemia in chronic users of PPIs. The rise in serum levels of gastrin has the potential to cause cellular hyperplasia in enterochromaffin-like cells, which caused discussions about the possibility of greater development of gastric neoplasia in these patients. There isn't, however, clear evidence that the prolonged use of these drugs predisposes the occurrence of cancers. The appearance of polyps on the gastric fundus region, on the other hand, is common in patients with hypergastrinemia, and they don't seem to have any potential for malignant transformation[23].

In conclusion, this study demonstrated an elevated number of inadequate IV omeprazole prescriptions in the studied hospital, a common problem in many other health institutions in the world, entailing a greater cost associated with the unnecessary administration of vials of this medication. These results prompt the implementation of multidisciplinary intervention strategies and medical education in order to minimize prescription errors, reducing costs and risks involved with IV PPI usage.

Authors' contribution

Bischoff LM and Faraco LSM: conception and design; analysis and interpretation of the data; drafting of the article; critical revision of the article for important intellectual content. Machado LV, Bialecki AVS and Almeida GM: analysis and interpretation of the data; critical revision of the article for important intellectual content. Becker SCC: conception and design; analysis and interpretation of the data; critical revision of the article for important intellectual content; final approval of the article.

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Bischoff LM, Faraco LSM, Machado LV, Bialecki AVS, Almeida GM, Becker SCC. Uso inapropriado de inibidores de bomba de prótons intravenosos e fatores associados em um hospital de alta complexidade no Brasil. Arq Gastroenterol. 2021;58(1):32-8.

RESUMO – **Contexto** – Atualmente, o uso intravenoso (IV) dos inibidores de bomba de prótons (IBPs) é indicado em poucas situações, como em casos de hemorragia digestiva alta ou impossibilidade de recebê-los via oral. Há diversos estudos mostrando o uso excessivo desse fármaco, na forma intravenosa, desnecessariamente e acarretando altos custos aos hospitais. **Objetivo** – Avaliar as indicações, posologias, duração do tratamento e custos das prescrições de omeprazol intravenoso. **Métodos** – Estudo retrospectivo de 333 pacientes que receberam omeprazol intravenoso entre julho a dezembro de 2018 em um hospital de alta complexidade no Brasil. **Resultados** – A prescrição de omeprazol intravenoso foi considerada totalmente adequada em apenas 23,4% das prescrições analisadas. O medicamento foi administrado principalmente em casos de suspeita de hemorragia digestiva alta (HDA) (19,1%) e profilaxia de úlcera de estresse em paciente com alto risco de HDA impossibilitado de receber via oral (18,7%). Foi observada associação estatisticamente significativa entre prescrição adequada e profilaxia de úlcera de estresse em paciente com alto risco de HDA impossibilitado de receber medicamento via oral; paciente em *nil per os* com indicação válida de IBPs; prescrição por médico da UTI; prescrição por médico do pronto atendimento; admissão na UTI; evolução a óbito; sepse; e traumatismo cranioencefálico (P<0,05). Já a prescrição inadequada teve associação estatisticamente significativa com prescrição por setor cirúrgico e a não evolução a óbito (P<0,05). O custo estimado do total de ampolas prescritas inadequadamente foi de US$1696,00. **Conclusão** – Houve um elevado número de prescrições de omeprazol intravenoso inadequadas no hospital estudado, acarretando um custo elevado para a instituição.

DESCRITORES – Inibidores da bomba de prótons. Úlcera péptica. Hemorragia gastrointestinal. Prescrição inadequada. Uso off-label. Endoscopia. Sobremedicalização.

REFERENCES

1. Shin JM, Sachs G. Pharmacology of proton pump inhibitors. Curr Gastroenterol Rep. 2008;10:528-34.
2. Sachs G, Shin JM, Howden CW. Review article: the clinical pharmacology of proton pump inhibitors. Aliment Pharmacol Ther. 2006;23(s2):2–8.
3. Food and Drug Administration. Highlights of prescribing information: PRILOSEC. [Internet]. [Access 2019 October 10]. Available from: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=medguide.page
4. Mayet A, Malhani A, Alshaikh M, Alsultan M. Pattern of intravenous proton pump inhibitors use in ICU and Non-ICU setting: A prospective observational study. Saudi J Gastroenterol. 2010;16:275-9.
5. Barletta JF, Bruno JJ, Buckley MS, Cook DJ. Stress Ulcer Prophylaxis. Crit Care Med. 2016;44:1395-405.
6. Anvisa [Internet]. Detalhe do Produto: OMEPRAZOL SÓDICO [Access 2020 June 15]. Available from: https://consultas.anvisa.gov.br/#/medicamentos/253510164320066/?nomeProduto=OMEPRAZOL%20S%C3%93DICO
7. Barkun AN. International Consensus Recommendations on the Management of Patients With Nonvariceal Upper Gastrointestinal Bleeding. Ann Intern Med. 2010;152:101-13.
8. Laine L, Jensen DM. Management of Patients With Ulcer Bleeding. Am J Gastroenterol. 2012;107:345-60.
9. Sachar H, Vaidya K, Laine L. Intermittent vs Continuous Proton Pump Inhibitor Therapy for High-Risk Bleeding Ulcers. JAMA Intern Med. 2014;174:1755-62.
10. Lau JYW, Sung JJY, Lee KKC, Yung MY, Wong SK, Wu JC, et al. Effect of Intravenous Omeprazole on Recurrent Bleeding after Endoscopic Treatment of Bleeding Peptic Ulcers. N Engl J Med. 2000 ;343:310-6.
11. Craig DGN, Thimappa R, Anand V, Sebastian S. Inappropriate utilization of intravenous proton pump inhibitors in hospital practice--a prospective study of the extent of the problem and predictive factors. QJM. 2010;103:327-35.
12. Kaplan GG, Bates D, McDonald D, Panaccione R, Romagnuolo J. Inappropriate Use of Intravenous Pantoprazole: Extent of the Problem and Successful Solutions. Clin Gastroenterol Hepatol. 2005;3:1207-14.
13. Perwaiz M, Posner G, Hammoudeh F, Schmidt F, Neupane N, Enriquez D, Gulati N. Inappropriate Use of Intravenous PPI for Stress Ulcer Prophylaxis in an Inner City Community Hospital. J Clin Med Res. 2010;215-9.
14. Lai PSM, Wong YY, Low YC, Lau HL, Chin K-F, Mahadeva S. Unexplained abdominal pain as a driver for inappropriate therapeutics: an audit on the use of intravenous proton pump inhibitors. PeerJ. 2014;2:e451.
15. Tsoi KKF, Hirai HW, Sung JJY. Meta-analysis: comparison of oral vs. intravenous proton pump inhibitors in patients with peptic ulcer bleeding. Aliment Pharmacol Ther. 2013;38:721-8.
16. Sung JJ, Suen B-Y, Wu JC, Lau JYW, Jessica Y L Ching 1, Vivian W Y Lee, et al. Effects of Intravenous and Oral Esomeprazole in the Prevention of Recurrent Bleeding from Peptic Ulcers after Endoscopic Therapy. Am J Gastroenterol. 2014;109:1005-10. Available from: https://insights.ovid.com/pubmed?pmid=24777150
17. Spiegel BMR, Dulai GS, Lim BS, Mann N, Kanwal F, Gralnek IM. The Cost-Effectiveness and Budget Impact of Intravenous Versus Oral Proton Pump Inhibitors in Peptic Ulcer Hemorrhage. Clin Gastroenterol Hepatol. 2006;4:988-97.e2. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1542356506004885
18. Enns R, Andrews CN, Fishman M, Hahn M, Atkinson K, Kwan P, Levy A. Description of prescribing practices in patients with upper gastrointestinal bleeding receiving intravenous proton pump inhibitors: a multicentre evaluation. Can J Gastroenterol. 2004;18:567-71.
19. Leontiadis GI, Sreedharan A, Dorward S, Barton P, Delaney B, Howden CW, et al. Systematic reviews of the clinical effectiveness and cost-effectiveness of proton pump inhibitors in acute upper gastrointestinal bleeding. Health Technol Assess. 2007;11:iii-iv, 1-164. doi: 10.3310/hta11510.
20. Lanas A. We Are Using Too Many PPIs, and We Need to Stop: A European Perspective. Am J Gastroenterol. 2016;111:1085-6. doi:10.1038/ajg.2016.166
21. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA. 2009;301:937-44. doi:10.1001/jama.2009.261
22. Yang YX, Metz DC. Safety of proton pump inhibitor exposure. Gastroenterology. 2010;139:1115-27. doi:10.1053/j.gastro.2010.08.023
23. Savarino E, Marabotto E, Zentilin P, et al. A safety review of proton pump inhibitors to treat acid-related digestive diseases. Expert Opin Drug Saf. 2018;17:785-94. doi:10.1080/14740338.2018.1497155

ORIGINAL ARTICLE

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Helicobacter pylori chronic gastritis on patients with premalignant conditions: OLGA and OLGIM evaluation and serum biomarkers performance

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ABSTRACT – **Background** – *H. pylori* chronic atrophic gastritis is a premalignant lesion, and its staging, according to OLGA and OLGIM systems aims to identify patients at increased risk of developing gastric cancer and optimize their follow-up. GastroPanel®, serum biomarkers panel including pepsinogen I (PGI), pepsinogen II (PGII), Gastrin 17 (G17) and anti- *H. pylori* antibodies is a noninvasive test for adenocarcinoma risk assessment in chronic *H. pylori* gastritis patients. **Objective** – Prospective study to evaluate the concordance between OLGA and OLGIM grading systems, as well as to evaluate GastroPanel´s performance in patients with premalignant lesions secondary to *H. pylori* chronic gastritis in Brazil. **Methods** – Patients with *H. pylori* chronic gastritis with premalignant lesions confirmed by histology were recruited from the gastrointestinal clinic of a University Hospital. All participants underwent endoscopic examination with biopsies which were reported according to updated Sydney system and premalignant lesions grading systems (OLGA and OLGIM). Blood samples were collected for biomarkers serological analysis (GastroPanel®, Biohit, Helsinki, Finland). The cut off values used to define high risk patients were those recommended by the manufacturer: PGI ≤30 µm/L and PGI/PGII ≤3. **Results** – 41 patients were recruited: 28 women, 13 men, mean age 67.3 (47-89, SD: 9.6) years. By OLGA system, were obtained: OLGA 0 (n=1), OLGA I (n=7), OLGA II (n=17), OLGA III (n=9), and OLGA IV (n=7). By OLGIM system, were obtained: OLGIM 0 (n=14), OLGIM I (n=5), OLGIM II (n=10), OLGIM III (n=10), and OLGIM IV (n=2). Regarding histological staging among patients staged as low risk (OLGA/OLGIM 0, I and II) and high risk (OLGA/OLGIM III and IV) for gastric cancer development, the concordance rate found between both classifications was 85.4%. Considering high risk patients, those patients thus included in at least one of the systems the final distribution of our sample considered 24 low-risk and 17 high-risk patients for the development of gastric cancer. To determine by GastroPanel® whether the patient would be at low or high risk of developing gastric cancer, PGI showed a sensitivity, specificity and accuracy of 0.47 (95%CI: 0.26–0.69), 0.67 (95%CI: 0.47–0.82), and 0.58 (95%CI: 0.43–0.72), respectively, while PGI/PGII showed sensitivity, specificity and accuracy of 0.06 (95%CI: 0.01–0.27), 0.83 (95%CI: 0.64–0.93) and 0.51 (95%CI: 0.36–0.66), respectively. **Conclusion** – The histological classifications OLGA and OLGIM presented a substantial concordance rate among themselves. Simultaneous use of both histological classification systems increased the identification's rate of high-risk patients. Biomarker analysis was not effective to distinguish low to high risk patients in the studied population. Further studies are needed to validate its use in clinical practice in Brazil.

HEADINGS – Atrophic gastritis, diagnosis. *Helicobacter pylori*. Severity of illness index. Biomarkers. Algorithms.

INTRODUCTION

Helicobacter pylori infection is already recognized as the main etiological factor of chronic gastritis, with an evolutionary potential for the development of peptic ulcer and gastric neoplasms (adenocarcinoma and MALT lymphoma)[1,2]. Although its presence evokes a local and systemic immune response, *H. pylori* infection, once acquired, persists indefinitely until it is properly treated. The accurate diagnosis of gastritis associated with *H. pylori* is confirmed by histopathological examination[3].

The sequence *H. pylori* infection → chronic gastritis → glandular atrophy → intestinal metaplasia is a set of associated changes that are very frequently observed. The risk of gastric adenocarcinoma is four to five times higher in patients with severe body atrophy compared to healthy patients. Among patients with severe atrophy of the antrum, there is an 18 times greater risk for development of gastric cancer, reaching 90 times in those with severe atrophy of body and antrum (pangastritis) when compared to healthy people[4-9].

Populational studies have been carried out to quantify the risk of this neoplasia in patients with premalignant gastric lesions in western world. In 2008, a cohort conducted in the Netherlands with 92,250 people with premalignant lesions estimated the following risks for developing gastric cancer, within a period of ten years after the initial diagnosis: 0.8% for people with atrophic gastritis; 1.8% for

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patients with intestinal metaplasia; 3.9% for patients with mild to moderate dysplasia and 32.7% for those with high-grade dysplasia[10]. In 2015, a cohort conducted in Sweden analyzed 405,172 individuals who underwent gastric biopsy for non-malignant indications from 1979 to 2011. The findings obtained allowed to predict that 1/256 patients with normal gastric mucosa, 1/85 with chronic gastritis, 1/50 with atrophic gastritis, 1/39 with intestinal metaplasia and 1/19 with dysplasia will develop gastric cancer within 20 years after identification of these lesions[11]. Such findings suggest that endoscopic follow-up implementation in patients with marked premalignant lesions could reduce mortality from gastric cancer if cost-benefit analysis proves favorable for a given population.

In order to provide prognostic/therapeutic useful information in premalignant gastric lesions patients management, histological systems have been developed for staging gastritis in this situation. In 2007, the Operative Link for Gastritis Assessment (OLGA) system was developed based on presence, extension and topography (antrum and/or gastric body) of atrophic changes[12]. Patients classified as stage III or IV are considered to be at high risk. The Operative link for Gastric Intestinal Metaplasia (OLGIM) Assessment system is also based on the same concept, but only considers the presence, extension and topography of lesions related to intestinal metaplasia[13]. The prognostic value of both systems has been documented in different studies, with different samples and populations, and its adoption is recommended by different consensus and guidelines meetings in different regions of the world[14-18].

To avoid excessive performance of invasive and costly procedures such as upper digestive endoscopy, there is a need for searching new non-invasive diagnostic methods, capable of detecting patients at risk of developing gastric cancer, such as patients with different phenotypes chronic gastritis, especially those associated with *H. pylori* infection. This is particularly relevant in countries with a high prevalence and incidence of *H. pylori* infection and gastric cancer in the population.

Pepsinogens, pro-enzymes of pepsin, are classified according to their biochemical and immunological properties into two types: pepsinogen I (PGI) and pepsinogen II (PGII). Both are produced by the gastric mucosa but in different locations. While PGI is produced exclusively by the chief cells and mucous cells of the gastric body, PGII is produced by these cells and also by mucous cells in the cardiac region, pyloric glands, and Brunner glands in the duodenal mucosa. Both pro-enzymes are excreted mainly into the gastric lumen, but a minimal portion (around 1%) diffuses into the bloodstream and can be measured[19,20]. PGI and PGII are increased in patients with *H. pylori* chronic gastritis. However, as atrophy of the body's mucosa occurs, due to the reduction of oxyntic glands, there is a more significant reduction in PGI than in PGII in mild atrophy phase, since this is also produced in other regions of the stomach. Sometimes the mucosa's inflammation associated with *H. pylori* infection is so severe that, even in the presence of atrophy, the levels of PGI and PGII may be elevated[21]. To overcome this limitation, the PGI/PGII (RPG) ratio is used, and today is considered the best serological marker for gastric atrophy and already used as a tool for gastric cancer risk screening in Japan and, incipiently, in other countries[22-27]. To improve the accuracy of non-invasive diagnosis of gastric atrophy, the addition of other biomarkers to pepsinogens has been suggested. By associating anti-*H. pylori* antibodies to pepsinogens dosage (ABCD method), Asian researchers have shown that the method has the potential to stratify healthy adults from those at increased risk of developing gastric cancer[28]. Another association of biomarkers recently described (GastroPanel®, Biohit, Helsinki, Finland), involves, in addition to the determination of pepsinogens and anti-*H. pylori* antibodies, the determination of gastrin-17 (G17), all of them through the collection of a single blood sample. Two recent meta-analyses have evaluated the performance of GastroPanel®: Syrjänen K[29] analyzed the results of 8,654 patients from different countries, having found sensitivity of 70.2% and specificity of 93.9% in the diagnosis of body´s atrophic gastritis and sensitivity of 53.8% and specificity of 84.1% in the diagnosis of antrum atrophic gastritis. Zagari RM, et al.[30] analyzed 20 studies involving 4,241 participants, finding similar sensitivity (70.4%) for the diagnosis of body´s atrophic gastritis and sensitivity of 65.4% for the diagnosis of antrum atrophic gastritis.

There are still few studies that relate levels of pepsinogens to the diagnosis of gastric atrophy in Latin America countries[31,32]. The aim of this study was to perform a prospective study to evaluate the concordance between OLGA and OLGIM systems, as well as GastroPanel® performance in patients with premalignant lesions secondary to *H. pylori* chronic gastritis in Brazil.

METHODS

A consecutive series of adult patients of both sexes, with *H. pylori* chronic gastritis with associated premalignant conditions on histology, was recruited from November/2016 to April/2019 at the upper gastrointestinal outpatient clinic of University Hospital. Patients were excluded if they had coagulation disorders that should avoid gastric biopsies or if they had acute illnesses and indication for surgery or urgent treatment to control their symptoms. Were also excluded patients who used antibiotics on the 30 days preceding upper digestive endoscopy, proton pump inhibitor (PPI), and histamine H2 receptor antagonist on the 10 days preceding upper digestive endoscopy, and patients with chronic atrophic gastritis from proven autoimmune etiology.

All patients included in the study answered a pre-established clinical questionnaire and, after agreeing to participate in the study and signing the informed consent form, were submitted to blood collection for the serological panel and upper digestive endoscopy.

Serological panel (GastroPanel®)

Immediately before endoscopic examination, a cubital vein blood sample (4 mL) was collected, and after centrifugation, the plasma was immediately stored into a -80ºC freezer. All samples were processed for ELISA readings using Sprinter XL equipment (Euroimmun, Germany). Each marker was analyzed on an individual microplate, and the device's software was programmed according to the manufacturer's instructions, who provided the following reference values: pepsinogen I: 30–160 µm/L; pepsinogen II: 3–15 µm/L; gastrin 17: 1–7 pmol/L; *Helicobacter pylori* IgG ELISA: <30 EUI = negative. For the classification of patients into low and high risks for the development of gastric cancer, the following values were considered: PGI ≤30: high-risk patient; PGI >30: low-risk patient; PGI/PG II ≤3: high-risk patient; PGI/PGII> 3: low-risk patient.

Upper digestive endoscopy with gastric biopsies

During endoscopy, six gastric biopsies were collected (two biopsies from gastric antrum, one biopsy from angular notch and three from gastric body). All biopsies were fixed in 10%

formaldehyde and included in paraffin blocks separately and reported according to the Updated Sydney System for Gastritis Classification[33]. The histological study of the gastric mucosa was performed in histological sections of 4 µm thick by a single gastrointestinal pathologist. The histological stains used were hematoxylin-eosin (HE) for histopathological analysis and Giemsa for *H. pylori* infection presence. All patients had their histological findings staged according to the OLGA[34] and OLGIM[13] systems.

Statistics

Descriptive statistics techniques were used. Continuous variables were compared using Student's *t*-test, Mann-Whitney test (non-parametric data), and the chi-square test was used to quantitatively assess the relationship between results obtained. Considering the histological results of the OLGA-OLGIM set as the gold standard, sensitivity, specificity, positive predictive value, negative predictive value, accuracy and 95% confidence interval (CI) were calculated for the results obtained through serological biomarkers. Kappa values and 95%CI were determined to concordance analysys between OLGA and OLGIM systems. The Confidence Interval Analysis (CIA) program was used to calculate the confidence intervals using Wilson´s method. Statistical significance was recognized for values of $P<0.05$. All statistical analyzes were performed using the MINITAB statistical package (Minitab Inc., USA) version 16, Excel (Office 10). The study was approved by the *Universidade Federal de Minas Gerais* Research Ethics Committee. For sampling, the number of subjects required as low risk to develop gastric cancer (OLGA and OLGIM I or II) and high risk (OLGA or OLGIM III or IV) was calculated based on studies showing expected values for GastroPanel® reported in previous studies[29]. It was estimated with 80% power, 172 and 61 patients from low risk and high risk, respectively. As the amount of patients needed would take too long to be recruited, it was adopted a convenience, not probabilistic sample composed by the number of patients suitable for this preliminar study included during the two years and six months study period.

RESULTS

Initially, 46 patients were recruited and five patients were excluded: one by using oral anticoagulant, two due to thrombocytopenia, one due to erroneous inclusion (proven autoimmune gastritis), and one patient for refusing to participate in the study. Thus, a total of 41 patients participated in the study, and their demographic characteristics can be seen in TABLE 1.

Histological findings

TABLE 2 shows the observed distribution of all patients according to the histological staging of gastritis OLGA and OLGIM. FIGURE 1 shows concordance's rate between both histological systems regarding the staging of patients as low and high risk of the development gastric cancer. It can be seen that, among the 41 patients in the study, 24 were classified as low risk by both systems, 11 were classified as high risk by both systems and six patients presented discordant classifications, thus conferring 0.678 (95%CI: 0.440–0.916) kappa value and 85.4% concordance rate. Considering high-risk patients those patients thus included in at least one the histological staging systems, the final distribution of our sample considered 24 patients at low risk and 17 as high risk for the development of gastric cancer. Based on this distribution,

TABLE 1. Demographic characteristics of the 41 patients in the study.

Sex: male/female	13/28
Mean age (years): (SD)	67.3 (9.6)
Mean BMI (kg/m^2): (SD)	26.8 (5.1)
Alcoholism: n (%)	6 (15)
Smoking: n (%)	8 (20)
Education: n (%)	
Elementary School	19 (46.3)
High School	19 (46.3)
Higher Education	3 (7.4)
Comorbidities: n (%)	
None	1 (2.4)
Hypertension	29 (70.7)
Diabetes mellitus	13 (31.7)
NSAIDs: n (%)	3 (7.3)
Dyspepsia: n (%)	22 (53.7)
Family history of gastric cancer: n (%)	5 (12.2)

SD: standard deviation; BMI: body mass index; NSAIDs: nonsteroidal anti-inflammatory drugs.

TABLE 2. Distribution of patients according to OLGA and OLGIM histological grading systems (n=41).

	OLGA 0	OLGA I	OLGA II	OLGA III	OLGA IV
	1	7	17	9	7
Number of patients	**OLGIM 0**	**OLGIM I**	**OLGIM II**	**OLGIM III**	**OLGIM IV**
	14	5	10	10	2

OLGA: Operative Link for Gastritis Assessment; OLGIM: Operative link for Gastric Intestinal Metaplasia.

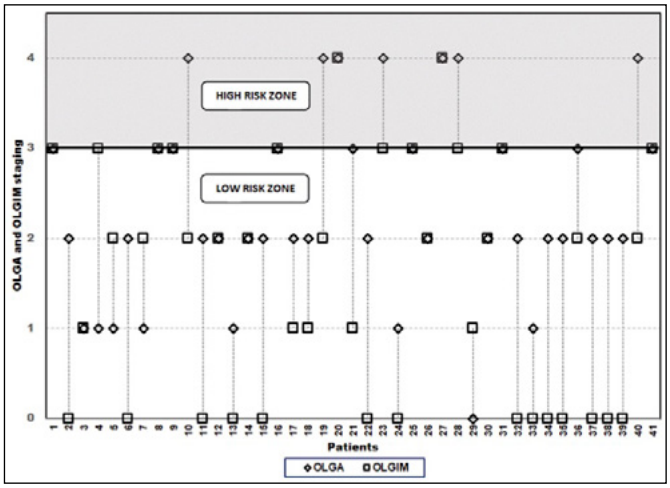

FIGURE 1. OLGA and OLGIM staging systems in 41 patients of the study.

TABLE 3 shows that the two groups do not present statistically significant differences on demographic variables, except for gender variable, with the female gender being predominant in high-risk group (P=0.02).

TABLE 3. Comparison of demographic characteristics in the 41 patients classified by the OLGA and OLGIM gastritis grading systems, as low and high risk for developing gastric cancer.

Variables	OLGA or OLGIM Low risk (n=24)	OLGA and/ or OLGIM High risk (n=17)	*P*-value
Mean age (years): SD	65.7 (10.1)	69.6 (8.6)	0.36*
Mean BMI (kg/m^2): SD	26.7 (4.4)	26.8 (6.1)	0.75*
Sex: male/female	11/13	2/15	0.02**
Education: ES/H	12/12	7/10	0.58**
Smoking: no/yes	19/5	14/3	0.80**
Alcoholism: no/yes	21/3	14/3	0.65**
Family history of gastric cancer: no/yes	22/2	14/3	0.37**
NSAIDs: no/yes	22/2	16/1	0.80**
Previous *H. pylori* treatment: no/yes	2/22	4/13	0.17**
H. pylori at histology: no/yes	18/6	16/1	0.11**
Dyspepsia: no/yes	12/12	7/10	0.58**

SD: standard deviation; OLGA: Operative Link for Gastritis Assessment; OLGIM: Operative link for Gastric Intestinal Metaplasia; ES: Elementary School; H: High School or Higher Education. *Mann Whitney test; **chi-square test.

Serological biomarkers

TABLE 4 shows PGI, PGI/PGII, and Gastrin-17 values obtained in patients with low and high risks for the development of gastric cancer by OLGA system, OLGIM system, and by OLGA-OLGIM set. No statistically significant differences were found in any of comparative analyses performed.

TABLE 5 shows the accuracy determinations of PGI biomarker and PGI / PGII ratio to determine whether the patient would be at low or high risk for developing gastric cancer. The PGI showed a sensitivity of 0.50, 0.42, and 0.47 for the OLGA, OLGIM and OLGA-OLGIM set, respectively. The observed specificity was 0.68, 0.62, and 0.67 for the OLGA, OLGIM and OLGA-OLGIM set, respectively and accuracy of 0.61, 0.56, and 0.58 for the OLGA, OLGIM, and OLGA-OLGIM set, respectively. The PGI / PGII showed sensitivity of 0.06, 0.00 and 0.06 for the OLGA, OLGIM, and OLGA-OLGIM set, respectively. The observed specificity was 0.84, 0.83, and 0.83 for the OLGA, OLGIM, and OLGA-OLGIM set, respectively, and accuracy of 0.49, 0.58 and 0.51 for the OLGA, OLGIM, and OLGA-OLGIM set, respectively.

Helicobacter pylori

FIGURE 2 shows previous *H. pylori* infection treatment information and histology and serology results. Only one patient had no informed previous infection's treatment and tested negative both by histology and serology.

TABLE 4. Comparative analysis of PGI levels, PGI / PGII, and Gastrin-17 observed in patients classified according to the risk of developing gastric cancer by the OLGA, OLGIM and by the OLGA and OLGIM set.

Variable	OLGA Low risk (n=25)	OLGA High risk (n=16)	*P*-value*
PG I (µg/L): mean (SD)	64.2 (67.6)	57.8 (64.9)	0.640
PGI/PGII: mean (SD)	17.9 (13.6)	17.8 (7.2)	0.659
Gastrin-17 (pmol/L) mean (SD)	12.4 (11.8)	29.5 (28.9)	0.317
Variable	**OLGIM Low risk (n=29)**	**OLGIM High risk (n=12)**	***P*-value**
PG I (µg/L): mean (SD)	64.2 (73.0)	50.1 (44.3)	0.808
PGI/PGII: mean (SD)	17.4 (13.4)	18.8 (4.1)	0.398
Gastrin-17 (pmol/L) mean (SD)	14.7 (18.4)	9.4 (19.3)	0.086
Variable	**OLGA and OLGIM Low risk (n=24)**	**OLGA and/or OLGIM High risk (n=17)**	***P*-value**
PG I (µg/L): mean (SD)	65.4 (68.8)	56.4 (63.1)	0.624
PGI/PGII: mean (SD)	17.9 (13.9)	17.8 (6.9)	0.701
Gastrin-17 (pmol/L) mean (SD)	9.3 (10.6)	18.6 (25.5)	0.317

OLGA: Operative Link for Gastritis Assessment; OLGIM: Operative link for Gastric Intestinal Metaplasia; SD: standard deviation. *Mann Whitney test.

TABLE 5. Accuracy measures of the PGI and PGI/PGII considering the OLGA, OLGIM gastritis grading systems and by the OLGA and OLGIM set.

	Accuracy measures	Gastritis grading systems		
		OLGA	OLGIM	OLGA and OLGIM
PGI	Sensitivity (95% CI)	0.50 (0.28–0.72)	0.42 (0.19–0.68)	0.47 (0.26–0.69)
	Specificity (95% CI)	0.68 (0.48–0.83)	0.62 (0.44–0.77)	0.67 (0.47–0.82)
	PPV (95% CI)	0.50 (0.28–0.72)	0.31 (0.14–0.56)	0.50 (0.28–0.72)
	NPV (95% CI)	0.68 (0.48–0.83)	0.72 (0.52–0.86)	0.64 (0.44–0.80)
	Accuracy (95% CI)	0.61 (0.46–0.74)	0.56 (0.41–0.70)	0.58 (0.43–0.72)
PGI/ PGII	Sensitivity (95% CI)	0.06 (0.01–0.28)	0.00 (0.00–0.24)	0.06 (0.01–0.27)
	Specificity (95% CI)	0.84 (0.65–0.94)	0.83 (0.65–0.92)	0.83 (0.64–0.93)
	PPV (95% CI)	0.20 (0.04–0.62)	0.00 (0.00–0.43)	0.20 (0.04–0.62)
	NPV (95% CI)	0.58 (0.42–0.73)	0.68 (0.50–0.80)	0.56 (0.40–0.70)
	Accuracy (95% CI)	0.49 (0.34–0.63)	0.58 (0.43–0.72)	0.51 (0.36–0.66)

OLGA: Operative Link for Gastritis Assessment; OLGIM: Operative link for Gastric Intestinal Metaplasia; PGI: pepsinogen I; PGII: pepsinogen II; PPV: positive predictive value; NPV: negative predictive value.

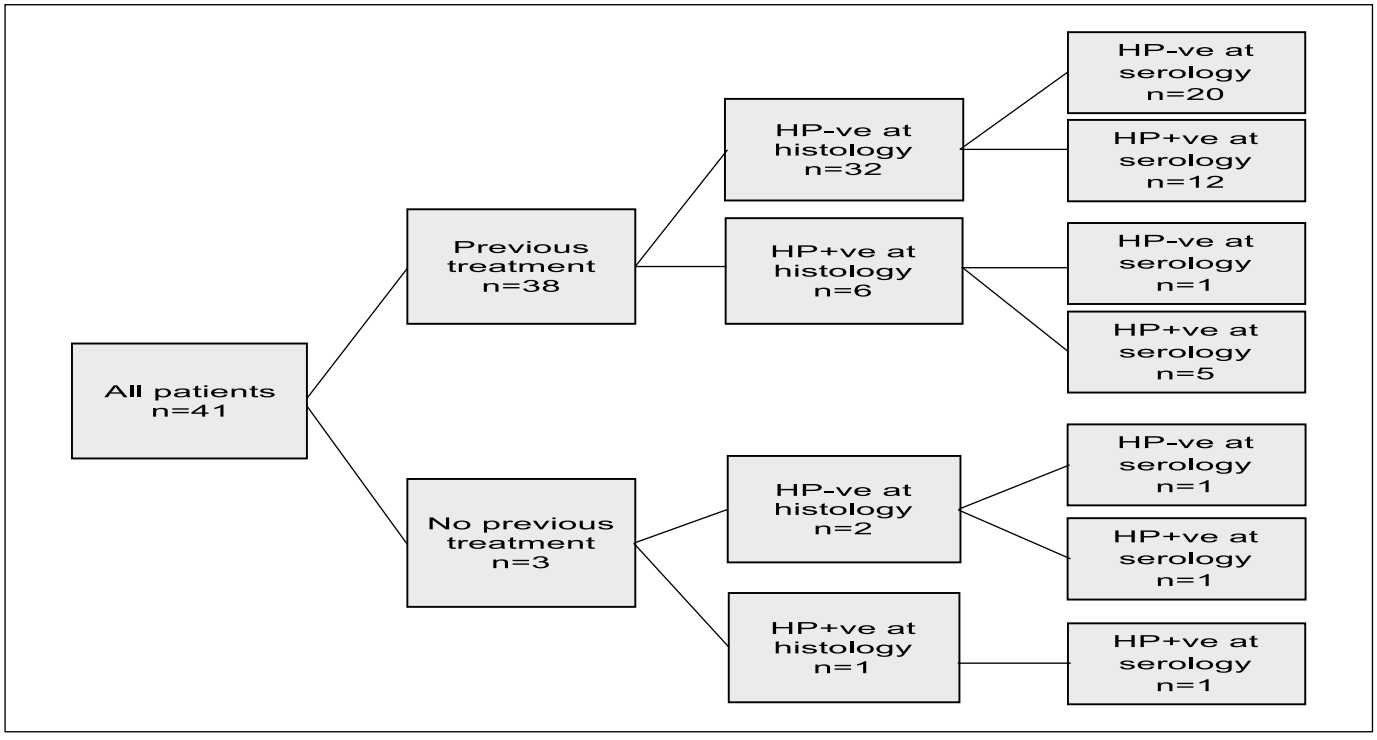

FIGURE 2. Findings regarding *H. pylori* infection (n=41) on previous treatment of the infection, the search for bacteria during histological examination and the serological findings of the presence of anti-*H. pylori* antibodies.

DISCUSSION

Our study's findings, obtained from 41 patients with *H. pylori* chronic gastritis and premalignant lesions, showed that 41.4% were high risk histologically classified. Although a recent systematic review about the prevalence of advanced gastric premalignant lesions in countries with low/moderate gastric cancer incidence (like Brazil), estimate 7.3% incidence (95%CI: 5.6–9.05) for atrophic gastritis and 7.7% (95%CI: 3.2–12.1) for intestinal metaplasia[35], these findings can be justified by origin bias, since our patients were recruited from a tertiary service, specialized on assisting patients with chronic esophageal-gastric-duodenal diseases. Besides the high mean age of patients, 67.3 (SD: 9.6) years, is another contributory factor, considering that premalignant lesions prevalence is three times higher in individuals over 40 when compared with those younger than 40 years old[35].

The analysis of OLGA and/ or OLGIM systems performance as a protocol to obtain gastric biopsies capable of increasing the premalignant lesion´s detection yield has shown promising results. In 2018, Yue H, et al.[36], carried out a systematic review and meta-analysis assessing the association between OLGA and OLGIM systems and the risk of gastric cancer, as well as the strength of this association. 2,700 patients included in six case-control studies (OLGA system used in all and OLGIM system in three) and two cohorts (one including OLGA system and another OLGIM system) were analyzed. Regarding the OLGA system, the analysis of cohort studies revealed that individuals staged as high risk had a 27.7 times greater risk of developing gastric cancer compared to their counterparts. The analysis of the only cohort analyzing the OLGIM system showed that patients classified as high-risk had RR 16.67 (95%CI: 0.80–327.53) for the development of gastric cancer or gastric dysplasia. The authors conclude that close and frequent monitoring of patients classified as high risk in OLGA or OLGIM systems is necessary to facilitate early diagnosis of gastric cancer.

Two recent cohorts have also analyzed the role of OLGA and OLGIM systems in assessing the concordance between intensity of pre-neoplastic lesions and gastric cancer development risk. Rugge M, et al.[37], in 2018, in Italy, followed 7,436 patients who underwent upper digestive endoscopy due to dyspeptic complaints. Histological evaluation was performed according to the OLGA system (OLGA 0: 80%, OLGA I: 12.6%, OLGA II: 4.3%, OLGA III: 2.0% and OLGA IV: 0.3%) and patients were followed for a median period of 6.6 years. 28 out of 7,436 patients included in the study developed neoplastic lesions: 17 patients with low-grade dysplasia, four with high-grade dysplasia, and seven with gastric cancer. According to OLGA staging at the time of study inclusion, 1/28 cases of gastric neoplasia were classified as OLGA 0, 2/28 patients as OLGA I, 3/28 patients as OLGA II, 17/28 patients as OLGA III and 5 / 28 as OLGA IV. Multivariate analysis of the study including gender, age, *H. pylori* presence and OLGA system determined upon admission to the study revealed, among these variables, only OLGA system as a predictor of neoplastic progression: being OLGA III: HR: 712.4 (95%CI: 92.543–5484.5) and OLGA IV: HR: 1450.7 (95%CI: 166.7–12626.0). In 2019, Den Hollander WJ et al.[38], prospectively analyzed 279 Dutch and Norwegian patients included in the study due to histological evidence of atrophic gastritis, intestinal metaplasia and/or dysplasia of the gastric mucosa at gastroscopy and staged according to the OLGIM system. After an average follow-up period of 57 months,

4/279 (1.4%) patients developed high-grade adenoma/dysplasia or gastric cancer. The progression to neoplasia occurred in one patient in low-risk group according to OLGIM system and in two patients in high-risk group (P=0.11). The authors conclude that, even in regions with a low incidence of gastric cancer, follow-up programs are able to detect gastric cancer in potentially curable stages, with a risk of neoplastic progression of 0.3% per year.

In our study, the two histological staging systems, OLGA and OLGIM, were concordant in 85.4% (kappa value: 0.678) and disagreement in 14.6% (n=6). The simultaneous use of both histological classification systems allowed 17 patients to be identified as high risk, 11 through identification by both systems, with five additional patients classified as high risk only by the OLGA system and one additional patient classified as high risk only by the OLGIM system. These findings coincide with those observed in a recent study and meta-analysis[36,37] suggesting that, although the OLGIM system provides easier identification of intestinal metaplasia and better interobserver reproducibility, it fails to identify an appreciable number of high-risk patients. Therefore, it is suggested that, for an accurate prediction of gastric cancer risk, both systems should be used simultaneously in daily pathological practice.

Studies have been performed to evaluate the concordance between OLGA and OLGIM systems in the staging of premalignant gastric lesions and their progression to gastric cancer. Both were developed from the Sydney System for classification and grading of gastritis[33], which is dependent on the histopathological findings from endoscopic biopsies. One limitation attributed to the OLGA system is related to the fact that its main parameter is the intensity and extent of atrophic gastritis, with studies by North American[39] and European[40] pathologists showing that interobserver agreement is low, even when using a visual analog scale. On the other hand, the OLGIM system, when proposing the use of intestinal metaplasia, the next step of the Pelayo Correa cascade for the development of gastric cancer[4], offers a more easily identifiable marker in the gastric mucosa and, consequently, with greater interobserver agreement[33,41]. Isajevs S, et al.[42], in 2014, compared the interobserver agreement between general pathologists and gastrointestinal specialized pathologists in the staging of gastritis by OLGA and OLGIM systems in 835 patients. The OLGIM system provided the highest interobserver agreement, however it was observed that a substantial proportion of high-risk individuals would not be detected if only the OLGIM system was adopted. In 2018, Mera RM, et al.[43], in a follow-up study for up to 16 years of 795 patients with pre-neoplastic gastric lesions, demonstrated that the probability of progression to gastric cancer among patients classified as high and low risk by the OLGIM system was twice as high as that observed in patients classified as high and low risk by the OLGA system.

In our study, the analysis of the demographic variables of patients classified in OLGA and OLGIM systems together as high and low risks showed statistical significance only for the gender variable, with the female gender being predominant in the high-risk population (P=0.02). The small sample of this study turns difficult to make greater inferences, requiring studies with greater sample power.

Our findings related to *H. pylori* infection showed that 38/41 (92.7%) patients underwent eradication treatment, with 6/38 (15.7%) still harboring the bacteria. The findings of 84.2% cure rate are consistent with *H. pylori* eradication rates obtained from the Brazilian and Latin American populations, in different studies[44]. A single patient in the study who denied previous anti-*H. pylori* treatment and whose histological and serological studies were negative showed a histological pattern of atrophic gastritis classified as OLGA IV. It is interesting to note that this situation has been described by ABCD Japanese serological classification, as being in group D, with a high risk for gastric cancer in which atrophy is so intense that it would turn gastric mucosa uninhabitable for *H. pylori*.[45].

For the prediction of premalignant changes identified by histology the analysis of PGI biomarkers and PGI/PGII ratio, based on OLGA-OLGIM set, showed 47% sensitivity for PGI (95%CI: 26–69) and 67% specificity (95%CI: 47–82), and for PGI/PGII ratio, the sensitivity was 6% (95%CI: 1-27) and 83% specificity (95%CI: 64–93). Regarding G-17 determination, based on OLGA-LGIM set, the mean values obtained in patients classified as low and high risk had no statistically significant difference from each other opposing a previous meta-analysis, with 13 studies, which described a 82% accuracy of G17 in identifying patients with chronic atrophic gastritis[46]. The values obtained here, unsatisfactory as discriminating patients with low and high risk of developing gastric cancer have also been observed in other studies. A prospective, multicenter, Spanish study analyzed 91 dyspeptic patients with the same serological panel used here. The values of PGI and PGI/PGII ratio did not show statistically significant differences when compared to histology, with 50% sensitivity (95%CI: 39–61) and 80% specificity (95%CI: 71–88)[47]. In Peru, Calarossi A, et al.[48] found sensitivity of 54% and specificity of 68% of serological panel to identify chronic atrophic gastritis. Recent meta-analysis analyzed the performance of the GastroPanel® in 20 studies including 4,241 patients and observed sensitivity of 74.7% (95%CI: 62–84.3) and specificity of 95.6% (95%CI: 92.6–97.4) of the serological panel to identify patients with chronic atrophic gastritis[30]. Another systematic review and meta-analysis study for the accuracy analysis of serological panel, including 27 studies, identified a sensitivity of 53.8% and specificity of 84.1% of the method to identify chronic atrophic gastritis[29].

Our results showed that GastroPanel® was not effective in identifying patients with pre-neoplastic lesions, and one of the factors that may have interfered on results is the mean age (67.3 years) of our studied population. In a review article Miki K[25], in 2006, considers sex, age, *H. pylori* infection, smoking and alcohol consumption as factors capable of influencing serum levels of pepsinogens and suggests that lower levels of pepsinogen tests found in the elderly population can be attributed to atrophic changes in the gastric mucosa and not exactly to the age itself. A recent Japanese guideline on the management of *Helicobacter pylori* infection also highlights the possibility that patients with gastric atrophy, mainly over 65 years of age, with an ongoing or past infection, often yield false-negative results on pepsinogen tests[49]. In this context, we assessed the presence of an association between advanced age (≥65 years) and higher levels of PGI and failed to find any statistically significant difference between the compared groups.

The present study has limitations that deserve consideration. Initially, although the study covered all patients with *H. pylori* chronic gastritis and associated pre-malignant lesions seen in a specialized outpatient clinic at university hospital for the period of the study, the small size hinders extrapolation of data obtained for the Brazilian population. The histopathological examination

performed by a single professional, although specialized in gastrointestinal pathology, became unable the determination of inter-observer concordance rates in the staging of OLGA and OLGIM systems. However, this preliminary study allows an approximate assessment of what is observed in real life. The use of endoscopic examinations with white light devices used on all our patients mimics the reality of endoscopic practice, not only in Brazil but also in countless countries around the world. However, it is undisputed to recognize the progressively increasing role of technological advances in endoscopic examinations as optical filters use (NBI®, narrow-band imaging) for image magnification of pre-malignant gastric lesions, with its use already recommended in recent guidelines in the area[49-51]. The serological evaluation of gastric atrophy presence through the use of biomarkers, in this study, restricted to a higher age group also constitute limitations of the present study and require future investigations.

In conclusion, the description of histological findings through OLGA and OLGIM staging systems in patients with *H. pylori* chronic gastritis associated with premalignant lesions showed substantial concordance and should be incorporated into daily practice. The simultaneous description of the two systems, OLGA and OLGIM, is more accurate than when described in isolation. The biomarkers PGI, PGI / PGII ratio, and G17, analyzed separately or together, showed low accuracy for diagnosis of premalignant lesions in the studied population. Further studies are needed to validate its use in clinical practice in Brazil.

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Authors' contribution

Coelho MCF, Coelho LGV and Barbosa AJA contributed to the conception and design of the study; Ribeiro HG, Gomes CGO, and Marinho FP contributed to recruit the patients and collection of the samples; Barbosa AJA and Coelho MCF analysed the biopsies; Coelho MCF and Coelho LGV analysed the data and wrote the paper.

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RESUMO – **Contexto** – Gastrite atrófica crônica por *H. pylori* constitui lesão pré-maligna e seu estadiamento de acordo com os sistemas OLGA e OLGIM, visa identificar pacientes com maior risco de desenvolver câncer gástrico e otimizar seu acompanhamento. GastroPanel® é um teste não invasivo composto por painel de biomarcadores séricos incluindo pepsinogênio I (PGI), pepsinogênio II (PGII), gastrina 17 (G17) e anticorpos anti- *H. pylori* para avaliação de risco de adenocarcinoma gástrico em pacientes com gastrite crônica por *H. pylori*. **Objetivo** – Estudo prospectivo para avaliar a concordância entre os sistemas de classificação OLGA e OLGIM, bem como avaliar o desempenho do GastroPanel® em pacientes com lesões pré-malignas secundárias à gastrite crônica por *H. pylori* no Brasil. **Métodos** – Pacientes com gastrite crônica por *H. pylori* portadores de lesões pré-malignas confirmadas por histologia (gastrite atrófica e metaplasia intestinal) foram recrutados no ambulatório de gastroenterologia de um hospital universitário. Todos os participantes foram submetidos a exame endoscópico com biópsias de antro e corpo gástricos analisadas de acordo com o Sistema Sydney atualizado e estadiadas pelos Sistemas OLGA e OLGIM de classificação das gastrites. Amostras de sangue foram coletadas para análise sorológica de biomarcadores (GastroPanel®, Biohit, Helsinki, Finlândia). Os valores de corte utilizados para definir pacientes de alto risco para desenvolvimento de câncer gástrico foram os recomendados pelo fabricante: PGI ≤30 μm e PGI/PGII ≤3. **Resultados** – Foram recrutados 41 pacientes: 28 mulheres, 13 homens, idade média 67,3 (47–89, DP: 9,6) anos. Pelo sistema OLGA, foram obtidos: OLGA 0 (n=1), OLGA I (n=7), OLGA II (n=17), OLGA III (n=9) e OLGA IV (n=7). Pelo sistema OLGIM, foram obtidos: OLGIM 0 (n=14), OLGIM I (n=5), OLGIM II (n=10), OLGIM III (n=10) e OLGIM IV (n=2). Em relação ao estadiamento histológico entre os pacientes de baixo risco (OLGA/OLGIM 0, I e II) e alto risco (OLGA/OLGIM III e IV) para o desenvolvimento de câncer gástrico, a taxa de concordância encontrada entre as duas classificações foi de 85,4%, com valor kappa=0,678 (IC95%: 0,440–0,916). Considerando como pacientes de alto risco, aqueles assim estadiados em pelo menos um dos sistemas, a distribuição final de nossa amostra encontrou 24 pacientes de baixo risco e 17 de alto risco para o desenvolvimento de câncer gástrico. Na determinação pelo GastroPanel® para classificação do paciente como de baixo ou alto risco para desenvolvimento de câncer gástrico, PGI mostrou sensibilidade, especificidade e acurácia de 0,47 (IC95%: 0,26–0,69), 0,67 (IC95%: 0,47–0,82) e 0,58 (IC95%: 0,43–0,72), respectivamente, enquanto a razão PGI/PGII mostrou sensibilidade, especificidade e acurácia de 0,06 (IC95%: 0,01–0,27), 0,83 (IC95%: 0,64–0,93) e 0,51 (IC95%: 0,36–0,66), respectivamente. **Conclusão** – As classificações histológicas OLGA e OLGIM apresentaram taxa de concordância substancial entre si. O uso simultâneo de ambos os sistemas de classificação histológica aumentou a taxa de identificação de pacientes de alto risco para desenvolvimento de câncer gástrico. Os resultados do GastroPanel® não foram eficazes para distinguir pacientes de baixo e alto risco para desenvolvimento de câncer gástrico na população estudada. Mais estudos são necessários para validar seu uso na prática clínica no Brasil.

DESCRITORES – Gastrite atrófica, diagnóstico. *Helicobacter pylori*. Índice de gravidade de doença. Biomarcadores. Algoritmos.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1:1311-5.
2. No authors listed. Schistosomes, liver flukes and Helicobacter pylori. IARC. Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1-241.
3. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med. 2002;347:1175-86.
4. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process – First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res. 1992;52:6735-40.
5. Wyatt JI. Histopathology of gastroduodenal inflammation. The impact of Helicobacter pylori. Histopathology. 1995;26:1-15.
6. Genta RM, Rugge M. Assessing risks for gastric cancer: new tools for pathologists. World J Gastroenterol. 2006;12:5622-7.
7. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura K, et al. Progression of chronic atrophic gastritis associated with Helicobacter pylori infection increases risk of gastric cancer. Int J Cancer. 2004;109:138-43.
8. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA. 2004;291:187-94.
9. Sipponen P, Kekki M, Haapakoski J, Ihamäki T, Siurala M. Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. Int J Cancer. 1985;35:173-7.
10. de Vries AC, van Grieken NC, Looman CW, Casparie MK, de Vries E, Meijer GA, et al. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. Gastroenterology. 2008;134:945-52.
11. Song H, Ekheden IG, Zheng Z, Ericsson J, Nyrén O, Ye W. Incidence of gastric cancer among patients with gastric precancerous lesions: observational cohort study in a low risk Western population BMJ. 2015;351:h3867.
12. Rugge M, Meggio A, Pennelli G, Piscioli F, Giacomelli G, De Pretis G, et al. Gastritis staging in clinical practice: the OLGA staging system. Gut. 2007;56:631-6.
13. Capelle LG, de Vries AC, Haringsma J, Borg FT, de Vries RA, Bruno MJ, et al. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. Gastrointest Endosc. 2010;71:1150-8.
14. Satoh K, Osawa H, Yoshizawa M, Nakano H, Hirasawa T, Kihira K, et al. Assessment of atrophic gastritis using the OLGA system. Helicobacter. 2008;13:225-9.
15. Marcos-Pinto R, Carneiro F, Dinis-Ribeiro M, Wen X, Lopes C, Figueiredo C, et al. First-degree relatives of patients with early-onset gastric carcinoma show even at young ages a high prevalence of advanced OLGA/OLGIM stages and dysplasia. Aliment Pharmacol Ther. 2012;35:1451-9.
16. Cho SJ, Choi IJ, Kook MC, Nam BH, Kim CG, Lee JY, et al. Staging of intestinal- and diffuse-type gastric cancers with the OLGA and OLGIM staging systems. Aliment Pharmacol Ther. 2013;38:1292-302.
17. Coelho LG, Marinho JR, Genta R, Ribeiro LT, Passos MCF, Zaterka S, et al. IV Brazilian Consensus Conference on Helicobacter pylori infection. Arq Gastroenterol. 2018;55:97-121.
18. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66:6-30.
19. Samloff IM. Pepsinogens I and II: purification from gastric mucosa and radioimmunoassay in serum. Gastroenterology 1982;82:26-33.
20. di Mario F, Cavallaro LG. Non-invasive tests in gastric diseases. Dig Liv Dis. 2008;40:523-30.
21. Iijima K, Sekine H, Koike T, Imatani A, Ohara S, Shimosegawa T. Serum pepsinogen concentrations as a measure of gastric acid secretion in Helicobacter pylori- negative and positive- Japanese subjects. J Gastroenterol. 2005;40:938-44.
22. Graham DY, Nurgalieva ZZ, El-Zimaity HM, Opekun AR, Campos A, Guerrero L, et al. Noninvasive versus histologic detection of gastric atrophy in a Hispanic population in North America. Clin Gastroenterol Hepatol. 2006;4:306-14.
23. Bornschein J, Selgrad M, Wex T, Kuester D, Malfertheiner P. Serological assessment of gastric mucosal atrophy in gastric cancer. BMC Gastroenterol. 2012;12:10.
24. Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen. 2004;11:141-7.
25. Miki K. Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer. 2006;9:245-53.
26. Tong Y, Wu Y, Song Z, Yu Y, Yu X. The potential value of serum pepsinogen for the diagnosis of atrophic gastritis among the health check-up populations in China: a diagnostic clinical research. BMC Gastroenterology. 2017;17:88.
27. Leja M, Kupcinskas L, Funka K, Sudraba A, Jonaitis L, Ivanauskas A, et al. The validity of a biomarker method for indirect detection of gastric mucosal atrophy versus standard histopathology. Dig Dis Sci. 2009;54:2377-84.
28. Terasawa T, Nishida H, Kato K, Miyashiro I, Yoshikawa T, Takaku R, et al. Prediction of gastric cancer development by serum pepsinogen test and Helicobacter pylori seropositivity in Eastern Asians: a systematic review and meta-analysis. PloS One. 2014;9:e109783.
29. Syrjänen K. A panel of serum biomarkers (GastroPanel®) in non-invasive diagnosis of atrophic gastritis. Systematic review and meta-analysis. Anticancer Research. 2016;36:5133-44.
30. Zagari RM, Rabitti S, Greenwood DC, Eusebi LH, Vestito A, Bazzoli F. Systematic review with meta-analysis: diagnostic performance of the combination of pepsinogen, gastrin-17 and anti-Helicobacter pylori antibodies serum assays for the diagnosis of atrophic gastritis. Aliment Pharmacol Ther. 2017;46:657-67.
31. Fernández F JI, de Aretxabala UX, Santander DR, Sabah ST, Maira JS, Navarro AR, et al. Detección de lesiones preneoplásicas gástricas mediante niveles séricos de pepsinógeno en población chilena. Rev Méd Chile. 2007;135:1519-25.
32. Fahey MT, Hamada GS, Nishimoto IN, Kowalski LP, Iriya K, Gama-Rodrigues JJ, et al. Ethnic differences in serum pepsinogen levels among Japanese and non-Japanese Brazilian gastric cancer patients and controls. Cancer Detect Prev. 2000;24:564-71.
33. Dixon MF, Genta RM, Yardley JH. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161-81.
34. Rugge M, Correa P, Di Mario F, El-Omar E, Fiocca R, Geboes K, et al. OLGA staging of gastrites: a tutorial. Dig Liver Dis. 2008;40:650-8.
35. Marques-Silva L, Areia M, Elvas L, Dinis-Ribeiro M. Prevalence of gastric precancerous conditions: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2014,26:378-87.
36. Yue H, Shan L, Bin L. The significance of OLGA and OLGIM staging systems in the risk assessment of gastric cancer: a systematic review and meta-analysis. Gastric Cancer. 2018;21:579-87.
37. Rugge M, Genta RM, Fassan M, Valentini E, Coati I, Guzzinati S, et al. OLGA Gastritis Staging for the prediction of gastric cancer risk: a long-term follow-up study of 7436 patients. Am J Gastroenterol. 2018;113:1621-8.
38. den Hollander WJ, Holster IL, den Hoed CM, Capelle LG, Tang TJ, Anten MP, et al. Surveillance of premalignant gastric lesions: a multicentre prospective cohort study from low incidence regions.Gut. 2019;68:585-93.
39. el-Zimaity HM, Graham DY, al-Assi MT, Malaty H, Karttunen TJ, Grahan DP, et al. Interobserver variation in the histopathological assessment of Helicobacter pylori gastritis. Hum Pathol. 1996;27:35-41.
40. Offerhaus GJ, Price AB, Haot J, ten Kate FJ, Sipponen P, Fiocca R, et al. Observer agreement on the grading of gastric atrophy. Histopathology. 1999;34:320-5.
41. Guarner J, Herrera-Goepfert R, Mohar A, Sanchez L, Halperin D, Ley C, et al. Interobserver variability in application of the revised Sydney classification for gastritis. Hum Pathol. 1999;30:1431-4.
42. Isajevs S, Liepniece-Karele I, Janciauskas D, Moisejevs G, Putnins V, Funka K, et al. Gastritis staging: interobserver agreement by applying OLGA and OLGIM systems. Virchows Arch. 2014; 464:403-7.

43. Mera RM, Bravo LE, Camargo MC, Bravo JC, Delgado AG, Romero-Gallo J, et al. Dynamics of Helicobacter pylori infection as a determinant of progression of gastric precancerous lesions: 16-year follow-up of an eradication trial. Gut. 2018;67:1239-46.
44. Coelho LG, Coelho MC. Clinical management of Helicobacter pylori: the Latin American perspective. Dig Dis. 2014;32:302-9.
45. Watabe H, Mitsushima T, Yamaji Y, Okamoto M, Wada R, Kokubo T, et al. Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut. 2005;54:764-76.
46. Wang X, Ling L, Li S, Qin G, Cui W, Li X, et al. The Diagnostic Value of Gastrin-17 Detection in Atrophic Gastritis: A Meta-Analysis. Medicine. 2016;95:e3599.
47. McNicholl AG, Forné M, Barrio J, De la Coba C, González B, Rivera R, et al. Accuracy of GastroPanel for the diagnosis of atrophic gastritis. Eur J Gastroenterol Hepatol. 2014;26:941-8.
48. Colarossi A, Inga R, Prochazka R, Reyes U, Bussalleu A, Léon-Barúa R. Pepsinógeno y gastrina en el diagnóstico no invasivo de la atrofia gástrica: un estudio caso-control en población peruana. Rev Gastroenterol Peru. 2011;31:110-15.
49. Kato M, Ota H, Okud M, Kikuchi S, Satoh K, Shimoyama T, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. Helicobacter. 2019;24:e12597.
50. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. Endoscopy. 2019;51:365-88.
51. Banks M, Graham D, Jansen M, Gotoda T, Coda S, di Pietro M, et al. British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma. Gut. 2019;68:1545-75.

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Body iron status indicators and inflammation indicators during inflammatory bowel disease therapy in children and adolescents

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ABSTRACT – **Background** – The treatment of patients with inflammatory bowel disease (IBD) consists of the induction and maintenance remission of the disease. Iron status indicators would be useful for the diagnosis of iron deficiency anemia, whereas the inflammation indicators would be for the diagnosis of chronic disease anemia. **Objective** – To assess body iron status indicators and inflammation indicators during the treatment of IBD, consisted of conventional or infliximab therapy in children and adolescents. **Methods** – A case-control study of a sample of 116 individuals, of which 81 patients with IBD, 18 of them receiving conventional therapy, 20 infliximab therapy, and 43 who were in remission of the disease, and 35 healthy (control group) children and adolescents. Iron status and inflammation indicators were investigated at baseline, and 2 and 6 months of both therapies – conventional and infliximab. **Results** – The mean age was 12.1±4.3 years. At baseline, both groups – conventional therapy and infliximab – presented significant differences in most markers studied compared to the control group. After 2 months of conventional therapy, hemoglobin and serum iron levels were lower than those of the control group; and red cells distribution width (RDW), total iron-binding capacity, transferrin receptor/ferritin ratio, and interleukin-6 were higher than the control group. After 2 months of infliximab treatment, hemoglobin and serum iron levels were lower than those of the control group; and RDW, soluble transferrin receptor, soluble transferrin receptor/ferritin ratio, and interleukin-6 were higher than the control group. After 6 months of conventional therapy, hemoglobin and serum iron levels were lower than those of the control group, and RDW and interleukin-6 were higher than those of the control group. After 6 months of infliximab treatment, the hemoglobin and serum iron levels were lower than the control group, and RDW, soluble transferrin receptor, soluble transferrin receptor/ferritin ratio, erythrocyte sedimentation rate, and platelets were higher than the control group. Regarding patients under treatment for at least one year (remission group), all markers studied, except transferrin, were similar to the control group. **Conclusion** – In conclusion, there were some contradictions among the different body iron status indicators and inflammation indicators at two and 6 months of treatment with conventional and infliximab therapy, however after one year of treatment, as shown by the remission group, all indicators studied, except transferrin, were similar to healthy children and adolescents.

HEADINGS – Inflammatory bowel diseases, therapy. Iron-deficiency anemia. Child. Adolescent.

INTRODUCTION

Crohn disease and ulcerative colitis are chronic diseases associated with a high risk of complications[1-3], with a consequent need for surgery and hospitalization[4]. The treatment of patients with inflammatory bowel disease (IBD) consists of the induction and maintenance remission of the disease, aimed at the healing of mucosa and improving intestinal and extra-intestinal manifestations[5-7] as low bone mineral density, growth deficit, pubertal delay, and anemia.

In order to assess anemia in IBD, the most useful body iron status indicators are transferrin saturation[8-10], soluble transferrin receptor (sTfR)[11-21], serum iron[11,12,17,22-24], soluble transferrin receptor/ferritin ratio (sTfR-SF)[12,14,16,17,25] and transferrin[17,26,27]; while erythrocyte sedimentation rate (ESR)[11,17,22,23,26,28], interleukin-6 (IL-6)[22,24,26], ferritin[11,12,16,17,23-30], albumin[24] and platelets[31] are considered inflammation indicators. The iron status indicators would be useful for the diagnosis of iron deficiency anemia, whereas inflammation indicators would be for the diagnosis of anemia of the chronic disease, also known as anemia of inflammation[32].

Literature about the influence of conventional or biologic therapy – infliximab – in anemia of patients with IBD is scarce. In adults with IBD, one study demonstrated that, after 14 weeks of treatment with infliximab, the occurrence of anemia decreased due to the control of disease activity, according to the parameters studied – ESR, ferritin and C-reactive protein (CRP)[28]. Another study in children and adolescents with Crohn disease, which assessed hemoglobin, hematocrit, mean corpuscular volume (MCV), and serum iron, found an increased concentration of serum iron after conventional three-month therapy (corticosteroid and/or thiopurine)[33].

Due to lack of studies that analyzed iron status indicators and inflammation indicators, during the treatment of IBD in pediatric patients, we decided to do this study aiming to assess body iron status indicators and inflammation indicators during the treatment of IBD, consisted of conventional or infliximab therapy in children and adolescents.

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METHODS

Patient population and study design

A case-control study, from July 2007 to January 2013, that consecutively included all outpatients followed up at a specialized clinic of pediatric IBD, 2–20 years old incomplete, of both genders, at the start of treatment with conventional therapy – corticosteroid and/or azathioprine and/or mesalazine – (conventional therapy group) or biologic treatment with infliximab (infliximab group) for IBD, confirmed by the set of clinical, endoscopic, pathological and imaging criteria. All the patients included in these groups with Crohn disease[34] presented Pediatric Crohn's Disease Activity Index (PCDAI) greater than 30, and those with ulcerative colitis[35] presented Pediatric Ulcerative Colitis Activity Index (PUCAI) greater than 65, at the baseline. We also included, consecutively, all outpatients with a confirmed diagnosis of IBD under treatment with conventional or infliximab therapy for more than one year who were in remission of disease (remission group). All the patients of this group presented disease activity indexes, PCDAI and PUCAI, lower than 10. As for exclusion criteria, patients with IBD who presented any other chronic associated disease were excluded.

We also studied a control group of healthy children and adolescents, who did not present either chronic disease or anemia, from a full-time school, located in the south of the city of São Paulo, that was the same location of the outpatient clinic of this study. This group's selection consisted of detailed clinical evaluation for signs of anemia – skin pallor, conjunctiva pallor, lip, and tongue pallor[36]. Therefore, patients without clinical signs of anemia or chronic disease were matched for gender and age with the study group and invited to participate in the project. Those who accepted the invitation were scheduled for blood collection at their school.

Therefore, the sample of this study comprised 116 individuals as follows: 18 patients in the conventional therapy group (7 with Crohn disease and 11 with ulcerative colitis); 20 patients in the infliximab group (all with Crohn disease); 43 patients in the remission group – 21 patients receiving conventional therapy and 22 receiving infliximab therapy (all with Crohn disease), (28/43 with Crohn disease and 15/43 with ulcerative colitis), and 35 healthy individuals in the control group.

Methods

In addition to the hemoglobin and red cells distribution width (RDW), the following indicators were assessed in all groups: transferrin saturation, sTfR, serum iron, total iron-binding capacity (TIBC), sTfR-SF, transferrin, ESR, IL-6, ferritin, albumin, and platelets. In addition to these laboratory parameters, we also assessed the intake and absorption of iron. It is interesting to note that the formula to calculate the sTfR-SF is bellow[37]:

$$\text{sTfR-SF} = \log \frac{\text{soluble transferrin receptor (µ/L)}}{\text{ferritin (µ/L)}}$$

Right after the collection of blood sampling for the tests described before, at the baseline, with the patient still in the fasting state, the iron absorption was examined. So, the ferrous sulfate was administered orally (1 mg of elemental iron/kg – maximum 25 mg of elemental iron), and, after two hours, the serum iron was measured again[38-40]. It was arbitrarily established that the iron absorption test was the difference between serum iron after iron overload and initial serum iron.

The patients of the conventional therapy and infliximab groups were assessed at the baseline (just before treatment starts), after 2 months and 6 months of treatment. The patients of the remission group were assessed after at least one year of treatment, and the control group was investigated only once.

PCDAI and PUCAI indices were assessed in all the patients of the conventional and infliximab therapy groups, after 6 months of the treatment start. According to the classification of Paris, patients were assessed regarding the location of the disease[41]. The methodology used in the analysis of laboratory tests is described below. Blood counts were performed by automated analysis using ADVIA 120 analyzer, and serum iron through the colorimetric method using ADVIA 1650 analyzer. Total iron-binding capacity was established using the formula: *total iron-binding capacity* = (*transferrin – serum iron*). The ferritin concentration was obtained by the chemiluminescence method using the ADVIA Centaur unit, and the measurement of transferrin was determined by turbidimetry using ADVIA 1650 analyzer. Transferrin saturation was determined using the following formula:

$$\text{transferrin saturation} = \frac{\text{serum iron}}{\text{TIBC}} \times 100$$

The dosage of the sTfR was made in duplicate and obtained using the ELISA test (Kit for measurement of soluble human transferrin R DTFR 1 – LGC Scientific Supply). The concentration of IL-6 was determined using the ELISA test (R&D Systems – Quantikine D6050 ELISA Kit; Minneapolis, MN), in duplicate, and the methodology was followed as determined by the manufacturer. ESR was obtained by automated analysis using the Spife device, and protein electrophoresis was determined by electrophoretic fractionation on agarose gel with densitometry reading.

All the anemic patients used iron medication in therapeutic doses of the elemental iron. The intake of dietary iron was assessed using the dietary survey called "usual daily diet" in the infliximab and conventional therapy groups at baseline and after 2 and 6 months of treatment. The dietary iron intake was calculated by computer program NutWin® version 2.5 (NutWin Software, Nutrition Support Program, Escola Paulista de Medicina, São Paulo, Brazil, 2002). We used Dietary Reference Intake (DRI)[42] as the reference standard for evaluating the recommended values.

Statistical analysis

There was no normal distribution of all quantitative variables (Kolmogorov Smirnov test), and, therefore, we used the Kruskal-Wallis test when comparing the means of the four groups; if there were any differences between them, multiple comparisons were made using the Tukey test. The comparison of the prevalence of anemia among the groups was assessed using Fischer's test.

Statistical calculations were performed using the SPSS version 11.5.0 programs (SPSS Inc., Chicago, IL), and the significance level was set at 0.05 or 5% rejection level of the null hypothesis.

Ethical considerations

The Research Ethics Committee of the Federal University of Sao Paulo approved the project – CEP/ UNIFESP n:0524/07 and n:1233/09, and all those responsible for the patients freely agreed to the terms of the consent/assent form with all questions being answered beforehand.

RESULTS

TABLE 1 shows the demographic and baseline characteristics of the population. The mean age of the patients was 12.1±4.3 years. By the classification of Paris[41], all patients with Crohn disease, at baseline, presented ileocolonic disease (L3), non-stenotic, and nonpenetrating type (B1), while all patients with ulcerative colitis presented pancolitis (E4) and severe type (S1).

Considering the limits of anemia by age, by the WHO[32], the proportion of anemic patients in the conventional therapy group at baseline was 61% (11/18), after 2 months of treatment was 33.3% (6/18) and after 6 months of treatment was 27.7% (5/18). In the infliximab group, 50% (10/20) of the patients presented anemia at baseline, 50% (10/20) of the patients presented anemia after 2 months of treatment, and 60% (12/20) of the patients presented anemia after 6 months of treatment. Therefore, there was no statistically significant difference between the proportion of anemic patients: at baseline of the conventional therapy group and baseline of the infliximab group (P=0.718); after 2 months of the treatment with conventional therapy and after 2 months of the treatment with infliximab (P=0.478); nor after 6 months of treatment with conventional therapy and after 6 months of treatment with infliximab (P=0.095). In the remission group, the proportion of anemia was equal to 18.6% (8/43). The proportion of anemic patients of the infliximab group after 6 months of treatment was statistically higher than that of anemic patients in the remission group (P=0.003). In contrast, there was no statistically significant difference in the proportion of anemic patients of the conventional therapy group after 6 months of treatment and of the remission group (P=0.649).

As shown in TABLE 1, at baseline, both groups – conventional therapy and infliximab – presented significant differences in respect of most markers studied compared to the healthy group.

After 2 months of conventional therapy (TABLE 2), hemoglobin and serum iron levels were lower than those of the control group; and RDW, TIBC, sTfR-SF, and IL-6 were higher than the control group. After 2 months of infliximab treatment (TABLE 2), hemoglobin and serum iron levels were lower than those of the control group; and RDW, sTfR, sTfR-SF, and IL-6 were higher than the control group.

The TABLE 3 demonstrated that after 6 months of conventional therapy, hemoglobin and serum iron levels were lower than those of the control group, and RDW and IL-6 were higher than those of the control group. After 6 months of infliximab treatment (TABLE 3), the hemoglobin and serum iron levels were lower than the control group, and RDW, sTfR, sTfR-SF, ESR, and platelets were higher than the control group. All patients of the conventional therapy group and the infliximab group, after 6 months of treatment, presented PCDAI and PUCAI lower than 10, so all of them were in remission of disease, according to these activity indices.

Regarding patients under treatment for at least one year (remission group), all markers studied, except transferrin, were similar to the control group (TABLE 1).

TABLE 1. Demographic and baseline characteristics of body iron status indicators and inflammation indicators of the patients with inflammatory bowel disease, according to the four studied groups.

Variable	Conventional Therapy (CT) N=18	Infliximab (I) N=20	Remission (R) N=43	Control (C) N=35	*P*	*P* – Tukey test
Age (year)	10.2 (4.4)	11.9 (4.5)	13.2 (4.6)	11.8 (3.2)	0.078	
Gender (F/M)	12/18	14/20	22/43	20/35	0.465	
Hemoglobin (g/dL)	11.2 (2.0)	11.2 (2.3)	12.8 (1.6)	13.7 (1.0)	<0.001	CT<R (P=0.004), CT<C (P=0.000), I<R (P=0.002), I<C (P=0.000)
RDW (red cells distribution width) (%)	16.1 (1.9)	17.0 (4.7)	14.6 (2.0)	13.5 (0.5)	<0.001	CT>C (P=0.002), I>R (P=0.004), I>C (P=0.000)
Transferrin saturation (%)	16.3 (11.9)	54.7 (74.3)	19.7 (131.2)	32.8 (13.5)	0.439	NS
Soluble transferrin receptor (mg/L)	36.5 (18.2)	37.4 (21.9)	21.0 (11.5)	21.6 (5.1)	<0.001	CT>R (P=0.001), CT>C (0.002), I>R (P=0.000) and I>C (P=0.001)
Serum iron (µg/dL)	37.4 (28.0)	69.5 (61.4)	82.5 (43.8)	98.0 (39.8)	<0.001	CT<R (P=0.003) and CT<C (P=0.000)
Total iron binding capacity (mg/dL)	247.4 (64.5)	197.5 (102.6)	177.1 (53.6)	202.6 (44.4)	0.003	CT>R (P=0.001)
Soluble transferrin receptor/ ferritin ratio (µg/dL)	0.3 (0.7)	1.5 (0.3)	-0.2 (0.5)	-0.3 (0.3)	<0.001	CT<I (P=0.000), CT>R (P=0.012), CT>C (P=0.001), I>R (P=0.000), I>C (P=0.000)
Iron intake (mg)	14.7 (5.4)	14.6 (4.7)	16.0 (6.0)	18.4 (17.3)	0.519	NS
Iron absorption (µg/dL)	157.7 (97.4)	181.2 (119.3)	192.8 (84.2)	203.4 (71.1)	0.351	NS
Transferrin (mg/dL)	288.5 (60.7)	272.2 (66.6)	262.0 (38.8)	300.6(22.5)	0.002	R<C (P=0.002)
Ferritin (ng/dL)	40.0 (69.6)	38.8 (51.6)	45.0 (50.5)	45.3 (25.4)	0.949	NS
Albumin (g/dL)	3.5 (0.6)	3.5 (0.7)	4.0 (0.5)	3.9 (0.2)	<0.001	CT<R (P=0.001), CT<C (P=0.021), I<R (P=0.001), I<C (P=0.013)
Erythrocyte sedimentation rate (mm^3/hour)	39.5 (37.4)	29.2 (29.9)	19.1 (19.7)	12.8 (9.8)	0.001	CT>R (P=0.012), CT>C (P=0.001)
Interleukin – 6 (pg/mL)	9.1 (4.8)	6.6 (6.8)	4.5 (6.0)	2.2 (3.3)	<0.001	CT>R (P=0.013), CT>C (P=0.000), I>C (P=0.019)
Platelets (mm^3)	513500 (252110)	461300 (164407)	323326 (110663)	339371 (79732)	<0.001	CT>R (P=0.000), CT>C (P=0.000), I>R (P=0.003), I>C (P=0.016)

Values correspond to the number of patients and means (SD). NS: non-significant.

TABLE 2. Characteristics, after two months of treatment, of body iron status indicators and inflammation indicators of the patients with inflammatory bowel disease, according to the four studied groups.

Variable	Conventional Therapy (CT) N=18	Infliximab (I) N=20	Remission (R) N=43	Control (C) N=35	*P*	*P* – Tukey Test
Hemoglobin (g/dL)	12.4 (2.0)	11.1 (2.7)	12.8 (1.6)	13.7 (1.0)	<0.001	I<R (*P*=0.002), CT<C (*P*= 0.047) and I<C (*P*=0.000)
RDW (red cells distribution width) (%)	16.6 (2.0)	17.1 (3.8)	14.6 (2.0)	13.5 (0.5)	<0.001	CT>R (*P*=0.007), CT>C (*P*=0.000), I>R (*P*=0.000) and I>C (*P*=0.000)
Transferrin saturation (%)	20.3 (12.2)	-3.0 (107.0)	19.7 (131.2)	32.8 (13.5)	0.001	*
Soluble transferrin receptor (mg/L)	35.3 (21.1)	37.1 (24.2)	21.0 (11.5)	21.6 (5.1)	0.003	I>R (*P*= 0.009) and I>C (*P*=0.019)
Serum iron (µg/dL)	58.0 (33.9)	57.2 (41.4)	82.5 (43.8)	98.0 (39.8)	0.002	CT<C (*P*=0.005) and I<C (*P*=0.003)
Total iron binding capacity (mg/dL)	252.4 (62.6)	206.2 (83.0)	177.1 (53.6)	202.6 (44.4)	0.001	CT>R (*P*=0.000) and CT>C (*P*=0.021)
Soluble transferrin receptor/ferritin ratio (µg/dL)	0.2 (0.4)	1.5 (0.4)	-0.2 (0.5)	-0.3 (0.3)	<0.001	CT<I (*P*=0.000), CT>R (*P*=0.042), CT>C (*P*= 0.006), I>R (*P*=0.000) and I>C (*P*=0.000)
Iron intake (mg)	14.2(5.0)	14.0 (5.0)	16.0 (6.0)	18.4 (17.3)	0.538	NS
Transferrin (mg/dL)	307.8 (64.5)	261.6 (61.9)	262.0 (38.8)	300.6(22.5)	<0.001	CT>I (*P*=0.010), CT>R (*P*=0.002), I<C (*P*=0.013) and R<C (*P*=0.001)
Ferritin (ng/dL)	25.1 (16.8)	76.4 (235.2)	45.0 (50.5)	45.3 (25.4)	0.001	*
Albumin (g/dL)	6.4 (12.3)	3.3 (0.7)	4.0 (0.5)	3.9 (0.2)	<0.001	*
Erythrocyte sedimentation rate (mm^3/hour)	24.1 (24.5)	25.6 (21.7)	19.1 (19.7)	12.8 (9.8)	0.114	NS
Interleukin – 6 (pg/mL)	7.0 (4.0)	5.8 (3.9)	4.5 (6.0)	2.2 (3.3)	<0.001	CT>C (*P*=0.003) and I>C (*P*=0.036)
Platelets (mm^3)	429722 (191904)	418550 (179688)	323326 (110663)	339371.4 (79732)	0.016	CT>R (*P*=0.026) and I>R (*P*=0.045)

Values correspond to the number of patients and means (SD). * It was not possible to detect any statistical difference. NS: non-significant.

TABLE 3. Characteristics, after six months of treatment, of body iron status indicators and inflammation indicators of the patients with inflammatory bowel disease, according to the four studied groups.

Variable	Conventional Therapy (CT) N=18	Infliximab (I) N=20	Remission (R) N=43	Control (C) N=35	*P*	*P* – Tukey Test
Hemoglobin (g/dL)	12.3 (1.8)	11.6 (2.1)	12.8 (1.6)	13.7 (1.0)	<0.001	CT<C (*P*=0.010), I<R (*P*=0.021) and I<C (*P*=0.000)
RDW (red cells distribution width) (%)	16.1 (4.1)	15.6 (1.8)	14.6 (2.0)	13.5 (0.5)	<0.001	CT>C (*P*=0.000) and I > C (*P*=0.003)
Transferrin saturation (%)	27.1 (17.5)	19.0 (23.4)	19.7 (131.2)	32.8 (13.5)	<0.001	*
Soluble transferrin receptor (mg/L)	30.3 (15.6)	32.8 (22.0)	21.0 (11.5)	21.6 (5.1)	0.003	I>R (*P*=0.009) and I>C (*P*=0.019)
Serum iron (µg/dL)	68.1 (34.8)	47.7 (35.4)	82.5 (43.8)	98.0 (39.8)	<0.001	CT<C (*P*=0.053), I<R (*P*=0.009) and I<C (*P*=0.000)
Total iron binding capacity (mg/dL)	218.0 (62.8)	241.9 (59.2)	177.1 (53.6)	202.6 (44.4)	0.001	CT>R (*P*=0.040) and I>R (*P*=0.000)
Soluble transferrin receptor/ ferritin ratio (µg/dL)	0.0 (0.5)	1.4 (0.3)	-0.2 (0.5)	-0.3 (0.3)	<0.001	CT<I (*P*=0.000), I>R (*P*=0.000) and I>C (*P*=0.000)
Iron intake (mg)	18.9 (5.3)	15.5 (5.7)	16.0 (6.0)	18.4 (17.3)	0.792	NS
Transferrin (mg/dL)	282.9 (44.2)	283.0 (43.8)	262.0 (38.8)	300.6(22.5)	<0.001	R<C (*P*=0.000)
Ferritin (ng/dL)	35.7 (38.9)	29.9 (34.9)	45.0 (50.5)	45.3 (25.4)	0.006	*
Albumin (g/dL)	3.7 (0.5)	3.6 (0.9)	4.0 (0.5)	3.9 (0.2)	0.005	I<R (*P*=0.021)
Erythrocyte sedimentation rate (mm^3/hour)	13.5 (12.8)	30.7 (21.2)	19.1 (19.7)	12.8 (9.8)	0.004	CT<I (*P*=0.010), I>R (*P*=0.056) and I>C (*P*=0.001)
Interleukin – 6 (pg/mL)	20.6 (13.5)	6.6 (6.7)	4.5 (6.0)	2.2 (3.3)	<0.001	CT>I (*P*=0.000), CT>R (*P*=0.000) and CT>C (*P*=0.000)
Platelets (mm^3)	334000 (116816)	456650 (159543)	323326 (110663)	339371 (79732)	0.001	CT<I (*P*=0.007), I>R (*P*=0.000) and I>C (*P*=0.002)

Values correspond to the number of patients and means (SD). * It was not possible to detect any statistical difference. NS: non-significant.

There was no statistically significant difference among the four groups studied concerning the means of iron absorption (TABLE 1) and iron intake (TABLE 1, 2, and 3). Therefore, the consumption of iron was similar among these groups at all studied times.

DISCUSSION

According to our knowledge, this is the first study that evaluated the influence of conventional therapy and therapy with infliximab in children and adolescents with IBD, analyzing almost all body iron status indicators and inflammation indicators.

Patients under treatment for at least one year (remission group) were similar to those of the control group for all markers studied except for transferrin, suggesting that at least one year of conventional therapy or therapy with infliximab would be sufficient to normalize iron status indicators and inflammation indicators.

At baseline, concerning the conventional therapy group, the concentrations of hemoglobin, serum iron, and albumin were lower when compared with the control group; whereas RDW, sTfR, sTfR-SF, ESR, IL-6, and platelets presented higher concentration than those of the control group. Regarding the infliximab group, hemoglobin and albumin levels were lower compared to those of the control group; however, RDW, sTfR, sTfR-SF, IL-6, and platelets showed higher levels than those of the control group. Therefore, at baseline, the markers' behavior in the conventional and infliximab groups was different when compared with a healthy population (control), showing the presence of anemia due to iron deficiency and anemia of inflammation.

In the course of treatment, after 2 months, for the conventional therapy group, the concentration of hemoglobin and serum iron was lower when compared with that of the control group; whereas, RDW, TIBC, sTfR-SF, and IL-6 demonstrated higher levels than in the control group. As for the infliximab group, levels of hemoglobin, serum iron, and transferrin were lower than those of the control group; however, RDW, sTfR, sTfR-SF, and IL-6 presented higher levels than the control group. Therefore, the behavior of markers after 2 months of treatment, either with conventional therapy or infliximab was different from that of the control group, indicating that this time of therapy was still not enough to normalize the markers studied, persisting the occurrence of anemia due to iron deficiency and anemia of inflammation.

After 6 months of treatment, regarding the conventional therapy group, the concentration of hemoglobin and serum iron was lower than that of the control group; whereas RDW and IL-6 were higher than those in the control group. As for the infliximab group, levels of hemoglobin and serum iron were lower when compared to those of the control group; however, RDW, sTfR, sTfR-SF, ESR, and platelets presented higher levels than those of the control group. Therefore, after 6 months, the performance of markers remained virtually the same both in conventional treatment as in the treatment with infliximab, also noting alteration of inflammation indicators, which suggests that anemia of inflammation was still present after this period with both therapies. However, there was an unexpected result for the treatment with infliximab, which was different from conventional treatment. The treatment with infliximab also demonstrated alterations of the body iron status indicators even after 6 months of treatment, which had not occurred with conventional therapy. Therefore, after 6 months of treatment with conventional therapy, there was still the presence of anemia of inflammation, whereas, after treatment with infliximab, there was the presence of anemia due to iron deficiency associated with anemia of inflammation. It would be discussed that the infliximab group was worst about the IBD compared to the conventional group and that even though the activity index indicated that the infliximab group was on remission, the anemia was not solved. So, in some patients, who presented iron deficiency anemia, the intravenous iron therapy, as shown by Venturieri, et al. study[43], would be indicated even though the patient presented normal iron absorption.

Considering that the iron absorption and iron intake in conventional therapy and infliximab groups were statistically similar to the control and remission groups, it can be inferred that the anemia was not due to low iron intake or iron absorption deficit. To the cause of anemia, the possible reason would be blood loss in the stool for the activity of IBD.

It is essential to highlight that after 6 months of treatment, the concentration of IL– 6 was higher in the conventional group compared with the control group, while there was no difference between infliximab and control group. Therefore, treatment with infliximab seems to be more effective in respecting anti-inflammatory power.

Regarding the sTfR, considered one of the best indicators of iron deficiency anemia[21,25], especially the sTfR-SF[25], it is interesting to note that there was higher concentration in both conventional and infliximab groups compared to remission and control group, at baseline, 2 and 6 months after treatment. One study with children[25] showed that sTfR and sTfR/log ferritin were significantly higher in the IBD group than the control group.

It is necessary to emphasize the lack of studies published regarding indicators of iron body status and inflammation indicators during the treatment of IBD in adults, children, and adolescents. It is also important to mention that these studies evaluated small among indicators. One study showed, after 3 months and a half of the treatment with infliximab, in adults with IBD, reduction in the occurrence of anemia of inflammation, according to the parameters of ESR, ferritin, and CRP[26]. The study in children and adolescents with Crohn disease treated with conventional therapy observed increased serum iron after 3 months of treatment[30].

Concerning iron absorption in patients with IBD, one study[23] using a different methodology from that of the present study, also concluded that the absorption was preserved in adults with activity IBD, and another[22], found decreased iron absorption in patients with Crohn disease, studying a sample from 5 to 25 years old.

One limitation of this study would be the size of the sample, which would be considered quite small, especially concerning the number of participants in each of the four groups: conventional therapy, infliximab, remission, and control. For example, the concentration of sTfR was higher in both groups – conventional and infliximab – compared to the remission and control groups, but there was an only statistical difference of the infliximab group.

CONCLUSION

In conclusion, there were some contradictions among the different body iron status indicators and inflammation indicators at 2 and 6 months of treatment with conventional and infliximab

therapy, however after one year of treatment, as shown by the remission group, all indicators studied, except transferrin, were similar to healthy children and adolescents.

Authors' contribution

Corrêa FF: development of the pre-project and all of the research stages (review of the literature, data collection, data analysis, and composition). Sdepanian VL: development of the pre-project and all of the research stages (review of the literature, data collection, data analysis, composition, supervision, and guidance).

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Corrêa FF, Sdepanian VL. Indicadores do estado corporal do ferro e indicadores de inflamação durante o tratamento da doença inflamatória intestinal em crianças e adolescentes. Arq Gastroenterol. 2021;58(1):48-54.

Resumo – **Contexto** – O tratamento de pacientes com doença inflamatória intestinal (DII) consiste na indução e manutenção da remissão da doença. Os indicadores do estado corporal do ferro seriam úteis para o diagnóstico da anemia por deficiência de ferro, enquanto os indicadores de inflamação para o diagnóstico da anemia da doença crônica. **Objetivo** – Avaliar os indicadores do estado corporal do ferro e os indicadores de inflamação durante o tratamento da doença inflamatória intestinal, com terapia convencional ou infliximabe em crianças e adolescentes. **Métodos** – Estudo de caso-controle de uma amostra de 116 indivíduos, sendo 81 pacientes com DII, dos quais 18 com terapia convencional, 20 infliximabe e 43 em remissão da doença, e 35 crianças e adolescentes saudáveis (grupo controle). Os indicadores do estado do ferro e os indicadores de inflamação foram avaliados no início, 2 e 6 meses de dois tipos de tratamento – terapia convencional e terapia com infliximabe. **Resultados** – A média de idade foi de 12,1±4,3 anos. No início do tratamento, ambos os grupos – terapia convencional e infliximabe – apresentaram diferenças significantes com relação à maioria dos marcadores estudados comparados ao grupo controle. Após 2 meses de terapia convencional, os níveis de hemoglobina e ferro sérico foram inferiores em comparação ao grupo controle; e amplitude de distribuição dos eritrócitos (RDW), capacidade total de ligação do ferro, razão entre o receptor de transferrina solúvel e ferritina e interleucina-6 foram superiores aos do grupo controle. Após 2 meses de tratamento com infliximabe os níveis de hemoglobina e ferro sérico foram inferiores em comparação ao grupo controle, e RDW, receptor de transferrina solúvel e interleucina-6 foram superiores aos do grupo controle. Após 6 meses de terapia convencional, os níveis de hemoglobina e ferro sérico foram inferiores aos do grupo controle, e RDW e interleucina-6 superiores aos do grupo controle. Após 6 meses de tratamento com infliximabe, os níveis de hemoglobina e ferro sérico foram inferiores comparados ao grupo controle, e RDW, receptor de transferrina solúvel, razão receptor de transferrina solúvel e ferritina, taxa de sedimentação de eritrócitos e plaquetas foram superiores ao do grupo controle. Quanto aos pacientes que estavam em tratamento há mais de um ano (grupo remissão), todos os marcadores, exceto a transferrina, foram similares ao grupo controle. **Conclusão** – Houve contradições entre os diferentes indicadores do estado corporal do ferro e dos indicadores de inflamação aos 2 e 6 meses de tratamento com terapia convencional e infliximabe, no entanto após um ano de tratamento, conforme observado pelo grupo em remissão, todos os indicadores estudados, exceto a transferrina, foram semelhantes aos das crianças e adolescentes saudáveis.

DESCRITORES – Doenças inflamatórias intestinais, terapia. Anemia ferropriva. Criança. Adolescente.

REFERENCES

1. Louis E, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. Gut. 2001;49:777-82.
2. 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;8:24450.
3. Hellers G, Bergstrand O, Ewerth S, Holmstrom B. Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. Gut. 1980;21:525-7.
4. Mekhjian HS, Switz DM, Melnyk CS, Rankin GB, Brooks RK. Clinical features and natural history of Crohn's disease. Gastroenterology. 1979;77:898-906.
5. Reenaers C, Belaiche J, Louis E. Impact of medical therapies on inflammatory bowel disease complication rate. World J Gastroenterol. 2012;18:3823-7.
6. Turner D, Ruemmele FM, Orlanski-Meyer E, Griffiths AM, Carpi JM, Bronsky J, Veres G, et al. Management of Paediatric Ulcerative Colitis, Part 1: Ambulatory Care-An Evidence-based Guideline from European Crohn's and Colitis Organization and European Society of Paediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2018;67:257-91.
7. Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis. 2014;8:1179-207.
8. Kuvibidila S, Warrier RP, Ode D, Yu L. Serum transferrin receptor concentrations in women with mild malnutrition. Am J Clin Nutr. 1996;63:596-601.
9. Stein J, Dignass AU. Management of iron deficiency anemia in inflammatory bowel disease - a practical approach. Ann Gastroenterol. 2013;26:104-13.
10. Kaitha S, Bashir M, Ali T. Iron deficiency anemia in inflammatory bowel disease. World J Gastrointest Pathophysiol. 2015;6:62-72.
11. Revel-Vilk S, Tamary H, Broide E, Zoldan M, Dinari G, Zahavi I, et al. Serum transferrin receptor in children and adolescents with inflammatory bowel disease. Eur J Pediatr. 2000;159:585-9.
12. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro F. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am J Clin Nutr. 2000;72:1191-5.
13. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta. 2003;329:9-22.
14. Jain S, Narayan S, Chandra J, Sharma S, Jain S, Malhan P. Evaluation of serum transferrin receptor and sTfR ferritin indices in diagnosing and differentiating iron deficiency anemia from anemia of chronic disease. Indian J Pediatr. 2010;77:179-83.
15. Pettersson T, Kivivuori SM, Siimes MA. Is serum transferrin receptor useful for detecting iron-deficiency in anaemic patients with chronic inflammatory diseases? Br J Rheumatol. 1994;33:740-4.
16. Yang Z, Dewey KG, Lönnerdal B, Hernell O, Chaparro C, Adu-Afarwuah S, et al. Comparison of plasma ferritina concentration with the ratio of plasma transferrin receptor to ferritin in estimating body iron stores: results of 4 intervention trials. Am J Clin Nutr.2008;87:1892-8.
17. Oustamanolakis P, Koutroubakis IE. Soluble transferrin receptor-ferritin indexis the most efficient marker for the diagnosis of iron deficiency anemia inpatients with IBD. Inflamm Bowel Dis. 2011;17:E158-9.

18. Stein J, Hartmann F, Dignass AU. Diagnosis and management of iron deficiency anemia in patients with IBD. Nat Rev Gastroenterol Hepatol. 2010;7: 599-610.
19. Karaskova E, Volejnikova J, Holub D, Velganova-Veghova M, Sulovska L, Mihal V, et al. Hepcidin in newly diagnosed inflammatory bowel disease in children. J Paediatr Child Health. 2018;54:1362-7.
20. Aljomah G, Baker SS, Schmidt K, Alkhouri R, Kozielski R, Zhu L, et al. Anemia in Pediatric Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr. 2018;67:351-5.
21. Daude S, Remen T, Chateau T, Danese S, Gastin I, Baumann C, et al. Comparative accuracy of ferritin, transferrin saturation and soluble transferrin receptor for the diagnosis of iron deficiency in inflammatory bowel disease. Aliment Pharmacol Ther. 2020;51:1087-95.
22. Semrin G, Fishman DS, Bousvaros A, Zholudev A, Saunders AC, Correia CE, et al. Impaired intestinal iron absorption in Crohn's disease correlates with disease activity and markers of inflammation. Inflamm Bowel Dis. 2006;12:1101-6.
23. Lomer MC, Cook WB, Jan-Mohamed HJ, Hutchinson C, Liu DY, Hider RC, et al. Iron requirements based upon iron absorption tests are poorly predicted by haematological indices in patients with inactive inflammatory bowel disease. Br J Nutr. 2012;107:1806-11.
24. Gasche C, Waldhoer T, Feichtenschlager T, Male C, Mayer A, Mittermaier C, et al; Austrian Inflammatory Bowel Diseases Study Group. Prediction of response to iron sucrose in inflammatory bowel disease-associated anemia. Am J Gastroenterol. 2001;96:2382-7.
25. Krawiec P, Pac-Kożuchowska E. Soluble transferrin receptor and soluble transferrin receptor/log ferritin index in diagnosis of iron deficiency anemia in pediatric inflammatory bowel disease. Dig Liver Dis. 2019;51:352-7.
26. Hyams JS, Fitzgerald JE, Treem WR, Wyzga N, Kreutzer DL. Relationship of functional and antigenic interleukin 6 to disease activity in inflammatory bowel disease. Gastroenterology. 1993;104:1285-92.
27. Bartels U, Pedersen NS, Jarnum S. Iron absorption and serum ferritin in chronic inflammatory bowel disease. Scand J Gastroenterol. 1978;13:649-56.
28. Bergamaschi G, Di Sabatino A, Albertini R, Ardizzone S, Biancheri P, Bonetti E, et al. Prevalence and pathogenesis of anemia in inflammatory bowel disease. Influence of anti-tumor necrosis factor-alpha treatment. Haematologica. 2010;95:199-205.
29. Kangaspunta M, Haapamäki J, Färkkilä M, Arkkila P. Inflammatory bowel disease and anemia: intravenous iron treatment. Scand J Gastroenterol. 2018;53:430-4.
30. Aksan A, Wohlrath M, Iqbal TH, Farrag K, Dignass A, Stein J. Serum Hepcidin Levels Predict Intestinal Iron Absorption in Patients with Inflammatory Bowel Disease. Clin Lab. 2019;65(3).
31. Danese S, Motte Cd Cde L, Fiocchi C. Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. Am J Gastroenterol. 2004;99:938-45.
32. World Health Organization (WHO). Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. Geneva, 2001.
33. Pytrus T, Flis A, Iwańczak F, Iwańczak B. [The frequency of anemia in children with newly diagnosed Crohn's disease in children]. Pol Merkur Lekarski. 2013;34:263-8.
34. Hyams JS, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, et al. Development and validation of a pediatric Crohn's disease activity index. J Pediatr Gastroenterol Nutr. 1991;12:439-47.
35. 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.
36. Sdepanian VL, Silvestrini WS, de Morais MB. [Diagnostic limitations of the physical examination in the identification of children with anemia]. Rev Assoc Med Bras. 1996;42:169-74.
37. Malope BI, MacPhail AP, Alberts M, Hiss DC. The ratio of serum transferrin receptor and serum ferritin in the diagnosis of iron status. Br J Haematol. 2001;115:84-9.
38. Mattar RH, de Azevedo RA, Speridião PG, Neto UF, Morais MB. Estado nutricional e absorção intestinal de ferro em crianças com doença hepática crônica com e sem colestase. J Pediatr. 2005;81:317-24.
39. Gross SJ, Stuart MJ, Swender PT, Oski FA. Malabsorption of iron in children with iron deficiency. J Pediatr. 1976;88:795-9.
40. De Vizia B, Poggi V, Vajro P, Cucchiara S, Acampora A. Iron malabsorption in giardiasis. J Pediatr. 1985;107:75-8.
41. Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis. 2011;17:1314-21.
42. Institute of medicine. National Research Council. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academy Press 2000.
43. Venturieri MO, Komati JTS, Lopes LHC, Sdepanian VL. Treatment with Noripurum EV(®) is effective and safe in pediatric patients with inflammatory bowel disease and iron deficiency anemia. Scand J Gastroenterol. 2019;54:198-204.

ORIGINAL ARTICLE

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RNAm expression and DNA methylation of *DKK2* gene in colorectal cancer

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ABSTRACT – **Background** – Colorectal cancer is the third most common neoplasm in the world. Methylation of tumor related genes in CpG islands can cause gene silencing and been involved in the development of cancer. The potential role of *DKK2* as a biomarker for early diagnosis of colorectal cancer remains unclear. **Objective** – The aim of the study was to evaluate the profile of methylation and RNAm expression of *DKK2* as potential predictors of colorectal cancer diagnosis and prognosis. **Methods** – Expression of mRNAs encoding *DKK2* in 35 colorectal cancer tissues was quantified using real-time polymerase chain reaction analysis. The DNA methylation was studied by high resolution melting analysis. The general characteristics of the patients were collected. DKK2 methylation and expression were compared to clinical, pathological aspects and overall survival. **Results** – Among the 35 patients studied, 18 were male, 10 were on right colon and 25 on left colon. Among the 20 patients with high hypermethylation, 15 of them had mRNA low expression of *DKK2*. There was no significant association between *DKK2* promoter methylation and *m*RNA *DKK2* expression and clinical or pathological features. *DKK2* promoter methylation (P=0.154) and *DKK2* RNA expression (P=0.345) did not show significant correlation with overall survival. **Conclusion** – *DKK2* promoter methylation and *DKK2* RNA status appear to be biomarkers of cancer diagnosis but not predictors of prognosis.

HEADINGS – Colorectal neoplasms. Tumor biomarkers. Methylation. Gene silencing. Histocytochemistry.

INTRODUCTION

Colorectal cancer (CRC) is the most common malignant neoplasm of the gastrointestinal tract, with approximately 1,800,000 new cases diagnosed yearly[1] (Bray et al.), being the third cause of cancer. In Brazil, CRC is the second cause of cancer in men and women[2] (INCA, 2020). According to Globocan, in 2018, there were more than 1.8 million new cases of colorectal cancer (CRC) with 881 thousand deaths.

Ageing with more than 50 years, high ingestion of fat and low in fiber, tobacco, inflammatory bowel disease and presence of adenomas are considered risk factors for CRC[3].

DNA alterations may be inherited in 15–20%[4] of the patients or caused mainly by interaction of environment and lifestyle[3]. Several mutations in the same clone of cells transform normal epithelial cells in cancer cells, with increased proliferation and loss of apoptosis and growth control.

Early screening denotes a central importance in the diagnosis of adenomas and early CRC[5]. There are a large number of studies focused on finding potential biomarkers through promoter methylation of tumor-related genes and expression of tumor suppressor genes in gastrointestinal cancers and precancerous lesions, suggesting their involvement in the progression of multiple levels of colorectal carcinogenesis[6].

High methylation of tumor related genes in CpG islands can cause gene silencing and been involved in the development of cancer[6,7]. Alterations of the Wnt/β-catenin signaling pathway are common in tumorigenesis of CRC and epigenetic modifications may be involved in the regulation of this pathway cancer[8,9].

The Dickkopf family (DKK) is composed by four glycoproteins (DKK1–4), secreted with two cysteine-rich domains, separated by a binding region. *DKK2* is a Wnt signaling inhibitor that is generally supraregulated in human cancers, including CRC. Studies on expression and methylation of *DKK2* in CRC suggest that *DKK2* functions as a tumor suppressor and Wnt / β-catenin pathway regulator[6].

A study already published in CRC fresh tumor tissue, by our group showed that all tumors cancer was methylated compared to normal tissues[10] (Silva). However, the circulating free DNA (cfDNA) of *DKK2 i*n peripheral blood of these patients was increased in only 25% of them[11].

The main goal of this study was to evaluate *DKK2* gene expression and methylation as a biomarker of diagnosis and prognosis in colorectal tumors and analyze the influence of *DKK2* methylation in mRNA expression.

METHODS

The study was approved by the Ethical Committee of the Federal University of São Paulo (CAAE no 58914716.0.0000.5505) and all subjects have given their informed written consent. The 35 biological samples were obtained during surgical resection of the tumor at Hospital Sao Paulo, UNIFESP, Brazil. Patients with familiar history of cancer, inflammatory bowel disease, radiotherapy,

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chemotherapy treatment, or concurrent or history of other types of cancers were excluded. DNA and RNA was isolated from the surgically removed colorectal cancer tumors.

Extraction of genomic DNA

Around 25 mg of each tumor tissue was mechanically disrupted using a Tissue Ruptor® (Qiagen, Venlo, Netherlands). The genomic DNA extracted and purified from fresh-frozen colorectal cancer samples using QIAmp DNA Mini kit (Qiagen, Milan, Italy) according to the manufacturer's instruction. The extracted DNA was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Bisulfite modification

Bisulfite conversion of isolated 200ng of genomic DNA from each sample were treated with sodium bisulfite using Cells-to-CpG™ Bisulfite Conversion Kit (Thermo Scientific, Wilmington, USA), according to the manufacturer's protocol. Briefly, Sodium bisulphite converts unmethylated cytosines to uracils, whilst 5-methylcytosines are left unchanged.

DNA methylation by high resolution melting (HRM) analysis

For the MS-HRM of *DKK2* gene we used methylation independent primers (MIP), based on previous study[11]. Briefly, primers for the DKK2 gene were designed to amplify methylated DNA and unmethylated DNA using Methyl Primer Express v 1.0 (Applied Biosystems, Foster City, CA, USA). The primer sequences for *DKK2* HM forward and reverse was AGGTATCGTTGCGTTGGTAGC and AAATCAAAAAACGTCCCCGA respectively. The primer sequences for *DKK2* HM forward and reverse was UM forward and reverse respectively was TTTAGGTATTGTTGTGTTGGTAGT and AAATCAAAAAACATCCCCAAACC.

The PCR analyses were run using the following conditions: one cycle of 95°C for 15 min, 60 cycles of 95°C for 15 s, 63°C annealing temperature for 30 s and 72°C for 30 s; followed by an HRM step of 95°C for 10 s and 50°C for 1 min, 70°C for 15 s, and continuous acquisition to 95°C at one acquisition per 0.1°C. The PCR reaction was set up in a final volume of 20 µL, containing 10–12,5 µL of MeltDoctor HRM master mix (Applied Biosystems, Foster City, CA USA), 10 pmol of each primer and 1 µL (almost 10–20 ng) of bisulfite modified DNA template. The experiments were performed in triplicate. FIGURE 1 shows the melting profiles of the two promoter regions analyzed. Control DNAs with varying the ratios of methylation: 0%, 25%, 50%, 75% and 100%, were prepared by mixing following the manufacturer's protocol (EpiTect methylated and unmethylated human control DNA, bisulfite converted, Qiagen, Milan, Italy). A standard curve with known methylation ratios was included in each assay to provide a quantitative analysis of DNA methylation profile of the promoter of *DKK2* gene. The profile of the aligned fluorescence intensity and melting temperature for serial dilutions of methylated DNA for the *DKK2* gene were plotted in the melting graph (FIGURE 1). The tumors with more than 25% of methylation were considered hypermethylated.

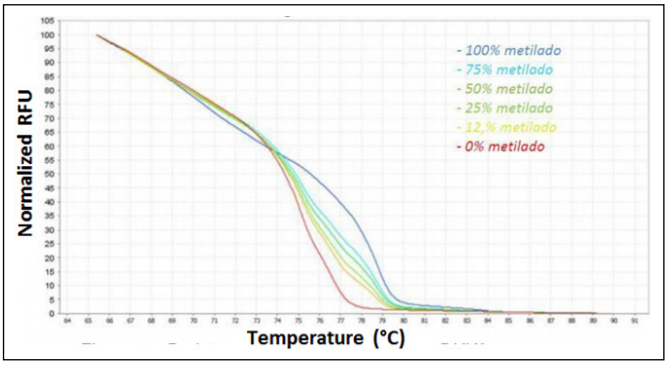

FIGURE 1. Fluorescence intensity profile aligned to the melting temperature for serial dilution of methylated DNA for the *DKK2* gene.

The reported relative fluorescence units (RFU) value within the melt curve was exported to Microsoft Excel and RFU average of each DNA standard was then used to obtain an interpolation curve. Finally, imputation of the RFU value (corresponding to the analyzed sample) to the polynomial function provided a precise (single value) estimate of the percentage of methylation.

Quantitative real-time reverse transcription PCR (qRT-PCR)

Reverse transcription reaction was performed using the Superscript VILO cDNA synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the manufacturer's protocol. Expression assays were performed by using TaqMan Gene Expression Assays for human *DKK2* under the inventory ID: Hs00205294_m1 [Amplicon Length: 88pb]. Human Endogenous control assays *ACTB* (encoding for beta actin, assay ID: Hs01060665_g1, amplicon length: 63pb) and *GAPDH* (encoding for glyceraldehyde-3-phosphate dehydrogenase, ID: Hs02786624_g1, amplicon length: 157pb) (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) served as references for normalization. TaqMan Gene Expression Master Mix according to the manufacturer's protocol (Applied Biosystems, Carlsbad, CA, USA). Reverse transcriptase reaction and Real-Time PCR were performed using the Step one plus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Briefly, the reactions were incubated in a 96-well plate at 95°C for 20 s, followed by 40 cycles of 95°C for 1 s and 60°C for 20 s. In each experiment, triplicate reactions were performed in each sample. Lastly, the relative expression was calculated as the ratio of *DKK2* to *ACTB* for each sample (calculated as the mean of triplicate). The relative quantity of mRNA for each sample were calculated by $2^{-\Delta\Delta CT}$, where ΔCT = (mean of triplicate CT_{ABCB1} − mean of triplicate CT_{ACTB}) and ΔΔCT = (ΔCT – mean ΔCT of all the samples). In pilot studies, the PCR amplicons were evaluated on agarose gels to verify product size.

Statistical analysis

The polynomial function determined by the polynomial regression curve of standards method estimates the percentage of methylation in each experiment. The measurements were performed in triplicate and data are expressed as mean ± standard deviation (SD). Pearson's correlation analysis was used to determine the relationship between mRNA *DKK2* expressions and DNA methylation profile of the promoter of *DKK2* gene. All other statistical differences were determinate with unpaired Student's *t*-test or one-way ANOVA followed by the Tukey test for multiple comparisons. Median survival was based on Kaplan-Meier curves and tested for significance by the log-rank test. The statistical analysis was performed using the Minitab V16.0 (State College, PA), or SPSS V 23 (SPSS Inc., Chicago, IL) or Office Excel for Windows 2010 (Microsoft, Redmond, WA). *P* value of <0.05 was considered statistically significant.

RESULTS

Thirty-five patients with colorectal tumors with 63.6±13.9 years, range 47–78 years, being 18 male and 20 with stage III or IV tumors were studied. Among these 10 were of right colon and 25 of left colon or rectum, called left colon.

This investigation extended the DNA methylation analysis of our previous study[11], that studied DNA methylation of *DKK2* gene by HRM method. The *DKK2* gene promoter unmethylated/hypomethylated and hypermethylated status are shown in FIGURE 2. Among the tumors 35 tumors, 20 are hypermethylated.

The relative quantity of mRNA expression was normalized to the relative quantity of ACTB for each sample and performed by qRT-PCR. The *DKK2* gene expression status is shown on FIGURE 3. Among the 35 tumors, 24 had low expression of RNAm of *DKK2*. Low expression of this gene and hypermethylation was observed in 15 (43%) patients.

The *DKK2* DNA methylation status and relative quantity of mRNA expressions and their relationship with clinical and pathological features in CRC patients are showed in TABLE 1. There is no significant association between genes methylation status and age, sex, tumor site, TNM stage and metastasis. There is also no significant association between DKK2 expression and clinical or pathological features (TABLE 2).

The results show that the expression level of the DKK2 mRNA was not correlated with the increasing or decreasing percentage of methylated *DKK2* gene in methylated samples.

The patients were followed for a period of 4.9±2.5 years. There was no difference on survival for *DKK2* promoter methylation (*P*=0.154) or DKK2 mRNA expression status (*P*=0.345).

DISCUSSION

The number of world CRC will increase during the next years, although in some countries the screening methods caused a decrease incidence in individuals with more than 50 years or earlier in patients with family history of CRC. In contrast, the number of younger patients with CRC are increasing with not elucidation of the cause[12].

Between the 35 patients with CRC included, 51.4% were men, the average age was 63.3±13.9 years, and most patients were over the age of 50 years (82.9%). These data are consistent with those published by INCA, which describes the incidence of CRC in Brazil[2] (INCA, 2020). The limitations of the study were the short number of tissues evaluated and the absence of adjacent free margin.

The *DKK2* gene is deleted in some cancers, but epigenetic alterations causing the methylation of the promoter region of the

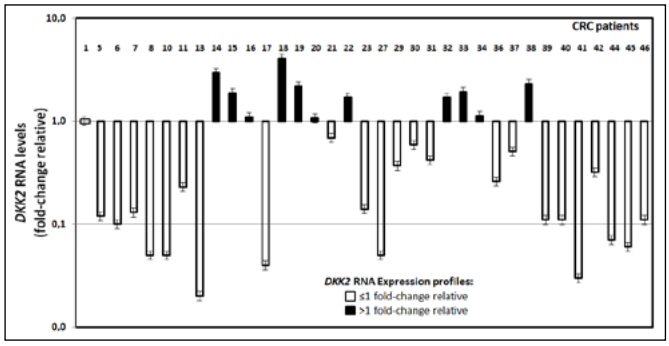

FIGURE 2. The methylation status of the *DKK2* gene of the colorectal cancer tumors.

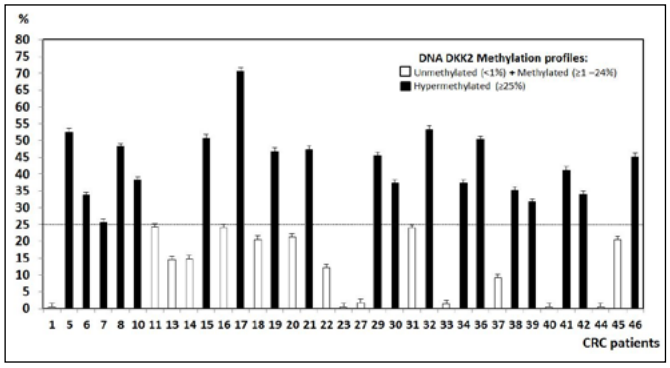

FIGURE 3. *DKK2* gene expression of the colorectal cancer tumors.

TABLE 1. *DKK2* DNA methylation according to the characteristics of the patients and the tumors.

Clinical aspects		N (%)	UnM+HypoM	HyperM	*P*	OR	95% CI
All cases		35 (100)	16 (45.7)	19 (54.3)	–	–	–
Age (years)	<65	19 (54.3)	7 (36.8)	12 (63.2)	0.250	1.00	–
	≥65	16 (45.7)	9 (56.2)	7 (43.8)		0.45	0.12–1.76
Gender	Female	17 (48.6)	8 (50.0)	9 (47.4)	0.877	1.00	–
	Male	18 (51.4	8 (50.0)	10 (52.6)		1.11	0.29–4.21
T location	Right	10 (28.6)	6 (37.5)	4 (21.1)	0.173	1.00	–
	Left	25 (71.4)	10 (62.5)	15 (78.9)		2.25	0.50–10.05
Stage	I+II	15 (42.9)	5 (31.3)	10 (52.6)	0.203	1.00	–
	III+IV	20 (57.1)	11 (68.7)	9 (47.4)		0.41	0.10–1.64
Metastasis	Mo	33 (94.3)	14 (87.5)	19 (100)	0.112	1.00	–
	M1	2 (5.7)	2 (12.5)	0		0.42	0.29–0.63

T location: tumor location; UnM+HypoM: unmethylated+ hypomethylated; HyperM: Hypermethylated; OR: odds ratio; CI: confidence interval. *qui square test.

TABLE 2. *DKK2* RNAm expression according to the characteristics of the patients and the tumors.

Clinical aspects		N (%)	≤1.0	>1.0	*P**	OR	95% CI
All cases		35 (100)	24 (68.6)	11 (31.4)	–	–	–
Age (years)	<65	19 (54.3)	13 (54.2)	6 (54.5)	0.983	1.00	0.23–4.13
	≥65	16 (45.7)	11 (45.8)	5 (45.5)		0	
Gender	Female	17 (48.6)	11 (45.8)	6 (54.5)	0.632	1.00	0.17–2.96
	Male	18 (51.4	13 (54.2)	5 (45.5)		0.70	
T location	Right	10 (28.6)	5 (20.8)	5 (45.5)	0.134	1.00	0.07–1.49
	Left	25 (71.4)	19 (79.2)	6 (54.5)		0.32	
Stage		15 (42.9)	10 (41.7)	5 (45.5)	0.833	1.00	0.20–3.61
	5 (20.8)	20 (57.1)	14 (58.3)	6 (54.5)		0.86	
Metastasis	5 (45.5)	33 (94.3)	23 (95.8)	10 (90.9)	0.536	1.00	0.13–40.54
		2 (5.7)	1 (4.2)	1 (9.1)		2.30	

T location: tumor location; OR: odds ratio; CI: confidence interval. *qui square test.

CpG islands may cause gene silencing that occurs by DNA binding proteins, a blocking of transcriptional regulators and a consequent decreased transcription[13].

Although the molecular mechanism of DKK2 is not well defined, some authors suggest that *DKK2* functions as a tumor suppressor gene and a regulator of the Wnt/β-catenin pathway in CRC[6,7]. Maehata et al., demonstrated in gastrointestinal cancer cell lines that CpG island hypermethylation is associated with gene silencing[14,15].

Hu et al.[16] described that the blockade of DKK2 by an antibody decreased tumor progression by immune cell activation and tumor angiogenesis suggesting that the combination of anti-DKK2 and anti-VEGFr can be a possible treatment of advancer CRC.

The methylation status of this gene and others is frequently analyzed by PCR, which evaluate qualitatively the presence or absence of methylation by bisulfite conversion-based methods that involve the conversion of non-methylated cytosines to uracil after treatment. This method did not allow the quantification of DNA methylated and cannot classify tissues with low methylation to others with higher methylation. In this assay, the methylation state was studied by HRM, which allows the quantitative determination. Some authors fail to associate methylation status with cancer, and others demonstrate that even methylated genes can continue to perform their functions normally. Based on this information it is important to discuss whether it is sufficient to describe the presence of methylation or whether it is necessary to go further and quantify the percentage[15,17,18].

DNA hypermethylation of *DKK2* was observed in 19 (54.3%) tumors and hipomethylated or unmethylated in the others (TABLE 1). In another study described by our group the hypermethylation *of DKK2* was described in 63% of the tumors tissues, but only in 4.45% of the patients with normal colonoscopy ($P<0.001$)[11]. The highest proportion of *DKK2* hypermethylated in CRC found in this study and in the Silva et al.[11], suggest that this gene is a biomarker of cancer. Jones and Takai found that patients with methylation of DNA in normal areas adjacent to the tumor had worst prognosis[19].

Epigenetic alterations occur during aging process and may be an explanation for the higher incidence of CRC after 60 years old. These alterations in older patients were described in human prostate cancer[20] and CRC.

The hypermethylation has been described in precancerous adenoma and can be serve as early detection markers[21-23]. In this study, we did not found difference in methylation status and pathological stages. These results reinforced that DNA methylation can serve as early detection markers.

Most of the tumors studied (68.6%) had lower expression of RNAm of *DKK2* gene and was not influenced by age, sex, site of the tumor or pathological stage.

Some studies published that hypermethylation of some genes in the promoter region may silence mRNA expression. Molnar et al. observed in several TP53 pathway genes, promoter methylation and RNAm expression alterations[24].

Although we did not find a correlation between hypermethylation of DNA and RNAm expression, methylation of the promoter gene may be the cause of the lower expression in 43% of the tumors. In these tumors downregulation or silencing of *DKK2* expression are associated with the hypermethylation status of its promoter. This fact can suggest the treatment of some cancer patients with demethylation drugs.

There are several molecular differences including DNA methylation between left and right colon[25], mainly in tumors with microsatellite instability (MSI) or microsatellite stability (MSS). In the present study, we examined DNA methylation and *DKK2* mRNA expression in patients without familiar history of cancer. In these patients, MSI is rare. Hypermethylation (78.9%) and lower expression of DKK2 (79.2%) was most prevalent in distal tumors. A previous study reported that specific cancer-related genes demonstrate differential methylation depending on colon location[26] in tumors with MSS. In a recent study[27] the authors described an increased DNA methylation levels of various genes, including *DKK2* in CRC tumors and adjacent mucosa with an MSS phenotype.

Univariate analysis by the Kaplan-Meier curves indicates that *DKK2* promoter methylation shows no significant difference between methylation and prognostic factors in CRC patients. Similarly, there is no significant association between *DKK2* RNA expression status and overall survival in CRC patients.

In conclusion, the frequency of *DKK2* gene hypermethylation and low RNAm expression is frequent on colorectal cancer tissue independent of the stage suggesting that this gene may be a biomarker of cancer. However, hypermethylation or low expression of *DKK2 are* not markers of prognosis in the tissues examined.

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Authors' contribution

Mamelli RE, Felipe AV, Silva TD and Hinz V did the inclusion of the patients and performance of the lab's tests. Mamelli RE, Felipe AV and Silva TD contributed to the interpretation of the results and the statistical analysis. Forones NM contributed to the concept, design, and the finalization of the manuscript.

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Mamelli RE, Felipe AV, Silva TD, Hinz V, Forones NM. Expressão do RNAm e metilação do DNA do gene *DKK2* em câncer colorretal. Arq Gastroenterol. 2021;58(1):55-60.

RESUMO – **Contexto** – O câncer colorretal é a terceira neoplasia mais comum no mundo. A metilação de alguns genes nas ilhas CpG podem causar silenciamento gênico e estar envolvida no desenvolvimento de câncer. O potencial papel de DKK2 como um biomarcador no diagnóstico precoce de CCR permanece incerto. **Objetivo** – O objetivo do estudo foi avaliar o perfil de metilação e expressão de RNAm do gene *DKK2* para identificar preditores potenciais de diagnóstico e prognóstico de CCR. **Métodos** – A expressão de mRNAs que codificam DKK2 em 35 tecidos de câncer colorretal foi quantificada por reação em cadeia da polimerase em tempo real e a metilação do DNA foi verificada por análise de alta resolução. As características gerais dos pacientes foram coletadas. A metilação e expressão de DKK2 foram comparadas aos aspectos clínicos, patológicos e à sobrevida global. **Resultados** – Entre os 35 pacientes estudados, 18 eram do sexo masculino, 10 tumores eram do cólon ascendente ou transverso e 25 do descendente ou reto. Entre os 20 pacientes com hipermetilação, 12 deles apresentaram baixa expressão de RNAm do gene *DKK2*. Não houve associação significativa entre a metilação do promotor de DKK2 e a expressão de RNAm de DKK2 e características clínicas ou patológicas. A metilação do promotor de DKK2 e a expressão do RNA de DKK2 não mostraram correlação com sobrevida global dos pacientes com CCR. **Conclusão** – A metilação do gene promotor e a expressão do RNAm do gene *DKK2* parecem ser biomarcadores de diagnóstico de câncer, mas não se mostraram úteis na avaliação prognóstica.

DESCRITORES – Neoplasias colorretais. Biomarcadores tumorais. Metilação. Inativação gênica. Histocitoquímica.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: Cancer J Clin. 2018;68:394-424.
2. INCA National Institute of Cancer: Estimate/2020 – Cancer Incidence in Brazil. Available from: https://rbc.inca.gov.br/revista/index.php/revista/article/view/927.
3. Aleksandrova K, Pischon T, Jenab M, Bueno-de-Mesquita HB, Fedirko V, Norat T, et al., Combined impact of healthy lifestyle factors on colorectal cancer: a large European cohort study. BMC Med. 2014;2:168.
4. Hemminki K, Chen B. Familial risk for colorectal cancers are mainly due to heritable causes. Cancer Epidemiol Biomarkers Prev. 2004;13:1253-6.
5. Winawer SJ. Colorectal cancer screening. Best Pract Res Clin Gastroenterol. 2007;21:1031-48.
6. Wang C, Yue Y, Shao B, Qiu Z, Mu J, Tang J, et al. Dickkopf -related Protein 2 is epigenetically inactivated and suppresses colorectal cancer growth and tumor metastases by antagonizing Wnt/β-Catenin signaling. Cell Physiol Biochem. 2017;41:1709-24.
7. Okugawa Y, Grady WM, Goel A. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology. 2015;149:1204-25.
8. Kazanets A, Shorstova T, Hilmi K, Marques M, Witcher M. Epigenetic silencing of tumor suppressor genes: Paradigms, puzzles, and potential. Biochim Biophys Acta. 2016;1865:275-88.
9. Lee SK, Hwang JH, Choi KY. Interaction of the Wnt/β-catenin and RAS-ERK pathways involving co-stabilization of both β-catenin and RAS plays important roles in the colorectal tumorigenesis. Adv Biol Regul. 2018;68:46-54.
10. Silva TD, Vidigal VM, Felipe AV, De Lima JM, Neto RA, Saad SS, et al. DNA methylation as an epigenetic biomarker in colorectal cancer. Oncol Lett. 2013;6:1687-92.
11. Silva TD, Felipe AV, Pimenta CA, Barão K, Forones NM. DNA methylation profile of the DKK2 gene as a biomarker in patients with colorectal cancer. Genet Mol Res. 2012;11:3138-45. doi: 10.4238/gmr16039816
12. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin. 2017;67:177-193.
13. Lopez-Serra L, Esteller M. Proteins that bind methylated DNA and human cancer: Reading the wrong words. Br J Cancer. 2008;98:1881-5.
14. Maehata T, Taniguchi H, Yamamoto H, Nosho K, Adachi Y, Miyamoto N, et al. Transcriptional silencing of Dickkopf gene family by CpG island hipermethylation in human gastrointestinal cancer. World J Gastroenterol. 2008;14:2702-14.
15. Bae JM, Kim JH, Kang GH. Epigenetic alterations in colorectal cancer: the CpG island methylator phenotype. Histol Histopathol. 2013;28:585-95.
16. Hu J, Wang Z, Chen Z, Li Ao, Sun J, Zheng M, et al. DKK2 blockage-mediated immunotherapy enhances anti-angiogenic therapy of Kras mutated colorectal cancer. Biomed Pharmacother 2020;127:110299.
17. Hrašovec S, Hauptman N, Glavač D, Jelenc F, Ravnik-Glavac M. TMEM25 is a candidate biomarker methylated and down-regulated in colorectal cancer. Dis Markers. 2013;34:93-104.
18. Nilsson TK, Löf-Öhlin ZM, Sun XF. DNA methylation of the p14ARF, RASSF1A and APC1A genes as an independent prognostic factor in colorectal cancer patients. Int J Oncol. 2013;42:127-33.
19. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science. 2001;293:1068-70.
20. Damaschke NA, Yang B, Bhusari S, Svaren JP, Jarrard DF. Epigenetic Susceptibility Factors for Prostate Cancer with Aging. Prostate. 2013; 73: 1721–1730.
21. Galamb O, Kalmár A, Péterfia B, Csabai I, Bodor A, Ribli D, et al. Aberrant DNA methylation of WNT pathway genes in the development and progression of CIMP-negative colorectal cancer. Epigenetics. 2016;11:588–602.

22. Beggs AD, Jones A, El-Bahrawy M, Abulafi M, Hodgson SV, Tomlinson IP. Whole-genome methylation analysis of benign and malignant colorectal tumours. J Pathol. 2013;229:697-704.
23. Luo Y, Wong CJ, Kaz AM, Dzieciatkowski S, Carter KT, Morris SM, et al. Differences in DNA methylation signatures reveal multiple pathways of progression from adenoma to colorectal cancer. Gastroenterology. 2014;147:418-29.
24. Molnár B, Galamb O, Péterfia B, Wichmann B, Csabai I, Bodor A, et al. Gene promoter and exon DNA methylation changes in colon cancer development - mRNA expression and tumor mutation alterations. BMC Cancer 2018;18:695. doi 10.1186/s12885-018-4609-x.
25. Horii J, Hiraoka S, Kato J, Harada K, Kuwaki K, Fujita H, et al. Age-related methylation in normal colon mucosa differs between the proximal and distal colon in patients who underwent colonoscopy. Clin Biochem. 2008;41:1440-8.
26. Sugai T, Habano W, Jiao Y-F, Tsukahara M, Takeda Y, Otsuka K, et al. Analysis of molecular alterations in left- and right-sided colorectal carcinomas reveals distinct pathways of carcinogenesis: proposal for new molecular profile of colorectal carcinomas. J Mol Diagn. 2006;8:193-201.
27. Sugai T, Yoshida M, Eizuka M, Uesugii N, Habano W, Otsuka K, et al. Analysis of the DNA methylation level of cancer-related genes in colorectal cancer and the surrounding normal mucosa. Clin Epigenetics. 2017;9:55. doi 10.1186/s13148-017-0352-4.

ORIGINAL ARTICLE

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Indocyanine green fluorescence imaging in robotic surgery: state of art, tips and tricks in current applications

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ABSTRACT – **Background** – Fluorescent imaging with indocyanine green is an emerging technology whose benefits are put in perspective. **Objective** – This article reports essential principles and approaches of intraoperative fluorescence in general surgery bringing familiarity to its practical usage. Our group describes possible pitfalls and provides tips and tricks for training surgeons making their attempts easier and reproducible during practice. **Methods** – This study overviews the most structured concepts, practical applications and its tricks in robotic fluorescence guided imaging surgery with indocyanine green. Possible pitfalls are emphasized and emerging fields of application are put in a perspective. **Results** – Guided information and practical applications in several surgical fields are described for a safe and reproducible indocyanine green fluorescence imaging use. **Conclusion** – Robotic assisted surgery combined to fluorescence imaging technology represents a logical evolution in image guided surgery and technology familiarity with guided information may represent a wider and safer spectrum of use in surgeons' hands.

HEADINGS – Robotics. Digestive system surgical procedures. Indocyanine green.

INTRODUCTION

Indocyanine green (ICG) is a fluorescent molecule whose incident infrared light of wavelength 780 nm provokes detectable photon emissions to dedicated optical systems[1]. Initially used in photography, its medical related applications have been documented since the mid-1950s for hepatic and cardiac function[2]. Intravascularly its half-life is about 2.4 minutes by binding to plasma lipoproteins until hepatic uptake and exclusively bile excretion[3].

Very low rates of adverse effects step up its virtually nontoxic using a standard dose of less than 2 mg/kg in non-iodine allergic patients.

Fluorescence-guided imaging has evolved the last few years however its firsts descriptions date decades ago. Historically, Moore[4] reported the ICG usage in differentiation of normal and malignant tissues applied to neurosurgery. Through implementations of advanced technologies, more complex interventions in the visceral surgery field became feasible with a minimally invasive concept and more recently intraoperative imaging applications are put in a perspective. ICG has found applications in several surgical fields enabling real-time visualization of structures of interests and information assessment that normally are uncertain under naked eyes. Tissue perfusion assessment, anatomic distinction, lymphography and others implementations have been described in general, gynecology, urology, colorectal surgery and surgical oncology practice[5-7].

Even more notorious, robotic-assisted surgery is spreading quickly and has shown to overcome the intrinsic limitations of laparoscopy. High definition, three-dimensional stereoscopic vision and magnification, a stable and surgeon guided camera, improved ergonomics, superior range of motion and motion scaling are remarkable advantages[8]. Also available, fluorescence imaging was integrated in 2010 in the Da Vinci Robotic Systems through infrared emission enhancing ICG molecules and providing real-time images of fluorescent structures (Firefly™ Fluorescence Imaging Scope; Intuitive Surgical, Sunnyvale, CA). Robotic assisted surgery combined to fluorescence imaging technology represents a logical evolution in image guided surgery and its benefits are still in progress.

Intraoperative fluorescence imaging is safe and reproducible among surgeons, however, each application demands a complete understanding of the topic. ICG biochemical properties, fluorescence technology devices, goals of usage, correct timing, location of application as well as patients conditions and possible pitfalls may interfere with your information assessment. The purpose of this article is to summarise the current usage of ICG fluorescence imaging in robotic general surgery and assure information and its correct application with tips and tricks regarding each different area of interest.

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METHODS

A literature narrative review was conducted regarding characterization of ICG fluorescence imaging applications in the robotic surgery field. Current context and practical experience was also described aiming for better guidance and reaching the goals of usage due to common unknown pitfalls and multiple areas of interest.

Fluorescence imaging technology and ICG

Fluorescence is caused by incident light that excites the target and causes light emission of a particular wave-length. The ICG molecule is a water-soluble fluorophore that when injected intravenously it quickly becomes protein-bound, confined to the intravascular compartment. When excited between 750 and 800 nm, fluorescence is viewed around the maximum peak of 832 nm[3]. The fluorescent emitted light passes through a sensor in the optical device filtering out other wavelengths, displaying the green light in real-time visualization. Firefly™ is the da Vinci integrated fluorescence capability that uses near-infrared technology activated at the surgeon console. The dosage and time point of administration, the start of the imaging process and detection of the signal varies depending on the area of interest.

Preparation of ICG for administration

Under sterile conditions, 25 mg of ICG normally disposed of in one vial is diluted using 10 mL sterile water for a 2.5 mg/mL solution vial. This solution for injection must be used within 6 hours after reconstitution otherwise precipitation may be present requiring discard of the solution. For intravenous purposes, administration followed by an immediate intravenous flush of 10 mL of an isotonic solution is encouraged to allow the dye to reach the intravascular compartment quickly and not be retained in the venous access. ICG administration may be performed via a central or peripheral venous line and multiple doses can be administered as required, up to the maximum recommended total dose of dye, kept below 2 mg/kg. In situ tissue administration is also applied for fluorescence lyphograpy or anatomic enhancement and its doses and methods are discussed separately.

APPLICATIONS

Tissue perfusion assessment

Based on the ability of ICG to become fluorescent, real-time intraoperative organ perfusion evaluation has been used in several clinical applications. The optimal time to detect a fluorescence signal varies between 25–60 seconds after administration and the signal peak is around 30–40 seconds after administration, losing intensity within 2 minutes. Literature reports variable dosages of ICG usage depending on patients bodyweight, ranging most of the studies from 2.5–10 mg. A 7.5 mg dose followed by a 10 mL bolus of saline in a coordinated administration should be performed by the anesthesiologist whenever asked by the surgeon and the area of interest must be already exposed and targeted by the surgeon's endoscope. On the console display, the surgeon activates the Firefly™ mode for enabling infrared emission to promote excitation and fluorescence of the desired tissue (FIGURE 1). Intensity peak and washout may be affected by the patient's circulatory condition as well as cardio-circulatory inotropes. A weak signal might be present if a long distance between the camera and the tissue is present. Also, a slow injection of the dye or a lack of flushing afterwards may contribute to fluorescence misinterpretation.

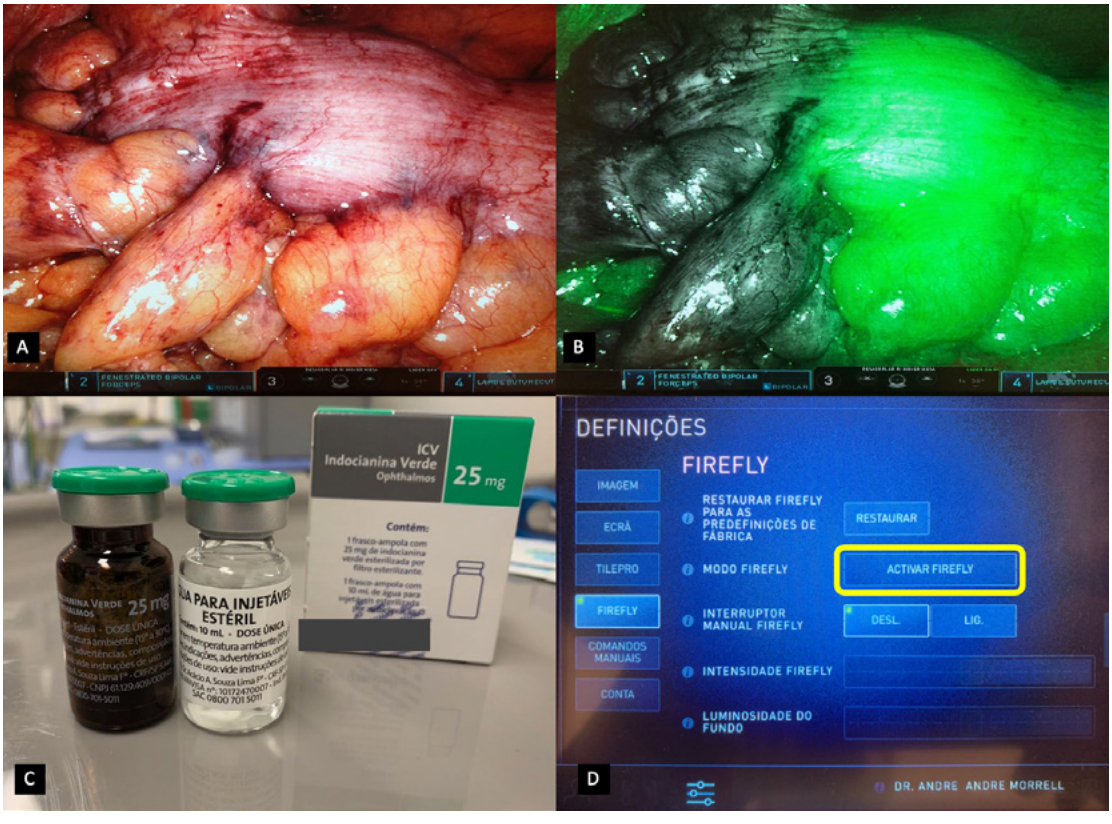

FIGURE 1. A. Image spectrum as usual in robotic optics. **B.** Fluorescence through indocyanine green Firefly™ technology activated. **C.** Indocyanine green bottles to create the greenish solution. **D.** Activation of Firefly™ mode with surgeon's console command.

Upper GI tract surgery

ICG tissue angiography might guide the identification of the optimal resection site and help estimate the blood supply of upper GI tissue and visceral anastomosis. Regarding esophageal surgery field, fluorescence imaging has been documented to early assess the graft perfusion and its vascularity web. Esophageal reconstruction is typically done with a tubularized gastric graft perfused by the right gastroepiploic arcade and the perfusion in proximal portion of the it, area where the anastomosis is typically created, is variable and often tenuous because the arcade rarely reaches its top. ICG image guided technology intraoperatively encourages correlation between adequate perfusion assessment and reduction anastomotic leak rates in these procedures[9,10]. Zehetner et al.[11] also described lower leakage rates in patients following esophagectomies when the anastomosis was placed in an area of good perfusion after fluorescence image. Intraoperative assessment of the conduit perfusion should be done by peripheral or central-line injection of 7.5 mg ICG immediately followed by a 10 mL flushing with saline, expecting its peak within 30 seconds. Kumagai et al.[12] recently reported a 90-second rule advocating a safer method to assure adequate tissue perfusion. Anastomosis blood supply may also be performed robotically whenever opting for an intrathoracic technique.

Bariatric surgery also represents a novel branch for this technology, especially to estimate the risk of leak after sleeve gastrectomies, gastric bypass anastomosis and revisional procedures (FIGURE 2.A, B). Di furia et al.[13] described a 43 patients analysis of minimally invasive sleeve gastrectomies with ICG perfusion assessment however without a well established correlation between gastric leak and sleeve conduit blood supply. Larger series and prospective trials still are needed in order to provide higher levels of evidence supporting ICG usage for tissue assessment in standard procedures. As for revisional bariatric surgeries, due to heterogeneity of cases, controlled studies still lack in literature.

Colorectal surgery

ICG perfusion assessment has found a broad field in colorectal surgery, used mainly to assess the perfusion of performed anastomosis and sites before deciding where to resect the bowel (FIGURE 2.C, D). The prospective multicenter study PILLAR II trial, reported lower anastomosis leakage rates compared to literature, 1.4% to 3–15% respectively in patients undergoing left sided colectomies and low anterior resections[14]. Also, systematic review data step up for the same results, stating half of leakage rates in patients utilizing ICG fluorescence image technology compared to its absence[15]. The latest meta-analysis regarding colon and rectal cancer surgeries recruited more than 1300 patients with significant reduction of anastomotic leak associated with ICG angiography. Not only important for checking anastomosis integrity, the real-time fluorescence guided image has also demonstrated changes in the operative technique and bowel transection area[16].

For an efficient angiography during colorectal surgery, the ICG usage should be divided in two different steps: first, the planned point of proximal and distal transection area just before bowel resection, to assure a correct transection line and well perfused remnant bowel (FIGURE 3.A, B). Second, after completion of the anastomosis, another course of ICG injection is encouraged to visualize the integrity of anastomosis and its vascularity (FIGURE 3.C, D). If a low colorectal anastomosis is performed, a third optional step by visualization of the rectum and anastomosis mucosa may be achieved with an additional Firefly™ integrated endoscope via proctoscopy.

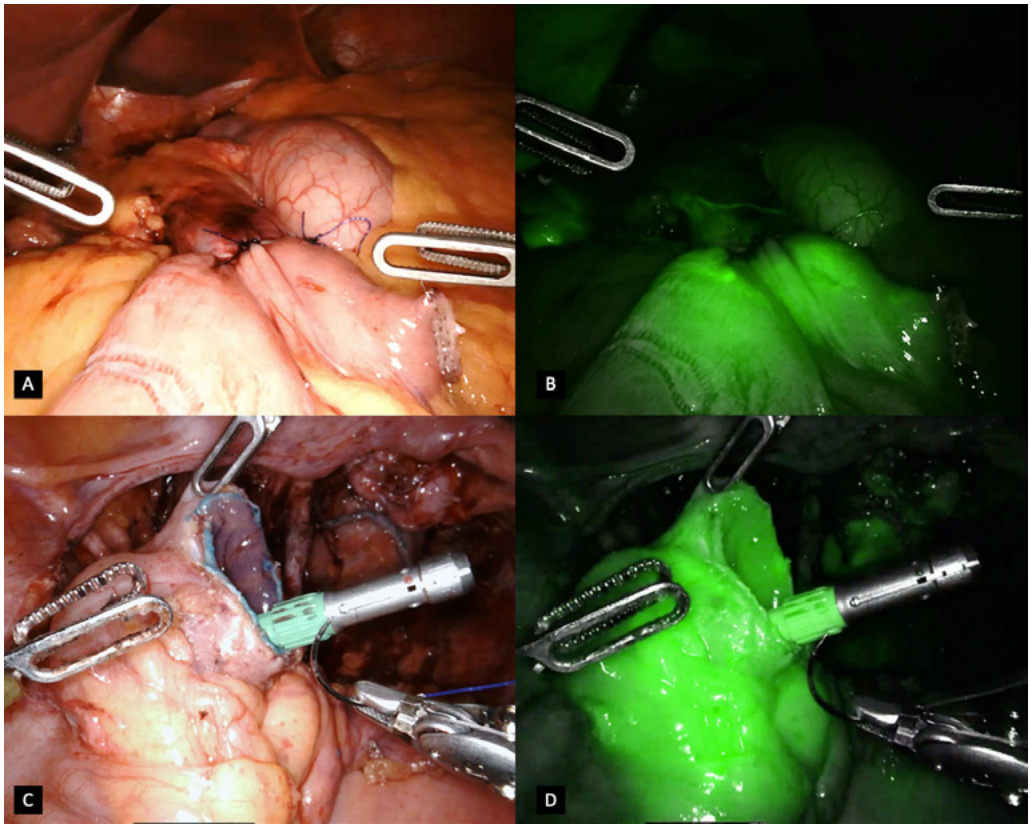

FIGURE 2. A. Spectrum of images in the usual form of gastrointestinal anastomosis. **B.** Gastrointestinal anastomosis seen with indocyanine green image guided fluorescence, with adequate tissue perfusion. **C.** Spectrum of images in the usual form of colonic segment before a colorectal anastomosis. **D.** Colon segment visualized with direct guided fluorescence imaging showing good blood supply.

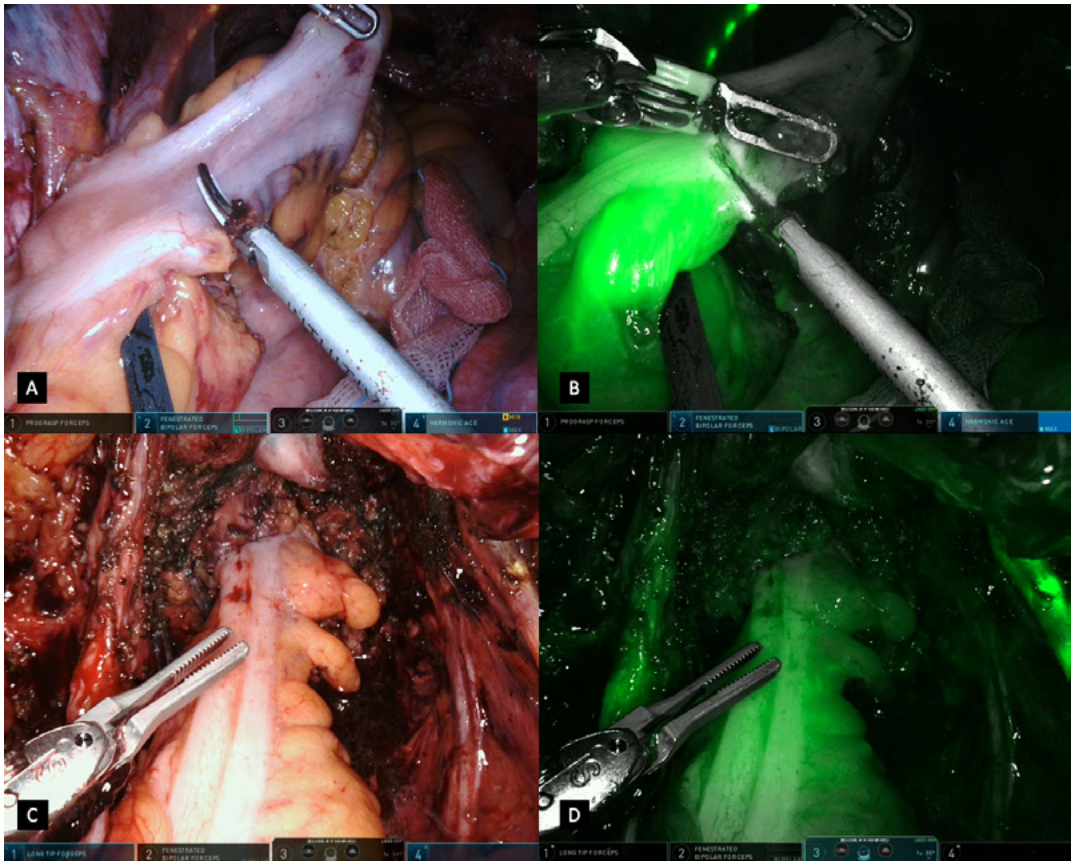

FIGURE 3. A. Bowel segment after mesenteric ligation in the usual color spectrum of the robotic endoscope. **B.** Bowel segment in real time detailed visualization of the well-perfused and ischemic segment. **C.** Colorectal anastomosis with usual visualization. **D.** Colorectal anastomosis with the Firefly™ mode activated, emitting direct fluorescence and showing good perfusion.

During the first step, proximal and distal margins should be already exposed and mesocolic or mesenteric vessels should be already dissected and ligated, assuring the most reliable bowel vascularity without further manipulation. The endoscope should be close to the area of interest, not exceeding 10 cm of distance which may affect signal detection of fluorescence and the ICG injection performed by the anesthesiologist should be coordinated and in quick flush followed by a push of 10 mL of saline. The surgeon at the console should change the optical status to the Firefly™ technology filter and wait for approximately 30 to 45 seconds for the optimal angiography signal. We recommend utilizing 7.5 mg of ICG in each step performed for a safe administration and well visualization of fluorescence. Bowel transection may be performed through the ICG endoscope filter, in real-time visualization of the enhanced green areas and not colored structures for a more accurate transection line. The second step of evaluation is achieved after completion of the anastomosis. The surgical field should be clear and the endoscope pointing directly to the structure of evaluation. A second bolus of 7.5 mg ICG intravenously should be administered according to surgeons command, waiting the same half minute period before enhancement is optimal. The third step may be concluded by inserting the endoscope into the anus using a transanal trocar and advancing to the staple line of the anastomosis, followed by a third bolus of 7.5 mg of ICG intravenously.

It's important to remember that not only colorectal anastomosis can be assessed, also possible the angiography whenever performing jejuno-jejunostomies or ileocolic anastomosis (FIGURE 4.A, B). Also, literature reports most of colectomies from oncological procedures however the tissue perfusion assessment is expanded to all others colorectal conditions, such as diverticulitis and diverticular disease, crohn and ulcerative colitis resections, deep infiltrating endometriosis and bowel affections.

Hepatobiliary surgery

Regarding tissue perfusion assessment, in liver surgery, ICG is still mainly used as a reagent for the evaluation of hepatic function. ICG elimination depends on hepatic blood flow, hepatocellular function and biliary excretion a due to pharmaco characteristics, its elimination considered as a useful dynamic test describing liver function and perfusion in the perioperative setting, in liver surgery and transplantation, as well as in critically ill patients. Also, ICG accumulates in the cancerous tissues of hepatocellular carcinoma (HCC) and in the noncancerous hepatic parenchyma around adenocarcinoma foci, which may be used to increase detection. Tumor boundaries and residual lesions are also achieved through real-time fluorescence.

Whenever using the ICG fluorescence for hepatic surgeries, some important aspects must be addressed. Liver fluorescence may be achieved from intravenous peripheral or central access administration or by a local intraoperative puncture injection such as portal vein or right gastric vessels. If ICG administration is done by peripheral or central venous access, liver fluorescence is expected in 5minutes; different from local intra abdominal injection whose liver enhancement develops rapidly within 1–2 min. A 0.25 mg/kg concentration of ICG should be injected and after administration

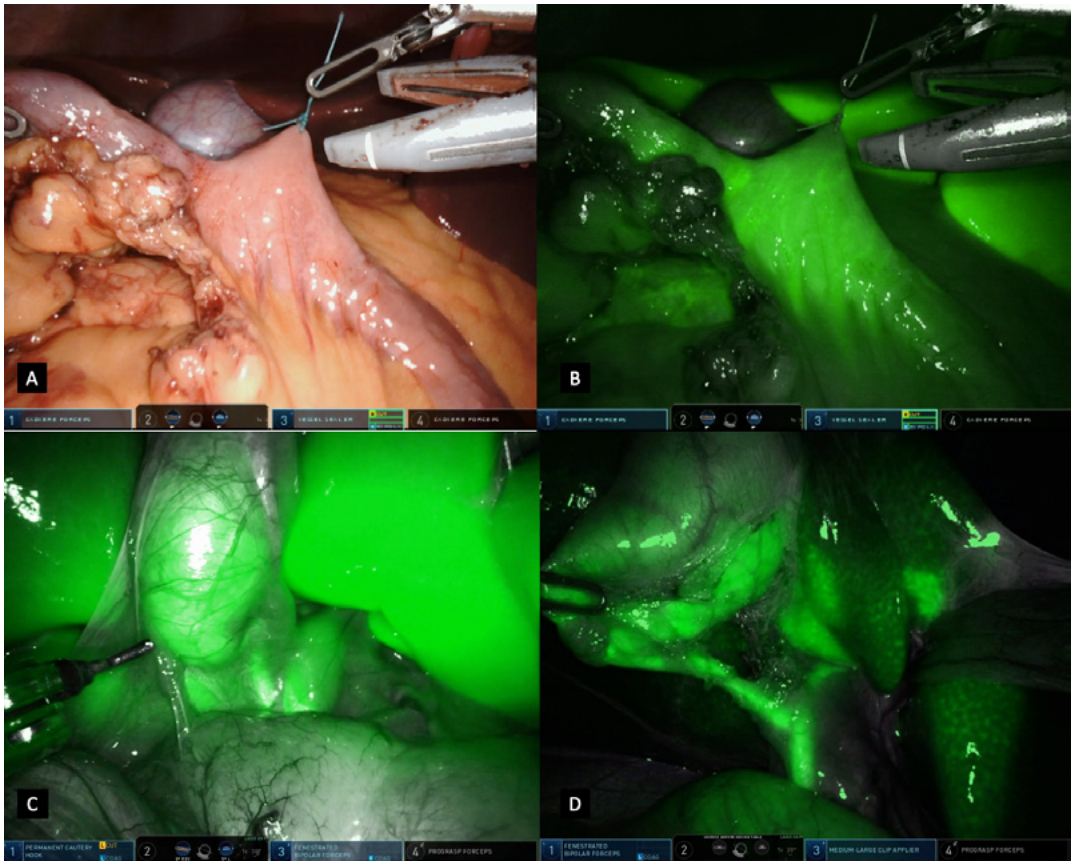

FIGURE 4. A. bowel segment before section under normal visualization. **B.** Bowel segment under direct visualization with fluorescence and tissue perfusion assessment. **C.** Biliary tree visualized with fluorescence mode made with indocyanine green injected intraoperatively. **D.** Biliary tree highlighted under fluorescence image with indocyanine green injected 4 hours prior to surgical procedure.

of the drug, tumors will appear as a shadow with a Firefly™ mode, as compared with normal liver tissues. Cirrhotic tissues are used to develop slowly and not uniform patterns of enhancement, a pitfall if not previously known.

A second manner of enhancing the visualization of lesions is by injecting the ICG the day before surgery, which will provide clearance of the substance in the normal hepatic parenchyma with residual stain in the altered tissue area. Also, for a better hepatic transection line visualization and perfusion enhancement in major hepatectomies, after clamping or ligation the portal pedicle and arterial branch, ICG administration also will enlighten the remnant liver tissue in contrast to the non well perfused.

Regarding pancreatic surgery, ICG fluorescence can be useful to identify pancreas tumors in patients undergoing pancreas resection, specifically neuroendocrine tumors and cystic neoplasms. A low dose of 2.5 mg can be injected intraoperatively; having neuroendocrine tumors enhanced with a higher fluorescence signal compared to pancreatic tissue; opposed to cystic neoplasms, which will display lesser fluorescence intensity compared to normal tissue.

Urgent surgery

ICG fluorescence angiography could be an objective and noninvasive method to assess intestinal viability to determine the extent of ischemia correctly. This application may be useful in bowel related cases, such as incarcerated or strangulated hernias, mesocolic or mesenteric dissection in adherences and obstructive diseases or even acute mesenteric ischemia. Similar to bowel perfusion assessment described previously, ICG administration by central ou peripheral catheter may help decision-making in challenging cases whenever naked eyes cannot assure optimal analysis.

ANATOMIC IDENTIFICATION

Safety during surgery has always been a matter of concern during advanced minimally invasive procedures. Identifying the correct anatomical plane and surrounding structures can be extremely demanding, requiring extensive knowledge and foresight. However, even in experienced hands, some challenging situations whereas anatomical variations, acute inflammation or adhesions may contribute to misunderstanding of the usual landmarks. Thus, fluorescence imaging enhances and helps visualization of some anatomic structures, aiming for better identification and prevention of inadvertent injuries.

Biliary anatomy

ICG fluorescence imaging to visualize and elucidate biliary anatomy represents one of the most established applications of this technology in abdominal surgery. Due to ICG excretion into bile entirely by the liver, its mechanism of enhancement became obvious. A special attention should be addressed to the time of injection when opting for the optimal biliary tree anatomy contrast during surgery. Different from what happens when aiming tissue perfusion assessment, the ICG administration should be performed at least 4 hours prior to surgery. Whenever possible, ICG injection may be achieved even 24h before the procedure. A premature administration of the dye will allow hepatic parenquima metabolize the

pigment and secrete into bile; avoiding green stains in liver tissue only accumulation of ICG inside the bile ducts (FIGURE 4.C, D). Injecting the ICG during intraoperative dissection for better biliary tree identification is one of the most important pitfalls in image guided fluorescence technology.

A 0.25 to 0.35 mg/kg dose of ICG should be administered minimum 4 hours prior to surgery, not exceeding 2 mg/kg maximum dosage, in peripheral or central venous access for the optimal bile ducts visualization. Once instruments and scope are ready, no additional injection of ICG is normally required during the procedure. Using the Firefly™ technology, the triangle of calot is easier exposed for common bile visualization as well as cystic duct whenever doing a cholecystectomy. The structures containing residual bile would be highlighted by the green stain and others structures such vascular or parenquima tissue will remain dark. If an acute cholecystitis with obliteration of the cystic duct caused by a stone is seen, the ICG fluorescence may not make the whole cystic duct and the gallbladder stained, due to inability of the dye to achieve that space. In this scenario, injection of ICG directly into the gallbladder may help elucidating the anatomy from a reverse perspective. Special care should be taken not to leak out the ICG in the surgical field, otherwise the dye will blush the non-biliary structures making impossible the optimal visualization.

Ureter identification

Intraoperative identification of the ureters can be challenging in some pelvic surgeries due to neoplastic diseases, previous surgery with distortion of natural surgical planes, inflammatory bowel conditions, deep infiltrating endometriosis or adhesions. For accurate and easier localization of ureters during minimally invasive surgery, fluorescence of ureters can be achieved through some methods.

In one possibility, a real time cystoscopy-guided, ureteral cannulation is done with 6-Fr ureteral catheter and a retrograde instillation of 5 mg ICG dye is performed preoperatively followed by 10 mL saline flush. Greenish fluorescence guided image in the ureters will occur due to ICG binding to the proteins of the ureteric epithelium (FIGURE 5.A, B). During the procedure, turning the Firefly™ mode active by the surgeon at the console will make the structure enlightened compared to the darkened tissues aside. No intraoperative or intravenous administration of ICG is necessary for this purpose. Another possibility to assure ureter identification intraoperatively is by placing lighted ureteral stents (FIGURE 5.C, D). Also through a preoperative cystoscopy-guided cannulation, both ureters may have the illuminated catheter positioned to a better identification and overcome some limitations of tactile feedback.

Leak tests

Leak tests using air or methylene blue are often performed to assess anastomosis or suture integrity in upper GI (gastrointestinal) procedures, such as bariatric gastric bypass, sleeve or oncological gastrectomies, or even urgent cases such as gastric or duodenal suture due to ulcer perforation. Similar to the methylene blue dye, however easily visible even in small amounts and fluorescent with laser excitement, the ICG has shown to be an excellent agent for leak testing through fluorescence image guided technology.

Both upper GI tract and colorectal procedures may use ICG to

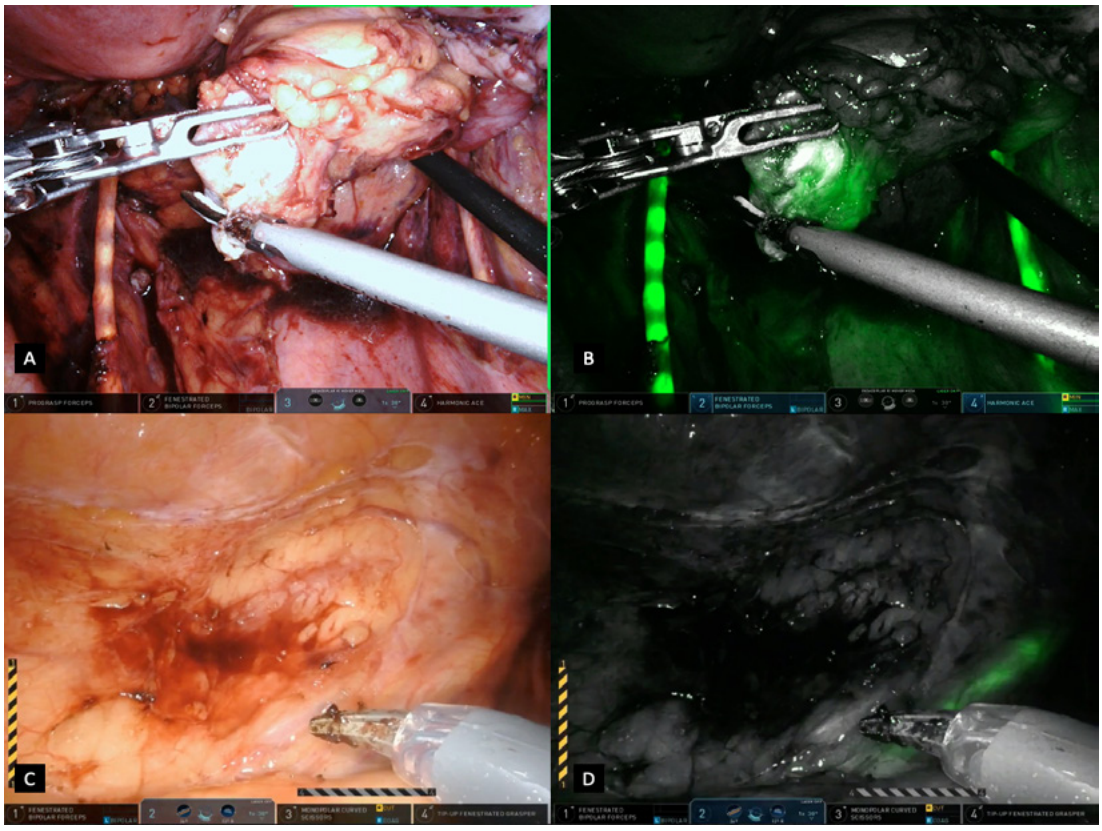

FIGURE 5. A. Ureter visualized after dissection in pelvic surgery. **B.** Bilateral ureteral enhancement through fluorescence with indocyanine green. **C.** Left ureter covered by the peritoneal flap under usual visualization. **D.** Left ureteral enhancement in real time with Firefly™ mode activation during colorectal surgery.

test anastomosis or suture integrity. By diluting the 25 mg of ICG to 250 mL of sterile water, this green stained solution is administered through a nasogastric tube or rectal catheter. Whenever performing the leak test in the upper GI tract, the nasogastric tube should be placed according to the desired landmark and distal bowel or gastric tissue need to be bluntly occluded to assure anastomosis pressure (FIGURE 6.A). After correct occlusion of the distal part, the surgeon in the console orders the anesthesiologist to administer the ICG and Firefly™ mode should be activated. Greenish leak is evident whenever present. In colorectal anastomosis, the patient should be placed in a trendelenburg position and the proximal bowel occluded bluntly by the surgeon, asking the bedside assistant to inject retrograde through the rectal stent the ICG dye, followed by activation of the Firefly™ technology.

Assessment of bile leak test is also a possibility of ICG fluorescence imaging in robotic hepatic surgery (FIGURE 6.B, C, D). By inserting a 5Fr or 6Fr soft tube into the common bile duct through the stump of the cystic, a solution of 25 mg of ICG diluted to 100 mL of sterile water is injected before liver parenchymal transection. Distal common bile duct should be clamped to avoid outflow of the dye to the duodenum and provide a higher pressure in the biliary tree. Activating the Firefly™ filter, bile leaks from the transected liver surface are able to be identified under real-time visualization with greenish enhancement of the parenquimal area. Chyle leak test through ICG administration can be also performed to obtain real-time fluorescent images of lymph flow. An ICG lymphography is achieved by subcutaneous injection of ICG into nearby lymph node regions, then the chyle flow image will be visualized in a greenish fluid intraoperatively when active in the Firefly™ technology.

Endometriosis

The diagnosis of peritoneal endometriosis during minimally invasive procedures may be difficult due to the polymorphic aspects of the lesions. The need for better visualization led to a rise in literature the use of contrast agents, such as ICG fluorescence imaging. Only few studies have reported evident improvement in detecting the endometrial tissue, although the ICG applications for a safe deep infiltrating endometriosis surgery and bowel resection is widely known[6]. Limited data is available to support the ICG fluorescence guided image to assure correct visualization of endometriosis in the peritoneal cavity.

Vascular anatomy

Vascular structures identification is one of the most important steps in every surgical procedure. By injecting the ICG solution intravenously, a real-time green fluorescence image is shown in blood vessels followed by tissue perfusion. Several abdominal surgeries may be helped in difficult decision-making dissections due to vascularity assessment. In colorectal procedures, identifying the inferior mesenteric artery (IMA) or left colic vessels during robotic rectal resection can be easily performed through ICG fluorescence technology. In upper GI tract surgeries, fluorescence can assure better identification of vascular anatomy of the infrapyloric artery in pylorus-preserving gastrectomies, or anatomy highlight of the hepatic and gastroduodenal arteries for a safe lymphadenectomy. In urologic field, selective arterial clamping with ICG fluorescence image provides an intraoperative renal angiogram so that he can selectively clamp minor arteries instead of clamping the main renal artery. Additionally, robotic assisted prostatectomies can be supplemented by ICG imaging to identify the prostatic neurovas-

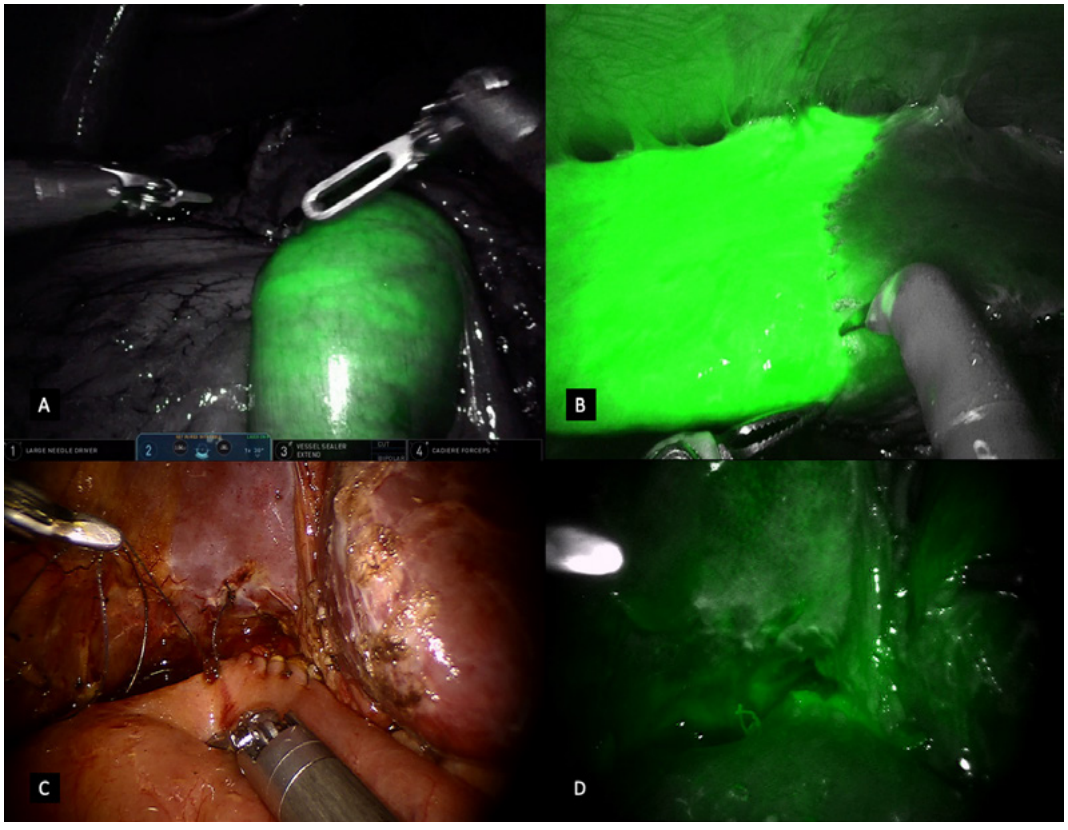

FIGURE 6. A. Leak test in gastrojejunal anastomosis with infusion of indocyanine green via endoluminal catheter. **B.** Enhancement of the well-perfused liver parenchyma in contrast to non-vascularized tissue. **C.** Biliodigestive anastomosis under habitual view. **D.** Bile leak test in hepatojejunostomy under fluorescence guided image.

cular bundles. Angiography for vascular identification is similarly performed as previously described for tissue perfusion assessment, with 7.5 mg of ICG followed by a 10 mL saline flush coordinated between surgeon and anesthesiologist.

FLUORESCENCE LYMPHOGRAPHY AND LYMPH NODE NAVIGATION

ICG has the property of lymphatic tropism and after submucosal or subserosal injection, it can follow the lymphatic vessels and accumulate in the lymph nodes. For an accurate lymphatic mapping, the dye should be administered submucosally and draining lymph nodes will be visualized intraoperatively. Whenever performing oncological procedures, guided lymphatic mapping may assure a more precise resection, staging or lymphadenectomy. A 2.5 mg/mL solution of ICG should be prepared as previously described and administered according to the area of interest. In colorectal diseases, greenish enhancement through fluorescence is achieved by a colonoscopy-guided submucosal injection of 2.5 mg of ICG solution in each four cardinal sites around the tumour the day before surgery, representing 4 mL in total. For a real-time visualization, the surgeon must turn on the Firefly™ mode for excitement of ICG molecules and standard fluorescence. Intraoperative ICG administration through subserosal injection around the lesion is also possible, however if any spill occurs the surgical field becomes blushed and fluorescence image is compromised. In rectal cancer, the lymphography may be helpful especially to detect the lymphatic drainage across the lateral lymph nodes and guide its removal. Also, in colon cancer whenever opting for a complete mesocolic excision technique, the fluorescence assessment could be useful for a safe and optimal D3 lymphadenectomy.

In the upper GI tract, an endoscopy-guided injection is also performed in 4 sites of the submucosa tissue around the gastric or esophageal lesion the day before surgery. Fluorescence guided image can be visualized as previously described and the use of ICG may offer some advantages in lymph node visualization over other modalities of dyes or radioisotopes. Under real-time lymphography, lymph node stations are accessed having a better visualization regarding disease staging as well as safety and completeness of the lymphadenectomy.

DISCUSSION

Indocyanine-green fluorescence imaging is an emerging technology and has several applications in the surgical field for information assessment and intraoperative decision-making. Current clinical practice has a broad spectrum of use and its real benefits are put in perspective more recently. In general surgery, different manners of use are reported as tissue perfusion assessment, anatomic identification and lymphography from upper GI tract to colorectal procedures[5,6,9,10]. Information like the correct dosage and dilution, the way of application whether fractionated or in bolus, and the exact time of use due to its degradation and kinetics are not evident in most of the available studies. A guided description of practical usage with well defined concepts and tricks could overcome limitations of unfamiliarity to the technology due to heterogeneity of methods reported.

The imaging of fluorescence emitted by ICG is a fast, simple, and relatively inexpensive tool without side effects. The ICG tissue angiography concept described in this report provides the surgeon a clear guide and advice on how to exactly perform an intraoperative ICG fluorescence angiography. Bringing familiarity to the dosage and practical measures, as well as pointing out possible pitfalls could help its use in training surgeons. Several studies have proven the efficacy of ICG fluorescence imaging in assessing bowel perfusion[17,18]. Uncoordinated administration or image capturing long after/or even before injecting the dye might lead to acquisition of false data causing misinterpretation of the tissue perfusion. To assess intestinal perfusion most studies suggest 2.5–10 mg of ICG depending on the body weight. A change in the transection line was performed in up to 40% of patients undergoing colorectal resections described by Hellan et al.[19]. Also, a case–control study has already reported lower rates of anastomotic leak compared to non-ICG related procedures[14].

Regarding upper GI tract, ICG has also been extended to a wide surgical field, with more concrete results in esophageal reconstruction procedures. A meta-analysis by Ladak et al.[9] recently evidenced a 69% reduction in anastomotic leaks in esophageal procedures with ICG fluorescence combined to intraoperative interventions. Due to encouraging results, fluorescence has been also extended for bariatric surgeries aiming for better outcomes. Its worldwide know that gastric bypass and sleeve gastrectomies are not free from adverse events; having the most worrying complications anastomosis and gastric leaks. Few studies in literature describes ICG use in perfusion assessment during bariatric procedures and preliminary results with clinical benefits still inconclusive[13,20].

In the hepatobiliary field, a unanimous consensus in the literature pertains to anatomic real-time visualization of the biliary tree. Comparative studies and systematic reviews are robust when describing its benefits in what is perhaps the most established ICG fluorescence guided image in recognition of anatomy used by general surgeons[21,22,23]. As previously mentioned, the optimal common bile duct fluorescence identification in cholecystectomies depends on the dosage and administration interval of ICG. It is reported to have better visualization if injected hours before the surgery than at the time of the procedure, with different patterns of contrast of the bile duct in relation to background normal liver tissue[24].

Although not an intraoperative fluorescence technology, another interesting ICG application is regarding perioperative efforts. Decades ago Hemming et al.[25] reported in cirrhotic patients that preoperative ICG elimination kinetics were independent predictors of postoperative mortality. The ICG plasma disappearance rate could be used to help decision making prior to major liver resections in order to minimize the risk of postoperative liver failure. More specific to surgical oncology hepatobiliary context, a recent meta-analysis showed that additional ICG fluorescence does decrease operative time, blood loss, hospital stay, and postoperative complications[26]. Also, some oncological cases may have benefits from ICG properties since its discovery of accumulation in the cancerous tissues of neoplastic lesions, such as hepatocellular carcinoma and in the noncancerous hepatic parenchyma around adenocarcinoma foci by Ishizawa et al.[27]. In clinical practice, improvement in detection of lesions is reported however further substantial data regarding sensibility and outcomes is still required.

In the surgical oncology field, the potential benefit associating ICG fluorescence imaging in gastrointestinal lymphography is another important topic explored in literature. Although studies demonstrate ICG as being superior to some radioactive tracers

and dyes in node navigation, its real clinical benefits are still in perspective. Herrera-Almario et al.[28] reported its application in oncological gastric resections providing real time intraoperative feedback regarding lymph node identification with a surgical time increase of less than 10 min. More recently, Cianchi et al.[29] in a matched cohort study described additional node detection during robotic surgery for gastric cancer. Despite a higher number of total lymph nodes harvested in the ICG group, its results failed to show good selectivity for metastatic occurence. In the hepato-pancreatobiliary scenario, Machado et al.[30] even described an intraoperative localization of the ampulla of Vater performed with Firefly™ fluorescence defining the superior margin of the resection in a robotic resection of the uncinate process of the pancreas. In colorectal oncology, lymphography has also been reported both in colon and rectal cancer[31]. In selective colonic cases, ICG fluorescence adoption in complete mesocolic excisions could allow a more reliable lymphadenectomy. Also, in rectal cancer surgery, extending lymphadenectomy to lateral pelvic nodes could help in reduction rates of nodal recurrence[32].

Although recent, the robotic ICG intraoperative fluorescence imaging with ICG has spread into numerous interesting applications in general surgery. Several other surgical fields are still in perspective such as fluorescent tumor-binding agents, peritoneal carcinomatosis diagnosis and even indocyanine green nanoparticles[33,34]. An important issue pertains to the costs, reproducibility and safety of this technology. The major positive feature is that training required is minimal and the learning curve is short, having a real-time visualization activated by a simple button click in the console. Costs also stand as minimal values once the robotic platform has Firefly™ technology already integrated and ICG solutions present affordable prices. In summary ICG fluorescence combined with robotic surgery has shown to be an important game changer in the surgeons armamentarium.

This report presents an overview of current ICG fluorescence imaging technological concepts and tips and tricks of its applications in robotic-assisted general surgery. Guided information and well known steps could help bring familiarity to training surgeons and make their attempts easier and more reproducible during practice. Although numerous interesting applications and encouraging outcomes, it remains to be proven whether ICG increments procedure-specific advances or real clinically substantial benefits in some yet unclear surgical fields.

CONCLUSION

Fluorescence-guided surgery is one of the most innovative examples of surgery integrated to images; which may hopefully provide better healthcare. The robotic platform integrated to ICG intraoperative fluorescence imaging has found numerous interesting applications in visceral surgery more recently, raising the hope that future studies will allow us to perform more precise surgery with less perioperative complications. Guided steps could assure an even wider spectrum of this resource use and safety in training surgeons, whose applications are vast and evidence is emerging showing another weapon in the robotic surgeon's armamentarium yielding tangible clinical benefits.

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Authors' contribution

Morrell ALG: conceived of the presented idea. Morrell AC: developed the theory and performed the computations. Mendes JM and Morrell-Junior AC: verified the analytical methods. Tustumi F and Morrell AG: supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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RESUMO – **Contexto** – A imagem fluorescente com verde de indocianina (VI) é uma técnica cirúrgica emergente na cirurgia robótica. **Objetivo** – Este artigo relata princípios e abordagens essenciais da fluorescência intraoperatória para sua prática em cirurgia geral. Nosso grupo descreve possíveis armadilhas e apresenta dicas e truques para treinar cirurgiões, tornando o uso do VI reprodutível. **Métodos** – Este estudo apresenta uma visão geral dos conceitos e aplicações práticas da imagem guiada por fluorescência com VI na cirurgia robótica. As possíveis armadilhas são enfatizadas e os campos de aplicação emergentes são colocados em perspectiva. **Resultados** – Aplicações práticas em vários campos cirúrgicos são descritas para um uso seguro e reprodutível de imagens de fluorescência com VI. **Conclusão** – A cirurgia assistida por robótica combinada à tecnologia de imagem de fluorescência representa uma evolução lógica na cirurgia guiada por imagem e a familiaridade desta técnica pode representar um ganho da qualidade cirúrgica.

DESCRITORES – Robótica. Procedimentos Cirúrgicos do sistema digestório. Verde de indocianina.

REFERENCES

1. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. J Appl Physiol. 1976;40:575-83.
2. Yannuzzi L. Indocyanine green angiography: a perspective on use in the clinical setting. Am J Ophthalmol. 2011;151:745-51.
3. Alander JT, Kaartinen I, Laakso A, Pätilä T, Spillmann T, Tuchin VV. A review of indocyanine green fluorescent imaging in surgery. Int J Biomed Imaging. 2012;2012:940585.
4. Moore GE. Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues. Science. 1947;106:130-1.
5. Boni L, Fingerhut A, Marzorati A, Rausei S, Dionigi G, Cassinotti E. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case- matched study. Surg Endosc. 2017;31:1836-40.
6. Morrell ALG, Ribeiro GMPAR, Santos TP, Chamie LP, Frare N, Serafini PC, Ribeiro DMFR. Robotic Natural Orifice Specimen Extraction with Totally Intracorporeal Anastomosis Associated with Firefly Fluorescence: Bowel Resection for Deep Infiltrating Endometriosis. Journal of Gynecologic Surgery. 2020. 128-35.
7. Bates AS, Patel VR. Applications of indocyanine green in robotic urology. J Robot Surg. 2016;10:357-9.
8. Damle A, Damle RN, Flahive JM, Schlussel AT, Davids JS, Sturrock PR, et al. Diffusion of technology: Trends in robotic-assisted colorectal surgery. Am J Surg. 2017;214:820.
9. Ladak F, Dang JT, Switzer N, Mocanu V, Tian C, Birch D, et al. Indocyanine green for the prevention of anastomotic leaks following esophagectomy: a meta-analysis. Surg Endosc. 2019;33:384-94.
10. Karampinis I, Ronellenfitsch U, Mertens C, Gerken A, Hetjens S, Post S, et al. Indocyanine green tissue angiography affects anastomotic leakage after esophagectomy. A retrospective, case-control study. Int J Surg. 2017;48: 210-4.
11. Zehetner J, DeMeester SR, Alicuben ET, Oh DS, Lipham JC, Hagen JA, et al. Intraopera- tive Assessment of Perfusion of the Gastric Graft and Correlation With Anastomotic Leaks After Esophagectomy. Ann Surg. 2015;262:74-8.
12. Kumagai Y, Hatano S, Sobajima J, Ishiguro T, Fukuchi M, Ishibashi KI, et al. Indocyanine green fluorescence angiography of the reconstructed gastric tube during esophagectomy: efficacy of the 90-second rule. Dis Esophagus. 2018;31(12). doi: 10.1093/dote/doy052.
13. Di Furia M, Romano L, Salvatorelli A, Brandolin D, Lomanto D, Cianca G, et al. Indocyanine Green Fluorescent Angiography During Laparoscopic Sleeve Gastrectomy: Preliminary Results. Obes Surg. 2019;29:3786-90.
14. Jafari MD, Wexner SD, Martz JE, McLemore EC, Margolin DA, Sherwinter DA, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. J Am Coll Surg. 2015;220:82-92.
15. Degett TH, Andersen HS, Gögenur I. Indo- cyanine green fluorescence angiography for intraoperative assessment of gastrointestinal anastomotic perfusion: a systematic review of clinical trials. Langenbecks Arch Surg. 2016 Sep;401: 767-75.
16. Ris F, Liot E, Buchs NC, Kraus R, Ismael G, Belfontali V, et al.; Near-Infrared Anastomotic Perfusion Assessment Network VOIR. Multicentre phase II trial of near-infrared imaging in elective colorectal surgery. Br J Surg. 2018;105:1359-67.
17. Morales-Conde S, Alarcón I, Yang T, et al. Fluorescence angiography with indocyanine green (ICG) to evaluate anastomosis in colorectal surgery: where does it have more value? Surg Endosc. 2020;34:3897-907. doi 10.1007/s00464-019-07159-1.
18. Shapera E, Hsiung RW. Assessment of Anastomotic Perfusion in Left-Sided Robotic Assisted Colorectal Resection by Indocyanine Green Fluorescence Angiography. Minim Invasive Surg. 2019;2019:3267217.
19. Hellan M, Spinoglio G, Pigazzi A, Lagares-Garcia JA. The influence of fluorescence imaging on the location of bowel transection during robotic left-sided colorectal surgery. Surg Endosc. 2014;28:1695-702.
20. Frattini F, Lavazza M, Mangano A, Amico F, Rausei S, Rovera F, et al. Indocyanine green-enhanced fluorescence in laparoscopic sleeve gastrectomy. Obes Surg. 2015;25:949-50.
21. Pesce A, Piccolo G, La Greca G, Puleo S. Utility of fluorescent cholangiography during laparoscopic cholecystectomy: A systematic review. World J Gastroenterol. 2015;21:7877-83.
22. Vlek SL, van Dam DA, Rubinstein SM, Lange-de Klerk ESM, Schoonmade LJ, Tuynman JB, et al. Biliary tract visualization using near-infrared imaging with indocyanine green during laparoscopic cholecystectomy: results of a systematic review. Surg Endosc. 2017;31:2731-42.
23. Machado MA, Surjan RC, Ardengh AO, Makdissi F. Robotic Left Hepatectomy and Roux-en-Y Hepaticojejunostomy After Bile Duct Injury. Ann Surg Oncol. 2019;26:2981-4.
24. Verbeek FP, Schaafsma BE, Tummers QR, van der Vorst JR, van der Made WJ, Baeten CIM, et al. Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. Surg Endosc. 2014;28:1076-82.
25. Hemming AW, Scudamore CH, Shackleton CR, Pudek M, Erb SR. Indocyanine green clearance as a predictor of successful hepatic resection in cirrhotic patients. Am J Surg. 1992;163:515-8.
26. Qi C, Zhang H, Chen Y, Su S, Wang XF, Huang XQ, et al. Effectiveness and safety of indocyanine green fluorescence imaging-guided hepatectomy for liver tumors: A systematic review and first meta-analysis. Photodiagnosis Photodyn Ther. 2019;28:346-53.
27. Ishizawa T, Fukushima N, Shibahara J, Koichi Masuda, Sumihito Tamura, Taku Aoki, et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer. 2009;115:2491-504.
28. Herrera-Almario G, Patane M, Sarkaria I, Strong VE. Initial report of near-infrared fluorescence imaging as an intraoperative adjunct for lymph node harvesting during robot-assisted laparoscopic gastrectomy. J Surg Oncol. 2016;113:768-70.
29. Cianchi F, Indennitate G, Paoli B, Ortolani M, Lami G, Manetti N, et al. The Clinical Value of Fluorescent Lymphography with Indocyanine Green During Robotic Surgery for Gastric Cancer: a Matched Cohort Study. J Gastrointest Surg. 2020;24:2197-203.1007/s11605-019-04382-y.
30. Machado MAC, Surjan R, Basseres T, Makdissi F. Robotic resection of the uncinate process of the pancreas. J Robot Surg. 2019;13:699-702.
31. Liberale G, Bohlok A, Bormans A, Bouazza F, Galdon MG, El Nakadi I, et al. Indocyanine green fluorescence imaging for sentinel lymph node detection in colorectal cancer: A systematic review. Eur J Surg Oncol. 2018;44:1301-6.
32. Nishigori N, Koyama F, Nakagawa T, Nakamura S, Ueda TU, Inoue T, et al. Visualization of Lymph/Blood Flow in Laparoscopic Colorectal Cancer Surgery by ICG Fluorescence Imaging (Lap-IGFI). Ann Surg Oncol. 2016;23 (Suppl 2):S266-S274.
33. Filippello A, Porcheron J, Klein JP, Cottier M, Barabino G. Affinity of Indocyanine Green in the Detection of Colorectal Peritoneal Carcinomatosis. Surg Innov. 2017;24:103-8.
34. Egloff-Juras C, Bezdetnaya L, Dolivet G, Lassalle HP. NIR fluorescence-guided tumor surgery: new strategies for the use of indocyanine green. Int J Nanomedicine. 2019;14:7823-38.

ORIGINAL ARTICLE

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Sphincterotomy alone versus sphincterotomy and biliary stent placement in the treatment of bile leaks: 10 year experience at a quaternary hospital

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ABSTRACT – **Background** – Hepatobiliary surgery and hepatic trauma are frequent causes of bile leaks and this feared complication can be safely managed by endoscopic retrograde cholangiopancreatography (ERCP). The approach consists of sphincterotomy alone, biliary stenting or a combination of the two but the optimal form remains unclear. **Objective** – The aim of this study is to compare sphincterotomy alone versus sphincterotomy plus biliary stent placement in the treatment of post-surgical and traumatic bile leaks. **Methods** – We retrospectively analyzed 31 patients with the final ERCP diagnosis of "bile leak". Data collected included patient demographics, etiology of the leak and the procedure details. The treatment techniques were divided into two groups: sphincterotomy alone vs. sphincterotomy plus biliary stenting. We evaluated the volume of the abdominal surgical drain before and after each procedure and the number of days needed until cessation of drainage post ERCP. **Results** – A total of 31 patients (18 men and 3 women; mean age, 51 years) with bile leaks were evaluated. Laparoscopic cholecystectomy was the etiology of the leak in 14 (45%) cases, followed by conventional cholecystectomy in 9 (29%) patients, hepatic trauma in 5 (16%) patients, and hepatectomy secondary to neoplasia in 3 (9.7%) patients. The most frequent location of the leaks was the cystic duct stump with 12 (38.6%) cases, followed by hepatic common duct in 10 (32%) cases, common bile duct in 7 (22%) cases and the liver bed in 2 (6.5%) cases. 71% of the patients were treated with sphincterotomy plus biliary stenting, and 29% with sphincterotomy alone. There was significant difference between the volume drained before and after both procedures ($P<0.05$). However, when comparing sphincterotomy alone and sphincterotomy plus biliary stenting, regarding the volume drained and the days needed to cessation of drainage, there was no statistical difference in both cases ($P>0.005$). **Conclusion** – ERCP remains the first line treatment for bile leaks with no difference between sphincterotomy alone vs sphincterotomy plus stent placement.

HEADINGS – Fístula biliar. Sphincterotomy. Stents. Endoscopic retrograde cholangiopancreatography. Cholecystectomy.

INTRODUCTION

Bile leaks are a potentially serious complication that can occur after cholecystectomy, liver transplantation, partial hepatectomy, and hepatic damage secondary to abdominal trauma[1,2]. Although post cholecystectomy surgery is responsible for most of biliary injuries, it occurs with an incidence of only 1%[3,4]. In hepatic resections and liver transplantations the incidence of bile duct injury ranges from 2% to 25%[3]. Regarding hepatic trauma, the non-operative management of hemodynamically stable patients has become the standard practice in most trauma centers achieving 85% success rate[5,6].

Conventional cholecystectomy has been replaced with laparoscopic cholecystectomy in the treatment of gallstones over the past three decades[7,8]. The laparoscopic approach is safe, effective and allows early discharge from the hospital as well as a faster recovery and better cosmetic results[7,9,10]. Despite the advantages of the laparoscopic approach this procedure is also related with higher rates of iatrogenic biliary injury (about 1% of all laparoscopic x 0.2% of conventional cholecystectomy)[7,9]. The principal causes of these injuries are the inadequate clipping of the cystic duct, misidentification of the anatomy, leaks from the gall bladder bed and thermal strictures[7].

Despite the improvements in perioperative treatment, hepatobiliary surgery is still associated with a high level of morbidity, especially after liver resection with morbidity higher than 20% and bile leaks occurring in approximately 7% of these procedures[11]. Unlike post cholecystectomy bile leaks, hepatobiliary surgery associated bile leaks can occur in different locations such as the anastomosis, or in the cut surface of the liver[3].

Bile leaks secondary to blunt or penetrating hepatic trauma are also a common and potentially a severe complication. The non-operative management of stable patients as well as the "damage control" techniques for unstable patients has been improving survival even in severe extensive hepatic parenchymal damage. As a result of the improved survival, bile leaks have become a frequent secondary complication[5].

Endoscopic retrograde cholangiopancreatography (ERCP)

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is the standard method used to diagnose bile leaks (BL), with the capacity to determine the location of the leak in more than 95% of the cases[3,12,13]. An important advantage of ERCP is the possibility of diagnosing and treating the leak in the same procedure. The goal of the endoscopic treatment is to eliminate the transpapillary pressure by performing a sphincterotomy, placing a biliary stent or the combination of both, lowering the pressure on the bile duct which allows healing the leak[1,3]. Large and refractory bile leaks are often treated with multiple plastic stents or self-expandable metal stents (SEMS), and usually stents are removed after 4 to 8 weeks[2].

The aims of this study are to determine efficacy of endoscopic treatment of bile leaks secondary to cholecystectomy, hepatectomies, and hepatic trauma and to compare the currently employed ERCP approaches to biliary leaks: endoscopic sphincterotomy alone and sphincterotomy plus biliary stenting, regarding the number of days needed to remove the abdominal surgical drain or cessation of drainage after each technique.

METHODS

This study was approved by the Ethics and Research Committee of *Hospital Santa Marcelina*, São Paulo. *Hospital Santa Marcelina* is a quaternary teaching hospital, trauma and cancer referral center, with a high number of surgeries performed per year. We retrospectively analyzed all ERCPs performed in the hospital between the years of 2009 and 2019. All procedures were performed by an advanced endoscopy fellow under the supervision of one experienced interventional endoscopist. Deep sedation, or general anesthesia were used according to the patient status. More than 1200 cases were analyzed and all patients who had a previous ERCP were excluded. From 1200 cases analyzed, we found 31 cases with a final diagnosis of "Bile Leak", and these were included in this study. Data collected included age, gender, etiology, and localization of the leak, primary catheterization, biliary dilatation, necessity of reoperation for suture the leak after the ERCP, and finally if the endoscopy treatment was therapeutic.

Etiology of the leaks were divided into four groups: conventional cholecystectomy, laparoscopic cholecystectomy, hepatectomy due to neoplasia and hepatic trauma. The locations of the leaks were divided in four major groups: cystic duct stump, common bile duct, hepatic common duct, and hepatic bed.

Our aim was to compare sphincterotomy alone with sphincterotomy plus stent placement. The decision to perform sphincterotomy alone or sphincterotomy plus stent placement was made by the endoscopist. In all stent cases, plastic stents were used measuring 8.5 or 10 Fr according to availability. Therapeutic success was determined when there was no need for further surgery or radiological interventions after the ERCP was performed.

Before initiating data analysis, we applied Shapiro-Wilk test to verify the normality of the data (95% CI). After verification of normality we applied the non-parametric test.

Data was analyzed by using the IBM-SPSS software version 22 for Windows. All the tests were realized with statistical significance of 5% (P values ≤0.05). To compare the volume drained before and after each technique (Stent x Sphincterotomy), were applied Wilcoxon tests for selected variables and the U de Mann-Whitney test for independent variable, both with 95% CI. To analyze days until the drain has been solved after each treatment the U de Mann-Whitney were applied with 95% CI.

RESULTS

A total of 31 patients undergoing ERCP between 2009 and 2019 with the final diagnosis of "bile leak" were analyzed. Amongst the 31 patients, 18 (58%) were men and 13 (42%) were women. The mean age was 51.5 years (range 24–87). The clinical variables are seen in TABLE 1. Laparoscopic cholecystectomy was the etiology of the leak in 14 of the 31 (45%) cases, followed by conventional cholecystectomy with 9 (29%) cases, hepatic trauma with 5 (16%) cases and hepatectomy with 3 (9.7%) cases. Primary catheterization of the papilla was possible in 24 (77%), while in the remaining 7 (22%) cases fistulotomy (infundibulotomy) was successfully performed.

TABLE 1. Clinic variables.

Variables	n	%
Etiology of the leak		
Conventional cholecystectomy	9	29.0%
Laparoscopic cholecystectomy	14	45.2%
Partial hepatectomy due to neoplasia	3	9.7%
Hepatic trauma	5	16.1%
Location of the leak		
Cystic duct stump	12	38.6%
Common bile duct	7	22.6%
Common hepatic duct	10	32.3%
Hepatic bed	2	6.5%
Primary catheterization		
No	7	22.6%
Yes	24	77.4%
Biliary dilatation		
No	23	74.2%
Yes	8	25.8%
Reoperation for suture the leak		
No	30	96.8%
Yes	2	6.5%
Endoscopy was therapeutic?		
No	2	6.5%
Yes	29	93.5%

The most frequent location of the leaks were the cystic duct stump with 12 (38.6%) cases, followed by hepatic common duct with 10 (32%) cases, common bile duct with 7 (22%) cases and the liver bed with 2 (6.5%) cases. The post laparoscopic cholecystectomy most frequent leak localization was the cystic duct stump (42% of the cases), followed by common bile duct (35%), hepatic common duct (14%) and hepatic bed (7%). Regarding conventional cholecystectomy, the most frequent localization was the cystic duct stump (66%), followed by common bile duct (22%), and hepatic bed (11%). Interestingly there were no cases of hepatic common duct leaks in conventional cholecystectomy cases. In all cases secondary to hepatectomy (three) and hepatic trauma (five) the localization of the leak was the common hepatic duct.

Of the 31 patients, 23 (74%) did not have biliary dilatation and 8 (26%) had. Only two of the 31 cases needed to have surgery after the ERCP to suture the leak (6.5%) and the procedure was not considered therapeutic. Both were from common hepatic leaks. In the first case the guidewire progressed only to the site of leak and

stent placement could not be placed. In the other case, although sphincterotomy and stenting were successfully realized, it was not enough to treat the leak. In both cases further surgery was necessary.

Considering adverse events related to the procedure, from the 31 cases, 1 (3.2%) had mild pancreatitis, with good resolution. There was one case in which ERCP was not therapeutic and evolved with abdominal sepsis after the procedure, with good resolution after antibiotics and surgery. There were no cases of perforation, bleeding, or death related to the procedure.

Regarding the treatment used, 71% of the patients were treated with sphincterotomy plus stent placement, and 29% with papillotomy alone (or fistulotomy when necessary). Data including volume of the abdominal surgical drainage before and after each procedure (measured 48 h after ERCP) are shown at TABLE 2. Comparative analysis between the volume of drainage before and after each technique can be seen in FIGURES 1 and 2, respectively. We also evaluated the days until the drain was removed or cessation of drainage occurred, after ERCP as seen at TABLE 3. There was significant difference between the volume drained before and after both procedures (P<0.05). However, when compared sphincterotomy alone and sphincterotomy plus stent placement there was no statistical difference between them (P>0.05). In sphincterotomy alone group the average drained volume before ERCP was 211±102 and after 35±41 (P=0.018) while in stent group was 356±217 and 98.67±106.21 (P=0.001) respectively. FIGURE 3 shows comparative analysis regarding days needed for cessation of drainage or removal of the abdominal surgical drain after each treatment. Analyzing both techniques as the days needed to remove the abdominal surgical drain or cessation of drainage after ERCP, there was no statistical difference (P>0.05). In stent group the average were 8 days, while in sphincterotomy alone group 11 days were needed until the drain could be removed.

TABLE 2. Comparative analysis between the volume of the surgical abdominal drain before and after each treatment.

Drained volume	Stent placement	Sphincterotomy alone	*P*-value[B]
Drained volume before ERCP	356.33±217.78	211.43±102.34	0.078
Drained volume after ERCP	98.67±106.21	35.71±41.07	0.298
P-value[A]	0.001**	0.018**	

ERCP: endoscopic retrograde cholangiopancreatography. A: Wilcoxon test (95% confidence). B: Mann-Whitney U test (95% confidence). **Statistical significance (95% confidence).

FIGURE 1. Volume of surgical drain before endoscopic retrograde cholangiopancreatography (ERCP) (in milliliters).

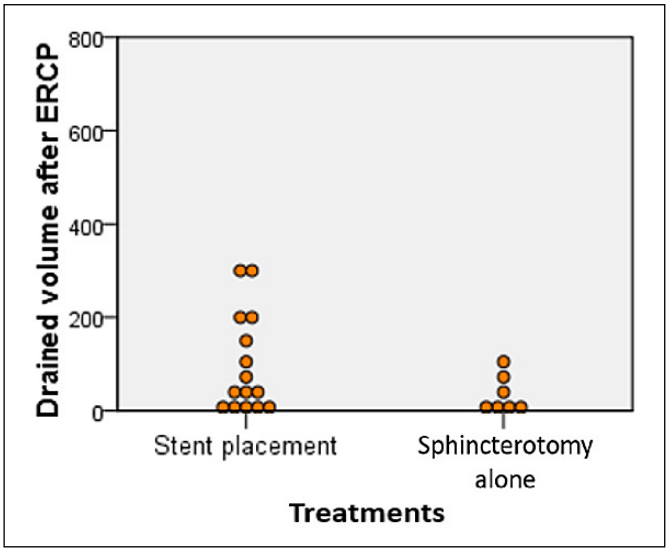

FIGURE 2. Volume of surgical drain after endoscopic retrograde cholangiopancreatography (ERCP) (in milliliters).

TABLE 3. Analysis of variation regarding days needed for cessation of surgical drainage after each treatment.

Days needed for cessation of drainage	Average	Standard deviation	*P*-value*
Stent placement	8	6	0.764
Sphincterotomy alone	11	16	

* Mann-Whitney U test (95% confidence).

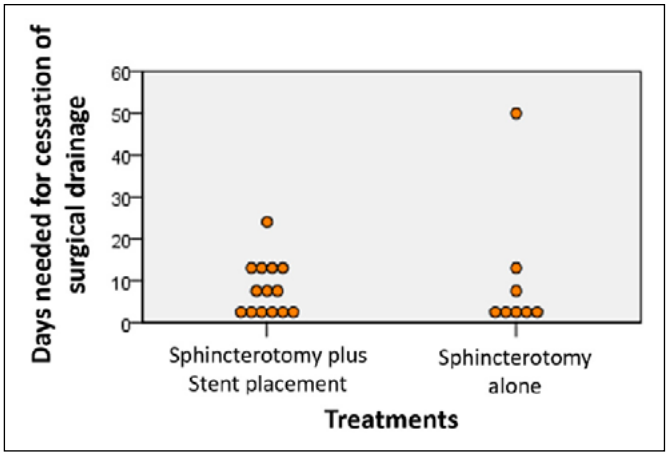

FIGURE 3. Days needed for cessation of surgical drainage.

DISCUSSION

Bile leaks are a concerning complication after hepatobiliary surgery. Less commonly, abdominal trauma with hepatic damage can also be the etiology of bile leaks. Cholecystectomy is one of

the most common types of abdominal surgery and bile leaks post cholecystectomy can result in increased morbidity, mortality, hospital expenditures and a decreased quality of a patient´s life[14]. Other frequent causes of bile leaks include biliopancreatic surgery, liver transplants, hepatectomies and hepatic trauma[12]. ERCP is the standard diagnostic and treatment modality for bile leaks[15]. They are associated with less morbidity than surgery and are a good alternative to radiological interventions. The mechanism of resolution of the leakage is lowering the transpapillary pressure, with a sphincterotomy, biliary stent placement or combination of both[16]. ERCP will lead to a decrease in the flow resistance of bile duct facilitating leak sealing. Although there is no need to seal the leak orifice with the stent, stent placement is based on the preference of the endoscopist and there is no consensus on whether this is beneficial.

The grade of the bile leak is an important concern when considering ERCP techniques as the treatment. Sandha et al.[13] reviewed 207 patients with bile leak diagnostic that undergone open or laparoscopic cholecystectomy. The leaks were classified as: low grade (leak identified only after intrahepatic opacification) and high grade (leak observed before intrahepatic opacification). In the same study was showed that sphincterotomy alone is a safe and effective treatment for low grade leaks and regarding high grade leaks the recommendation remains biliary stenting[13,17]. However, this is not a consensus. Dolay et al.[18] concluded that biliary stenting seems to be a more effective method than sphincterotomy in post cholecystectomy bile leaks without CBD dilatation[12,18]. Kaffes et al. analysis indicates that stent insertion, may be superior to sphincterotomy alone in patients with post cholecystectomy bile leak, independent of the size of the stent[19]. FIGURE 4 shows an ERCP performed after a laparoscopic cholecystectomy with a bile leak from common bile duct. After 8 weeks of biliary stenting, the procedure was repeated with total seal of the leak, as shown in FIGURE 5 with no extravasation of the contrast.

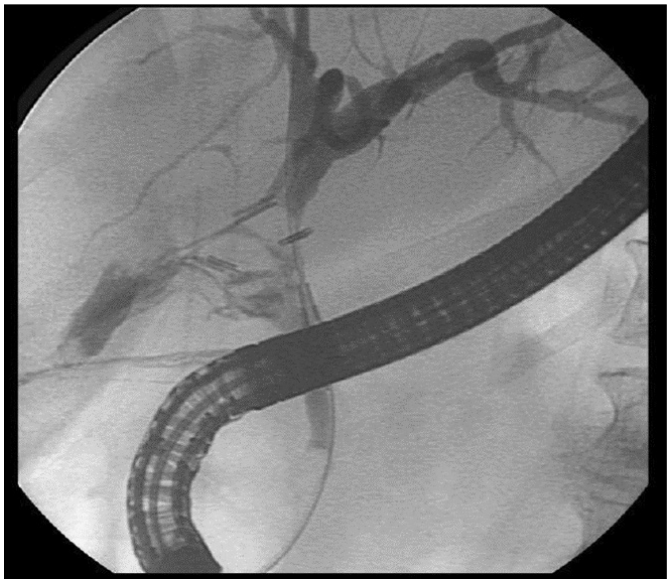

FIGURE 4. Post Laparoscopic Cholecystectomy leak: it´s possible to observe extravasation of contrast from common bile duct (courtesy of Dr Everson L.A. Artifon).

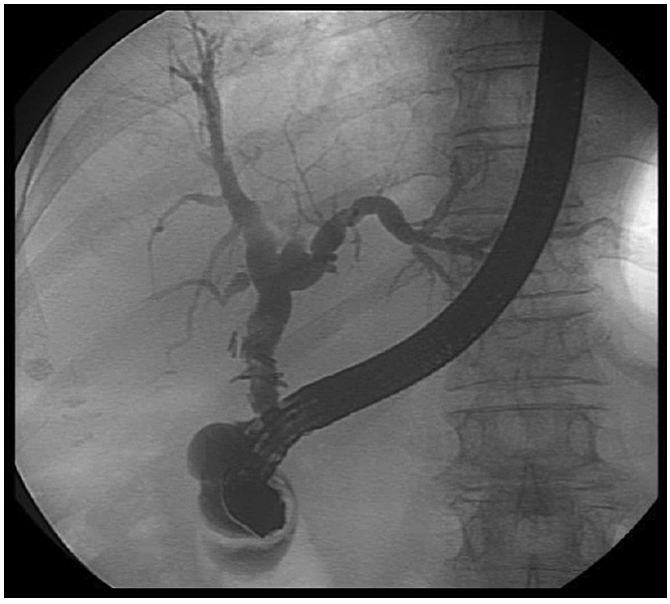

FIGURE 5. Same case from figure 7: Cholangiogram after 8 weeks at stent removal(courtesy of Dr Everson L.A. Artifon).

The liver and spleen are the most common organs injured after abdominal trauma[20]. 85–89% of hemodynamically stable patients with liver injury from blunt abdominal trauma can be managed safely without surgery[5,6]. The first concern in patients with hepatic trauma is hemorrhage, and stabilizing patients hemodynamically is critical. If this is not possible, surgery is most likely indicated. Conservative treatment includes arteriography and selective embolization, computed tomography guided drainage of collections and ERCP with sphincterotomy and biliary stent placement[5,20]. Recent studies have shown that ERCP is an effective treatment for bile leaks secondary to traumatic liver injuries, with more than 80% success rate[21]. In all five cases of bile leak secondary to hepatic trauma ERCP was successfully used in treatment with a decrease in abdominal surgical drainage volume after the procedure, and cessation of the drainage in 1–7 (minimum 1 and maximum 7) days, concluding that in our casuistic ERCP was effective and safe to treat bile leaks after hepatic complex trauma.

Despite the technical advances in hepatic surgery, bile leaks remain a feared complication of these surgeries. In Martin et al. study nearly 8% of all liver resections developed bile leak after hepatectomy and when bile duct reconstruction was present, this rate importantly increased to 29%[11]. ERCP success rates after liver resections are lower than in post cholecystectomy (75% versus 70 to 100% respectively), and other techniques as Rendezvous may be necessary. In our study we analyzed three hepatectomies secondary to due to neoplasia, and all the procedures were concluded with sphincterotomy. Two of the three cases were successfully treated with ERCP and resolution of the leak. In the third case, despite ERCP with sphincterotomy and stent placement was concluded, the patient had a major bile leak from common hepatic duct, and the ERCP was not capable to solve the leak, with necessity of further surgery.

When comparing the overall success between sphincterotomy and sphincterotomy plus stent placement there was no statistical difference. We compared the volume of the abdominal drain before and after ERCP, with a significant amount of drainage after both

procedures, with no difference in drainage between them. Similarly, there was not a significant difference in the number of days needed to remove the abdominal drain between the two techniques.

Self-expandable metal stents (SEMS) are another option used in the treatment of bile leaks. Because of its larger diameter, they may more effectively divert bile away from the site of the leak[3]. In Wang et al. study the use of SEMS was analyzed in the treatment of complex bile leaks after cholecystectomy or liver transplantation, showed efficacy at resolving bile leaks, however associated with ulcerations, choledocholithiasis and stenosis[22].

Our study, as a retrospective single center analysis, has its limitations, there may be a selection bias on witch cases the stent was placed, limiting the comparisons the can be made on this study between the two approaches on the treatment of biliary leaks. The chosen technique was decided by the senior endoscopist during the procedure according to operator experience. Stent placement without a sphincterotomy should be considered, principally in young patients, due to the risk of late development of bile duct cancer after sphincterotomy. Hakamada et al. showed in his study a 7.4% prevalence of cholangiocarcinoma among 108 cases of sphincterotomy at intervals 1 to 20 years and considered the chronic cholangitis as the probably causative factor of this development, suggesting that these patients should be closely monitored[23]. Another reason to consider stenting without sphincterotomy in young patients, is because this is considered a group of risk for post-ERCP pancreatitis (PEP)[24]. FIGURE 6 shows an example of biliary stenting without sphincterotomy, with success and good drainage of bile.

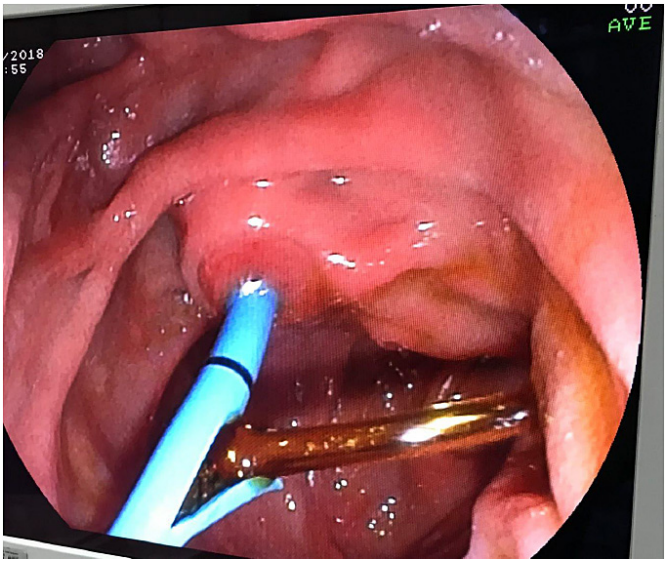

FIGURE 6. Suggestion of treatment for bile leaks in young patients: Stenting without sphincterotomy may prevent late biliary cancer development.

Despite the limitations presented, our study analyzed the different causes of bile leaks, comparing their treatments and results, different from the most other studies that analyzed just one etiology of bile leaks. A prospective study would take much longer to perform and will likely need to include multiple centers. We considered the success of ERCPs when it could be used to resolve the leak, without any further surgery or intervention. Among the 31 ERCPs performed, two were not successful procedures. In the first unsuccessful procedure, ERCP was not technically possible because it was a major bile leak, and in the second unsuccessful procedure, despite the successful ERCP the patient needed further interventions to resolve the leak. The two unsuccessful cases were common hepatic duct leaks, reinforcing studies that show that the location of the leak predicts the success of the ERCP[3,25]. Tewani et al. suggests that ERCP is 3.3 times more likely to be successful in bile leaks in the cystic duct stump or duct of Luschka, than in other locations[3].

CONCLUSION

In conclusion, we suggest the treatment of bile leaks with ERCP as the standard of care, independent of the etiology of the bile leak. Leaks from the cystic duct stump are more likely to resolve with ERCP. We conclude that both sphincterotomy and stent placement are highly effective in the resolution of the leaks with no significant difference in outcomes. We also conclude that leaks of the common hepatic duct are less likely to achieve success with endoscopic procedures. In summary, ERCP remains the first line treatment in the diagnosis and treatment of bile leaks, and both sphincterotomy alone and biliary sphincterotomy with biliary stent placement are safe and effective treatments.

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Authors' contribution

Flumignan VK: conceptualization, methodology, project administration, writing-original draft, and writing-review and editing. Sachdev AH: writing-review and editing. Nunes JPS, Silva PF, Pires LHB, Andreoti MM: data curation.

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Flumignan VK, Sachdev AH, Nunes JPS, Silva PF, Pires LHB, Andreoti MM. Esfincterotomia isolada versus esfincterotomia associada a passagem de prótese biliar no tratamento de fístulas biliares: 10 anos de experiência de um hospital quaternário. Arq Gastroenterol. 2021;58(1):71-6.

RESUMO – **Contexto** – Cirurgia hepatobiliar e trauma hepático são causas frequentes de fístulas biliares, e esta temida complicação pode ser manejada de forma segura através da colangiopancreatografia retrógrada endoscópica (CPRE). O procedimento consiste em esfincterotomia isolada, passagem de prótese biliar ou combinação das duas técnicas, porém a forma ideal permanece incerta. **Objetivo** – O objetivo desse estudo é comparar a realização de esfincterotomia isolada versus locação de prótese biliar no tratamento de fístulas pós-cirúrgicas e traumáticas. **Métodos** – Foram analisados de forma retrospectiva 31 CPREs com diagnóstico final de "fístula biliar". A informação colhida incluía dados demográficos dos pacientes, etiologia das fístulas e detalhes dos procedimentos. As técnicas de tratamentos foram divididas em dois grupos: esfincterotomia isolada vs esfincterotomia associada a locação de prótese biliar. Foram analisados os volumes dos drenos abdominais cirúrgicos antes e depois de cada procedimento e o número de dias necessários para que ocorresse cessação da drenagem pelo dreno abdominal cirúrgico após a CPRE. **Resultados** – Um total de 31 pacientes (18 homens e 3 mulheres; idade média de 51 anos) com fístulas biliares foram avaliados. Colecistectomia laparoscópica foi a etiologia da fístula em 14 (45%) casos, seguida de colecistectomia convenvional em 9 (29%) pacientes, trauma hepático em 5 (16%) pacientes, e hepatectomia secundária a neoplasia em 3 (9,7%) pacientes. As localizações mais frequentes das fístulas foram: coto do ducto císticos com 12 (38,6%) casos, seguido de ducto hepático comum em 10 (32%) casos, ducto colédoco em 7 (22%) cases e leito hepático em 2 (6,5%) casos. 71% dos pacientes foram tratados com esfincterotomia associada a passagem de prótese biliar e 29% com esfincterotomia isolada. Houve diferença estatística em relação ao volume drenado antes e depois de ambos os procedimentos (P<0,05). Entretanto, quando comparada esfincterotomia isolada e esfincterotomia associada a passagem de prótese biliar, em relação ao volume drenado e ao número de dias necessários para cessação da drenagem, não houve diferença estatística em ambos os casos (P>0,005). **Conclusão** – A CPRE permanece como tratamento de primeira linha no tratamento de fístulas biliares, sem diferença entra a esfincterotomia isolada versus esfincterotomia associada a passagem de prótese biliar.

DESCRITORES – Fístula biliar. Esfincterotomia. Stents. Colangiopancreatografia retrógrada endoscópica. Colecistectomia.

REFERENCES

1. Adler DG, Papachristou GI, Taylor LJ, McVay T, Birch M, Francis G, et al. Clinical outcomes in patients with bile leaks treated via ERCP with regard to the timing of ERCP: a large multicenter study. Gastrointest Endosc. 2017;85:766-72. doi:10.1016/j.gie.2016.08.018
2. Vlaemynck K, Lahousse L, Vanlander A, Piessevaux H, Hindryckx P. Endoscopic management of biliary leaks: a systematic review with meta-analysis. Endoscopy. 2019;51:1074-81. doi:10.1055/a-0835-5940
3. Tewani SK, Turner BG, Chuttani R, Pleskow DK, Sawhney MS. Location of bile leak predicts the success of ERCP performed for postoperative bile leaks. Gastrointest Endosc. 2013;77:601-8. doi:10.1016/j.gie.2012.11.026
4. Abbas A, Sethi S, Brady P, Taunk P. Endoscopic management of postcholecystectomy biliary leak: When and how? A nationwide study. Gastrointest Endosc. 2019;90:233-241.e1. doi:10.1016/j.gie.2019.03.1173
5. Lubezky N, Konikoff FM, Rosin D, Carmon E, Kluger Y, Ben-Haim M. Endoscopic sphincterotomy and temporary internal stenting for bile leaks following complex hepatic trauma. Br J Surg. 2006;93:78-81. doi:10.1002/bjs.5195
6. Croce MA, Fabian TC, Menke PG, et al. Nonoperative management of blunt hepatic trauma is the treatment of choice for hemodynamically stable patients. Results of a prospective trial. Ann Surg. 1995;221:744-55. doi:10.1097/00000658-199506000-00013
7. Sharma H, Bird G. Endoscopic management of postcholecystectomy biliary leaks. Frontline Gastroenterol. 2011;2:230-3. doi:10.1136/flgastro-2011-100031
8. Cohen JT, Charpentier KP, Beard RE. An Update on Iatrogenic Biliary Injuries: Identification, Classification, and Management. Surg Clin North Am. 2019;99:283-99. doi:10.1016/j.suc.2018.11.006
9. Rustagi T, Aslanian HR. Endoscopic management of biliary leaks after laparoscopic cholecystectomy. J Clin Gastroenterol. 2014;48:674-8. doi:10.1097/MCG.0000000000000044
10. Bergman JJ, van den Brink GR, Rauws EA, et al. Treatment of bile duct lesions after laparoscopic cholecystectomy. Gut. 1996;38:141-7. doi:10.1136/gut.38.1.141
11. Martin AN, Narayanan S, Turrentine FE, et al. Clinical Factors and Postoperative Impact of Bile Leak After Liver Resection. J Gastrointest Surg. 2018;22:661-7. doi:10.1007/s11605-017-3650-4.
12. Rainio M, Lindström O, Udd M, Haapamäki C, Nordin A, Kylänpää L. Endoscopic Therapy of Biliary Injury After Cholecystectomy. Dig Dis Sci. 2018;63:474-80. doi: 10.1007/s10620-017-4768-7.
13. Sandha GS, Bourke MJ, Haber GB, Kortan PP. Endoscopic therapy for bile leak based on a new classification: results in 207 patients. Gastrointest Endosc. 2004;60:567-74. doi:10.1016/s0016-5107(04)01892-9
14. Pandit N, Yadav TN, Awale L, Deo KB, Dhakal Y, Adhikary S. Current Scenario of Postcholecystectomy Bile Leak and Bile Duct Injury at a Tertiary Care Referral Centre of Nepal. Minim Invasive Surg. 2020;2020:4382307. doi:10.1155/2020/4382307
15. Fasoulas K, Zavos C, Chatzimavroudis G, Trakateli C, Vasiliadis T, Ioannidis A, et al. Eleven-year experience on the endoscopic treatment of post-cholecystectomy bile leaks. Ann Gastroenterol. 2011;24:200-5.
16. Yun SU, Cheon YK, Shim CS, Lee TY, Yu HM, Chung HA, et al. The outcome of endoscopic management of bile leakage after hepatobiliary surgery. Korean J Intern Med. 2017;32:79-84. doi:10.3904/kjim.2015.165
17. Ghazanfar S, Qureshi S, Leghari A, Taj MA, Niaz SK, Quraishy MS. Endoscopic management of post operative bile duct injuries. J Pak Med Assoc. 2012;62:257-62.
18. Dolay K, Soylu A, Aygun E. The role of ERCP in the management of bile leakage: endoscopic sphincterotomy versus biliary stenting. J Laparoendosc Adv Surg Tech A. 2010;20:455-9. doi:10.1089/lap.2009.0308
19. Kaffes AJ, Hourigan L, De Luca N, Byth K, Williams SJ, Bourke MJ. Impact of endoscopic intervention in 100 patients with suspected postcholecystectomy bile leak. Gastrointest Endosc. 2005;61:269-75. doi:10.1016/s0016-5107(04)02468-x
20. Brillantino A, Iacobellis F, Festa P, Mottola A, Acampora C, Corvino F, et al. Non-Operative Management of Blunt Liver Trauma: Safety, Efficacy and Complications of a Standardized Treatment Protocol. Bull Emerg Trauma. 2019;7:49-54. doi:10.29252/beat-070107
21. Khamaysi I, Suissa A, Yassin K, Gralnek IM. Traumatic bile leak in war-injured Syrians: five patients treated by ERCP. Endoscopy. 2015;47 (Suppl 1 UCTN):E426-E427. doi:10.1055/s-0034-1392656.
22. Wang AY, Ellen K, Berg CL, Schmitt TM, Kahaleh M. Fully covered self-expandable metallic stents in the management of complex biliary leaks: preliminary data - a case series. Endoscopy. 2009;41(9):781-786. doi:10.1055/s-0029-1215050
23. Hakamada K, Sasaki M, Endoh M, Itoh T, Morita T, Konn M. Late development of bile duct cancer after sphincteroplasty: a ten- to twenty-two-year follow-up study. Surgery. 1997;121:488-92. doi:10.1016/s0039-6060(97)90101-x
24. Rustagi T, Jamidar PA. Endoscopic retrograde cholangiopancreatography (ERCP)-related adverse events: post-ERCP pancreatitis. Gastrointest Endosc Clin N Am. 2015;25:107-21. doi:10.1016/j.giec.2014.09.006.
25. Yabe S, Kato H, Mizukawa S, Akimoto Y, Uchida D, Seki H, et al. Predictive factors for outcomes of patients undergoing endoscopic therapy for bile leak after hepatobiliary surgery. Dig Endosc. 2017;29:353-61. doi:10.1111/den.12798.

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Prevalence and time of development of systemic arterial hypertension in patients after liver transplantation

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ABSTRACT – **Background** – The use of immunosuppressive drugs after liver transplantation (LT) is associated with the development of systemic arterial hypertension (SAH), in addition to other comorbidities of metabolic syndrome. **Objective** – Therefore, the purpose of this study was to analyze the time after use immunosuppressive drugs the patient progresses to SAH, as well as to identify its prevalence and the factors that may be correlated to it. **Methods** – A retrospective and longitudinal study was conducted, based on the analysis of medical records of 72 normotensive patients, attended in the transplant unit of a university hospital, between 2016 and 2019. **Results** – It was observed, on average, 9±6.98 months after immunosuppressive use, the patients were diagnosed with hypertension, and the prevalence of transplanted patients who evolved to SAH in this study was 59.64% (41 patients). In addition, there was a correlation between serum dosage of tacrolimus and the development of SAH (P=0.0067), which shows that tacrolimus has a significant role in the development of SAH. Finally, it was noticed that the development of post-transplantation hypertension indicates a higher risk of the patient presenting the other parameters of metabolic syndrome, as well as a higher impairment in its renal function (P=0.0061). **Conclusion** – This study shows that the patients evolved to SAH in an average of 9±6.98 months after immunosuppressive drug use. We have also found high prevalence of systemic arterial hypertension (59.64%) in patients after liver transplantation, who used calcineurin inhibitors, especially when associated with the use of tacrolimus.

HEADINGS – Liver transplant. Hypertension. Prevalence. Immunosuppressive agents, adverse effects. Tacrolimus.

INTRODUCTION

The first attempt of liver transplants was performed by Starzl et al. in 1963 in the United States, and in Brazil, this type of transplantation has only occurred for the first time in 1985[1]. Initially, however, the survival of patients in the first year was low, only after the discovery of the immunosuppressive drug, cyclosporine, there has been a change in the scenario[2]. Currently, the main etiologies of liver diseases that lead to liver transplantation are cirrhosis related to viral hepatitis (HCV, HBV), alcohol, non-alcoholic fatty liver disease and chronic hepatitis C[3]. Even with recent advances in surgical techniques and immunosuppression therapies, recent studies[4,5] have shown that patients who have undergone liver transplants tend to have a higher risk of having metabolic syndrome, which include obesity, dyslipidemia, systemic arterial hypertension and hyperglycemia[4].

Systemic arterial hypertension (SAH) manifested after liver transplantation is associated with the use of isolated or associated immunosuppressants, such as calcineurin inhibitors (cyclosporine and tacrolimus), corticosteroids, mTOR (mammalian target of rapamycin inhibitors), in addition to other factors such as alteration of renal function and steatosis[6,7]. Calcineurin inhibitors cause widespread arterial vasoconstriction and this promotes sodium reabsorption and, consequently, higher volume of water, which results in increased volemia and thus leads to increased blood pressure[8] Thereby, systemic arterial hypertension is a complication in liver transplants recipients and can have severe influences on quality of life and even on morbidity and mortality of individuals[6,8].

This study, therefore, aimed to verify the prevalence of SAH in patients after liver transplantation, in a university hospital, as well as to analyze the factors that could be associated with the presence of hypertension, such as, immunosuppressive drugs and metabolic syndrome.

METHODS

It concerns a retrospective, longitudinal, cohort study based on the analysis of 213 medical records of patients undergoing liver transplantation in a university hospital from 2015 to 2018. The Research Ethics Committee of FAMERP approved the research project

Were excluded from the research those patients who had previous systemic arterial hypertension or who died within the first five months after transplantation, temporal cutout performed for analysis of the immunosuppressive action. Patients with systolic blood pressure >140 mmHg and/or diastolic blood pressure >90

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mmHg and/or in antihypertensive use were considered hypertensive. Of the 213 records checked, 141 were excluded because they had previous SAH or died within the first five months after the transplant. Thus, for this research, were studied 72 patients who underwent liver transplantation and met the inclusion criteria. These patients were divided into two groups: Group A (n=41) which included patients who developed systemic arterial hypertension after transplantation and Group B (n=31) which included patients who did not develop systemic arterial hypertension after transplantation.

Demographic and clinical data were analyzed such as age, gender, Child-Pugh (C-P) classification, Model for End-stage Liver Disease, transplant indication. In addition, the prevalence of systemic arterial hypertension after liver transplantation was verified, as well as the time after transplantation in which SAH developed and the classification according to degree.

Furthermore, body weight variation on the day of admission for the surgery and the body weight 5 months after transplantation were analyzed to verify if there was a statistical difference. The immunosuppressants studied were those prescribed in the first months after transplantation. It was also evaluated the development of diabetes mellitus and the dosage of patients' creatinine at three different times (1 day after transplant, in addition 3 months and 6 months after transplantation).

The information obtained was inserted into Excel spreadsheet. All statistical analyses were performed with a significance level = 0.05. The Mann-Whitney test was used to compare both groups since the data were not parametric, and Spearman's Linear Correlation was used for the correlations.

RESULTS

Demographic and clinical data of the patients studied are presented in TABLE 1.

TABLE 1. Demographic and clinical data.

Variables	Groups	N	Mean and SD	%	*P* value
Age	A	41	55.02±10.51		0.3871
	B	31	52.03±10.40		
Gender	A	41		Male – n= (75.61%)	0.9185
	B	31		Male – n=23 (74.20%)	
C-P	A	41		A – n=9 (21.95%) B – n=13 (31.70%) C – n=19 (46.34%)	0.3750
	B	31		A – n=5 (16.12%) B – n=8 (25.80%) C – 18 (58.06%)	
MELD	A	41	23.78±4.43	MELD > 20 n=28 (68.29%)	0.8556
	B	31	23.54±4.02	MELD > 20 n=23 (74.19%)	
Etiologies	A	41		Cirrhosis ALD – n=9 (21.95%) Cirrhosis ALD + HCC + VHC – n=6 (4.63%) Cirrhosis ALD + HCC – n=4 (9.75%) Cirrhosis ALD + VHC – n=3 (7.31%) Cryptogenic cirrhosis – n=3 (7.31%) NASH cirrhosis – n=3 (7.31%) Cirrhosis ALD + HCC + VBH – n = 2 (4.31%) Cirrhosis VHC+ HCC – n=2 (4.31%) Autoimmune cirrhosis – n=2 (4.31%) Fulminant hepatitis – n=2 (4.31%) Others – n=5 (12.19%)	
	B	31		Cirrhosis ALD – n=9 (29.03%) Cirrhosis VHC + HCC – n=4 (12.90%) Hemochromatosis cirrhosis – N=9 (9.67%) Cryptogenic cirrhosis – n=2 (6.45%) Cirrhosis AIH + HPS – n=2 (6.45%) NASH Cirrhosis – n=2 (6.45%) Primary sclerosing cholangitis – n=2 (6.45%) Others – n=7 (22.58%)	–

SD: standard deviation; MELD: Model for End-stage Liver Disease; ALD: alcoholic liver disease; HCC: hepatocellular carcinoma; VHC: viral hepatitis cirrhosis; VBH: virus B hepatitis; AIH: autoimmune hepatitis; HPS: hepatopulmonary syndrome; C-P: Child-Pugh.

The presence of systemic arterial hypertension was observed, on average, 9±6.98 months after the use of immunosuppressive drugs, and the prevalence of SAH was 59.64% (41 patients). In addition, there was no statistical difference between the ages of the groups analyzed (*P*=0.3871), as well as, there were no differences between the other clinical parameters, allowing to observe that the use of immunosuppressive drugs was the predominant factor for the development of comorbidity analysed.

The mean blood pressure measured in patients who developed SAH was systolic blood pressure of 149±10.88 mmHg and diastolic blood pressure 92±8.82 mmHg. 63.41% (26) of the patients who developed SAH, were already diagnosed in stage I of systemic arterial hypertension, while 36.59% were diagnosed in more advanced stages, as shown in TABLE 2.

TABLE 2. Arterial pressure classification.

Classification	%
Hypertension stage I	63.41 % (n=26)
Hypertension stage II	29.26 % (n=12)
Hypertension stage III	7.31 % (n=3)

According to TABLES 3 and 4, it can be verified that there was a statistical difference between the doses of Tacrolimo prescribed when compared to the groups that developed SAH and those that did not develop SAH, as well as, we can observe the correlation between the dosage of Tacrolimo and the development of SAH.

TABLE 3. Difference between groups in relation to the type of immunosuppressive drugs used.

Medications	Groups	Mean ± SD dosage	*P* value
Tracolimo	A – n=35	6.65±2.60	**0.0067**
	B – n=27	4.96±2.18	
Mycophenolate	A – n=35	871±204	0.3022
	B – n=29	733±289	
Cyclosporine	A – n=3	258±100	–
	B – n=0	0	
Everolimo	A – n=6	2.58±1.23	0.4555
	B – n=4	2±0.70	
Azathioprine	A – n=6	58.33±18.63	–
	B – n=0	0	

SD: standard deviation. *P*<0.05 = statistically significant difference.

TABLE 4. Correlation between immunosuppressant dosage and the presence of post-transplant hypertension.

Medications	Groups	*P* value	R value
Tacrolimo	A – n=35	**0.0050**	**0.0067**
	B – n=27		
Mycophenolate	A – n=35	0.2784	0.1322
	B – n=29		
Cyclosporine	A – n=3	–	–
	B – n=0		
Everolimo	A – n=6	0.4711	0.2583
	B – n=4		
Azathioprine	A – n=6	–	–
	B – n=0		

P<0.05 = statistically significant difference.

Furthermore, statistical analyses were performed regarding patients' body weight variation and presence of diabetes, as well as the evolution of creatinine in order to analyze renal injury due to hypertension, as shown in TABLE 5.

TABLE 5. Comparisons of different variables between groups.

Variables	Groups	Mean and sd	*P* value
Body weight variation	A – n=41	3.65±9.98	**0.0459**
	B – n=31	-1.61±9.34	
Diabetes	A – n=41	nine pretransplant patients, eight post-transplant patients	–
	B – n=31	six pretransplant patients, three post-transplant patients	
Creatinine – month 0	A – n=41	1.36±0.9	0.4529
	B – n=31	1.22±0.74	
Creatinine – month 6	A – n=41	1.325±0.79	**0.0061**
	B – n=31	1.27±1.45	
Creatinine – month 12	A – n=41	1.26±0.55	0.1085
	B – n=31	1.22±0.88	

SD: standard deviation. *P*<0.05 = statistically significant difference.

DISCUSSION

Few studies have demonstrated the time when patients were diagnosed with systemic arterial hypertension

they only show that the earlier the recognition, prevention and treatment, the better the impact on the patient's survival[9]. Thus, it was observed in this study that after 9±6.98 months of transplantation and onset of the immunosuppressant, the patients were diagnosed and that 63.41% were in stage I of SAH; 29.26% in stage II and 7.31% in stage III.

In addition, from the data obtained, it is observed that 59.64% of patients acquired systemic arterial hypertension after liver transplantation, a value three times higher than what is expected in the general population[10,11], but within the values found in other studies, such as Aparicio LS et al.[12], which observed that the rates of systemic arterial hypertension after liver transplantation were 50–80%.

The development of systemic arterial hypertension in the transplanted patient is associated with the use of immunosuppressive drugs, and many studies relate it to the use of cyclosporine and tacrolimus, which are calcineurin-inhibiting drugs[12-14], since they cause endothelial dysfunction and compromises the vasodilator response, besides producing vasoconstrictor substances and activating the renin angiotensin aldosterone system. Cyclosporine is the immunosuppressive drugs which is most associated with systemic arterial hypertension[15,16] when compared to tacrolimus. According to the article by Canzanello VJ et al.[17], only 33% of patients who used tacrolimus developed SAH, against those who used cyclosporine, in which 82% of patients became hypertensive. However, we observed that tacrolimus has a great correlation with systemic arterial hypertension, as 86% of transplanted patients used this drug and among them, 56% evolved to systemic arterial hypertension.

Although there are data that correlate mTOR (mammalian target of rapamycin inhibitors) with systemic arterial hypertension, this was not observed in our study, since the number of patients who used this drug was very small (Di Stefano C et al.[7]).

According to the research by Pérez MJ et al.[6], patients with immunosuppressive drugs use and presence of metabolic syndrome may evolve to renal failure due to reduced glomerular filtration and microalbuminuria. One of the biomarkers used for renal evaluation is the serum creatinine dosage. Therefore, this study analyzed the creatinine dosage in three moments. The first evaluation was the creatinine dosage soon after liver transplantation, in which there was no statistical difference between the groups, since both were slightly above the reference value to renal injury, which is common to occur after surgery[18]. The second evaluation occurred 6 months after the transplantation and statistical difference between the groups can be seen, inasmuch as Group A (systemic arterial hypertension group) presented higher values, when compared to Group B, this fact is in agreement with those of Pérez MJ et al.[6], which reports that the presence of metabolic syndrome due to the use of immunosuppressant increased the risk of developing renal failure. The third evaluation, in turn, showed no statistical difference between the groups, which may be associated with the onset of systemic arterial hypertension treatment and adequacy of immunosuppressive drugs levels.

Another statistical difference found between the groups was in relation to body weight variation, in which systemic arterial hypertension patients had an increase in their weight, while non-hypertensive patients had a decrease in body mass, this may be due to the fact that hypertensive patients have a higher risk for the other parameters of metabolic syndrome[19]. This higher probability of presenting the other metabolic syndrome topics can be observed in the fact that 9 hypertensive patients also had diabetes mellitus, while with the non-hypertensive patients only 3 developed diabetes mellitus.

CONCLUSION

This study shows that patients evolved to SAH on average 9±6.98 months after using the immunosuppressant. We also found a high prevalence of systemic arterial hypertension (59.64%) in patients post liver transplantation, who used calcineurin inhibitors, and that he use of tacrolimus has a great influence on the development of this disease, which is little evidenced in other studies.

Moreover, it has also been proved that the development of systemic arterial hypertension after transplantation indicates a greater risk for the patient to present the other parameters of metabolic syndrome as well as to evolve to kidney problems, what aggravate the morbidity and mortality of these individuals.

Authors' contribution

Lemos BO: conceptualization, data collection, formal analysis and writing of the manuscript. Silva RCMA: conceptualization, formal analysis and writing of the manuscript. Silva RF: conceptualization, supervision and writing of the manuscript.

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Lemos BO, Silva RCMA, Silva RF. Prevalência e tempo de desenvolvimento da hipertensão arterial sistêmica em pacientes após transplante de fígado. Arq Gastroenterol. 2021;58(1):77-81.

RESUMO – **Contexto** – O uso de imunossupressores pós-transplante de fígado (TF) está associado ao desenvolvimento de hipertensão arterial sistêmica (HAS), além de outras alterações da síndrome metabólica. **Objetivo** – Sendo assim, o objetivo deste estudo foi analisar a partir de quando tempo após o uso do imunossupressor o paciente evolui para HAS, assim como, identificar a sua prevalência e outros fatores que podem estar relacionados, como injuria renal. **Métodos** – Realizou-se um estudo retrospectivo, longitudinal, baseado em análise de 72 prontuários de pacientes, atendidos na unidade de transplante de um hospital universitário, que não apresentavam hipertensão arterial prévia, entre período de 2016 a 2019. **Resultados** – Observou-se que, em média, 9±6,98 meses após uso do imunossupressor, os pacientes foram diagnosticados com hipertensão arterial sistêmica, sendo que a prevalência de pacientes transplantados que evoluíram para HAS, neste estudo, foi de 59,64% (41 pacientes). Além disso, verificou-se uma correlação entre a dosagem sérica de tacrolimus e o desenvolvimento de HAS (P=0,0067), o que evidencia que o tacrolimus tem uma atuação significativa no desenvolvimento da hipertensão arterial sistêmica. Por fim, percebeu-se que o desenvolvimento de HAS pós-transplante indica um maior risco de paciente apresentar os outros parâmetros da síndrome metabólica, como também maior prejuízo na sua função renal (P=0,0061). **Conclusão** – Este estudo mostra que os pacientes evoluíram para HAS em média 9±6,98 meses após o início do uso do imunossupressor. Verificou-se também alta prevalência de hipertensão arterial sistêmica (59,64%) em pacientes pós-transplante de fígado, que usavam inibidores de calcineurina, principalmente, quando associado ao uso de tacrolimus.

DESCRITORES – Transplante de fígado. Hipertensão. Prevalência. Imunossupressores, efeitos adversos. Tacrolimo.

REFERENCES

1. Mies S. Liver Transplantation. Rev. Assoc. Med. Bras., São Paulo, v. 44, n. 2, p. 127-134, June 1998. doi: 10.1590/s0104-42301998000200011.
2. Starzl TE, Klintmalm GBG, Porter KA, Iwatsuki S, Schroter GP. Liver transplantation with use of cyclosporin-A and prednisone. N Engl J Med. 1981;305:266-9.
3. Chagas AL, Felga GEG, Diniz MA, Silva RF, Mattos AA, Silva RCMA, et al. Hepatocellular carcinoma recurrence after liver transplantation in a Brazilian multicenter study: clinical profile and prognostic factors of survival. Eur J Gastroenterol Hepatol. 2019;31:1148-56. doi: 10.1097/MEG.0000000000001448.
4. Watt KDS, Charton MR. Metabolic syndrome and liver transplantation: A review and guide to management. J Hepatol. 2010;53:199-206. doi: 10.1016/j.jhep.2010.01.040.
5. Zheng J, Wang WL. Risk factors of metabolic syndrome after liver transplantation. Hepatobiliary Pancreat Dis Int. 2015;14:582-7. doi: 10.1016/s1499-3872(15)60037-6.
6. Pérez MJ, Grande RG, Gusmán EO, Trillo VA, López JMR. Metabolic complications in liver transplant recipients. World J Gastroenterol. 2016;22:6416-23. doi: 10.3748/wjg.v22.i28.6416.
7. Di Stefano C, Vanni E, Mirabella S, Younes R, Boano V, Mosso E. Risk factors for arterial hypertension after liver transplantation. J Am Soc Hypertens. 2018;12:220-9. doi: 10.1016/j.jash.2018.01.002.
8. Luca L, Westbrook R, Tsochatzis EA. Metabolic and cardiovascular complications in the liver transplant recipient. Ann Gastroenterol. 2015;28:183-92.
9. Watt KD, Charlton MR. Metabolic syndrome and lver transplantation: a review and guide to management, J. Hepatol. 2010;53:199-206. doi 10.1016/i.jhep.2010.01.040
10. Passos VMA; Assis TD; Barreto SM. Arterial hypertension in Brazil: prevalence estimate from population-based studies. Epidemiol. Serv. Saúde, Brasília. 2006;15(1):35-45. doi: 10.5123/S1679-49742006000100003.
11. Lobo LAC, Canuto R, Dias da Costa JS, Pattussi MT. [Temporal trend in the prevalence of systemic arterial hypertension in Brazil]. [Article in Portuguese]. Cad. Saúde Pública. 2017;33:e00035316. doi:10.1590/0102-311X00035316
12. Aparicio L, Alfie J, Barochiner J, Cuffaro P, Rada M, Morales M, Galarza C, Waisman G. Hypertension: The Neglected Complication of Transplantation. ISRN Hypertension. 2013. doi: 10.5402/2013/165937.
13. Friedrich C. Luft, How calcineurin inhibitors cause hypertension, Nephrology Dialysis Transplantation. 2012;27:473-5. doi: 10.1093/ndt/gfr679
14. Textor SC, Taler SJ, Canzanello VJ, Schwartz L, Augustine JE. Posttransplantation Hypertension Related to Calcineurin Inhibitors Liver Transpl. 2000; 6:521-30.
15. Canzanello VJ, Textor SC, Taler SJ, Schwartz LL, Porayko MK, Wiesner RH, Krom RA. Late hypertension after liver transplantation: a comparison of cyclosporine and tacrolimus (FK 506). Liver Transpl. 1998;4:328-34.
16. Gojowy D, Adamczak M, Dudzicz S, Gazda M, Karkoszka H, Wiecek A. High Frequency of arterial hypertension in patients after liver transplantation. Transplant Proc. 2016;48:1721-4. doi: 10.1016/j.transproceed.2015.11.043.
17. Canzanello VJ, Schwartz L, Taler SJ, Textor SC, Wiesner RH, Porayko MK, Krom RA. Evolution of cardiovascular risk after liver transplantation: a comparison of cyclosporine A and tacrolimus (FK506). Liver Transpl Surg. 1997;3:1-9.
18. Ersoy Z, Ozdemirkan A, Zeyneloglu P, Pirat A, Torgay Adnam, Haneral M. Incidence of acute kidney injury following liver transplantation. Transplatation; 2018;102:S855. doi 10.1097/01.tp.0000543927.74859.54
19. Laish I, Braun M, Mor E, Sulkes J, Harif Y, Ben Ari Z. Metabolic syndrome in liver transplant recipients: prevalence, risk factors, and association with cardiovascular events. Liver Transpl. 2011;17:15-22. doi: 10.1002/lt.22198.

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Results of immunohistochemistry in the differential diagnosis of early hepatocellular carcinoma and nodules with high-grade dysplasia in patients with cirrhosis

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ABSTRACT – **Background** – Hepatocellular carcinoma (HCC) is the most frequent primary cancer of the liver and cirrhosis is considered a pre-malignant disease. In this context, the evolutionary sequence from low grade dysplastic nodule and high grade dysplastic nodule (HGDN) to early HCC and advanced HCC has been studied. The differential diagnosis between HGDN and early HCC is still a challenge, especially in needle biopsies **Objective** – To evaluate an immunohistochemistry panel to differentiate dysplastic nodules and HCC. **Methods** – Patients with cirrhosis who underwent surgical resection or liver transplantation were included. The sensitivity, specificity and accuracy for the diagnosis of neoplasia were analyzed by evaluating five markers: heat shock protein 70, glypican 3, glutamine synthetase, clathrin heavy chain and beta-catenin. $P \leq 0.05$ was considered statistically significant. **Results** – One hundred and fifty-six nodules were included; of these, 57 were HCC, 14 HGDN, 18 low grade dysplastic nodules and 67 regenerative macronodules. Sensitivity of HCC diagnosis was 64.9% for glypican 3 and 77.2% for glutamine syntetase, while specificity was 96.0% and 96.0% respectively. When the panel of four markers was considered (excluding beta catenin), the specificity ranged from 87.9% for one positive marker to 100% for at least three markers. The best accuracy for HCC diagnosis was obtained with at least two positive markers, which was associated with a sensitivity of 82.5% and specificity of 99%. **Conclusion** – Differential diagnosis of dysplastic nodules and HCC by morphological criteria can be challenging. Immunomarkers are useful and should be used for the differential diagnosis between HCC and HGDN.

HEADINGS – Hepatocellular carcinoma. Glutamate synthase. Glypicans.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer and the second major cause of death among malignant neoplasms. Moreover, there is a projection of increased prevalence of this neoplasia in the next 10 years worldwide[1-4]. Liver cirrhosis is considered a pre-malignant disease, with a risk of developing HCC[3]. In this regard, the following evolutionary sequences have been demonstrated: low grade dysplastic nodule (LGDN), high grade dysplastic nodule (HGDN), early HCC and advanced HCC[5-9]. The differential diagnosis between HGDN and early HCC has been the subject of several studies. Histological differentiation by morphological analysis alone is not possible most of the time, especially in needle biopsies[7,10,11].

Di Tommaso et al.[12], demonstrated the validity of heat shock protein 70 (HSP70), glypican 3 (GPC3) and glutamine synthetase (GS) as immunohistochemical markers in this setting. Using the three markers' panel the positivity for at least two of the three markers, regardless of which one resulted in a sensitivity of 72% and a specificity of 100% for the diagnosis of early HCC. When a fourth immunohistochemical marker, clathrin heavy chain (CHC), was added to the panel, there was an increase in sensitivity and diagnostic accuracy of this neoplasm[13]. A prospective study carried out subsequently validated the role of the immunomarker panel, however, the panel only slightly increases the diagnostic accuracy in an expert setting[14]. More recently, Uthamalingam et al. evaluating a population in a non-western country, failed to confirm these results, mainly in patients without cirrhosis, and showed low sensitivity for routine diagnosis of HCC[15].

Another promising immunohistochemical marker in the identification of HCC is the anti-beta catenin antibody. The mutation in beta catenin exon three has been detected in this neoplasm and in the adenoma with a risk of malignant transformation[16,17]. Furthermore, current data suggest that mutations

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predicted to activate the beta catenin pathway were associated with maintenance of tumor initiating cells, tumor progression, metastasis and drug resistance, especially innate resistance to immune checkpoint blockade[18,19].

Therefore, the aim of the present study was to evaluate the sensitivity, specificity and accuracy of the GS, GPC3, HSP70 and CHC markers and to study the influence of beta catenin, added to the panel of four markers, in the diagnosis of HCC.

METHODS

Consecutive patients with hepatic cirrhosis who underwent surgical resection or liver transplantation were studied at the *Hospital Irmandade Santa Casa de Misericórdia de Porto Alegre* (ISCMPA), a tertiary hospital in Southern Brazil. The diagnosis of HCC, prior to the procedure, was performed through imaging according to established criteria or by a liver biopsy[20].

Surgical specimens and explanted livers were fixed in a 10% formalin solution and subjected first to macroscopic analysis. Macro-nodule was defined when the size or texture of the nodule differs from the background cirrhotic nodules, when reaching 5 mm or more[21]. Subsequently, the macro-nodules were designated, embedded in paraffin, sectioned and stained with hematoxylin and eosin and classified into regenerative macro-nodules (RMN), LGDN, HGDN, early HCC and advanced HCC[22]. HCCs were identified according to the Japanese classifications of histologically as well, moderately or poorly differentiated[23]. The gold standard to define HCC, was histopathology, mainly presence of stromal invasion and the loss of reticulin framework. Other findings are the grade of nuclear atypia, high nuclear to cytoplasmic ratio and architectural atypia[9]. Patients with HCC beyond Milan criteria that were awaiting liver transplantation undergoing transarterial chemoembolization (TACE) as a bridge to transplant. Nodules with 100% necrosis were excluded.

To perform the immunohistochemistry, the blocks were sectioned in thicknesses of three microns, dewaxed and rehydrated. The Reveal HPR System, SPRING® Kit was used to detect proteins: anti-beta catenin (E247) at a dilution of 1/200 (ABCAM®), anti-GS at a dilution of 1/400 (ABCAM®), anti-HSP70 at a dilution of 1/300 (ABCAM®), anti-CHC at a 1/1000 dilution (ABCAM®) and anti-GPC3 (1G12) at a 1/400 dilution (ABCAM®). Antigenic recovery was performed with sodium citrate (pH 6.0) for 40 minutes. Endogenous peroxidase activity was blocked using two baths of 10-minute hydrogen peroxide (H_2O_2) 30 volumes, at 5% volumes in methanol. Blocking of nonspecific activities was performed with a 1% bovine albumin serum for one hour. Incubation with the primary antibodies was performed overnight at 4°C. Incubation with secondary antibodies was performed for 30 minutes at room temperature. For negative control of the technique, the same tissues were used incubated with the same antibodies, except the primary one which was replaced by a 1% BSA (bovine serum albumin). The antigen-antibody binding was visualized with the chromogen DAB (diaminobenzidine). Counter staining was done with Harris hematoxylin; The slides were dehydrated and mounted with a synthetic resin. Cases were considered positive when at least 5% of cells showed staining and were classified according to the intensity (weak, moderate or accentuated) and its classification as focal or diffuse.

For statistical analysis SPSS software (StatisticalPackage for Social Sciences) version 17.0 was used. Quantitative variables were described using mean and standard deviation (symmetric distribution) or median (asymmetric distribution). The sensitivity, specificity and accuracy for the diagnosis of HCC were analyzed by first evaluating the five markers (GS, GPC3, HSP70, CHC and beta-catenin) and then the four markers (excluding beta catenin). The value of $P \leq 0.05$ was considered statistically significant.

Informed consent was obtained from each patient included in the study and the study protocol is in accordance of ethical guidelines from the National Health Council of the Ministry of Health (Brazil- Resolution 466/2012) and the 1975 Declaration of Helsinki. The study was approved by the ISCMPA Research Committee.

RESULTS

Fifty-one patients were included. Seventeen of these underwent liver resection and 34 were submitted to orthotopic liver transplantation. Thirty-six patients (70.6%) were male. The mean age of the patients was 59.7 and the median was 64.0 (ranging from 42 to 75 years).

One hundred and fifty-six nodules were evaluated after the exclusion of two nodules due to complete necrosis. Patients submitted to surgical resection had a single nodule, all classified as HCC, with a diameter varying from 1.0 cm to 3.2 cm with a median of 1.9 cm. Patients submitted to liver transplantation had a mean number of nodules per patient of 3.18 and median 2.0 (ranging from 1 to 6 nodules) with a diameter varying from 0.7 cm to 4.0 cm with a median of 2.0 cm. Of these, 40 were HCC, 14 HDGN, 18 LGDN and 67 RMN.

Regarding HCC, histological classification identified 22 nodules with well differentiated HCC and 35 with moderately differentiated / poorly differentiated HCC.

Individual sensitivity in cases of HCC diagnosis was 18.5% for beta catenin, 45.6% for HSP70, 61.4% for CHC, 64.9% for GPC3 and 77.2% for GS. Positive cases of HCC with the most important markers are shown in FIGURE 1.

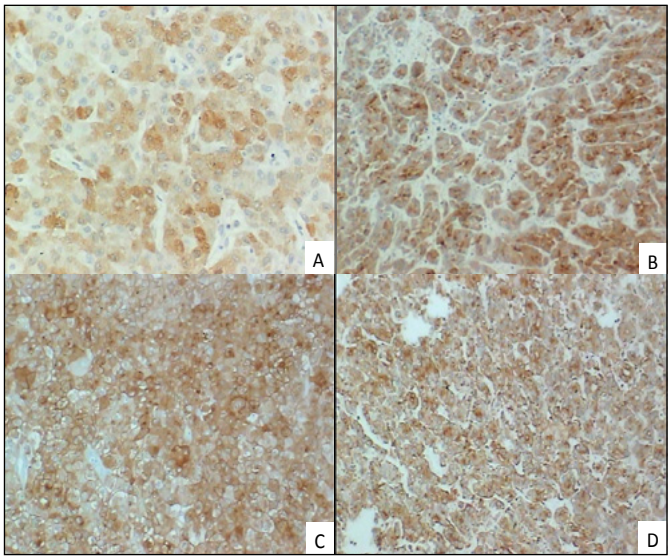

FIGURE 1. Hepatocellular carcinoma. Positive markers: **(A)** glutamine synthetase [GS]; **(B)** glipican 3 [GPC3]; **(C)** heat shock protein 70 [HSP70]; **(D)** clathrin heavy chain [CHC].

When the panel of four markers was considered (excluding beta catenin in view of its low sensitivity when performed in isolation; in fact, just in one case, beta catenin was the only positive marker, but the diagnosis was HGDN), the sensitivity ranged from 10.5% for positivity of all markers to 96.5% for the positivity of only one marker. Specificity ranged from 87.9% for one marker to 100% for at least three markers. The diagnostic accuracy ranged from 67.3% when all four markers were considered to 92.9% when considering at least two positive markers. The best accuracy was obtained when considering at least two positive markers, which was associated with a sensitivity of 82.5% and specificity of 99% (TABLE 1).

TABLE 1. Sensitivity, specificity and accuracy for the diagnosis of HCC with four markers.

Positive markers	Non HCC (n=99)	HCC (n=57)	HCC		
			Sensitivity (%)	Specificity (%)	Accuracy (%)
4M Panel					
All four	0	6	10.5	100	67.3
At least 3	0	26	45.6	100	80.1
At least 2	1	47	82.5	99.0	92.9
At least 1	12	55	96.5	87.9	91.0
GS	4	44	77.2	96.0	89.1
GPC3	4	37	64.9	96.0	84.6
HSP70	3	26	45.6	97.0	78.2
CHC	2	27	61.4	97.9	86.3

HCC: hepatocellular carcinoma; GS: Glutamine Synthetase; GPC3: glypican 3; HSP70: Heat Shock Protein 70; CHC: Clathrin heavy chain; 4M Panel: panel with 4 immunomarkers.

DISCUSSION

The development of HCC is more frequent in patients with HDGN as compared to LGDN[24]. A clinical follow-up study demonstrated that HGDN shows a malignant transformation risk of approximately 30% to 40% in 24 months[25]. Evidence of malignant transformation of HGDN is the fact that some of these nodules exhibit a well differentiated HCC microscopic focus[26]. In the study by Borzio et al., 31% of HGDNs exhibit malignant transformation at a mean follow-up of 33 months[27]. Similarly, the study by Kobayashi et al. demonstrated that the relative risk of developing HCC from HGDN was 46.2%, 61.5% and 80.8% at 1, 3 and 5 years respectively[28]. More recently, these findings have been confirmed, with dysplastic nodules being considered high-risk pre-malignant lesions[29].

Regarding HCC in a patient with hepatic cirrhosis, it is recommended that the patient be submitted to screening and surveillance every 6 months. When nodules larger than 1 cm are found, dynamic imaging study for diagnosis should be performed. If necessary for a better diagnostic clarification a liver biopsy is recommended[20,30].

Some studies have shown that the non-invasive diagnosis of HCC may present false positive results[31-33]. Hayashi et al.[31] demonstrated that in 8 of 30 (27%) patients transplanted by HCC, neoplasia was not confirmed in the explant, which resulted in an incorrect organ allocation in these patients. Wiesner et al.[32] showed that 31% of patients who underwent liver transplantation for nodules smaller than or equal to 1.9 cm and 9% of patients with nodules between 2 and 5 cm had no evidence of neoplasia in the explanted liver. Similar results were also found in a French study, where the false-positive diagnosis of HCC in pre-transplants occurred in 20% of the patients[33]. On the other hand, a false-positive rate <3% was detected in a cohort of Asian patients after liver resection[34].

We want to emphasize here that the danger of invasive treatments in lesions misdiagnosed is greater than the minimal risks of liver biopsy[35]. Therefore, especially in non-typical cases, a biopsy is critical for diagnostic elucidation.

Furthermore, biopsy can also assess prognostic parameters like tumor differentiation and is crucial for differential diagnosis with intra-hepatic cholangiocarcinoma. On the other hand, from the morphological point of view, the differentiation of HGDN and early HCC by needle biopsy presents a diagnostic challenge and is sometimes impossible to establish. Both HGDN and HCC may present cell population enlargement, cytoplasmic basophilia, hyperchromasia and nuclear atypia, altered nucleus cytoplasm ratio, reduced number of portal spaces, macrotrabecula and pseudoacinar transformation[9,22]. The only characteristic that differentiates HCC from HGDN is stromal invasion, which is difficult to detect in needle biopsy[36].

The recent identification of immunomarkers in this differentiation has been extremely useful for a more accurate diagnosis[37,38]. GPC3 has been the most studied marker; literature shows a sensitivity between 75.7% and 94.8% and specificity of 96% to 97%[39-44]. On the other hand, the negativity for GPC3 does not exclude the diagnosis of HCC, especially in cases of needle biopsy, since immuno staining can be heterogeneous. With respect to dysplastic lesions, Wang et al.[39] demonstrated that 10.6% of these nodules exhibited GPC3. In the study by Coston et al.[41], the GPC3 was present in 7% of LGDN and in 23% of HGDN. In the present study, the sensitivity of GPC3 for the diagnosis of HCC was approximately 65% and the specificity was 96%.

Di Tommaso et al.[13] showed that CHC was the most sensitive isolated marker for the diagnosis of well differentiated HCC, demonstrating sensitivity of 58.8%, versus GS (41.2%), HSP70 (17.6%) and GPC3 (11.8%). In the present study, the isolated marker with the highest sensitivity and specificity was GS with 77.2% sensitivity, 96% specificity and 89.1% accuracy. The specificity for each marker alone was above 95%, with the exception of the beta-catenin marker, which also had a very low sensitivity. The mutation of beta catenin may be present in HCC, but some authors have demonstrated its presence in the minority of patients, which confirms our findings[17,45]. On the other hand, some researches have shown the presence of changes in the beta-catenin pathway in about 50% of the analyzed tumors, with prognostic and therapeutic importance[46-49]. Thus, the use of beta-catenin in the histological diagnosis of HCC does not play a prominent role, which differs from the perspectives of HCC treatment.

In the study by Di Tommaso et al.[12], performed on surgical biopsies, analyzing 52 non-malignant nodules and 53 HCC, the negativity for all the markers (HSP70, GPC3 and GS) was found in 100% of the cases of regenerative nodules. In contrast, positiv-

ity for all markers was present in less than half of the early HCCs. The positivity for 2 out of 3 markers had a sensitivity of 70% and a specificity of 100%. Similarly, a study using this panel of needle biopsies demonstrated an accuracy for the diagnosis of HCC of 78.4% (2 positive markers) with 100% specificity[50]. Including the CHC, the panel of 4 markers demonstrated that positivity for at least 2 markers obtained an accuracy of 97% for HCC[13]. In the present study, analyzing 99 non-malignant nodules and 57 HCC, the best diagnostic accuracy for HCC was also related to the positivity of at least two markers (92.9%) with a specificity of 99%.

It is noteworthy that Sherman[51], in an editorial, questions the real importance of these immunomarkers in the differential diagnosis of HCC and HGDN, especially because the diagnosis of neoplasm is performed according to morphological criteria. In fact, the most important apply of the immunomarkers are nodules less than 2 cm, but can be of value in greater nodules, mainly if they are well-differentiated.

The possible limitations of this study were the retrospective designed and inclusion of moderate and poor differentiated neoplasia in the differential diagnosis of hepatic nodules.

CONCLUSION

The fact that most pathologists do not have expertise in the differential diagnosis of dysplastic nodules and HCC by morphological criteria, makes the immunohistochemical markers of great value. Thus, we conclude that the HSP70, GPC3, GS and CHC markers are useful and should be used mainly for the differential diagnosis between HCC and HGDN.

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Authors' contribution

Coral GP: contributed for analysis and interpretation of data, statistical analysis, drafting of the manuscript, critical revision of the manuscript for important intellectual content and approval of the final version of the manuscript. Branco F: contributed for the study concept and design, analysis and interpretation of data and approval of the final version of the manuscript. Meurer R: contributed for acquisition of data, analysis and interpretation of data and approval of the final version of the manuscript. Marcon PS: drafting of the manuscript, critical revision of the manuscript and approval of the final version of the manuscript. Fontes PRO: contributed for the study concept and design and approval of the final version of the manuscript. Mattos AA: contributed for the study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content and approval of the final version of the manuscript.

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Coral GP, Branco F, Meurer R, Marcon PS, Fontes PRO, Mattos AA. Papel da imunohistoquímica no diagnóstico diferencial do carcinoma hepatocelular precoce e dos nódulos com displasia de alto grau em pacientes com cirrose. Arq Gastroenterol. 2021;58(1):82-6.

RESUMO – **Contexto** – O carcinoma hepatocelular (CHC) é o câncer primário do fígado mais frequente e a cirrose é considerada uma doença pré-maligna. Nesse contexto, a sequência evolutiva do nódulo displásico de baixo grau e nódulo displásico de alto grau (NDAG) para CHC precoce e CHC avançado tem sido estudada. O diagnóstico diferencial entre NDAG e CHC precoce ainda é um desafio, principalmente em biópsias por agulha. **Objetivo** – Avaliar um painel de imunohistoquímica para diferenciar nódulos displásicos de CHC. **Métodos** – Foram incluídos pacientes com cirrose submetidos à ressecção cirúrgica ou transplante de fígado. A sensibilidade, especificidade e acurácia para o diagnóstico da neoplasia foram analisadas avaliando cinco marcadores: proteína de choque térmico 70kDa, glipican 3, glutamina sintetase, clatrina de cadeia pesada e beta-catenina. $P \leq 0,05$ foi considerado estatisticamente significativo. **Resultados** – Cento e cinquenta e seis nódulos foram incluídos; destes, 57 eram CHC, 14 NDAG, 18 nódulos displásicos de baixo grau e 67 macronódulos regenerativos. A sensibilidade do diagnóstico de CHC foi de 64,9% para glipican 3 e 77,2% para glutamina sintetase, enquanto a especificidade foi de 96,0% e 96,0%, respectivamente. Quando o painel de quatro marcadores foi considerado (excluindo beta catenina), a especificidade variou de 87,9% para um marcador positivo a 100% para pelo menos três marcadores. A melhor acurácia para o diagnóstico de CHC foi obtida com pelo menos dois marcadores positivos, o que foi associado a uma sensibilidade de 82,5% e especificidade de 99%. **Conclusão** – O diagnóstico diferencial de nódulos displásicos e CHC por critérios morfológicos pode ser desafiador. Imunomarcadores são úteis e devem ser usados para o diagnóstico diferencial entre CHC e NDAG.

DESCRITORES – Carcinoma hepatocelular. Glutamato sintase. Glipicanas.

REFERENCES

1. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. Hepatology. 2020. doi: 10.1002/hep.31288.
2. Sayiner M, Golabi P, Younossi ZM. Disease Burden of Hepatocellular Carcinoma: A Global Perspective. Dig Dis Sci. 2019;64:910-7.
3. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol. 2019;16:589-604.
4. Valery PC, Laversanne M, Clark PJ, Petrick JL, McGlynn KA, Bray F. Projections of primary liver cancer to 2030 in 30 countries worldwide. Hepatology. 2018;67:600-11.
5. Park YN. Update on precursor and early lesions of hepatocellular carcinomas. Arch Pathol Lab Med. 2011;135:704-15.
6. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2020;17:139-52.
7. Roskams T, Kojiro M. Pathology of early hepatocellular carcinoma: conventional and molecular diagnosis. Semin Liver Dis. 2010;30:17-25.
8. Roncalli M, Borzio M, Di Tommaso L. Hepatocellular dysplastic nodules. Hepatol Res. 2007;37 (Suppl 2):S125-34.
9. Wanless IR. International consensus on histologic diagnosis of early hepatocellular neoplasia. Hepatol Res. 2007;37 (Suppl 2):S139-41.

10. Roncalli M, Terraccianob L, Di Tommaso L, David E, Colombo M; Gruppo Italiano Patologi Apparato Digerente (GIPAD); Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Dig Liver Dis. 2011;43 (Suppl 4):S361-72.
11. Quaglia A. Hepatocellular carcinoma: a review of diagnostic challenges for the pathologist. J Hepatocell Carcinoma. 2018;5:99-108.
12. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, et al. Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. Hepatology. 2007;45:725-34.
13. Di Tommaso L, Destro A, Fabbris V, Spagnuolo G, Laura Fracanzani A, Fargion S, et al. Diagnostic accuracy of clathrin heavy chain staining in a marker panel for the diagnosis of small hepatocellular carcinoma. Hepatology. 2011;53: 1549-57.
14. Tremosini S, Forner A, Boix L, Vilana R, Bianchi L, Reig M, et al. Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. Gut. 2012;61:1481-7.
15. Uthamalingam P, Das A, Behra A, Kalra N, Chawla Y. Diagnostic Value of Glypican3, Heat Shock Protein 70 and Glutamine Synthetase in Hepatocellular Carcinoma Arising in Cirrhotic and Non-Cirrhotic Livers. J Clin Exp Hepatol. 2018;8:173-80.
16. Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. Am J Pathol. 2000;157:763-70.
17. Suarez IM, Uribe D, Jaramillo CM, Osorio G, Perez JC, Lopez R, et al. Wnt/beta catenin signaling pathway in hepatocellular carcinomas cases from Colombia. Ann Hepatol. 2015;14:64-74.
18. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, et al. Prospective Genotyping of Hepatocellular Carcinoma: Clinical Implications of Next-Generation Sequencing for Matching Patients to Targeted and Immune Therapies. Clin Cancer Res. 2019;25:2116-26.
19. Wang W, Smits R, Hao H, He C. Wnt/β-Catenin Signaling in Liver Cancers. Cancers (Basel). 2019;11:926.
20. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018;69:182-236.
21. Wanless IR. Liver biopsy in the diagnosis of hepatocellular carcinoma. Clin Liver Dis. 2005;9:281-5.
22. International Working Party. Terminology of nodular hepatocellular lesions. Hepatology. 1995;22:983-93.
23. Kojiro M. Pathology of early HCC-progression from early to advanced. Hepatogastroenterology. 1998;45:1203-5.
24. Iavarone M, Manini MA, Sangiovanni A, Fraquelli M, Forzenigo LV, Di Tommaso L, et al. Contrast-enhanced computed tomography and ultrasound-guided liver biopsy to diagnose dysplastic liver nodules in cirrhosis. Dig Liver Dis. 2013;45:43-9.
25. Di Tommaso L, Sangiovanni A, Borzio M, Park YN, Farinati F, Roncalli M, et al. Advanced precancerous lesions in the liver. Best Pract Res Clin Gastroenterol. 2013;27:269-84.
26. Kojiro M. Focus on dysplastic nodules and early hepatocellular carcinoma: an eastern point of view. Liver Transpl. 2001;10 (2 Suppl 1):S3-8.
27. Borzio M, Fargion S, Borzio F, Fracanzani AL, Croce AM, Stroffolini T, et al. Impact of large regenerative, low grade and high grade dysplastic nodules in hepatocellular carcinoma development. J Hepatol. 2003;39:208-14.
28. Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, et al. Dysplastic nodules frequently develop into hepatocellular carcinoma in patients with chronic viral hepatitis and cirrhosis. Cancer. 2006;16:636-47.
29. Sato T, Kondo F, Ebara M, Sugiura N, Okabe S, Sunaga M, et al. Natural history of large regenerative nodules and dysplastic nodules in liver cirrhosis: 28-year follow-up study. Hepatol Int. 2015;9:330-6.
30. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD Guidelines for the Treatment of Hepatocellular Carcinoma. Hepatology. 2018;67:358-80.
31. Hayashi PH, Trotter JF, Forman L, Kugelmas M, Steinberg T, Russ P, et al. Impact of pretransplant diagnosis of hepatocellular carcinoma on cadveric liver allocation in the era of MELD. Liver Transpl. 2004;10:42-8.
32. Wiesner RH, Freeman RB, Mulligan DC. Liver transplantation for hepatocellular cancer: the impact of the MELD allocation policy. Gastroenterology. 2004;127 (5 Suppl 1): S261-7.
33. Compagnon P, Grandadam S, Lorho R, Turlin B, Camus C, Jianrong Y, et al. Liver transplantation for hepatocellular carcinoma without a preoperative tumor biopsy. Transplantation. 2008;86:1068-76.
34. Lee H, Yoon JH, Kim H, Yi NJ, Hong SK, Yoon KC, et al. False Positive Diagnosis of Hepatocellular Carcinoma in Liver Resection Patients. J Korean Med Sci. 2017;32:315-20.
35. Boyum JH, Atwell TD, Schmit GD, Poterucha JJ, Schleck CD, Harmsen WS, et al. Incidence and Risk Factors for Adverse Events Related to Image-Guided Liver Biopsy. Mayo Clin Proc. 2016;91:329-35.
36. International Consensus Group for hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology. 2009;49:658-64.
37. Lo RC, Ng IO. Hepatocellular tumors: immunohistochemical analyses for classification and prognostication. Chin J Cancer Res. 2011;23:245-53.
38. Nguyen T, Phillips D, Jain D, Torbenson M, Wu TT, Yeh MM, et al. Comparison of 5 Immunohistochemical Markers of Hepatocellular Differentiation for the Diagnosis of Hepatocellular Carcinoma. Arch Pathol Lab Med. 2015;139:1028-34.
39. Wang HL, Anatelli F, Zhai QH, Adley B, Chuang ST, Yang XJ. Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. Arch Pathol Lab Med. 2008;132:1723-8.
40. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology. 2003;125:89-97.
41. Coston WMP, Loera S, Lau SK, Ishizawa S, Jiang Z, Wu CL, et al. Distinction of hepatocellular carcinoma from benign hepatic mimickers using glypican-3 and CD34 immunohistochemistry. Am J Surg Pathol. 2008;32:433-44.
42. Libbrecht L, Severi T, Cassiman D, Vander Borght S, Pirenne J, Nevens F, et al. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. Am J Surg Pathol. 2006;30:1405-11.
43. Liu H, Li P, Zhai Y, Qu CF, Zhang LJ, Tan YF, et al. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. World J Gastroenterol. 2010;16:4410-5.
44. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncol. 2009;34:649-56.
45. Okabe H, Kinoshita H, Imai K, Nakagawa S, Higashi T, Arima K, et al. Diverse basis of β-catenin activation in human hepatocellular carcinoma: implications in biology and prognosis. PloS One. 2016;11:e0152695.
46. Khalaf AM, Fuentes D, Morshid AI, Burke MR, Kaseb AO, Hassan M, et al. Role of Wnt/β-catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. J Hepatocell Carcinoma. 2018;5:61-73.
47. Choi WT, Ramachandran R, Kakar S. Immunohistochemical approach for the diagnosis of a liver mass on small biopsy specimens. Hum Pathol. 2017;63:1-13.
48. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, et al. Prospective Genotyping of Hepatocellular Carcinoma: Clinical Implications of Next-Generation Sequencing for Matching Patients to Targeted and Immune Therapies. Clin Cancer Res. 2019;25:2116-26.
49. Di Tommaso L, Spadaccini M, Donadon M, Personeni N, Elamin A, Aghemo A, et al. Role of liver biopsy in hepatocellular carcinoma. World J Gastroenterol. 2019;25:6041-52.
50. Di Tommaso L, Destro A, Seok JY, Balladore E, Terracciano L, Sangiovanni A, et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol. 2009;50:746-54.
51. Sherman M. Histological diagnosis of early hepatocellular carcinoma. Hepatology. 2011;53:1427-9.

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Long term management of glycogen storage disease type 1b: a Brazilian tertiary center experience

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ABSTRACT – **Background** – Glycogen storage disease (GSD) type 1b is a multisystemic disease in which immune and infectious complications are present, in addition to the well-known metabolic manifestations of GSD. Treatment with granulocyte-colony stimulating factor (G-CSF) is often indicated in the management of neutropenia and inflammatory bowel disease. **Objective** – To report on the demographics, genotype, clinical presentation, management, and complications of pediatric patients with glycogen storage disease type 1b (GSD 1b), with special attention to immune-related complications. **Methods** – Retrospective case series of seven patients with GSD 1b diagnosed and followed at a tertiary university hospital in Brazil, from July/2000 until July/2016. **Results** – Mean age at referral was fourteen months. Diagnosis of GSD 1b was based on clinical and laboratory findings and supported by genetic studies in five cases. All patients presented suffered from neutropenia, managed with G-CSF – specifically Filgrastim. Hospitalizations for infections were frequent. Two patients developed inflammatory bowel disease. Six patients remained alive, one died at age 14 years and 9 months. The mean age at the end of the follow-up was 11.5 years. Compliance to treatment was suboptimal: poor compliance to medications, starch and dietetic management of GSD were documented, and outpatient appointments were frequently missed. **Conclusion** – Managing GSD 1b is challenging not only for the chronic and multisystemic nature of this disease, but also for the additional demands related dietary restrictions, use of multiple medications and the need for frequent follow-up visits; furthermore in Brazil, the difficulties are increased in a scenario where we frequently care for patients with unfavorable socioeconomic status and with irregular supply of medications in the public health system.

HEADINGS – Glycogen storage disease type I. Neutropenia. Inflammatory bowel diseases. Immune system diseases. Granulcyte colony-stimulating factor. Filgrastim. Pediatrics.

INTRODUCTION

Glycogen storage diseases (GSD) are a group of rare inherited disorders caused by different enzymatic defects in the glycogen metabolism. The severity of the disease is highly variable according to type and degree of the enzyme activity impairment. GSD type 1 has a reported incidence of 1/100.000 live births[1], with two major subtypes: the most frequent is 1a, which corresponds to 80% of cases and is determined by mutations in the glucose-6 phosphatase gene[2], and GSD type 1b is the second most important and frequent subtype, and it is caused by mutations in the glucose-6 phosphatase translocase gene (SLC37A4 gene, also known as G6PT1 gene)[2]. To this date, no Brazilian long term follow-up studies on GSD 1b have been published – there are, however, Brazilian studies on the molecular and clinical characterization of GSD 1a[3] and a cross-sectional analysis on GSD types 1a and 1b[4].

In glycogen storage disease type 1b (GSD 1b), besides the clinical and laboratorial manifestations that are common to GSD type 1a (hepatomegaly, the characteristic "doll-like" face with "fat cheeks", short stature, hypoglycemia, lactic acidosis, hypertriglyceridemia, and hyperuricemia), patients also develop neutropenia and neutrophil dysfunction[5-9], leading to increased susceptibility to infections. The pathogenesis of neutropenia in GSD 1b remains poorly understood. Different mechanisms have been proposed: increased production of reactive oxygen species[10], abnormal neutrophil differentiation[6-11], shorter neutrophil lifespan[6,12] and abnormal cytokine profiles[8,13]. Furthermore, neutrophils from patients with GSD 1b present abnormal mobility, chemotaxis, calcium mobilization, respiratory burst and phagocyte activity[7,14].

Granulocyte colony-stimulating factor (G-CSF), a hematopoietic growth factor essential for the neutrophil differentiation and function, has been used for approximately 3 decades as pharmacological agent to increase neutrophil count, lifespan, and activation. Its efficacy and safety as a long term therapy for patients with GSD 1b was proven: it significantly reduces the rates of recurrent bacterial infections and enterocolitis in this particularly susceptible population[15].

The aim of the present study was to report on the demographics, genotype, clinical presentation and management of pediatric patients presenting with GSD 1b, as well as to characterize complications and clinical course on their follow-up.

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METHODS

A retrospective chart review of all pediatric patients with GSD 1b, who were diagnosed and/or followed at the Clinics Hospital, University of Campinas – São Paulo, Brazil, from July/2000 to July/2016, was conducted. Subjects were identified from a patient registry kept by the senior author. The study was approved by the Research Ethics Committee of University of Campinas Clinics Hospital under Certificate number 61819516.8.00005404, approval number 1.846.888.

Two investigators (Takao MMV and Sandy NS) collected data from the medical records including: gender, date of birth, age and type of initial manifestations, genotype, laboratorial data (alanine aminotransferase, blood glucose, lactate, triglycerides, uric acid, and neutrophil count), number of infections requiring hospitalization, use of G-CSF use of antibiotic prophylaxis, and comorbidities. Summary statistics was used to describe the data.

RESULTS

Seven patients were included in the study, five were male, and two patients were siblings. The median age at referral was eleven months. The diagnosis of GSD 1b was based on clinical and laboratory findings, as well as liver biopsy, and later supported by next generation sequencing DNA analysis (confirmed by Sanger sequencing) in five cases. Genetic testing was not performed for all patients, since this is not routinely available in our public healthcare system – this type of request is reserved for selected/ atypical cases, upon clear justification of its need, and approval of the indication. Gender, age of symptoms onset, age of referral, symptoms of presentation and genotype (when available) data is summarized in TABLE 1. There was a delay between symptoms onset and referral. Hypoglycemia was universally reported at presentation, in association with seizures in five cases.

During long term follow-up, there was significant variation in terms of metabolic control: hypoglycemia, hypertriglyceridemia, hyperlactatemia, hyperuricemia and elevated transaminases were often document. TABLE 2 summarizes the broad variation in the main laboratory test results – critically abnormal results can be noted.

Persistent and severe neutropenia (defined as less than 500 neutrophils per microliter in peripheral blood) was treated with G-CSF, specially Filgrastim, in all cases. The median age for initiation of G-CSF was 5 years. None of the patients developed any serious adverse G-CSF side effect requiring discontinuation of this treatment: one patient developed splenomegaly as side effect, managed with reduction of dosage. Four of the seven patients also received prophylactic antibiotics. Despite those measures, hospitalizations due to recurrent infections were frequent. Worst documented neutropenia, age of G-CSF initiation, number and causes of hospitalizations due to infections and use of antibiotic prophylaxis are summarized on TABLE 3.

TABLE 1. Gender, age of symptoms onset, age of referral, symptoms of presentation and genotype.

Patient	Gender	Age of symptoms onset	Age of referral	Presentation	Genetics – nucleotide change and exon
#1	Male	1 month	9 months	Hepatomegaly and hyypoglycemia	c.[654G>A]: c.[654G>A] Exon 4
#2	Female	6 months	11 months	Increased abdominal girth, hypoglycemia and seizures	
#3	Male	2 months	1 year 1 month	Hypoglycemia and seizures	c.[1042_1043delCT]; c.[1042_1043delCT] Exon 11
#4	Male	1 month	11 months	Hepatomegaly, hyypoglycemia, seizures, recurrent infections	c.[899G>A]; c.[899G>A] Exon 9
#5 *	Female	At birth	3 years 5 months	Increased abdominal girth, hypoglycemia and recurrent infections	c.[703_705delGTG];[1042_1043delCT] Exons 7 and 11
#6 *	Male	1 month	7 months	Hepatomegaly, hypoglycemia, seizures, hypotonia	c.[703_705delGTG]; [1042_1043delCT] Exons 7 and 11
#7	Male	4 months	7 months	Hepatomegaly, hyypoglycemia, seizures and developmental delay	

* Patients #5 and #6 are siblings.

TABLE 2. Summary of the range of variation of the main metabolic laboratory results.

Patient	Lactate (mg/dL)	Alanine Aminotransferase (U/L)	Uric acid (mg/dL)	Triglycerides (mg/dL)	Glycemia (mg/dL)
#1	1.2–5.3	2–68	3.7–15.3	57–1447	3–108
#2	1.9–48.2	14–81	5.6–9.6	120–600	56–112
#3	1.9–6.9	26–76	2.5–6.3	269–1192	3–181
#4	2.2–7.4	11–214	4.3–7.5	99–390	48–11
#5	3.3–9.3	29–113	6.2–8	185–562	22–142
#6	2.3–11.7	51–1556	5.5–10.4	71–1361	15–91
#7	1.2–14.9	7–92	5.9–14.1	91–666	38–150

TABLE 3. Most severe neutropenia, age of G-CSF initiation, history of hospitalizations due to infections, use of antibiotic prophylaxis.

Patient	Most severe neutropenia (cells/microliter)	Age of G-CSF initiation	Number of hospitalizations due to infections, and causes	Use of prophylactic antibiotics
#1	270	9 years 8 months	Four: Gastroenteritis (2) and Sepsis of unknown source (2)	No
#2	190	8 years 5 months	One: Pneumonia	TMP-SMX (poor compliance) Amoxicillin (current)
#3	110	7 years 2 months	Three: Gastroenteritis (2) and Pneumonia	No
#4	60	2 years 1 month	Three: Gastroenteritis, Pneumonia, and Laryngitis/Tonsillitis	TMP-SMX (for 1 year 11 months) Amoxicillin (current)
#5	219	5 years	Four: Gastroenteritis (2) and Pneumonia (2)	Amoxicillin (current)
#6	220	1 years 11 months	Eleven: Bronchiolitis, Varicella, Pneumonia, Cellulitis, Laryngitis/Tonsillitis, pseudo membranous colitis, Dengue, Sepsis of unknown source (2), Gastroenteritis (2)	Azithromycin (poor compliance) Amoxicillin (current)
#7	60	1 year 7 months	Five: Gastroenteritis 2), Pneumonia (2), skin abscess	TMP-SMX (current)

G-CSF: granulocyte colony-stimulating factor; TMP-SMX: trimethoprim-sulfamethoxazole.

In our study population, compliance was sub-optimal follow-up appointments were frequently missed, medications were irregularly administered, poor compliance to starch and dietetic restrictions were documented. In most cases, socioeconomic vulnerability was an important aggravating factor related to poor compliance. Two patients developed Crohn-like Inflammatory Bowel Disease, confirmed by colonoscopy and were mainly managed with G-CSF, but also required intermittent use of systemic corticosteroids. Additionally, atopic manifestations were frequently noted: three patients developed allergic rhinitis and asthma, and one also presented atopic dermatitis. All patients were monitored with annual ultrasounds as part of surveillance for hepatic adenoma, a well-known complication in GSD1[16]: detected in one of our patients, who as asymptomatic and did not develop any complications. The tumor was better characterized with a computed tomography study: solid hyperdense lesion with arterial hypervascularization noted in the hepatic segment V, measuring 1.3 x 1.2 cm. This lesion has been monitored with ultrasound every six months and no significant growth was noted thus far. The first patient diagnosed at our Institution died at age 14 years and 9 months, due to sepsis of unknown origin. At the end of the study follow-up, six patients remained alive, with a median age of 11 years. Compliance, associated conditions, and age at the end of follow-up period are summarized on TABLE 4.

DISCUSSION

To this date, more than a hundred mutations in the SLC37A4 determining GSD 1b have been described, according to The Human Gene Mutation Database®, and novel mutations continue to be reported. With a broader use of molecular techniques to support the diagnosis and management of GSD, a genotype-phenotype correlation in regards to the severity of the neutropenia has been explored, but not well described so far[15,17]. All mutations noted in our patients have been previously described, and no inference in terms genotype-phenotype correlation could be established in our case series.

TABLE 4. Compliance, associated conditions, and age at the end of follow-up period.

Patient	Description of compliance	Associated conditions	Age at the end of follow-up
#1	Poor: frequently missed follow-up visits, irregular use of G-CSF.	IBD Cataract Osteoporosis Severe malnutrition	Died at 14 years 9 months
#2	Regular: missed some follow-up visits, irregular timing in the use of starch, poor compliance to some medications.	Asthma Allergic rhinitis Constipation	15 years
#3	Regular: periodically irregular routine with the use of starch and medications, but never missed follow-up visit; predominantly suboptimal metabolic control.	Asthma Allergic rhinitis Atopic dermatitis	13 years
#4	Regular: irregular use of G-CSF.	IBD Urticaria	10 years
#5	Poor: frequently missed follow-up visits, predominantly poor metabolic control.	None	12 years
#6	Poor: frequently missed consultations; predominantly poor laboratory control; irregular compliance to starch and dietary recommendations; refusal to use prophylactic antibiotics.	Anemia	9 years
#7	Good	Asthma Allergic rhinitis	7 years

G-CSF: granulocyte colony-stimulating factor; IBD: inflammatory bowel disease.

The primary treatment for GSD type 1 is diet therapy: fructose, lactose, and sucrose are avoided, while raw cornstarch is given enterally to prevent hypoglycemia. In GSD 1b, the abnormal neutrophil count and function adds another layer of complexity to the management of the disease: a variety of infectious complications are frequent in patients with GSD 1b, including infections affecting the upper and lower respiratory tracts, gastrointestinal and genitourinary tracts, skin and mucous membranes, deep abscesses and sepsis of unknown origin. The main agents involved are staphylococci, streptococci and *Escherichia coli*[5,18]. In the present study, the infections that most often required hospitalizations for treatment were pneumonia and gastroenteritis.

Prophylactic antibiotics are often used in patients with recurrent infections and neutropenia. The benefits of such intervention have been broadly demonstrated outside of GSD 1b. It became an official recommendation of the European 2002 Consensus Guideline for the management of GSD 1, even though it was recognized that these benefits were not specifically systematically studied in GSD[18]. More recently published, the practice guideline of the American College of Medical Genetics and Genomics did not provide any recommendations in that specific subject[16]. Cotrimoxazole or trimethoprim-sulfamethoxazole (TMP-SMX) is typically the antibiotic of choice[18]. In the present case series, the use of TMP-SMX was initially used in three of the seven cases, however due to poor compliance and mainly complaints of gastrointestinal intolerance, it was changed to Amoxicillin in some cases.

On a different note, there is a growing interest in the use of probiotics in GSD 1b, but the evidence remains anecdotal[19]. More studies are needed to first more broadly the changes in the composition of the gut microbiota in GSD 1b, and later identify whether there will be any specific indications for probiotics in this group of patients. A national study has reported dysbiosis in GSD[20], and this finding was later corroborated in an Italian study[21]. As the knowledge in this area is still evolving, probiotics may be costly and are not routinely available in our public health system in Brazil, none of the patients in the present cohort used probiotics.

Granulocyte colony-stimulating factor is one of the cornerstones in the management of GSD 1b. Its use not only has positive impact in the neutrophil count and severity of recurrent infections (particularly recurrent bacterial infections and enterocolitis)[15], but it also constitutes an important component in the treatment of the Inflammatory Bowel Disease in GSD 1b[8]. In the present study, we observed laboratory and clinical response in all patients. In our cohort, the mean age of initiation of G-CSF was 5 years, in keeping with previous studies that reported development of severe neutropenia at the median age of 4.5 years[17]. With prolonged use of G-CSF in neutropenic patients, the following complications have been reported: 1) hypersplenism, splenomegaly[18], and splenic rupture[22,23] – probably resulting from extramedullary hematopoiesis; 2) bone health related complications – transient or persistent bone pain, and osteopenia/osteoporosis[24,25], 3) transient hematologic complications – monocytosis, eosinophilia, reticulocytosis, and thrombocytopenia; 4) risk of neoplasms – prolonged stimulation of the bone marrow may predispose to leukemic transformation[26]; 5) loss of response to treatment; 6) and other less often reported complications – chronic urticaria, hepatic adenomas, polycystic ovaries, increased uric acid, leukocytoclastic vasculitis; blood-proliferative glomerulonephritis. Thus, the use of this medication requires careful surveillance and constant re-evaluation of the risks and benefits[27], and there is a growing interest in G-CSF sparing strategies. In this case series, as in other studies[17], no serious adverse events related to the use of G-CSF were observed, as recommended, all patients were regularly monitored by clinical and laboratory evaluations.

It has been suggested that vitamin E also has a role in improving neutrophil count and reducing the frequency and severity of infections, and its effectiveness in reducing the frequency of infection and improving neutropenia has been reported in GSD 1b patients[28]. Although it is easy to argue that vitamin E supplementation would have many advantages over the use of G-CSF, in terms of route of administration (oral vs subcutaneous, respectively) and safety/adverse effects, its use as a strategy to reduce or avoid G-CSF has not been systematically studied, and the current evidence is considered insufficient to recommend routine use of vitamin E supplementation alone as a G-CSF sparring strategy. In the GSD 1 practice guideline of the American College of Medical Genetics and Genomics vitamin E is mentioned under "other dietary considerations", but it is not officially recommended. Considering the relative low cost and safety of this intervention, all our patients have been on daily vitamin E (doses of 400 UI/day), however in none of the cases that intervention has clearly led to significant weaning of G-CSF. Assessment of vitamin E levels in our patients was not routinely done, as unfortunately this specific laboratory assessment is not customarily available in our public health system. Another therapy recently explored is the off-label use of an SGLT2-inhibitor, empagliflozin, typically used to treat type 2 diabetes. It was reported to successfully treat neutropenia and neutrophil dysfunction in GSD 1b, allowing weaning and even discontinuation of G-CSF[29]. This therapy was not attempted in any of our patients.

Besides neutropenia and neutrophil dysfunction, patients with GSD 1b may experience lymphopenia, impaired regulatory T cell function ant autoimmunity. The exact mechanism for autoimmunity in these patients is still not well described[30], but several studies have reported an increased risk for autoimmune disorders, including Inflammatory Bowel Disease (IBD), thyroid autoimmunity, and myasthenia gravis. Two of our patients developed IBD (Crohn disease–like enterocolitis): one of the patients presented with recurrent episodes of vomiting and diarrhea at six years of age, that later progressed to chronic diarrhea, while the second patient presented chronic diarrhea at 18 months. The association between GSD 1b and IBD has been reported since the late 1970s, and later a high prevalence of IBD was noted in GSD (up to 77%)[8]. G-CSF is the mainstay therapy for IBD in GSD 1b, but not all patients respond to it[16]. Other therapies, such as salicylates, mesalamine, sulfasalazine and prednisone may be necessary[15]. In our two cases, steroids were temporarily used (prednisone up to 1mg/kg), with no associated complications/side effects with a short term course of this medication. No other autoimmune diseases were noted in our cohort of patients.

GSD 1b management requires frequent follow-up appointments, regular use of medications and a restricted diet, with scheduled use of cornstarch. Education about the disease has a fundamental role for treatment compliance. Outpatient visits in our institution are coordinated to include at least a dietician and the pediatric Immunology and Hepatology medical teams at each visit. Furthermore, a psychologist is available for patients and caregivers on demand or when recommended by the medical teams. Annual educational and social events for patients/caregivers have been organized. Despite all those measures, suboptimal compliance is the

reality in most of the cases. More than the well-known difficulties of caring for a chronic disease, the irregular supply of medications by our public health system, and unfavorable socioeconomic conditions had a negative impact on treatment compliance.

Our study has the well-known limitations of retrospective design, and additionally our cohort encompasses almost two decades of care, when there were significant changes in the understanding and therefore in the management of GSD 1b. Nonetheless, this is the first long term follow-up pediatric GSD 1b case series in Brazil, in which we report a wide spectrum of disease severity, complications and associated conditions, as well as we discuss current recommendations for GSD 1b treatment. Severe neutropenia was universally present, and all patients were treated with G-CSF, nevertheless, infectious diseases requiring hospitalizations were relatively common. The early recognition and medical management of sepsis is fundamental in GSD 1b to avoid a potentially fatal outcome, as in these patients not only neutrophil count is affected, but also neutrophil function is impaired. In the present cohort of patients, managing GSD 1b was noted to be challenging not only for the chronic and multisystemic nature of this disease, requiring multiple medical and dietary interventions, but also complicated by non-medical risk factors, namely socioeconomic vulnerability.

Authors' contribution

Takao MMV and Sandy NS conceptualized the article, obtained REB approval, collected the data, drafted the initial manuscript, reviewed and revised the manuscript, approved the final draft as submitted. Riccetto AGL contributed to the manuscripts, reviewed and revised the manuscript, approved the final draft as submitted. De Tommaso AMA supervised throughout all the different phases of this study, analyzed the data, reviewed and revised the manuscript, and approved the final draft as submitted.

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Takao MMV, Sandy NS, Riccetto AGL, De Tommaso AMA. Manejo em longo prazo de glicogenose tipo 1b: experiência de um centro terciário brasileiro. Arq Gastroenterol. 2021;58(1):87-92.

RESUMO – **Contexto** – Glicogenose (GSD) tipo 1b é uma doença multissistêmica em que complicações imunológicas e infecciosas estão presentes, além das manifestações metabólicas bem conhecidas da GSD. O tratamento com fator estimulador de colônias de granulócitos (G-CSF) é frequentemente indicado no tratamento da neutropenia e doença inflamatória intestinal. **Objetivo** – Relatar sobre a dados demográficos, genótipo, apresentação clínica, manejo e complicações de pacientes pediátricos com GSD tipo 1b (GSD 1b), com atenção especial às complicações relacionadas ao sistema imunológico. **Métodos** – Série de casos retrospectiva de sete pacientes com GSD 1b diagnosticados e acompanhados em um hospital universitário terciário no Brasil, de julho/2000 a julho/2016. **Resultados** – A idade média no encaminhamento foi de 14 meses. O diagnóstico de GSD 1b foi baseado em achados clínicos e laboratoriais e apoiado por estudos genéticos em cinco casos. Todos os pacientes apresentaram neutropenia, tratada com G-CSF – especificamente Filgrastim. As hospitalizações por infecções foram frequentes. Dois pacientes desenvolveram doença inflamatória intestinal. Seis pacientes permanecem vivos, um morreu aos 14 anos e 9 meses de idade. A média de idade ao final do acompanhamento foi de 11,5 anos. A adesão ao tratamento foi sub-ótima: má adesão aos medicamentos, amido e manejo dietético de GSD foram documentados, e consultas ambulatoriais foram frequentemente perdidas. **Conclusão** – O manejo da GSD 1b é um desafio, não apenas pela natureza crônica e multissistêmica desta doença, mas também pelas demandas adicionais relacionadas a restrições dietéticas, uso de múltiplos medicamentos e a necessidade de consultas de acompanhamento frequentes; no Brasil, isso ainda é dificultado em um cenário em que frequentemente atendemos pacientes com situação socioeconômica desfavorável e com oferta irregular de medicamentos no sistema público de saúde.

DESCITORES – Doença de depósito de glicogênio tipo I. Neutropenia. Doenças inflamatórias intestinais. Doenças do sistema imunitário. Fator estimulador de colônias de granulócitos. Filgrastim. Pediatria.

REFERENCES

1. Chou JY, Jun HS, Mansfield BC. Type I glycogen storage diseases: disorders of the glucose-6-phosphatase/glucose-6-phosphate transporter complexes. J Inherit Metab Dis. 2015;38:511-9.
2. Chou JY, Mansfield BC. Mutations in the glucose-6-phosphatase-alpha (G6PC) gene that cause type Ia glycogen storage disease. Hum Mutat. 2008;29:921-30.
3. de CRF, Caldas HC, Norato DY, Schwartz IV, Giugliani R, Burin MG, Sartorato EL. Glycogen storage disease type Ia: molecular study in Brazilian patients. J Hum Genet. 2001;46:146-9.
4. Santos BL, Souza CF, Schuler-Faccini L, Refosco L, Epifanio M, Nalin T, et al. Glycogen storage disease type I: clinical and laboratory profile. J Pediatr (Rio J). 2014;90:572-9.
5. Melis D, Fulceri R, Parenti G, Marcolongo P, Gatti R, Parini R, et al. Genotype/phenotype correlation in glycogen storage disease type 1b: a multicentre study and review of the literature. Eur J Pediatr. 2005;164:501-8.
6. Chou JY, Jun HS, Mansfield BC. Neutropenia in type Ib glycogen storage disease. Curr Opin Hematol. 2010;17:36-42.
7. Jun HS, Weinstein DA, Lee YM, Mansfield BC, Chou JY. Molecular mechanisms of neutrophil dysfunction in glycogen storage disease type Ib. Blood. 2014;123:2843-53.
8. Visser G, Rake JP, Fernandes J, Labrune P, Leonard JV, Moses S, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease type I. J Pediatr. 2000;137:187-91.
9. Choi R, Park HD, Ko JM, Lee J, Lee DH, Hong SJ, et al. Novel SLC37A4 Mutations in Korean Patients With Glycogen Storage Disease Ib. Ann Lab Med. 2017;37:261-6.
10. Kim SY, Nguyen AD, Gao JL, Murphy PM, Mansfield BC, Chou JY. Bone marrow-derived cells require a functional glucose 6-phosphate transporter for normal myeloid functions. J Biol Chem. 2006;281:28794-801.
11. Sim SW, Weinstein DA, Lee YM, Jun HS. Glycogen storage disease type Ib: role of glucose-6-phosphate transporter in cell metabolism and function. FEBS Lett. 2020;594:3-18.

12. Kuijpers TW, Maianski NA, Tool AT, Smit GPA, Rake JP, Roos D, Visser G. Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b). Blood. 2003;101:5021-4.
13. Dieckgraefe BK, Korzenik JR, Husain A, Dieruf L. Association of glycogen storage disease 1b and Crohn disease: results of a North American survey. Eur J Pediatr. 2002;161 (Suppl 1):S88-92.
14. Kim SY, Jun HS, Mead PA, Mansfield BC, Chou JY. Neutrophil stress and apoptosis underlie myeloid dysfunction in glycogen storage disease type Ib. Blood. 2008;111:5704-11.
15. Dale DC, Bolyard AA, Marrero T, Kelley ML, Makaryan V, Tran E, et al. Neutropenia in glycogen storage disease Ib: outcomes for patients treated with granulocyte colony-stimulating factor. Curr Opin Hematol. 2019;26:16-21.
16. Kishnani PS, Austin SL, Abdenur JE, et al. Diagnosis and management of glycogen storage disease type I: a practice guideline of the American College of Medical Genetics and Genomics. Genetics in Medicine. 2014;16(11):e1-e1.
17. Sarajlija A, Djordjevic M, Kecman B, Skakic A, Pavlovic S, Pasic S, Stojiljkovic M. Impact of genotype on neutropenia in a large cohort of Serbian patients with glycogen storage disease type Ib. Eur J Med Genet. 2019;63:103767.
18. Visser G, Rake JP, Labrune P, Leonard JV, Moses S, Ullrich K, et al. Consensus guidelines for management of glycogen storage disease type 1b - European Study on Glycogen Storage Disease Type 1. Eur J Pediatr. 2002;161 (Suppl 1):S120-123.
19. Carnero-Gregorio M, Molares-Vila A, Corbalán-Rivas A, Villaverde-Taboada C, Rodríguez-Cerdeira C. Effect of VSL#3 Probiotic in a Patient with Glycogen Storage Disease Type Ia and Irritable Bowel Disease-like Disease. Probiotics Antimicrob Proteins. 2019;11:143-9.
20. Colonetti K, Bento Dos Santos B, Nalin T, Moura de Souza CF, Triplett EW, Dobbler PT, et al. Hepatic glycogen storage diseases are associated to microbial dysbiosis. PLoS One. 2019;14:e0214582.
21. Ceccarani C, Bassanini G, Montanari C, Casiraghi MC, Ottaviano E, Morace G, et al. Proteobacteria Overgrowth and Butyrate-Producing Taxa Depletion in the Gut Microbiota of Glycogen Storage Disease Type 1 Patients. Metabolites. 2020;10:133.
22. Masood N, Shaikh AJ, Memon WA, Idress R. Splenic rupture, secondary to G-CSF use for chemotherapy induced neutropenia: a case report and review of literature. Cases J. 2008;1:418.
23. Benguerfi S, Thepault F, Lena H, Ricordel C. Spontaneous splenic rupture as a rare complication of G-CSF injection. BMJ Case Rep. 2018;2018: bcr2017222561. doi: 10.1136/bcr-2017-222561.
24. Kokai Y, Wada T, Oda T, Kuwabara H, Hara K, Akiyama Y, et al. Overexpression of granulocyte colony-stimulating factor induces severe osteopenia in developing mice that is partially prevented by a diet containing vitamin K2 (menatetrenone). Bone. 2002;30:880-5.
25. Sekhar RV, Culbert S, Hoots WK, Klein MJ, Zietz H, Vassilopoulou-Sellin R. Severe osteopenia in a young boy with Kostmann's congenital neutropenia treated with granulocyte colony-stimulating factor: suggested therapeutic approach. Pediatrics. 2001;108(3):E54.
26. Li AM, Thyagu S, Maze D, Schreiber R, Sirrs S, Stockler-Ipsiroglu S, et al. Prolonged granulocyte colony stimulating factor use in glycogen storage disease type 1b associated with acute myeloid leukemia and with shortened telomere length. Pediatr Hematol Oncol. 2018;35:45-51.
27. Rosenberg PS, Alter BP, Bolyard AA, Bonilla MA, Boxer LA, Cham B, et al. The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood. 2006;107:4628-35.
28. Melis D, Minopoli G, Balivo F, Marcolongo P, Parini R, Paci S, et al. Vitamin E Improves Clinical Outcome of Patients Affected by Glycogen Storage Disease Type Ib. JIMD Rep. 2016;25:39-45.
29. Wortmann SB, Van Hove JLK, Derks TGJ, Chevalier N, Knight V, Koller A, et al. Treating neutropenia and neutrophil dysfunction in glycogen storage disease IB with an SGLT2-inhibitor. Blood. 2020;136:1033-43.
30. Melis D, Carbone F, Minopoli G, Rocca CL, Perna F, De Rosa V, et al. Cutting Edge: Increased Autoimmunity Risk in Glycogen Storage Disease Type 1b Is Associated with a Reduced Engagement of Glycolysis in T Cells and an Impaired Regulatory T Cell Function. J Immunol. 2017;198:3803-8.

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Impact of aging in the surgical outcomes of gastric cancer patients

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ABSTRACT – **Background** – As age advances, a higher burden of comorbidities and less functional reserve are expected, however, the impact of aging in the surgical outcomes of gastric cancer (GC) patients is unknown. **Objective** – The aim of this study is to evaluate surgical outcomes of GC patients according to their age group. **Methods** – Patients submitted to gastrectomy with curative intent due to gastric adenocarcinoma were divided in quartiles. Each group had 150 patients and age limits were: ≤54.8, 54.9–63.7, 63.8–72, >72. The outcomes assessed were: postoperative complications (POC), 90-day postoperative mortality, disease-free survival (DFS) and overall survival (OS). **Results** – Major surgical complications were 2.7% in the younger quartile vs 12% for the others (P=0.007). Major clinical complications raised according to the age quartile: 0.7% vs 4.7% vs 5.3% vs 7.3% (P<0.042). ASA score and age were independent risk factors for major POC. The 90-day mortality progressively increased according to the age quartile: 1.3% vs 6.0% vs 7.3% vs 14% (P<0.001). DFS was equivalent among quartile groups, while OS was significantly worse for those >72-year-old. D2 lymphadenectomy only improved OS in the three younger quartiles. Age >72 was an independent risk factor for worse OS (hazard ratio of 1.72). **Conclusion** – Patients <55-year-old have less surgical complications. As age progresses, clinical complications and 90-day mortality gradually rise. OS is worse for those above age 72, and D2 lymphadenectomy should be individualized after this age.

HEADINGS – Stomach neoplasms. Aging. Survival. Postoperative complication. Gastrectomy.

INTRODUCTION

Although the incidence of gastric cancer (GC) is slowly diminishing, it remains as one of the most common and lethal neoplasms in the world[1]. Surgical resection remains as the main treatment and it is a morbid procedure even in reference centers[2-5]. The disease is mostly diagnosed at late age (64 and above) and with life expectancy increasing globally, gastrectomy in elder patients is expected to grow. As age advances, a higher burden of comorbidities and less functional reserve are expected[6], however, the impact of aging in the surgical outcomes of gastric cancer patients is unknown. Is there an age-specific risk? An age limit where D2 lymphadenectomy should not be performed? Perhaps the clinical performance is more important than age itself.

The purpose of this paper was to evaluate how aging affects the surgical outcomes of GC patients submitted to gastrectomy with curative intent.

METHODS

This is a retrospective cohort study from a single institution. Data came from prospective database. All patients submitted to gastrectomy with curative intent (D1 or D2 lymphadenectomy) due to gastric adenocarcinoma between 2009 and 2019 were considered for inclusion. Those operated in urgency or with metastatic disease in the pathology report were excluded.

Patients were divided in quartiles, the 4 age groups were: young age (YA), lower intermediate (LI), higher intermediate (HI) and advanced age (AA). Their characteristics, laboratorial and radiologic exams, pathologic report and follow-up were revised. Pre-operative laboratory tests were considered. Comorbidities were classified with the Charlson Comorbidity Index (CCI)[7] (GC was not considered in the score), American Society of Anesthesiologists Classification (ASA)[8] was used for preoperative clinical performance assessment. Surgery and lymphadenectomy were performed according to the Japanese guidelines[9], the specimen was fixed in Carnoy's solution[10] and the 8th edition of the TNM used for staging[11].

Postoperative complications were classified as minor and major (>II)[12] and divided in surgical (directly related to the surgical procedure) or clinical (indirectly related, e.g.: myocardial infarction, pneumonia, thrombosis). The outcomes assessed were postoperative complications (POC), postoperative surgical mortality (during hospital stay or until 30 days from surgery), 90-day mortality, disease-free survival and overall survival. The 90-day mortality was the chosen parameter to analyze more completely the short-term surgical results[13].

Adjuvant or perioperative chemotherapy (CMT) was performed (capecitabine and oxaliplatin/cisplatin or cisplatin and irinotecan or 5-fluorouracil and oxaliplatin) for T3-4 and N+ disease or at the oncologist's discretion.

This study was approved by the Hospital Ethics Committee and is registered online (www.plataformabrasil.com; CAAE: 30308620.1.0000.0068).

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Statistical analysis

For statistical analysis, nominal data will be presented in frequencies with percentages and numerical data in mean with standard deviation. The *t* test and squared-chi test were used for continuous and categorical variables, respectively.

The association of clinical and surgical variables with the occurrence of 90-day mortality and POC were analyzed by binary logistic regression, and odds ratios (ORs) with 95% confidence interval (95% CI) were calculated.

Survival curves were calculated for each quartile group starting at the day of the gastrectomy and estimated with the Kaplan-Meier method. Log-rank test was used to analyze the difference between the curves. Overall survival (OS) was considered from the date of the surgery until death or last follow-up and Disease-free survival (DFS) until disease recurrence or last follow-up.

Cox regression analysis was performed to evaluate prognostic factors associated with survival. A *P*-value ≤0.05 was considered statistically significant (*P*-values are two-tailed). Analyses were performed with SPSS version 20.0 (Inc, Chicago, IL, USA).

RESULTS

Among 1,157 patients undergoing surgical treatment for GC, 600 fulfilled the inclusion criteria. The mean age was 62.8 years (range 22.7 to 94.5), 58.7% were male. Subtotal gastrectomy and D2 lymphadenectomy were performed in 64.8% and 83% of cases, respectively. Major POC occurred in 14% of the patients and postoperative mortality was 5.3%.

According to the quartiles, the following age groups were obtained: YA≤54.8, LI=54.9–63.7, HI=63.8–72, AA>72. TABLE 1 presents the groups characteristics; 150 patients were included in

TABLE 1. Clinical and surgical characteristics of gastric adenocarcinoma patients operated with curative intent according to the age groups: young age (YA), lower intermediate (LI), higher intermediate (HI) and advanced age (AA).

Variables	YA n=150 (%)	LI n=150 (%)	HI n=150 (%)	AA n=150 (%)	*P*
Age (years)					—
Mean (SD)	45.9 (7.1)	59.6 (2.7)	67.8 (2.4)	77.9 (4.9)	
Gender					**0.037**
Female	77 (51.3)	55 (36.7)	59 (39.3)	57 (38)	
Male	73 (48.7)	95 (63.3)	91 (60.7)	93 (62)	
BMI (Kg/cm²)					0.270
Mean (SD)	24.5 (4.8)	24.6 (6.0)	25.3 (4.7)	24.1 (4.2)	
Hemoglobin (g/dL)					**0.013**
<13	74 (49.8)	77 (51.3)	86 (57.3)	100 (66.7)	
≥13	75 (50.3)	73 (48.7)	64 (42.7)	50 (33.3)	
Albumin (g/dL)					**0.003**
<3.5	16 (12.7)	23 (17.8)	13 (9.8)	34 (25.8)	
≥3.5	110 (87.3)	106 (82.2)	120 (90.2)	98 (74.2)	
Neutrophil lymphocyte ratio					0.491
Mean (range)	2.65 (3.13)	2.71 (2.53)	2.59 (2.42)	3.02 (2.53)	
Charlson–Deyo Comorbidity Index (CCI)					**<0.001**
0	119 (79.3)	97 (64.7)	81 (54)	93 (62)	
≥1	31 (20.7)	53 (35.3)	69 (46)	57 (38)	
ASA Classification					**<0.001**
I / II	135 (90)	110 (73.3)	103 (68.7)	103 (68.7)	
III / IV	15 (10)	40 (26.7)	47 (31.3)	47 (31.3)	
Surgical access					**0.010**
Open	114 (76)	119 (79.3)	126 (84)	135 (90)	
Laparoscopy	36 (24)	31 (20.7)	24 (16)	15 (10)	
Type of ressection					0.109
Subtotal	88 (58.7)	98 (65.3)	95 (63.3)	108 (72)	
Total	62 (41.3)	52 (34.7)	55 (37.7)	42 (28)	
Lymphadenectomy					**<0.001**
D1	7 (4.7)	20 (13.3)	17 (11.3)	58 (38.7)	
D2	143 (95.3)	130 (86.7)	133 (88.7)	92 (61.3)	
Tumor location					0.605
Lower	101 (67.3)	102 (68)	93 (62)	95 (63.3)	
Medial	31 (20.7)	30 (20)	35 (23.3)	32 (21.3)	
Upper	13 (8.87)	12 (8)	18 (12)	14 (9.3)	
Whole	1 (0.7)	4 (2.7)	0 (0)	5 (3.3)	
na	4 (2.7)	2 (1.3)	4 (2.7)	4 (2.7)	

SD: standard deviation; BMI: body mass index; ASA: American Society of Anesthesiologists; na: not applicable. *P*-values in bold are statistically significant.

each group. Female patients were more common in the YA group and the frequency of patients with lower hemoglobin and albumin rates increased with age. The groups HI and AA had higher burden of comorbidities and higher ASA score. In relation to surgery, D2 lymphadenectomy and laparoscopic surgery were more frequent in younger groups (YA and LI), while D1 was more frequent in the AA group.

Regarding pathological characteristics (TABLE 2), Lauren's diffuse tumors and poorly differentiated histology were more common in the YA group, while intestinal type was predominant in the AA patients. There was no significant difference in pT, pN and pTNM status between groups.

Short-term outcomes

Postoperative outcomes are presented in TABLE 3. Major complications raised progressively according to the age group. The frequency of major clinical complications according to the age quartile group was: 0.7% vs 4.7% vs 5.3% vs 7.3% (P<0.042). Surgical complications were less frequent in the YA group. Postoperative mortality (Clavien V) increased as age progressed (0.7% vs 4% vs 7.3% vs 9.3%, P=0.005) and the same occurred with 90-day mortality (1.3% vs 6.0% vs 7.3% vs 14%, P<0.001).

The length of hospital stay was higher for older patients; Chemotherapy (perioperative or adjuvant) was administered less frequently as age progressed (p<0.001). Recurrence rate was similar among groups (P=0.588)

Analysis of potential risk factors for POC and 90-day mortality is shown in TABLE 4. Age groups and ASA were independent risk factors for major POC, while advanced age group and ASA >II were independent risk factors for 90-day mortality.

Survival analysis

In a median follow-up of 31.1 months (mean of 36.9 months), 208 patients died and 129 had disease recurrence. The OS and DFS rates for the entire cohort were 57.2% and 71.4%, respectively.

Among the 129 patients who had recurrence (YA: 37, LI: 34, HI: 30, AA: 29), there was no difference concerning the site of relapse among groups (regional vs peritoneal vs distant). YA patients had one site of relapse in 94.6% of the times vs 73.5% (LI), 70% (HI) and 64.3% (AA) (P=0.019).

TABLE 2. Pathological characteristics of gastric adenocarcinoma patients operated with curative intent according to the age groups: young age (YA), lower intermediate (LI), higher intermediate (HI) and advanced age (AA).

Variables	YA n=150 (%)	LI n=150 (%)	HI n=150 (%)	AA n=150 (%)	*P*
Tumor size (cm)					0.051
Mean (range)	4.5 (2.8)	5 (3.2)	4.3 (2.4)	5.1 (3.3)	
Lauren type					**<0.001**
Intestinal	48 (32)	80 (53.3)	88 (58.7)	112 (74.7)	
Diffuse/mixed	102 (68)	70 (46.7)	62 (41.3)	38 (25.3)	
Histological grade					**<0.001**
Well/mod. differentiated	44 (29.3)	70 (46.7)	78 (52)	93 (62)	
Poorly differentiated	106 (70.7)	80 (53.3)	72 (48)	57 (38)	
Lymphatic invasion					0.315
No	81 (54)	68 (45.3)	83 (55.3)	77 (51.3)	
Yes	69 (46)	82 (54.7)	67 (44.7)	73 (48.7)	
Venous invasion					0.475
No	101 (67.3)	96 (64)	108 (72)	98 (65.3)	
Yes	49 (32.7)	54 (36)	42 (28)	52 (34.7)	
Perineural invasion					0.550
No	74 (49.3)	77 (51.3)	85 56.7)	83 (55.3)	
Yes	76 (50.7)	73 (48.7)	65 (43.3)	67 (44.7)	
Number of lymph nodes					0.118
Mean (SD)	41.1 (17.7)	42.6 (18.3)	41.4 (19.1)	37.8 (16.9)	
pT					0.908
T1/T2	60 (40)	64 (42.7)	66 (44)	62 (41.3)	
T3/T4	90 (60)	86 (57.3)	84 (56)	88 (58.7)	
pN					0.188
N0	62 (41.3)	57 (38)	70 (46.7)	74 (49.3)	
N+	88 (58.7)	93 (62)	80 (53.3)	76 (50.7)	
pTNM					0.665
I/II	80 (53.3)	83 (55.3)	87 (58)	90 (60)	
III	70 (46.7)	67 (44.7)	63 (42)	60 (40)	

SD: standard deviation. *P*-values in bold are statistically significant.

TABLE 3. Surgical outcomes of gastric adenocarcinoma patients operated with curative intent according to the age groups: young age (YA), lower intermediate (LI), higher intermediate (HI) and advanced age (AA).

Variables	YA n=150 (%)	LI n=150 (%)	HI n=150 (%)	AA n=150 (%)	*P*
Postoperative complications (POC)					**<0.001**
No POC	121 (80.7)	100 (66.7)	90 (60.0)	92 (61.3)	
Minor POC	24 (16)	26 (17.3)	34 (22.7)	29 (19.3)	
Major POC	5 (3.3)	24 (16)	26 (17.3)	29 (19.3)	
Major Clinical POC					**0.042**
No	149 (99.3)	143 (95.3)	142 (94.7)	139 (92.7)	
Yes	1 (0.7)	7 (4.7)	8 (5.3)	11 (7.3)	
Major Surgical POC					**0.007**
No	146 (97.3)	132 (88)	131 (87.3)	131 (87.3)	
Yes	4 (2.7)	18 (12)	19 (12.7)	19 (12.7)	
POC Clavien V					**0.005**
No	149 (99.3)	144 (96)	139 (92.7)	136 (90.7)	
Yes	1 (0.7)	6 (4)	11 (7.3)	14 (9.3)	
Length of hospital stay (days)					**0.001**
Mean (SD)	9.7 (5.5)	12.0 (8.7)	13.8 (11.5)	12.9 (10.5)	
Chemotherapy					**<0.001**
No	55 (36.7)	57 (38)	67 (44.7)	111 (74)	
Yes	95 (63.3)	93 (62)	83 (55.3)	39 (26)	
90-day mortality					**<0.001**
No	148 (98.7)	141 (94)	139 (92.7)	129 (86)	
Yes	2 (1.3)	9 (6)	11 (7.3)	21 (14)	

SD: standard deviation. *P*-values in bold are statistically significant.

TABLE 4. Multivariate analysis for major postoperative complications (POC) and 90-day mortality of gastric adenocarcinoma patients operated with curative intent.

Major POC	**Univariate**			**Multivariate**		
Variables	**OR**	**95%CI**	***P***	**OR**	**95%CI**	***P***
Male (vs female)	1.80	1.09–2.96	**0.021**	1.58	0.95–2.63	0.080
Age group (vs YA)						
LI	5.52	2.05–14.91	**0.001**	4.67	1.71–12.73	**0.003**
HI	6.08	2.27–16.31	**<0.001**	5.08	1.87–13.81	**0.001**
AA	6.95	2.61–18.51	**<0.001**	5.77	2.14–15.53	**0.001**
ASA III/IV (vs I/II)	2.37	1.46–3.84	**<0.001**	1.88	1.04–3.40	**0.038**
Charlson ≥1 (vs 0)	1.65	1.04–2.64	**0.035**	1.01	0.56–1.80	0.976
Total gastrectomy (vs subtotal)	1.16	0.72–1.87	0.545	—	—	—
Hemoglobin <13 (vs ≥13)	1.10	0.69–1.76	0.680	—	—	—
Albumin <3.5 (vs ≥3.5)	1.24	0.65–2.33	0.513	—	—	—
NLR ≥2.5 (vs <2.5)	1.20	0.75–1.93	0.442	—	—	—
90-day mortality	**Univariate**			**Multivariate**		
Variables	**OR**	**95%CI**	***P***	**OR**	**95%CI**	***P***
Male (vs female)	1.90	0.95–3.78	0.067	—	—	—
Age group (vs YA)						
LI	4.72	1.00–22.24	**0.050**	2.81	0.57–13.88	0.204
HI	5.86	1.27–26.89	**0.023**	3.05	0.62–14.92	0.168
AA	12.05	2.77–52.37	**0.001**	7.38	1.39–33.15	**0.009**
ASA III/IV (vs I/II)	4.85	2.56–9.18	**<0.001**	3.11	1.39–6.98	**0.006**
Charlson ≥1 (vs 0)	2.79	1.49–5.25	**0.001**	1.24	0.55–2.79	0.603
Total gastrectomy (vs subtotal)	0.79	0.40–1.54	0.483	—	—	—
Hemoglobin <13 (vs ≥13)	1.49	0.78–2.86	0.227	—	—	—
Albumin <3.5 (vs ≥3.5)	2.13	1.01–4.46	**0.046**	1.45	0.65–3.20	0.365
NLR ≥2.5 (vs <2.5)	2.00	1.07–3.73	**0.029**	1.36	0.67–2.71	0.406

YA: young age; LI: lower intermediate; HI: higher intermediate; AA: advanced age; ASA: American Society of Anesthesiologists; NLR: neutrophil lymphocyte ratio; CI: confidence interval; OR: odds ratio. *P*-values in bold are statistically significant.

DFS was equivalent among quartiles (p=0.91) (FIGURE 1). Considering OS, the AA group had significantly worse survival compared to the YA (*P*=0.007); LI and HI patients had similar OS compared to the YA group (*P*=0.179 and *P*=0.08, respectively).

OS was studied according to the extent of the lymphadenectomy (FIGURE 1). Analyzing the 3 younger quartiles together, D2 patients had better survival compared to D1 (*P*=0.005). In the AA group, there was no significant improvement in OS for patients who underwent D2 lymphadenectomy compared to D1 (*P*=0.065).

Univariate and multivariate analysis are showed in TABLE 5. The type of gastrectomy, histologic type, pT, pN and CMT were factors associated with DFS at the multivariate analysis. For OS, advanced age, ASA III/IV, total gastrectomy, pT3-4 and pN+ were independent risk factors related with worse survival. The hazard ratio for AA group was 1.72 (95%CI 1.15–2.57, *P*=0.008).

DISCUSSION

The incidence of malignant neoplasms (GC included) increases as age advances. DNA damage and cell exposition to carcinogens accumulate over time[14]. Besides, age-related changes to the DNA repair, immune and endocrine systems may promote or facilitate carcinogenesis[15]. With aging comorbidities raise and performance deteriorates. So, theoretically outcomes of a morbid procedure,

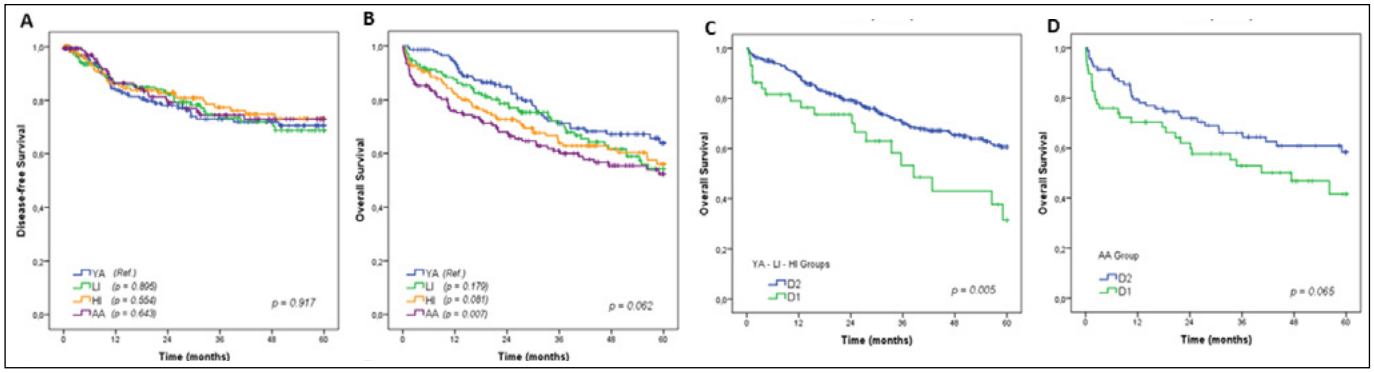

FIGURE 1. Disease-free survival and overall survival of gastric adenocarcinoma patients operated with curative intent according to their age groups and the extension of the lymphadenectomy.

TABLE 5. Univariate and multivariate analysis for disease-free survival (DFS) and overall survival (OS) of gastric adenocarcinoma patients operated with curative intent.

Disease-free survival		Univariate			Multivariate	
Variables	HR	95%CI	*P*	HR	95%CI	*P*
Male (vs female)	1.30	0.91–1.86	0.149	—	—	—
Age group (vs YA)						
LI	0.97	0.61–1.55	0.913	—	—	—
HI	0.86	0.53–1.39	0.539	—	—	—
AA	0.89	0.54–1.45	0.637	—	—	—
Total gastrectomy (vs subtotal)	2.35	1.66–3.32	**<0.001**	2.21	1.56–3.14	**<0.001**
Diffuse/mixed Laurén type (vs others)	1.61	1.14–2.28	**0.007**	1.45	1.01–1.06	**0.041**
pT3/pT4 status (vs pT1/pT2)	8.98	4.95–16.27	**<0.001**	5.14	2.64–10.01	**<0.001**
pN+ (vs pN0)	9.75	5.49–17.31	**<0.001**	5.98	3.14–11.40	**<0.001**
non-CMT vs (CMT)	0.64	5.49–17.31	**0.016**	2.59	1.77–3.81	**<0.001**
Overall Survival		**Univariate**			**Multivariate**	
Variables	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***P***
Male (vs female)	1.37	1.03–1.81	**0.032**	1.19	0.89–1.58	0.243
Age group (vs YA)				—	—	—
LI	1.31	0.87–1.97	0.193	1.23	0.82–1.87	0.319
HI	1.42	0.96–2.13	0.083	1.32	0.88–1.99	0.179
AA	1.71	1.15–2.53	**0.008**	1.72	1.15–2.57	**0.008**
Charlson 1 (vs 0)	1.30	0.98–1.72	0.063	—	—	—
ASA III/IV (vs I/II)	1.95	1.46–2.62	**<0.001**	1.70	1.26–2.29	**0.001**
Total gastrectomy (vs subtotal)	1.56	1.19–2.06	**0.001**	1.45	1.09–1.92	**0.010**
pT3/pT4 status (vs pT1/pT2)	2.51	1.83–3.42	<0.001	1.72	1.20–2.46	**0.003**
pN+ (vs pN0)	2.73	2.01–3.71	**<0.001**	1.98	1.39–2.82	**<0.001**
non-CMT vs (CMT)	1.09	0.83–1.44	0.520	—	—	—

YA: young age; LI: lower intermediate; HI: higher intermediate; AA: advanced age; ASA: American Society of Anesthesiologists; CMT: chemotherapy; CI: confidence interval; HR: hazard ratio. *P*-values in bold are statistically significant.

such as gastrectomy, worsen as age progresses. However, the impact of aging in the results of gastrectomy for GC is poorly addressed in the literature[16]. Two studies from a vast database suggest that older age associates with worse prognosis, however important limitations and bias are observed: information concerning margin status, resections with curative intent, and lymphadenectomy performed are all missing; also lymph node count was very low[17,18]. Other study included 448 patients and observed that age was an independent risk factor for gastric cancer-specific mortality (GCSM), with age 70–79 being associated with increased GCSM for stages I and II. Patients with stage IV disease, who underwent palliative treatment, and who received support care alone were included. Margins and lymphadenectomy performed and not reported[19].

In our cohort, aging significantly increased complications and shortened OS. Patients in the younger quartile (≤54.8) showed fewer major surgical complications, probably because they are more fit and with superior performance. Major clinical complications, postoperative and 90-day mortalities gradually raised as age quartile increased.

The independent risk factors that related to major POC were ASA score >II (OR: 1.88) and aging; the risk increased progressively according to the age quartile (OR: 4.67 vs 5.08 vs 5.77). The two independent risk factors for 90-day mortality were ASA >II and being in the advanced age quartile. The difference in 90-day mortality between the youngest and eldest was colossal (1.3% vs 14%) and demonstrates the importance of aging in the short-term results. It also indicates that after 72-year-old indication for surgery should be individualized. The 30-day mortality is commonly used to access the surgical risk, we considered the death rate until 90 days to have a better understanding of the surgical and oncological short-term results[20].

When we consider the long-term, DFS was not impacted by aging, but OS was worse for those with advanced age. These findings are with agreement with available data[21]. They also suggest that elders may not live long enough to show oncological benefit after radical procedure. At this moment, few countries in the world have life expectancy above 80-year-old. So, submitting elders who are at their final years to a morbid procedure is a difficult decision, especially when we consider that with aging complications increase and life expectancy shortens. D2 was less performed as age increased demonstrating the concern to limit surgical aggressiveness in elders. Advanced age was an independent risk factor for worse OS and the benefit of D2 lymphadenectomy in this quartile failed to achieve the statistical significance observed in the younger quartiles.

Despite being more aggressive, total gastrectomy did not correlate with major POC or 90-day mortality. However, it was associated with worse DFS and OS. Possible explanations are that compared to subtotal gastrectomy, recovery and nutritional status are more impaired and larger lesions, that have a worse prognosis per se, require total gastrectomy more frequently.

Our study has other interesting findings. As expected, the frequency of diffuse tumors was greater in younger patients[22]. Neutrophil lymphocyte ratio (NLR), a prognostic maker in GC, increased with aging suggesting less specific immunological response as age advances[23]. As age progressed, laparoscopic access and D1 were less frequent, the postoperative length of stay longer, and chemotherapy less used. The two main factors that influenced DFS were pN and pT status; while pN, pT, and advanced age had the greatest impact in OS.

Although data collection was prospective, our study has the limitations of its retrospective nature. Another limitation is the fact that, as expected, older patients received CMT less frequently. On the other hand, it is the first time that aging is correlated with the short-term outcomes, and that only patients submitted to potential curative resection were included in the cohort. Also, perioperative care and surgical technique changed significantly over the last decades, we considered a recent time frame from a unique center to make the cohort more uniform.

CONCLUSION

Patients younger than age 55 have less surgical complications. As age progresses clinical complications and 90-day mortality gradually rise. DFS is not impacted by aging, while OS is worse for those above age 72. Also, D2 dissection should be indicated with caution for those older than 72 years.

Authors' contribution

Study concept and design: Dias AR. Data analysis: Pereira MA. Data acquisition: Ramos MFKP. Manuscript preparation: Dias AR and Ribeiro Jr U. Manuscript editing: Zilberstein B. Final review: Cecconello I.

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RESUMO – **Contexto** – Conforme a idade avança, se esperam mais morbidades e menor reserva funcional. Entretanto não está claro qual o impacto do envelhecimento nos resultados cirúrgicos do câncer gástrico (CaG). **Objetivo** – O intuito deste estudo é avaliar os resultados cirúrgicos de pacientes com CaG de acordo com o grupo etário. **Métodos** – Pacientes submetidos a gastrectomia por adenocarcinoma gástrico com intuito curativo foram divididos em quartis. Cada grupo incluiu 150 indivíduos e os limites etários foram: ≤54,8; 54,9–63,7; 63,8–72; >72. Os resultados avaliados foram: complicações pós-operatórias (CPO), mortalidade em 90 dias, sobrevida livre de doença (SLD) e sobrevida global (SG). **Resultados** – Complicações cirúrgicas maiores ocorreram em 2,7% dos pacientes no quartil mais jovem vs 12% para os demais (P=0,007). A incidência de complicações clínicas maiores aumentou conforme o quartil: 0,7% vs 4,7% vs 5,3% vs 7,3% (P<0,042). A pontuação ASA e a idade foram fatores de risco independentes para CPO maiores. A mortalidade em 90 dias aumentou progressivamente conforme o quartil etário: 1,3% vs 6,0% vs 7,3% vs 14% (P<0,001). A SLD foi equivalente entre os quartis, enquanto a SG foi significativamente pior para os >72 anos de idade. Linfadenectomia D2 aumentou a SG apenas para os 3 quartis mais jovens. Idade > 72 foi fator independente de risco para pior SG (razão de chances de 1,72) **Conclusão** – Pacientes < 55 anos tem menos complicações cirúrgicas. Conforme a idade avança, as complicações clínicas e a mortalidade em 90 dias aumenta gradualmente. A SG é pior se >72 anos e a indicação de linfadenectomia D2 deve ser individualizada a partir dessa idade.

DESCRITORES – Neoplasias gástricas. Envelhecimento. Sobrevida. Complicações pós-operatórias. Gastrectomia.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68:394-424. doi:10.3322/caac.21492
2. Ramos M, Pereira MA, Yagi OK, Dias AR, Charruf AZ, Oliveira RJ, et al. Surgical treatment of gastric cancer: a 10-year experience in a high-volume university hospital. Clinics (Sao Paulo, Brazil). 2018;73 (Suppl 1):e543s. doi:10.6061/clinics/2018/e543s
3. Baiocchi GL, Marrelli D, Verlato G, Morgagni P, Giacopuzzi S, Coniglio A, et al. Follow-up after gastrectomy for cancer: an appraisal of the Italian research group for gastric cancer. Ann Surg Oncol. 2014;21:2005-11. doi:10.1245/s10434-014-3534-8
4. Nashimoto A, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, et al. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. Gastric Cancer. 2013;16:1-27. doi:10.1007/s10120-012-0163-4
5. Norero E, Vega EA, Diaz C, Cavada G, Ceroni M, Martinez C, et al. Improvement in postoperative mortality in elective gastrectomy for gastric cancer: Analysis of predictive factors in 1066 patients from a single centre. Eur J Surg Oncol. 2017;43:1330-6. doi:10.1016/j.ejso.2017.01.004
6. Ramos MFKP, Pereira MA, Dias AR, Yagi OK, Zaidan EP, Ribeiro-Júnior U, et al. Surgical outcomes of gastrectomy with D1 lymph node dissection performed for patients with unfavorable clinical conditions. Eur J Surg Oncol. 2019;45:460-5. doi:10.1016/j.ejso.2018.11.013
7. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-83.
8. Doyle DJ, Goyal A, Bansal P, Garmon EH (2017) American Society of Anesthesiologists Classification (ASA Class). In: StatPearls. StatPearls Publishing LLC., Treasure Island FL. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441940/
9. Japanese Gastric Cancer A. Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer. 2017;20:1-19. doi:10.1007/s10120-016-0622-4
10. Dias AR, Pereira MA, Mello ES, Zilberstein B, Cecconello I, Ribeiro Junior U. Carnoy's solution increases the number of examined lymph nodes following gastrectomy for adenocarcinoma: a randomized trial. Gastric Cancer. 2016;19:136-42. doi:10.1007/s10120-014-0443-2
11. Ajani JA, In H, Sano T, Gaspar LE, Erasmus JJ, Tang LH, et al. American Joint Committee on Cancer (AJCC). Cancer Staging Manual. 8th edition. Stomach. Springer. 2017;17:203-20.
12. Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. Ann Surg. 2004;240:205-13. doi:10.1097/01.sla.0000133083.54934.ae
13. Damhuis RA, Wijnhoven BP, Plaisier PW, Kirkels WJ, Kranse R, van Lanschot JJ. Comparison of 30-day, 90-day and in-hospital postoperative mortality for eight different cancer types. Br J Surg. 2012; 99 (8):1149-1154. doi:10.1002/bjs.8813
14. Arai T, Takubo K. Clinicopathological and molecular characteristics of gastric and colorectal carcinomas in the elderly. Pathol Int. 2007;57:303-14. doi:10.1111/j.1440-1827.2007.02101.x
15. Anisimov VN. Carcinogenesis and aging. Adv Cancer Res. 1983;40:365-424. doi:10.1016/s0065-230x(08)60684-3
16. Saito H, Osaki T, Murakami D, Sakamoto T, Kanaji S, Tatebe S, Tsujitani S, Ikeguchi M. Effect of age on prognosis in patients with gastric cancer. ANZ J Surg. 2006;76:458-61. doi:10.1111/j.1445-2197.2006.03756.x
17. Song P, Wu L, Jiang B, Liu Z, Cao K, Guan W. Age-specific effects on the prognosis after surgery for gastric cancer: A SEER population-based analysis. Oncotarget. 2016;7:48614-24. doi:10.18632/oncotarget.9548
18. Chen J, Xu Y, Long Z, Zhou Y, Zhu H, Wang Y, Shi Y. Impact of Age on the Prognosis of Operable Gastric Cancer Patients: An Analysis Based on SEER Database. Medicine (Baltimore). 2016;95:e3944. doi:10.1097/MD.0000000000003944
19. Lee JG, Kim SA, Eun CS, Han DS, Kim YS, Choi BY, et al. Impact of age on stage-specific mortality in patients with gastric cancer: A long-term prospective cohort study. PLoS One. 2019;14:e0220660. doi:10.1371/journal.pone.0220660
20. Damhuis RA, Wijnhoven BP, Plaisier PW, Kirkels WJ, Kranse R, van Lanschot JJ. Comparison of 30-day, 90-day and in-hospital postoperative mortality for eight different cancer types. Br J Surg. 2012;99:1149-54. doi: 10.1002/bjs.8813.
21. Xu Y, Wang Y, Xi C, Ye N, Xu X. Is it safe to perform gastrectomy in gastric cancer patients aged 80 or older?: A meta-analysis and systematic review. Medicine (Baltimore). 2019;98:e16092. doi:10.1097/MD.0000000000016092
22. Arai T, Esaki Y, Inoshita N, Sawabe M, Kasahara I, Kuroiwa K, Honma N, Takubo K. Pathologic characteristics of gastric cancer in the elderly: a retrospective study of 994 surgical patients. Gastric Cancer. 2004;7:154-9. doi:10.1007/s10120-004-0285-4
23. Szor DJ, Dias AR, Pereira MA, Ramos MFKP, Zilberstein B, Cecconello I, Ribeiro-Júnior U. Prognostic Role of Neutrophil/Lymphocyte Ratio in Resected Gastric Cancer: A Systematic Review and Meta-analysis. Clinics (Sao Paulo). 2018;73:e360. doi:10.6061/clinics/2018/e360

ORIGINAL ARTICLE

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Esophageal cancer mortality in Brazil: a time-series analysis from the global burden of disease study

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ABSTRACT – **Background** – In the world, around 450,000 new cases of esophageal cancer are diagnosed each year. **Objective** – To evaluate the trend of esophageal cancer mortality rates in Brazil between 1990-2017. **Methods** – A time series study using data on mortality from esophageal cancer in residents ≥30 years in Brazil from 1990 to 2017. Data was estimated by the Global Burden of Disease (GBD) study and analyzed according to sex, age group and federal unit of Brazil. The standardized rates according to age were calculated by the direct method using the standard GBD world population. Annual average percentage change and 95% confidence interval (95% CI) were calculated for mortality by Joinpoint regression. **Results** – The age-standardized mortality rate in males was 20.6 in 1990 and 17.6/100,000 in 2017, increasing according to age, being 62.4 (1990) and 54.7 (2017) for ≥70 years. In women, the age-standardized mortality rate was 5.9 in 1990 and 4.2/100,000 in 2017. There was a reduction in mortality rates in all age groups and both sexes with great variation among the states. **Conclusion** – Despite the high mortality rates for esophageal cancer in Brazil, the trend was decreasing, but with regional differences. Mortality was around four times higher in men.

HEADINGS – Esophageal neoplasms. Global burden of disease. Mortality. Epidemiological studies.

INTRODUCTION

In the world, around 450,000 new cases of esophageal cancer are diagnosed every year[1]. These incidences have been growing rapidly[2,3]. The estimative for 2018 indicated that it is ranked number seven as the cancer with the most incidents and the sixth biggest cause of death by cancer in the world[4]. In Brazil, 11,405 new cases of esophageal cancer were registered and 9,761 deaths, of which 7,645 (78%) were men[4]. In 2015, it was the fifth largest cause of death in male patients[5]. Studies point to differences regarding the histological type[6-8], with squamous cell carcinoma being the most predominant, especially in South America and Asia[9].

The literature indicates a predominance in males with a highest of incidence between the fifth and sixth decade of life[1,6]. Obesity is cited as a risk factor for esophageal cancer by predisposing to gastroesophageal reflux disease (GERD) and Barrett's esophagus, a preceding condition to adenocarcinoma[6]. Smoking is one of the most important risk factors for squamous cell carcinoma[7]. Besides that, the consumption of alcohol, a high fat diet[8], and the consumption of hot foods are associated with this type of cancer[10].

Patients with esophageal cancer have a reserved prognosis, in spite of the survival rate increase over the last five years that has been verified in studies, 5% in the 1960s and around 20% in the 2010s[11]. In the cases in which the diagnostic is done in the initial phase and due to the advancement of the endoscopic treatment with the minimally invasive technique of resection called endoscopic dissection of the submucosa, the survival in five years reaches 95%[12]. However, more than 30% of patients develop metastasis, lowering the survival rate in five years to 4.5%[1]. Differences in the outcome of treatment, in terms of survivability and recurrence, can be found according histological type, but it also depends on the state of the disease and the treatment done[13,14].

In Brazil, a study evaluated the mortality of cancer from 1990 until 2015 and estimated a significant reduction of approximately 14% in mortality by esophageal cancer with similar patterns among the states, except for Ceará and Paraíba, both in the Northeast region, which had a significant increase in the last decades[5]. On the other hand, another study evaluated the temporal trends of esophageal cancer and reported an increase of incidence between 2005 and 2015, while the death rate remained the same[15].

Considering the few studies on the subject and the divergence in the data presented, which could be related to the failure in the registering of the cause of death into the information systems, it is expected that the use of estimates which have a data source that was corrected and treated to generate standardized information, as it was done all over the world by the Global Burden of Diseases (GBD) study, can elucidate the epidemiological situation of esophageal cancer in Brazil and in each state. Thus, the objective of this study was to estimate the mortality rate of esophageal cancer in Brazil and in the states of the country and evaluate the tendency between 1990 and 2017.

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METHODS

This is a time series study that used data concerning mortality by esophageal cancer (the tenth revision of the International Classification of Diseases, ICD-10: C15) that occurred in residents starting at 30 years old in Brazil, between 1990 and 2017[16]. To do this, applied corrections to the mortality data were done as a correction of death register and the redistribution of incorrectly defined and unspecific codes, i.e. garbage codes, with the purpose of obtaining estimates that are more coherent with the national reality. The estimates were done by the GBD study, coordinated by the Institute of Health Metrics and Evaluation (IHME)[17].

The data referent to the frequency of deaths by esophageal neoplasia was analyzed according to the year and the territory considered in a population that is 30 years old or older. The specific rates were calculated by (30 to 49 years old, 50 to 69 years, and 70 or older), and the standard rate by age (30 years old or older), according to sex and the 26 states of Brazil and the Federal District. The standardized rates according to age were calculated by the direct method using the standard GBD world population[17]. The crude and standardized rates of mortality was calculated for 100,000 inhabitants. The average annual percentage of change (AAPC) and the respective 95% confidence intervals (95% CI) were estimated to evaluate the trends of mortality during 1990 and 2017 by use of the Joinpoint Regression Program software[18], version 4.7.0.0. The AAPC is the weighted average of the angular coefficients of the linear regression, with weights equal to lengths to each segment of the whole interval. An increase or decrease in the trend is statistically significant when different from 0 (P<0.05) and stable when equal to 0 (P>0.05).

This study respected the ethical preconceptions of research and specific Brazilian resolutions. Data was used in an aggregated manner without identifying individuals and causing any damage to them. The GBD study is compliant with the Guidelines for Accurate and Transparent Health Estimates Reporting statement. This study was approved by the Research Ethics Committee of *Universidade Federal de Minas Gerais* (CAAE no. 62803316.7.0000.5149).

RESULTS

In Brazil, between 1990 and 2017, the highest esophageal cancer mortality rates were ascribed to males (FIGURE 1). The mortality rates increased with age, thus the largest rates were found for people who were 70 years old or older for both sexes (FIGURE 2).

Among men in Brazil, the mortality rate between 30 and 49 years old (per 100,000 men) was 3.3 in 1990 and 3.0 in 2017, with a reduction of −0.3% per year; between 50 and 69 years old, the rate was 30.9 (1990) and 27.2 (2017) with a reduction of −0.4% per year. The standardized mortality rate according to age (30 years old or older) was 20.6 and 17.6 per 100,000 inhabitants in 1990 and 2017, respectively (TABLE 1).

The age group between 30 and 49 years old in 11 states, generally located in the Northeast region of Brazil, presented a trend of increase, while four states presented a trend of reduction. Between the ages of 50 and 69, eight states presented an increase and six other states showed a trend of decrease, and were generally located in the South and Southeast regions. Among men 70 years old or older, nine states had a trend of increase and six states had a reduction. To the standardized rate per age (30 years old or older), there was a trend of increase in seven states and a trend of decrease in nine

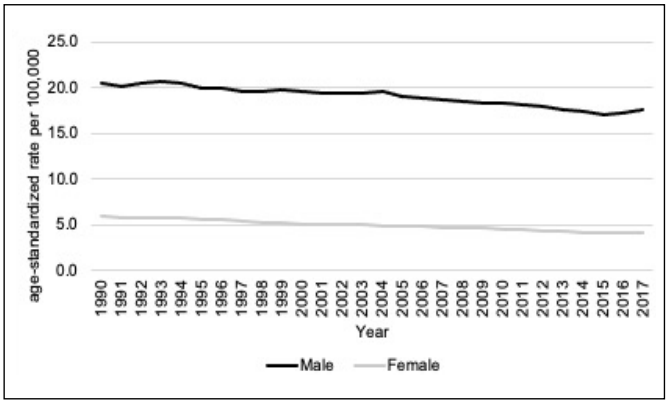

FIGURE 1. Temporal trends in esophageal cancer mortality, according to sex, in Brazil, in the period 1990-2017.

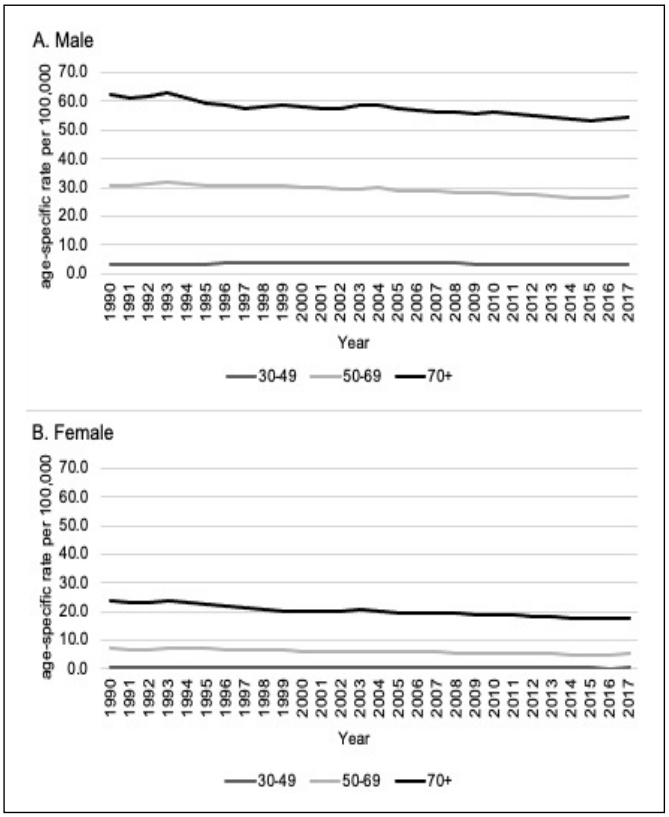

FIGURE 2. Temporal trends in esophageal cancer mortality, according to sex and age group, in Brazil, in the period 1990-2017.

states (TABLE 1). The increase trend is notable in Rio Grande do Norte and Bahia, among all age groups, as well as the reduction in the state of São Paulo. The trend of increase was verified, in general, in the Northeast region, and the reduction was verified in the states of the South and Southeast regions.

Among women, in Brazil, the mortality rate between 30 and 49 years old (per 100,000 women) was 0.7 in 1990, and 0.6 in 2017, with a reduction of −0.7% per year; in the age group between 50 and 69 years old, the rate was 7.0 (1990) and 5.2 (2017), with a decrease of −1.1% per year; and among 70 years old or older, the rate was 24.0 (1990) and 17.7 (2017), with a decrease of −1.1% per

TABLE 1. Esophageal cancer mortality rate and trend in male, by age-group, in states and Brazil, in the period 1990-2017.

Population	30-49 years old			50-69 years old			70+ years old			30+ years old		
	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)
	1990	2017		1990	2017		1990	2017		1990	2017	
Acre	1.5	1.6	0.1 (-0.4;0.7)	12.4	12.4	-0.1 (-0.6;0.5)	25.4	29.3	**0.5* (0.3;0.7)**	8.6	8.8	0.0 (-0.3;0.4)
Alagoas	1.8	1.9	0.2 (-0.3;0.6)	11.5	15.1	**0.9* (0.4;1.4)**	25.7	30.1	**0.7* (0.2;1.2)**	8.3	9.9	**0.6* (0.3;1.0)**
Amapá	1.2	1.6	**1.1* (0.8;1.3)**	11.1	13.2	**0.6* (0.4;0.8)**	26.2	32.3	**0.8* (0.6;1.0)**	8.0	9.6	**0.7* (0.5;0.9)**
Amazonas	1.5	1.4	-0.1 (-0.6;0.4)	12.5	13.2	0.2 (-0.1;0.6)	29.0	31.8	0.5 (-0.3;1.4)	9.0	9.3	0.2 (-0.2;0.6)
Bahia	2.1	3.3	**1.7* (1.2;2.2)**	16.8	28.1	**1.9* (1.7;2.0)**	35.7	52.6	**1.4* (0.9;1.9)**	11.4	17.8	**1.7* (1.4;1.9)**
Ceará	2.1	2.4	**0.6* (0.2;1.0)**	17.6	21.4	0.8 (-0.0;1.6)	35.9	50.2	**1.3* (0.9;1.7)**	11.5	14.7	**1.0* (0.5;1.4)**
Distrito Federal	2.6	2.0	**-1.0* (-1.8;-0.3)**	23.5	18.1	**-0.9* (-1.7;-0.2)**	56.2	52.1	-0.3 (-1.1;0.5)	18.2	14.9	**-0.8* (-1.5;-0.1)**
Espírito Santo	3.7	4.6	0.7 (-0.4;1.9)	37.0	36.0	-0.1 (-0.6;0.3)	83.8	71.8	-0.6 (-1.4;0.1)	26.9	23.6	**-0.5* (-0.9;-0.1)**
Goiás	2.4	2.3	-0.2 (-1.0;0.6)	20.2	19.3	-0.2 (-0.7;0.3)	39.7	40.2	0.0 (-0.8;0.8)	13.5	12.8	-0.2 (-0.8;0.3)
Maranhão	1.5	1.0	**-1.7* (-2.3;-1.1)**	8.8	8.3	-0.2 (-1.1;0.6)	14.6	19.1	**1.2* (0.4;2.1)**	5.8	5.7	0.0 (-0.9;1.0)
Mato Grosso	2.2	2.5	**0.5* (0.2;0.8)**	20.6	21.2	0.1 (-0.4;0.6)	41.8	44.0	0.2 (-0.3;0.7)	14.0	14.2	0.0 (-0.4;0.5)
Mato Grosso do Sul	2.9	3.4	0.6 (-0.3;1.6)	23.3	28.2	**0.8* (0.1;1.4)**	55.7	56.9	-0.1 (-0.4;0.2)	17.2	18.5	0.4 (-0.3;1.0)
Minas Gerais	4.0	5.0	**0.7* (0.0;1.5)**	36.7	35.7	-0.3 (-0.9;0.4)	78.8	68.0	**-0.6* (-1.0;-0.1)**	25.4	23.0	-0.5 (-1.1;0.1)
Pará	1.2	1.3	0.3 (-0.5;1.0)	11.2	12.4	0.5 (-0.4;1.3)	26.6	29.2	0.3 (-0.3;0.9)	8.0	8.6	0.3 (-0.4;1.0)
Paraíba	2.0	2.5	**0.8* (0.4;1.1)**	15.6	19.1	**0.8* (0.3;1.2)**	37.9	40.3	0.2 (-0.4;0.8)	11.1	12.8	0.6 (-0.0;1.1)
Paraná	4.4	4.0	-0.4 (-1.0;0.2)	46.0	34.0	**-1.1* (-1.4;-0.8)**	105.5	75.4	**-1.2* (-1.6;-0.9)**	32.7	23.0	**-1.3* (-1.7;-0.8)**
Pernambuco	1.6	2.3	**1.4* (0.9;1.9)**	14.1	19.8	**1.3* (0.8;1.7)**	32.2	44.5	**1.2* (0.7;1.7)**	10.1	13.5	**1.1* (0.6;1.6)**
Piauí	1.4	1.9	**1.0* (0.4;1.6)**	11.4	13.9	0.8 (-0.2;1.8)	26.7	27.7	0.1 (-0.7;1.0)	8.3	9.1	0.4 (-0.3;1.2)
Rio de Janeiro	3.6	2.9	**-0.9* (-1.5;-0.3)**	30.7	27.1	-0.5 (-1.1;0.2)	63.8	47.5	**-1.0* (-1.5;-0.5)**	21.2	16.5	**-0.9* (-1.4;-0.4)**
Rio Grande do Norte	1.7	2.6	**1.6* (1.0;2.2)**	12.4	20.3	**1.9* (0.8;3.0)**	31.9	45.8	**1.3* (0.3;2.3)**	9.1	14.0	**1.5* (1.0;2.1)**
Rio Grande do Sul	6.1	5.1	-0.6 (-1.2;0.0)	61.1	42.9	-0.6 (-1.2;0.0)	135.3	99.4	**-1.1* (-1.5;-0.8)**	42.9	29.5	**-1.4* (-1.6;-1.1)**
Rondônia	2.7	2.4	-0.4 (-1.4;0.6)	22.9	19.5	**-0.6* (-1.0;-0.2)**	49.6	47.2	-0.0 (-0.6;0.5)	16.8	14.0	**-0.6* (-1.1;-0.1)**
Roraima	1.5	1.5	0.1 (-0.5;0.7)	14.1	12.5	**-0.4* (-0.6;-0.2)**	31.1	32.1	0.1 (-0.3;0.4)	10.5	9.5	**-0.4* (-0.7;-0.1)**
Santa Catarina	4.1	3.8	-0.3 (-1.3;0.7)	50.5	33.1	**-1.6* (-2.0;-1.2)**	115.6	79.4	**-1.5* (-1.8;-1.1)**	35.5	23.4	**-1.5* (-2.1;-1.0)**
São Paulo	3.8	2.7	**-1.3* (-1.8;-0.8)**	36.2	28.4	**-1.3* (-1.8;-0.8)**	71.9	51.0	**-1.2* (-1.6;-0.8)**	24.4	17.4	**-1.2* (-1.5;-0.9)**
Sergipe	1.5	2.2	**1.3* (0.3;2.3)**	11.4	15.6	**1.3* (0.7;2.0)**	28.2	31.1	0.4 (-0.3;1.1)	8.3	10.4	**0.9* (0.0;1.7)**
Tocantins	1.4	1.6	**0.7* (0.4;0.9)**	10.5	11.3	0.2 (-0.2;0.6)	20.2	24.9	**0.8* (0.3;1.3)**	7.5	7.8	0.2 (-0.1;0.4)
Brazil	3.3	3.0	**-0.3* (-0.6;-0.1)**	30.9	27.2	**-0.4* (-0.6;-0.3)**	62.4	54.7	**-0.5* (-0.7;-0.2)**	20.6	17.6	**-0.6* (-0.8;-0.3)**

AAPC: average annual percentage of change; CI: confidence interval. *P-value <0.05.

year. The standardized mortality rate by age group was of 5.9 and 4.2 per 100,000 women in 1990 and 2017, respectively (TABLE 2).

In the age group of 30 and 49 years, 12 states presented a trend of decrease in mortality rate among women. The 50 to 69 years old group in two states in the Northeast region presented an increase, and 17 other states presented a decrease. Among those 70 years old or older, there was an increase trend (in Ceará, in the Northeast region) and 16 states had a reduction trend. At last, the standardized rate according to age (30 years old or older) presented an increase trend in one state (Ceará) and a trend of decrease in 20 states (TABLE 2).

TABLE 2. Esophageal cancer mortality rate and trend in female, by age-group, in states and Brazil, in the period 1990-2017.

Population	30-49 years old			50-69 years old			70+ years old			30+ years old		
	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)	Rate		AAPC (95% CI)
	1990	2017		1990	2017		1990	2017		1990	2017	
Acre	0.3	0.2	**-0.5* (-0.7;-0.2)**	2.6	2.1	**-0.9* (-1.2;-0.5)**	8.1	7.7	-0.2 (-0.7;0.4)	2.2	1.8	**-0.7* (-1.1;-0.2)**
Alagoas	0.4	0.4	-0.3 (-1.4;0.7)	3.3	3.3	0.0 (-0.4;0.5)	11.6	11.3	-0.1 (-0.7;0.5)	2.8	2.7	-0.1 (-0.6;0.4)
Amapá	0.3	0.3	-0.1 (-0.7;0.6)	3.2	3.0	-0.2 (-0.4;0.1)	11.4	9.6	**-0.7* (-1.0;-0.5)**	2.9	2.4	**-0.7* (-0.8;-0.5)**
Amazonas	0.3	0.3	-0.5 (-1.5;0.4)	3.3	2.6	**-0.9* (-1.1;-0.6)**	11.3	9.9	-0.4 (-1.1;0.2)	2.8	2.3	**-0.7* (-1.3;-0.2)**
Bahia	0.5	0.6	0.4 (-0.4;1.2)	4.5	5.0	**0.4* (0.1;0.7)**	15.1	14.4	-0.2 (-0.6;0.1)	3.7	3.7	-0.0 (-0.2;0.2)
Ceará	0.5	0.5	0.0 (-0.8;0.9)	4.2	5.4	**1.1* (0.7;1.6)**	17.7	20.5	**0.7* (0.1;1.3)**	3.9	4.6	**0.7* (0.0;1.4)**
Distrito Federal	0.6	0.4	**-1.8* (-2.4;-1.3)**	5.9	3.2	**-2.3* (-2.7;-1.9)**	21.6	14.6	**-1.4* (-1.8;-1.0)**	5.8	3.4	**-1.9* (-2.1;-1.8)**
Espírito Santo	1.0	0.8	**-0.8* (-1.4;-0.2)**	8.8	6.7	**-1.0* (-1.3;-0.7)**	32.9	24.2	**-1.2* (-1.7;-0.7)**	8.5	5.6	**-1.5* (-1.9;-1.1)**
Goiás	0.6	0.5	**-0.8* (-1.1;-0.6)**	5.5	4.4	**-0.8* (-1.3;-0.3)**	21.1	14.0	**-1.4* (-1.9;-0.9)**	5.6	3.5	**-1.6* (-2.1;-1.1)**
Maranhão	0.3	0.2	-0.5 (-1.2;0.2)	1.8	1.9	0.1 (-0.3;0.6)	6.2	5.5	-0.4 (-1.0;0.3)	1.6	1.4	-0.2 (-0.8;0.4)
Mato Grosso	0.5	0.5	-0.4 (-1.0;0.1)	4.9	4.0	**-0.8* (-1.3;-0.3)**	16.3	14.2	-0.5 (-1.2;0.2)	4.2	3.4	**-0.7* (-1.2;-0.2)**
Mato Grosso do Sul	0.7	0.6	-0.1 (-0.5;0.3)	6.6	5.0	**-1.0* (-1.6;-0.5)**	21.9	18.4	**-0.6* (-1.2;-0.0)**	5.8	4.3	**-1.0* (-1.6;-0.5)**
Minas Gerais	1.0	1.0	0.0 (-0.6;0.6)	10.0	7.0	**-1.3* (-1.8;-0.9)**	33.5	24.0	**-1.3* (-1.6;-0.9)**	8.6	5.7	**-1.6* (-1.9;-1.2)**
Pará	0.4	0.3	**-1.0* (-1.3;-0.6)**	3.0	2.4	**-0.8* (-1.3;-0.3)**	11.5	8.9	**-0.9* (-1.1;-0.6)**	2.7	2.1	**-0.9* (-1.1;-0.7)**
Paraíba	0.6	0.5	**-0.7* (-1.4;-0.1)**	4.9	4.2	**-0.5* (-0.8;-0.2)**	19.7	16.4	**-0.8* (-1.5;-0.1)**	4.5	3.6	**-0.8* (-1.5;-0.1)**
Paraná	1.1	0.8	**-1.3* (-2.2;-0.4)**	12.4	7.2	**-2.0* (-2.3;-1.6)**	40.2	25.5	**-1.6* (-2.0;-1.3)**	10.5	6.0	**-2.0* (-2.4;-1.7)**
Pernambuco	0.6	0.5	-0.4 (-1.1;0.3)	4.4	4.5	0.1 (-0.6;0.8)	15.1	17.3	0.6 (-0.1;1.4)	3.9	3.9	0.0 (-0.4;0.4)
Piauí	0.4	0.3	0.0 (-0.9;0.9)	3.0	3.2	0.4 (-0.5;1.3)	9.9	10.2	0.2 (-0.5;0.9)	2.5	2.5	0.2 (-0.4;0.9)
Rio de Janeiro	0.7	0.5	**-1.2* (-1.8;-0.7)**	7.3	5.0	**-1.4* (-1.8;-1.0)**	23.5	15.0	**-1.6* (-2.3;-0.9)**	6.0	3.7	**-1.7* (-2.3;-1.2)**
Rio Grande do Norte	0.4	0.5	0.4 (-0.0;0.9)	3.7	4.0	0.2 (-0.2;0.7)	13.4	14.6	0.3 (-0.2;0.8)	3.1	3.4	0.2 (-0.2;0.6)
Rio Grande do Sul	1.2	1.1	-0.2 (-1.3;0.9)	14.0	10.1	**-1.2* (-1.8;-0.6)**	51.5	35.2	**-1.4* (-1.9;-0.8)**	12.4	8.2	**-1.5* (-1.9;-1.1)**
Rondônia	0.6	0.5	**-1.0* (-1.5;-0.5)**	5.9	4.0	**-1.4* (-1.7;-1.1)**	18.7	16.4	**-0.5* (-0.8;-0.2)**	5.3	3.8	**-1.3* (-1.7;-0.9)**
Roraima	0.4	0.3	**-0.9* (-1.6;-0.2)**	4.1	3.0	**-1.1* (-1.5;-0.8)**	14.0	12.3	**-0.5* (-0.7;-0.2)**	4.0	3.0	**-1.1* (-1.3;-0.8)**
Santa Catarina	0.8	0.6	**-0.7* (-1.4;-0.0)**	8.4	5.7	**-1.4* (-2.3;-0.4)**	32.6	22.4	**-1.5* (-1.6;-1.4)**	7.9	5.1	**-1.5* (-2.3;-0.8)**
São Paulo	0.6	0.5	**-0.9* (-1.1;-0.7)**	6.1	4.3	**-1.3* (-1.7;-0.9)**	20.8	13.3	**-1.6* (-2.3;-0.9)**	5.3	3.3	**-1.7* (-2.2;-1.2)**
Sergipe	0.4	0.4	-0.6 (-1.5;0.4)	3.7	3.1	-0.5 (-1.1;0.0)	14.4	11.5	**-0.8* (-1.3;-0.3)**	3.2	2.7	**-0.7* (-1.2;-0.2)**
Tocantins	0.4	0.4	-0.1 (-1.0;0.8)	3.5	3.2	-0.4 (-0.9;0.1)	12.3	11.3	-0.4 (-0.7;0.0)	3.5	2.7	**-0.9* (-1.1;-0.7)**
Brasil	0.7	0.6	**-0.7* (-1.1;-0.3)**	7.0	5.2	**-1.1* (-1.4;-0.8)**	24.0	17.7	**-1.1* (-1.5;-0.7)**	5.9	4.2	**-1.2* (-1.6;-0.8)**

AAPC: average annual percentage of change; CI: confidence interval. **P*-value < 0.05.

DISCUSSION

The results of this study point to a reduction in the esophageal cancer rate throughout the country for all age groups, considering that the mortality rates observed of males was approximately four times bigger than females. The reduction of esophageal cancer is in accordance with the findings in rural China and also in some of the countries of Europe, such as France, Switzerland and Denmark, in similar periods[19,20].

The predominance of males was observed in a global study with ratios varying between 3.3:1 and 7:1[1,21,22]. While the reasons for this predominance may not be entirely known, the greater exposure of men is one of the main risk factors, such as smoking and alcohol consumption[23,24], which contribute to it. Furthermore, the role of androgen receptors in the pathogenesis of the disease have been studied in order to clarify the predominance of males[25]. Besides the lower rates, females had the biggest reduction in mortality when compared to males. Such data could be related to the better general

health condition of women, considering that women traditionally seek health attention more frequently[26]. In spite of the increase of patients seeking health care throughout the years, this increase is bigger among women[27].

The highest mortality in a given age group was after 70 years of age, as observed in the United States between 2009 and 2013[28]. This could be related to the cumulative character of the exposure to carcinogenic factors, especially being exposed to smoke in the past. Even though the mortality rate is regressing in this age group, this population is growing significantly with the phenomenon of population aging. It is estimated that in 1980, the population over 60 years old represented about 6% of the total population in Brazil, and that in 2010 this number was close to 11%[29]. In 2017, it was 14.6%[30]. Considering this piece of data, the social impact of esophageal cancer tends to increase.

The variability of the incidence rates and esophageal cancer mortality, even in small geographical areas, is described in the literature as an epidemiological characteristic of the disease[31]. In this study, the states were analyzed individually and a great disparity in the results was verified, with rates of higher magnitude located in the South of the country and an emphasis in Rio Grande do Sul, which is also the number one state in tobacco consumption[32]. Considering that the consumption of hot drinks is associated with a higher risk of esophageal cancer[10], another variable that contributes to the high rates of mortality in Rio Grande do Sul is a drink called *chimarrão*, which is a type of hot herbal tea that is highly consumed in this region.

The mortality rates decreased in the states with a higher development index, which are concentrated in the South and Southeast regions of the country, while less developed states, mainly located in the Northeast region, had an increase in rates. Such facts could be the result of a probable predominance in the carcinoma histological subtype of squamous cells, which have a relationship that is inversely proportional to the human development index[21]. Besides that, this type of cancer has demonstrated better survival rates and a lower rate of relapse in comparison to adenocarcinomas[14]. Inequality in the country persists, even though enhancements were verified in the last years[27]. The more developed regions are also those that have better access to quality health services to diagnose and provide the proper treatment to the disease. Additionally, the growing mortality rates in the less developed states agree with studies that show a higher incidence of esophageal cancer in urban and developed areas of the country[15].

The decrease of mortality in Brazil could be related to the reduction of incidence of the disease, which is related to the decrease of the prevalence of smokers of both sexes in the last decades. The prevalence of smokers 18 years old or older decreased from 43.3% in 1989 to 13.2% in 2017 for men, and 27% to 7.5% for women, in the same period[33]. These results express many regulatory policies that were adopted in the country, such as the ratification, in 2006, of the Framework Convention on Tobacco Control of the World Health Organization[34]. Among these implemented policies, the monitoring of the use of tobacco, the increase of taxes in these products, the prohibition of advertisement of tobacco products are highlighted; the law n. 12.546 in 2011 instituted places free of tobacco[35]; the decree nº 8.262 in 2014[36], which regulated these ambiances and determined an increase of places with warnings[37].

Besides the verified advancements in the Brazilian health system, specifically the access to these health services[27], another factor potentially related to the reduction of mortality is the advancement of medicine regarding the diagnosis and treatment of cancer, such as the target therapy and endoscopic resection of the injury in the initial stages of the disease[12,38]. However, the early diagnosis of esophageal cancer is a challenge in Brazil and in Western countries which lack tracking policies, even in high-risk patients[39].

The Global Burden of Disease (GBD) study dealt with the systems databases to obtain more adequate quality data. Among the corrections are those of the underreporting of deaths and redistribution of unspecified causes. This treatment makes available a standardized comparison between places and periods in which the quality indices are heterogeneous. In Brazil, that is not different and this is very useful, considering that the states present diversified situations related to the quality of mortality data. On the other hand, this applied analytic methodology done by the GBD studies have many modeling stages in which it presupposes and coefficient estimates must be elaborated, which results in different data of directly estimates from the national Vital Registration System. In this way, the data analysis done by GBD could be considered limited by the fact that it must accept premises and world inferences which could not be the most adequate to the reality in Brazil, since it did not use crude data registered in information system. However, it is important to note that the GBD study has been amply shared and used by researchers in different themes, bringing to the study the potential of allowing mortality comparisons between different states and regions in Brazil, as well as in other countries.

CONCLUSION

As expected, the esophageal cancer mortality rates increase with age, being higher in the ≥70 years old group. There was a trend to decrease the mortality rate in Brazil during the presented period in every age group and in both sexes, even though differences were identified among the states. In spite of the reduction in mortality rates throughout a significant part of the Brazilian states, these are still elevated when compared to the rest of the world. The expectation is that, with the increase of new therapies and early diagnosis, the impact of the disease will be minimized and the prognostic of the patients improved.

Authors' contribution

Oliveira MM: conceptualization, formal analysis, writing-review and editing. Silva IPB: data collection and organization, supporting formal analysis, writing-original draft. Teixeira R: data curation, formal analysis, writing-review and editing. Malta DC: conceptualization, supporting data collection and analysis, writing-review and editing. Iser BPM: conceptualization, supporting data collection and analysis, project administration, writing-review and editing.

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RESUMO – **Contexto** – No mundo, cerca de 450.000 novos casos de câncer de esôfago são diagnosticados a cada ano. **Objetivo** – Avaliar a tendência das taxas de mortalidade por câncer de esôfago no Brasil entre 1990–2017. **Métodos** – Estudo de série temporal utilizando dados de mortalidade por câncer de esôfago em residentes ≥30 anos no Brasil de 1990 a 2017. Os dados foram estimados pelo estudo *Global Burden of Disease* (GBD) e analisados segundo sexo, faixa etária e unidade federal de Brasil. As taxas padronizadas de acordo com a idade foram calculadas pelo método direto usando a população mundial padrão do GBD. Mudança percentual média anual e intervalo de confiança de 95% (IC 95%) foram calculados para mortalidade por regressão de *joinpoint*. **Resultados** – A taxa de mortalidade padronizada por idade no sexo masculino foi de 20,6 em 1990 e 17,6 / 100.000 em 2017, aumentando conforme a idade, sendo 62,4 (1990) e 54,7 (2017) para ≥70 anos. Nas mulheres, a taxa de mortalidade padronizada por idade foi de 5,9 em 1990 e de 4,2 / 100.000 em 2017. Houve redução das taxas de mortalidade em todas as faixas etárias e em ambos os sexos com grande variação entre os estados. **Conclusão** – Apesar das altas taxas de mortalidade por câncer de esôfago no Brasil, a tendência é decrescente, mas com diferenças regionais. A mortalidade foi cerca de quatro vezes maior nos homens.

DESCRITORES – Neoplasias esofágicas. Carga global da doença. Mortalidade. Estudos epidemiológicos.

REFERENCES

1. Tatarian T, Palazzo F. Chapter 35 − Epidemiology, Risk Factors, and Clinical Manifestations of Esophageal Cancer. In: Yeo CJ, editor. Shackelford's Surgery of the Alimentary Tract. 2 vol. 8th ed.. Philadelphia: Elsevier; 2019. p. 362-7. Available from: http://www.sciencedirect.com/science/article/pii/B9780323402323000352. Accessed in 2020 (Jun 16).
2. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. CA Cancer J Clin. 2012;62:118-28. doi: 10.3322/caac.20141.
3. Zhang Y. Epidemiology of esophageal cancer. World J Gastroenterol. 2013;19:5598-606. doi: 10.3748/wjg.v19.i34.5598.
4. World Health Organization. International Agency of Research on Cancer. Global Cancer Observatory. Cancer Today. 2018. [Accessed 2020 June 16]. Available from: https://gco.iarc.fr/.
5. Guerra MR, Bustamante-Teixeira MT, Corrêa CSL, et al. [Magnitude and variation of the burden of cancer mortality in Brazil and Federation Units, 1990 and 2015]. [Article in Portuguese, En]. Rev Bras Epidemiol. 2017;20(Suppl 1):102-15. doi: 10.1590/1980-5497201700050009.
6. Coleman HG, Xie SH, Lagergren J. The Epidemiology of Esophageal Adenocarcinoma. Gastroenterology. 2018;154:390-405. doi: 10.1053/j.gastro.2017.07.046.
7. Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of esophageal cancer by histological subtype in 2012. Gut. 2015;64:381-7. doi: 10.1136/gutjnl-2014-308124.
8. Huang FL, Yu SJ. Esophageal cancer: Risk factors, genetic association, and treatment. Asian J Surg. 2018;41:210-5. doi: 10.1016/j.asjsur.2016.10.005.
9. Esophageal cancer: epidemiology, pathogenesis and prevention. Nat Clin Pract Gastroenterol Hepatol. 2008;5:517-26. doi: 10.1038/ncpgasthep1223.
10. Chen Y, Tong Y, Yang C, Gan Y, Sun H, Bi H, et al. Consumption of hot beverages and foods and the risk of esophageal cancer: a meta-analysis of observational studies. BMC Cancer. 2015;15:449. doi: 10.1186/s12885-015-1185-1.
11. Lagergren J, Smyth E, Cunningham D, Lagergren P. Oesophageal cancer. Lancet. 2017;390:2383-96. doi: 10.1016/S0140-6736(17)31462-9.
12. Arantes V, Espinoza-Ríos J. [Early esophageal squamous cell carcinoma management through endoscopic submucosal dissection]. [Article in En, Spanish]. Rev Gastroenterol Mex. 2018;83:259-67. doi: 10.1016/j.rgmxen.2018.05.004.
13. Rustgi AK, El-Serag HB. Esophageal Carcinoma. N Engl J Med. 2014;371:2499-509. doi: 10.1056/NEJMra1314530.
14. Saigí M, Oliva M, Aliste L, Calvo M, Hormigo G, Serra Ò, et al. Clinical relevance of histologic subtypes in locally advanced esophageal carcinoma treated with pre-operative chemoradiotherapy: Experience of a monographic oncologic centre. PLoS One. 2017;12:e0184737. doi: 10.1371/journal.pone.0184737.
15. Amorim CA, De Souza LP, Moreira JP, Luiz RR, Carneiro AJV, De Souza HSP. Geographic distribution and time trends of esophageal cancer in Brazil from 2005 to 2015. Mol Clin Oncol. 2019;10:631-8. doi: 10.3892/mco.2019.1842.
16. World Health Organization. International statistical classification of diseases and related health problems, 10th rev. [Internet]. [Accessed 2020 June 16]. World Health Organization. 1994. Available from: https://apps.who.int/iris/handle/10665/38450.
17. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1736-88. doi: 10.1016/S0140-6736(18)32203-7.
18. National Cancer Institute. Joinpoint Regression Program - Surveillance Research Program. Joinpoint Trend Analysis Software. Statistical methodology and applications branch, Surveillance Research Program. 2019. [Internet]. [Accessed in 2020 June 16]. Available from: https://surveillance.cancer.gov/joinpoint/.
19. Gao X, Wang Z, Kong C, Yang F, Wang Y, Tan X. Trends of Esophageal Cancer Mortality in Rural China from 1989 to 2013: An Age-Period-Cohort Analysis. Int J Environ Res Public Health. 2017;14:218. doi: 10.3390/ijerph14030218.
20. Gupta B, Kumar N. Worldwide incidence, mortality and time trends for cancer of the oesophagus. Eur J Cancer Prev. 2017;26:107-18. doi: 10.1097/CEJ.0000000000000249.
21. Wong MCS, Hamilton W, Whiteman DC, Jiang JY, Qiao Y, Fung FDH, et al. Global Incidence and mortality of oesophageal cancer and their correlation with socioeconomic indicators temporal patterns and trends in 41 countries. Sci Rep. 2018;8:4522. doi: 10.1038/s41598-018-19819-8.
22. Queiroga RC, Pernambuco AP. [Esophageal Cancer: Epidemiology, Diagnosis, and Treatment]. [Internet]. [Accessed in 2020 June 16]. [Article in Portuguese]. Revista Brasileira de Cancerologia. 2006;52:173-8. Available from: https://rbc.inca.gov.br/site/arquivos/n_52/v02/pdf/revisao3.pdf.
23. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health. 2017;32:1018-36. doi: 10.1080/08870446.2017.1325890.
24. Abnet CC, Arnold M, Wei WQ. Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology. 2018;154:360-73. doi: 10.1053/j.gastro.2017.08.023.
25. Sukocheva OA, Li B, Due SL, Hussey DJ, Watson DI. Androgens and esophageal cancer: What do we know?. World J Gastroenterol. 2015;21:6146-56. doi: 10.3748/wjg.v21.i20.6146.
26. Malta DC, Bernal RTI, Lima MG, Araújo SSC, Silva MMA, Freitas MIF, et al. [Noncommunicable diseases and the use of health services: analysis of the National Health Survey in Brazil]. [Article in En, Portuguese]. Rev Saude Publica. 2017;51(Suppl 1):4s. doi: 10.1590/s1518-8787.2017051000090.
27. Nunes BP, Flores TR, Garcia LP, Chiavegatto Filho ADP, Thumé E, Facchini LA. [Time trend of lack of access to health services in Brazil, 1998-2013]. [Article in En, Portuguese]. Epidemiol Serv Saude. 2016;25:777-87. doi: 10.5123/S1679-49742016000400011.
28. Noone AM, Cronin KA, Altekruse SF, Howlader N, Lewis DR, Petkov VI, Penberthy L. Cancer incidence and survival trends by subtype using data from the Surveillance Epidemiology and End Results Program, 1992-2013. Cancer Epidemiol Biomarkers Prev. 2017;26:632-41. doi: 10.1158/1055-9965.EPI-16-0520.
29. Miranda GMD, Mendes ACG, Silva ALA da. O envelhecimento populacional brasileiro: desafios e consequências sociais atuais e futuras [Population aging in Brazil: current and future social challenges and consequences]. Rev Bras Geriatr Gerontol. 2016;19:507-19. doi: 10.1590/1809-98232016019.150140.
30. Instituto Brasileiro de Geografia e Estatísticas − IBGE. Pesquisa Nacional por Amostra de Domicílios Contínua − PNAD Contínua. Características gerais dos domicílios e dos moradores. 2018. Available from: https://www.ibge.gov.br/estatisticas/sociais/trabalho/17270-pnad-continua.html?edicao=24437&t=resultados. Accessed in 2020 (Jun 16).
31. Soares EO, Mattos IE, Monteiro GTR. Tendência da mortalidade por câncer de esôfago em capitais brasileiras, 1980-2002. Escola Nacional de Saúde Pública Sérgio Arouca (ENSP). 2008. Available from: http://www6.ensp.fiocruz.br/repositorio/resource/363454. Accessed in 2020 (Jun 16).

32. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Análise em Saúde e Vigilância de Doenças não Transmissíveis. Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico, Vigitel 2018. Brasília: Ministério da Saúde; 2019. [Accessed 2020 June 16]. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2018_vigilancia_fatores_risco.pdf.
33. Instituto Nacional do Câncer. Dados e números da prevalência do tabagismo. INCA − Instituto Nacional de Câncer. Observatório da Política Nacional de Controle do Tabaco. 2018. [Accessed 2020 June 16]. Available from: https://www.inca.gov.br/observatorio-da-politica-nacional-de-controle-do-tabaco/dados-e-numeros-prevalencia-tabagismo.
34. Brasil. Presidência da República. Casa Civil. Decreto no 5.658, de 2 de janeiro de 2006. [Accessed 2020 June 16]. Available from: http://www.planalto.gov.br/ccivil_03/_Ato2004-2006/2006/Decreto/D5658.htm.
35. Brasil. Presidência da República. Casa Civil. Subchefia para Assuntos Jurídicos. Lei nº 12.546, de 14 de dezembro de 2011. [Accessed 2020 June 16]. Available from: http://www.planalto.gov.br/CCIVIL_03/_Ato2011-2014/2011/Lei/L12546.htm.
36. Brasil. Presidência da República. Casa Civil. Decreto no 8.262, de 31 de maio de 2014. Altera o Decreto no 2.018, de 1o de outubro de 1996, que regulamenta a Lei no 9.294, de 15 de julho de 1996. [Accessed 2020 June 16]. Available from: http://presrepublica.jusbrasil.com.br/legislacao/121697845/decreto-8262-14.
37. Malta DC, Silva AG, Machado ÍE, De Sá ACMGN, Dos Santos FM, Prates EJS, Cristo EB. Trends in smoking prevalence in all Brazilian capitals between 2006 and 2017. J Bras Pneumol. 2019;45. doi: 10.1590/1806-3713/e20180384.
38. Parakh S, Gan HK, Parslow AC, Burvenich IJG, Burgess AW, Scott AM. Evolution of anti-HER2 therapies for cancer treatment. Cancer Treat Rev. 2017;59:1-21. PMID: 28715775; doi: 10.1016/j.ctrv.2017.06.005.
39. Arantes V, Forero Piñeros EA, Yoshimura K, Toyonaga T. [Advances in the management of early esophageal carcinoma]. Rev Col Bras Cir. 2012;39:534-43. doi: 10.1590/S0100-69912012000600015.

REVIEW

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Correlation between trough levels of infliximab and postoperative endoscopic recurrence in Crohn's disease patients submitted to ileocolonic resections: a systematic review

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ABSTRACT – **Background** – The rates of postoperative endoscopic recurrence (PER) in patients with Crohn's disease (CD) are consistent. Anti-TNF therapy has been increasingly used in the postoperative setting, despite the lack of robust data in the literature on the measurement of trough levels and consequences of their use. **Objective** – The aim of this review was to assess trough levels of infliximab (IFX) in CD patients after ileocolonic resections in correlation with the presence of PER. **Methods** – We searched for studies that evaluated trough levels of IFX in patients with CD, who underwent ileocaecal resections, and correlated them with the presence of PER. We used MEDLINE through PubMed and CENTRAL Cochrane library databases, and after matching the inclusion criteria, the studies were methodologically evaluated with qualitative analysis of the data. **Results** – A total of 155 studies were initially identified in the databases search and only four matched the inclusion criteria. They comprised one prospective cohort study, one randomized controlled trial and two retrospective cohort studies, the last one performed in pediatric patients. This evidence suggested the correlation of PER with low trough levels of IFX and the presence of antibodies to the drug. The quality of the evidence generated varied from very low to high, due to the heterogeneity found between the studies and the risks of bias that were identified. **Conclusion** – Low levels of IFX and the presence of antibodies to the drug were directly associated with increased PER rates in patients with CD, who underwent ileocolonic resections. Controlled and randomized clinical trials with adequate methodological quality are warranted to confirm the conclusions from this systematic review.

HEADINGS – Crohn disease. Recurrence. Infliximab. Drug monitoring.

INTRODUCTION

Crohn's disease (CD) is a chronic autoimmune inflammatory disorder, of unknown etiology, which can affect any segment of the digestive tract, with variable intensity and unpredictable clinical course. It is estimated that up to 80% of patients with CD will need surgery at some point in their disease course, with 40% going through more than one surgical intervention. Refractory symptoms to medical therapy (corticosteroids, immunomodulators and biological therapy), bowel obstruction due to strictures and formation of fistulas or abscesses are the main indications for surgery. The most common abdominal surgical procedure in CD is ileocolonic resection[1-3].

Despite providing a prolonged period of disease control, surgery is not curative, and recurrence rates are high. Recurrence can be histological, endoscopic, clinical or combined, and histological and endoscopic lesions usually precede and predict clinical symptoms[2]. The severity of endoscopic lesions after ileocolonic resections has been traditionally assessed using the Rutgeerts' score[4]. In the original study, ileocolonoscopy was performed 1 year after surgery and lesions in the neo-terminal ileum were scored as follows: i0 (without lesions), i1 (<5 erosions/aphthoid ulcers), i2 (>5 erosions/aphthoid ulcers with normal mucosa between lesions or large lesions confined to anastomosis), i3 (diffuse ileitis with erosions/aphthoid ulcers) and i4 (diffuse inflammation with ulcers, nodules and/ or luminal narrowing). Postoperative endoscopic recurrence (PER) is defined as a Rutgeerts score ≥2.

Studies demonstrated that in patients undergoing ileocolonic resections, endoscopic recurrence rates can be as high as 73% in 1 year and 85% in 3 years after surgery[3]. Some factors were associated with an increased risk of PER: male gender, active smoking, penetrating phenotype, extensive small bowel resection (>50cm) and previous use of corticosteroids[1].

The efficacy of tumor necrosis factor (TNF) alpha inhibitors in preventing endoscopic recurrence after ileocolonic resections has been established and demonstrated by several studies[2,5,6]. A recent systematic review demonstrated that anti-TNF therapies, both in mono or in combination with immunomodulators, appear to be the most effective agents to prevent PERin CD[3]. However, such agents are not used for all patients, in order to avoid overtreatment in cases of slower disease progression and for cost reasons. Thus, the indication for anti-TNFs after surgery is indeed reserved for cases of patients with risk factors for recurrence.

Serum infliximab (IFX) levels are associated with clinical

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response, disease remission and mucosal healing. In a systematic review, Moore et al.[7] demonstrated that during maintenance therapy, patients in clinical remission had significantly higher serum levels immediately before the next dose (trough levels) of IFX than individuals who were not in remission. Patients with an IFX level >2 μg/mL were more likely to be in clinical remission or achieve endoscopic remission than patients with levels <2 μg/mL. In a randomized study, Van de Casteele et al.[8] observed that trough levels of IFX were higher in patients with mucosal healing at endoscopic examination than in patients with active disease at endoscopy. In the study by Maser et al.[9] the rates of clinical remission were higher in patients with a detectable serum level of IFX as compared to patients in whom serum IFX was undetectable, including those without antibodies (82% versus 6%). In a similar scenario, Imaeda et al.[10] demonstrated that there was endoscopic improvement in patients with mean IFX levels >1.4 μg/mL as compared to patients in whom serum IFX was undetectable.

Although there is adequate evidence of the efficacy of anti-TNFs in preventing endoscopic recurrence after ileocolonic resections, the impact of serum drug levels on PER has been poorly explored. Due to the scarce evidence related to the topic in the literature and the potential for a therapeutic strategy to be studied, we conducted a systematic review in order to assess studies which correlated trough levels of IFX in CD patients that underwent ileocolonic resections with the presence or not of postoperative endoscopic recurrence.

METHODS

Search strategy

This review was carried out in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines[11,12]. The published studies were identified after search conducted in Medline electronic databases via PubMed https://www.ncbi.nlm.nih.gov/pubmed/ and CENTRAL Cochrane library [https://www.cochranelibrary.com/] in January 2020, using five different search instruments, with the terms: (Crohn's disease) and (recurrence) and (infliximab) and (levels); (Crohn's disease) and (recurrence) and (infliximab) and (serum); (Crohn's disease) and (recurrence) and (therapeutic drug monitoring); (Crohn's disease) and (endoscopic recurrence) and (therapeutic drug monitoring); (Crohn's disease) and (endoscopic recurrence) and (ileocolonic resection) and (infliximab).

PICO model

According to the PICO model (population; intervention; control; outcome)[13] we sought for studies that evaluated serum levels of IFX in patients with CD that underwent ileocolonic resection and were using this anti-TNF agent after surgery, correlating them with the presence or not of postoperative endoscopic recurrence.

Eligibility and inclusion and exclusion criteria

Studies to be considered eligible should meet the following inclusion criteria: analysis of patients with CD, submitted to ileocolonic resections and treated with IFX in the postoperative period; evaluation of endoscopic recurrence with colonoscopy; and assessment of serum levels (trough levels) of IFX after surgery. No restrictions were applied in relation to patients' age, date of publication or language. All studies classified under at least one of the following conditions were excluded: review articles, guidelines, consensus articles, editorials or case reports; studies involving patients with other diseases than CD; studies with other drugs than IFX; studies using animal models.

Study selection and data collection process

Through electronic databases or by manual search, studies were selected, independently by two reviewers (FSBB and PGK), after removing duplicate records. Any study whose title and abstract did not indicate inclusion in the eligibility criteria was excluded from further analyses. In all other studies, the full text was assessed to determine its inclusion or exclusion.

The following data were collected from the selected studies: journal, year of publication, name of the authors, type of article, number of patients, age, sex, disease location, duration of the disease, smoking, history of medications for CD before surgery, previous surgical procedures, concomitant use of immunomodulators, duration of treatment with IFX, postoperative colonoscopy and trough levels of IFX.

Evaluation of methodological quality of studies

The methodology of the selected studies was evaluated using the Cochrane Collaboration's tool[14] for randomized clinical trials (RCTs) and the Methodological Index for non-randomized studies (MINORS)[15] for non-randomized clinical trials and for observational studies. The risk of bias was judged as "low", "high" and "uncertain" when the Cochrane Collaboration's tool was used. When MINORS was used, the items were scored with "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.

Statistical analysis

The small number of studies and their respective methodologies allowed only a qualitative analysis of the data. In descriptive components, means, medians, standard deviations and percentages were calculated. A meta-analysis was not performed in view of the small number of patients, lack of control and heterogeneous nature of the studies.

RESULTS

Bibliographic search and study selection

FIGURE 1 summarizes the study selection process. The search in the electronic databases identified 145 studies; 10 others were added by the authors by manual search. Overall, 48 studies were immediately excluded because they were duplicated. After reviewing the title and abstract of the remaining 107 studies, 27 were considered for full text analysis. At the end of the review, four articles were included for qualitative analysis, while the other 23 were excluded for respective reasons described in the flowchart.

Methodological quality of included studies

FIGURE 2 describes the Cochrane Risk of bias tool for the randomized control study and the criteria used for its evaluation. TABLE 1 presents more information about the criteria used in MINORS, for non-randomized studies, in addition to the scores of each manuscript in this methodological quality assessment instrument.

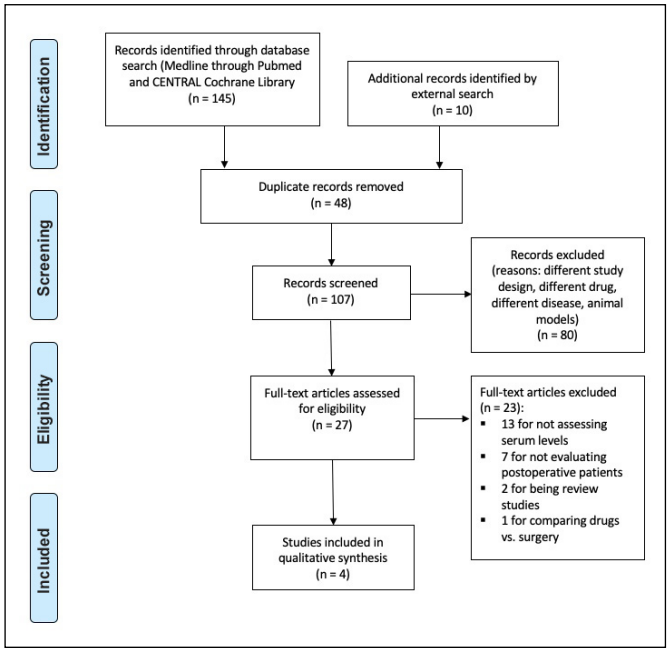

FIGURE 1. Systematic review PRISMA flow diagram.

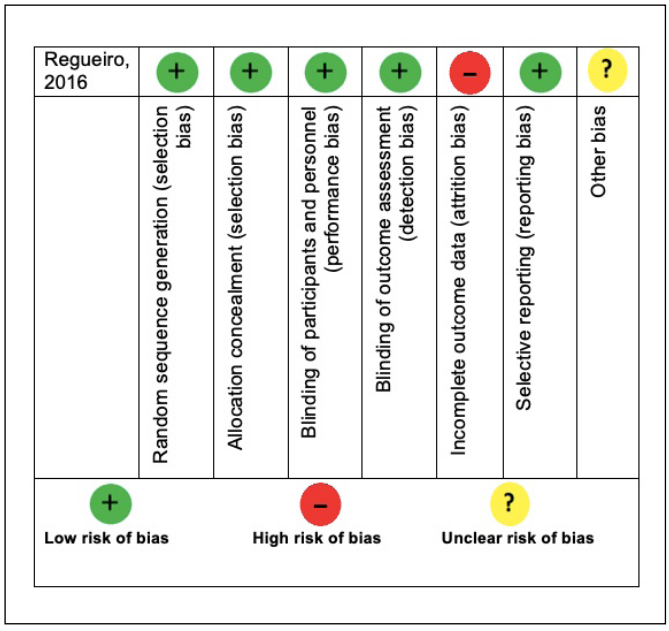

FIGURE 2. Cochrane Risk of Bias Tool for randomized trials.

Characteristics of the studies

Of the four articles included for the qualitative analysis, one was a prospective cohort study[16], one was a randomized clinical trial[17] and two were retrospective cohort studies[18,19], the last being exclusively in pediatric patients[19]. The number of patients ranged from 11 to 297, totalizing 404 patients included in the selected studies[16,17].

A study dosed serum levels of IFX and anti-IFX antibodies, in five consecutive patients who had clinical and endoscopic remission after one year of treatment with IFX at a dose of 3 mg/kg and compared with a control group of 6 patients who did not undergo surgery and were in remission with a dose of 5 mg/kg of IFX[16].

TABLE 1. Methodological Index for Non-randomized studies (MINORS) for evaluation of non-randomized clinical trials and observational studies included. Items are scored as 0 (not reported), 1 (reported, but inadequate) or 2 (reported and adequate). The global ideal score is 16 for non-comparative studies and 24 for comparative studies.

	Sorrentino, 2015	Fay, 2017	Van Hoeve, 2018
A clearly stated aim	2	2	2
Inclusion of consecutive patients	2	2	1
Prospective collection of data	2	1	1
Endpoints appropriate to the aim of the study	2	2	2
Unbiased assessment of the study endpoint	0	0	0
Adequate follow-up period to the aim of the study	2	0	2
Loss to follow-up <5%	2	0	0
Prospective calculation of the study sample	2	0	1
Additional criteria for comparative studies			
Adequate control group	2		
Contemporary groups	2		
Baseline equivalence of groups	2		
Adequate statistical analyses	2		
Total	22	7	9

The clinical trial assessed the effectiveness of IFX in preventing postoperative recurrence in 297 patients who were randomized into a group that received IFX 5 mg/kg and another that received placebo every 8 weeks for 200 weeks, with the primary outcome being assessed for clinical recurrence and the secondary, endoscopic recurrence.[17]

The retrospective cohort in adults included 73 consecutive postoperative patients treated with IFX (32 patients) or adalimumab (41 patients)[18]. For this qualitative analysis, only data from patients using IFX were used. Serum levels of the drug and anti-IFX antibodies were correlated to the Rutgeerts' score at colonoscopy, assessed after a period of at least 6 months after surgery, ranging from 7 to 43 months. The average time between the colonoscopy and the serum level of IFX dosage was 29 days, varying between 0 and 90 days.

The pediatric population study was a retrospective analysis of medical records, which included 52 children (33 with Crohn's disease and 19 with ulcerative colitis), with only 2 (3.8%) in the postoperative period who used IFX and had serum levels of the drug correlated with clinical, biochemical and endoscopic remission[19]. The results were not separated by disease, nor patients who did not undergo surgery were differentiated from those who underwent a surgical procedure.

TABLE 2 shows in detail the characteristics of the included studies, as well as the clinical and demographic aspects of the patients.

TABLE 2. Characteristics of selected studies and clinical and demographic profile of patients.

Journal	Sorrentino, 2015		Regueiro, 2016		Fay, 2017	Van Hoeve, 2018
	PLoS ONE		Gastroenterology		Inflammatory bowel diseases	Journal of Crohn's and colitis
Study design	Cohort prospective		Prospective, multicenter, randomized, double-blind CT		Cohort retrospective	Cohort, retrospective, pediatric patients
Number of patients, n / intervention	5 / IFX	6 / non postoperative patients using IFX	150 / Placebo	147 / IFX	73 32 / IFX 41 / ADA	52 / IFX 33: CD – 2 postoperative 19: UC
Male gender, n (%)	4 (80)	6 (100)	81 (54)	77 (52.4)	IFX / 18 (56.3)	23 (44)
Age in years, median (range)	44 (32-51)	34.5 (24-48)	34 (18-69)	35 (18-76)	IFX / 35 (29-40.6)	–
Disease duration in years, median	8	5.5	3.32	6.49	IFX / 10	4.5
Smoking n (%)	1 (20)	1 (16.7)	–	–	IFX / 14 (43.8)	–
Previous surgeries n (%)	5 (100)	1 (16.7) – perianal fistula	59 (39.4)	67 (45.5)	IFX / 8 (25)	–
Medications prior to surgery, n (%)						
Corticosteroid			96 (64)	104 (71.2)		
Mesalamine			101 (67.3)	100 (68.5)		
Azathioprine	–	–	77 (51.3)	73 (50)		
6-mercaptopurine			22 (14.7)	19 (13)		
Anti-TNF			30 (20)	37 (25.3)	IFX / 20 (62.5)	–
Concomitant immunosuppression, n (%)						
None			–	–	IFX / 22 (68.8)	
Thiopurines			27 (18)	21 (14.3)	IFX / 8 (25)	40 (70)
Methotrexate	5 (100)	6 (100)	0	4 (2.7)	IFX / 2 (6.3)	
Disease location, n (%)						
Ileal		2 (33.4)	146 (97.3)	144 (98.6)	IFX / 16 (50)	7 (21)
Colonic		1 (16.7)	76 (50.7)	89 (61)	IFX / 5 (15.6)	6 (18)
Ileocolonic	5 (100)	3 (50)			IFX / 11 (34.4)	20 (61)

CT: computed tomography; CD: Crohn's disease; UC: ulcerative colitis; IFX: infliximab; TNF: tumor necrosis factor; ADA: adalimumab.

Results per included study

Sorrentino et al. included patients underwent ileocolonic resection, who started IFX to prevent endoscopic recurrence, at a dose of 5 mg/kg[16]. After 3 years of surgery, colonoscopy was performed and no patient had recurrence, and IFX therapy was interrupted for all patients. After 4 months without treatment, 83% had endoscopic recurrence. Those with recurrence (n=10) restarted IFX in a dose optimization study performed by the same team, which demonstrated that the dose of 3 mg/kg was sufficient to induce and maintain endoscopic remission for 1 year in all patients[20]. Five of these ten patients participated in the current study. Anti-IFX antibodies and serum levels (trough levels) of the drug were measured immediately before all infusions, which occurred every 8 weeks, and were compared to those of patients with CD who did not undergo surgery and who were in remission in one standard dose of 5 mg/kg of IFX. After additional 18 months, patients treated with 3 mg/kg of IFX underwent colonoscopy.

The mean values of serum levels of IFX were 4.75±0.83 µg/mL in controls (with usual doses of 5 mg/kg), as compared to 2.0±0.3 µg/mL in patients treated with IFX 3 mg/kg (P<0.05). In four of the five patients treated with IFX 3 mg/kg, the trough level of IFX was lower than 3 µg/mL while in all controls the trough level was greater than 3 µg/mL. Antibodies were present in 2 of 5 patients treated with 3 mg/kg and in none of the controls. In the two patients with anti-IFX antibodies, their concentration was below 10 µg/mL. All five patients evaluated in the treatment group had a Rutgeerts' score at 18 months of i0 or i1. TABLE 3 summarizes the drug and antibody concentrations and Rutgeerts' score of the five patients included in the study. The global MINORS score for the study was 22, and its main bias was the heterogeneity of the sample, where the control group consisted of patients not submitted to ileocolonic resections, in addition to blinding bias.

TABLE 3. Infliximab (IFX) through levels, antibodies anti-IFX and Rutgeerts score of included patients[16].

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
IFX through level (µg/mL)	4.4±0.8	0.91±0.1	0.91±0.1	1.3±0.3	2.8±0.3
Antibody concentration (U/mL)	Negative	5.4±0.3	Negative	7.7±0.8	Negative
Rutgeerts score on colonoscopy	i0	i1	i1	i1	i0

The PREVENT multicenter study included 297 patients who underwent ileocolonic resection and were randomized in a 1:1 ratio into two groups: patients treated with intravenous infusion of IFX every 8 weeks or placebo[17]. Until week 76, endoscopic recurrence rates (Rutgeerts' score ≥i2) for the IFX and placebo groups were 22.4% and 51.3%, respectively (P<0.001). In patients treated with IFX, the serum level of the drug was measured at

week 72, and among patients who had endoscopic recurrence, 52.4% had levels lower than the lower limit of quantification (0.1 µg/mL); 31.3% levels between 0.1 and 1.85 µg/mL; 18.8% between 1,85 µg/mL and 4.44 µg/mL; 26.7% between 4.44 µg/mL and 7.77 µg/mL and 13.3% had levels greater than 7.77 µg/mL. Endoscopic recurrence until week 76 was observed in 64.7% (11 of 17), 46.7% (7 of 15) and 30.1% (22 out of 73) of patients positive, negative or with inconclusive levels for anti-IFX antibodies, respectively. The study showed a high risk of bias in relation to incomplete data on outcomes, but with a low risk of bias in all other determinants for assessing methodological quality.

Fay et al. demonstrated that among patients treated with IFX, lower trough levels (median, 1.1 [0–0.6] µg/mL versus 2.4 [0.45–4.1] µg/mL P=0.008) and presence of anti-IFX antibodies (1/18 [5.6%] versus 10/14 [71.4%], P=0.0001) were significantly related to the presence of PER[18]. Median levels of IFX were significantly higher in patients with a Rutgeerts' score of i0 as compared to those with a score of i4, as shown in TABLE 4. When limited to anti-TNF naive patients (n=20), the difference in IFX trough levels (2.3 [0.3–3.8] versus 1.1 [0.1–3.3] µg/mL, P=0.048) and antibodies (7.7% versus 60%, P=0.044) remained significant. The MINORS score for this study was 7, as it was a retrospective study and did not report on impartial analysis of the sample, follow-up period and loss of follow-up.

TABLE 4. Infliximab through levels stratified by Rutgeerts score of the included patients (P=0.037)[18].

	Rutgeerts i0	Rutgeerts i1	Rutgeerts i2	Rutgeerts i3	Rutgeerts i4
Through level (µg/mL)	3.1 (0.1–4.1)	1.1 (0.1–3.9)	1.1 (0.5–3.6)	0.4 (0.1–1.1)	0.1 (0.1–3)

The pediatric study by Van Hoeve et al. obtained 686 serum level measurements during IFX maintenance therapy in 52 pediatric patients with inflammatory bowel disease (IBD)[19]. Endoscopic data were available at 87 times in 40 different patients after a median of 12.6 (12.6–22.4) months. In 54% of visits to physicians' office, patients were in endoscopic remission at the time of the assessment (n=47). The IFX trough levels used for comparison were measured at the time of colonoscopy if the patient received an IFX infusion on the same day. In all other cases, IFX trough levels were measured at the time of the nearest IFX infusion. The median serum IFX levels during maintenance were significantly higher in children who were in endoscopic remission (6.5 [4.2–9.5] µg/mL) as compared with those active disease at endoscopy (3.2 [2.3–5.6] µg/mL, P=0.001). The same results were found when performing the diagnostic analysis (CD versus ulcerative colitis). The two postoperative patients who were included in the study did not have their data evaluated individually and did not provide conclusions about postoperative endoscopic recurrence related to serum IFX levels. The study presented a MINORS score of 9, as it is a retrospective cohort, with no data on impartial analysis, loss of follow-up and does not include consecutive patients.

DISCUSSION

Postoperative recurrence in CD has been studied since the early 90's. At that time, Rutgeerts et al. published their experience with patients who underwent ileocaecal resections and were followed for 8 years[4]. This study demonstrated the natural history of postoperative recurrence in CD and confirmed that endoscopic recurrence is the first to occur, in approximately 75% of patients after 1 year of surgery, preceding clinical symptoms, and showing the importance of controlling disease activity at an earlier stage.

Overall, rates of PER are vastly variable in the literature, suggesting approximately 10% in trials with biological therapy, and reaching up to 90% in patients without adequate postoperative management[5]. Anti-TNF therapy is increasingly used in the postoperative scenario in CD. However, data on the impact of serum levels and anti-drug antibodies in patients with CD after intestinal resection are lacking in relation to the risk of recurrence.

Two of the studies included in this review have a risk of bias related to the study population (reduced sample, absence of randomization, blinding and control group), heterogeneity between doses used, administration times, measurement of serum levels and even omission of the dose of the drug which was used, making the quality of the evidence not very robust[16,18]. Despite of that, the evidence found tends to confirm the correlation of endoscopic recurrence with low serum levels of IFX and the presence of anti-drug antibodies.

The PREVENT study was the first large multicenter, randomized, placebo-controlled trial on the use of biologicals in patients with CD after ileocolonic resection[17]. The rate of endoscopic recurrence 18 months after surgery in patients treated with IFX was 22.4%, remaining similar to most findings in the literature (which demonstrated endoscopic recurrence in 6 months of 21% in patients with anti-TNF therapy), against 51.3% in the placebo group. This study also demonstrated, with statistical significance, that lower serum IFX levels and the presence of antibodies against the drug are related to a higher risk of endoscopic recurrence. This study's data comprised the higher level of evidence published over the topic.

The pediatric study assessed exposure to IFX in children with IBD and its correlation with results of clinical, biological and endoscopic remission[19]. This study represented the largest cohort of serum IFX levels in a pediatric population, and demonstrated, for the first time, a clear association between IFX trough levels and mucosal healing. However, such a study did not analyze the results separately between CD and ulcerative colitis, in addition to presenting only two patients in the postoperative period, who also did not have the data independently assessed. Thus, it was not possible to extract the data that meet the established inclusion criteria with accuracy. However, the study has a strong impact in the pediatric area, bringing for the first time the conclusion that children with IBD, treated with IFX under maintenance regimen, and in clinical and endoscopic remission, had significantly higher serum IFX levels, supporting the importance of proactive therapeutic monitoring to improve long-term results.

Several points still need to be better defined with regards to the correlation between serum levels of IFX and endoscopic recurrence. The best time for dosing after surgery has yet to be pointed. In addition, patients can be reinduced with the drug after surgical procedures. The best time point to perform colonoscopy for recurrence analysis, usually between 6–12 months, also needs to be better defined. However, despite these questions, there seems to be an association between absence of recurrence and higher mean serum levels of the drug. The interval between dosage and colonoscopy also varied in different studies, and in an ideal scenario, it should be kept to a minimum (endoscopic examination at the day of infusion,

for example). However, current evidence in the literature seems to reflect in the postoperative scenario a significant relationship between higher levels and mucosal healing, in a similar way to luminal disease in patients who did not undergo surgery.

In the study by Fay et al., despite the significant difference between groups with or without PER, patients with no recurrence still had low serum levels of IFX (2.4 [0.45–4.1] µg/mL), as the lower cutoff is usually 3.0 µg/mL in the maintenance phase[6,18]. A similar pattern was also identified in the study by Sorrentino et al., despite the reduced sample of analyzed patients[16]. One can speculate that even IFX levels lower than 3.0 µg/mL can be associated with absence of recurrence, mostly if antibodies to the drug are not detected. This topic deserves special attention in future studies, as the real lower cutoff in the postoperative scenario can be slightly different than in luminal disease, with no previous surgery.

The methodological limitations of this review should be noted. Databases such as Embase and Scopus were not screened due to technical difficulties. Therefore, the occurrence of publication bias cannot be ruled out, although the authors have tried to minimize it. Only four studies were included, with low or moderate methodological quality, presenting several problems such as blinding as well as restricted and often heterogeneous population sample, which makes the evaluation of results limited, and these should be interpreted with caution. The interval between dosage of IFX serum levels and colonoscopy also varied in different studies, which represents an important limitation. In an ideal scenario, this interval should be kept to a minimum (endoscopic examination at the day of blood collection, for example).

Low serum levels of IFX and the presence of anti-IFX antibodies are associated with an increased probability of postoperative endoscopic recurrence in patients with CD who underwent ileocolonic resections. On the other hand, higher levels are associated to normal mucosal status and low Rutgeerts' score status (i0/i1). Strategies to optimize the dose of IFX, aiming at increased serum levels of the drug, should be better studied in the postoperative scenario to prevent or eliminate the problem of recurrence. Controlled and randomized clinical trials with adequate methodological quality are warranted to confirm the conclusions of this review.

Authors' contribution

Baraúna FSB and Kotze PG contributed to study conception and design; they also contributed to data collection and analysis, writing, editing, reviewing and gave final approval of the article.

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Baraúna FSB, Kotze PG. Correlação entre níveis séricos de infliximabe e recorrência endoscópica pós-operatória em pacientes com doença de Crohn submetidos a ressecções ileocólicas: uma revisão sistemática. Arq Gastroenterol. 2021;58(1):107-13.

RESUMO – **Contexto** – As taxas de recorrência endoscópica pós-operatória em pacientes com doença de Crohn (DC) são significativas. A terapia anti-TNF é cada vez mais usada no cenário pós-operatório, apesar da escassez de dados na literatura sobre dosagem de níveis séricos e anticorpos da droga. **Objetivo** – Realizou-se uma revisão sistemática com o intuito de se avaliar níveis séricos de infliximabe (IFX) em pacientes com DC submetidos a ileocolectomia e correlacionar com a presença ou não de recorrência endoscópica da doença. **Métodos** – Buscou-se por estudos que avaliaram o nível sérico do IFX em pacientes com DC, submetidos a ileocolectomias, correlacionando-os à presença de recorrência endoscópica pós-operatória. Utilizou-se as bases de dados MEDLINE via PubMed e CENTRAL *Cochrane Library,* e após atingirem os critérios de inclusão, os estudos foram avaliados metodologicamente e foi realizada análise qualitativa dos dados. **Resultados** – Um total de 155 estudos foram identificados e apenas quatro atingiram os critérios de inclusão. Um era estudo de coorte prospectivo, o segundo era um ensaio clínico randomizado e dois eram estudos de coortes retrospectivas, sendo o último exclusivamente em pacientes pediátricos. As evidências encontradas tendem a confirmar a correlação da recorrência endoscópica a baixos níveis séricos de IFX e presença de anticorpos anti-droga. A qualidade da evidência gerada variou de muito baixa a alta, devido à heterogeneidade encontrada entre os estudos e o risco de viés identificado. **Conclusão** – Baixos níveis séricos do IFX e presença de anticorpos contra a droga estão associados a probabilidade aumentada de recorrência endoscópica pós-operatória nos pacientes com DC submetidos a ileocolectomias. Ensaios clínicos controlados e randomizados com adequada qualidade metodológica são necessários para confirmar as conclusões desta revisão.

DESCRITORES – Doença de Crohn. Recidiva. Infliximab. Monitoramento de medicamentos.

REFERENCES

1. de Barcelos IF, Kotze PG, Spinelli A, Suzuki Y, Teixeira FV, Albuquerque IC. Factors affecting the incidence of early endoscopic recurrence after ileocolonic resection for Crohn's disease: a multicentre observational study. Colorectal Dis. 2017;19:O39-O45.
2. Armuzzi A, Felice C, Papa A, Marzo M, Pugliese D, Andrisani G, et al. Prevention of postoperative recurrence with azathioprine or infliximab in patients with Crohn's disease: An open-label pilot study. J Crohn's Colitis. 2013;7: e623-9.
3. Burr NE, Hall B, Hamlin PJ, Selinger CP, Ford AC, et al. Systematic review and network meta-analysis: Medical therapies to prevent recurrence of post-operative Crohn's disease. J Crohn's Colitis. 2018. Available from: https://academic.oup.com/ecco-jcc/advance-article/doi/10.1093/ecco jcc/jjy216/5250061.
4. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. Gastroenterology 1990;99: 956–63.
5. Kotze PG, Yamamoto T, Danese S, Teixeira V, Albuquerque IC, Saad-Hossne R, et al. Direct Retrospective Comparison of Adalimumab and Infliximab in Preventing Early Postoperative Endoscopic Recurrence After Ileocaecal Resection for Crohn's Disease: Results from the MULTIPER Database. J Crohn's Colitis. 2015;541-7.
6. Mitrev N, Vande Casteele N, Seow CH, Andrews JM, Connor SJ, Moore GT, et al. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. Aliment Pharmacol Ther. 2017;46:1037-53.
7. Moore C, Corbett G, Moss AC. Systematic review and meta-analysis: Serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. J Crohn's Colitis. 2016;10:619-25.
8. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148:1320-9.e3.

9. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of Trough Serum Infliximab to Clinical Outcome After Scheduled Maintenance Treatment for Crohn's Disease. Clin Gastroenterol Hepatol. 2006;4:1248-54.
10. Imaeda H, Bamba S, Takahashi K, Fujimoto T, Ban H, Tsujikawa T, et al. Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. J Gastroenterol. 2014;49:674-82.
11. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009;62:e1-34.
12. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097
13. Brasil. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Departamento de Ciência e Tecnologia. Diretrizes metodológicas: elaboração de revisão sistemática e metanálise de ensaios clínicos randomizados. Brasília : Editora do Ministério da Saúde, 2012. 92 p.: il. – (Série A: Normas eManuais Técnicos) ISBN 978-85-334-1951-3. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/diretrizes_metodologicas_elaboracao _sistematica.pdf.
14. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD. The Cochrane Collaboration ' s tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928 doi: 10.1136/bmj.d5928
15. 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;73:712-716.
16. Sorrentino D, Marino M, Dassopoulos T, Zarifi D, Del Bianco T. Low Dose Infliximab for Prevention of Postoperative Recurrence of Crohn's Disease: Long Term Follow-Up and Impact of Infliximab Trough Levels and Antibodies to Infliximab. PLoS One. 2015;10:e0144900.
17. Regueiro M, Feagan BG, Zou B, Johanns J, Blank MA, Chevrier M, et al. Infliximab Reduces Endoscopic, but Not Clinical, Recurrence of Crohn's Disease After Ileocolonic Resection. Gastroenterology. 2016;150:1568-78.
18. Fay S, Ungar B, Paul S, Levartovsky A, Yavzori M, Fudim E, et al. The Association Between Drug Levels and Endoscopic Recurrence in Postoperative Patients with Crohn's Disease Treated with Tumor Necrosis Factor Inhibitors. Inflamm Bowel Dis. 2017;23:1924-9.
19. Van Hoeve K, Dreesen E, Hoffman I, Van Assche G, Ferrante M, Gils A, et al. Higher Infliximab Trough Levels Are Associated With Better Outcome in Paediatric Patients With Inflammatory Bowel Disease. J Crohns Colitis 2018;12(11):1316-25.
20. Sorrentino D, Paviotti A, Terrosu G, Avellini C, Geraci M, Zarifi D. Low-dose maintenance therapy with infliximab prevents postsurgical recurrence of Crohn's disease. Clin Gastroenterol Hepatol. 2010;8:591-9.

REVIEW

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Helicobacter pylori and colorectal neoplasms: a concise review

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ABSTRACT – *Helicobacter pylori* is the main etiological agent of all malignant tumors caused by an infectious disease. It is a major, at times dominant, factor in the pathogenesis of a large spectrum of diseases such as acute and chronic gastritis, gastric and duodenal ulcers, gastric carcinoma, and lymphoma. Epidemiological and experimental studies suggest that *H. pylori* chronic infection may be related to different extragastric diseases, including colorectal neoplasms. This concise review aims to explore the association of *H. pylori* infection with colorectal cancer and adenoma, including the recent epidemiological findings, the diagnostic methods employed to detect *H. pylori* and virulent factors, and the potentially involved mechanisms. Furthermore, is attempted to establish the current data integration for causal inference using the Bradford-Hill causality criteria. The weak, although global, strength of the epidemiological positive association between *H. pylori* infection and colonic neoplasms associated to new mechanisms postulated to explain this interaction, including intestinal dysbiosis, should stimulate future studies. Prospective confirmatory studies to establish the role of *H. pylori* eradication in the process of carcinogenic transformation of the colonic epithelium may define its eventual role in the treatment and prevention of colonic neoplasms.

HEADINGS – *Helicobacter* infections. Colorectal neoplasms. Adenoma. Epidemiologic factors.

INTRODUCTION

It is estimated today that at least 13% of all malignant tumors are caused by an infectious agent, and *Helicobacter pylori* (*H. pylori*) is the main etiological agent and responsible for approximately 810,000 cases of gastric cancer worldwide each year[1]. After *H. pylori* was identified by Marshall and Warren in 1983 in Australia, this microorganism has been found in at least 50% of the world´s population[2]. It is a major, at times dominant, acquired environmental factor in the pathogenesis of a large spectrum of diseases such as acute and chronic gastritis, gastric and duodenal ulcers, gastric carcinoma, and lymphoma. Epidemiological and experimental studies suggest that *H. pylori* chronic infection may be related to different extragastric diseases, including colorectal cancer (CRC) and precancerous lesions[3,4] (FIGURE 1).

CRC is a multifactorial disease of global concern, being the third most commonly diagnosed cancer with nearly 1.4 million new cases in 2018[5]. A majority of CRC is sporadic; both genetic and environmental factors like nutritional practices, cigarette smoking, physical activity, obesity, and heavy alcohol consumption play an important part in the etiology of CRC[6]. Besides, increasing evidence has established a role for the intestinal microbiota in the development of colorectal cancer[7].

This paper aims to make a concise review of the association of *H. pylori* infection with colorectal cancer and adenoma, including the main epidemiological findings and potentially involved mechanisms. For these purposes, a PubMed search up to May 2020 was performed using a combination of the following keywords: *Helicobacter pylori*, colorectal cancer, colon cancer, adenoma, and colonic polyps. Also, the reference lists of all relevant articles were reviewed.

Potential associations among *H. pylori* infection and colorectal neoplasms

Four recent meta-analyses demonstrated a positive association between *H. pylori* infection and the risk of CRC and colorectal adenoma, presenting OR (95%CI) varying from 1.27 (1.17–1.37) to 1.44 (1.26–1.65) regarding CRC and 1.49 (1.37–1.62) to 1.66 (1.39–1.97) to colorectal adenoma[8-11] (TABLE 1). The studies involved in these meta-analyses came from 16 countries situated in Asia (38%), Europe (33%), America (mostly the USA) (20%), Middle-East (7%), and Oceania (2%).

H. pylori infection

H. pylori can be detected in the gastric mucosa by different methods, either in fragments removed during endoscopy and used for histopathological, microbiological, biochemical and molecular studies or by non-invasive tests that include searching for *H. pylori* anti-antibodies in a blood sample, breath tests using carbon-13 labeled urea or investigating fecal antigens[12].

Although most studies analyzed mainly the presence of serum antibodies to *H. pylori*, it is important to consider some factors that may contribute to the heterogeneity among the studies results and their relationship with CRC. Among them, the presence of current or previous infection and the presence or absence of bacterial virulence factors such as the cag pathogenicity island, which encodes the oncogenic effector protein cagA and the allelic variation in the vacuolating cytotoxin A (vacA) were not mentioned[13]. Likewise, studies didn´t inform the presence or absence of chronic sequelae of *H. pylori* infection in gastric epithelium inducing premalignant changes (atrophic gastritis and intestinal metaplasia), the increased level of serum gastrin promoting epithelial cell

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Extragastric digestive diseases: NAFLD, NASH, autoimune pancreatitis, pancreatic cancer, and colorectal neoplams.
Cardiovascular diseases: coronary atherosclerotic disease and myocardial infarction.
Neurological diseases: stroke, Alzheimer's disease, Parkinson's disease, Guillain-Barré syndrome, and migraine.
Dermatological diseases: rosacea and chronic urticaria.
Hematologic diseases: iron deficiency anemia, pernicious anemia, and primary immune thrombocytopenia.
Ophthalmological diseases: open-angle glaucoma, central serous chorioretinitis, and neuromyelitis optica.
Otorhinolaryngological diseases: chronic rhinosinusitis.
Endocrinology disorders: metabolic syndrome, type 2 diabetes.

FIGURE 1. *Helicobacter pylori* infection and potentially linked extragastric diseases.
NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis.

TABLE 1. Studies included on the meta-analyses about the association between *H. pylori* and colorectal cancer and colorectal adenoma.

Meta-analysis Author (year)	Number of studies	Colorectal cancer OR (95% CI)	Colorectal adenoma OR (95% CI)
Wu (2013)[8]	27	1.39 (1.18–1.64)	1.66 (1.39–1.97)
Zhao (2016)[9]	14	1.33 (1.01–1.77)	
Yang (2019)[10]	27	1.27 (1.17–1.37)	
Choi (2020)[11]	48	1.44 (1.26–1.65)	1.49 (1.37–1.62)

growth and proliferation, and hypochlorhydria that might lead to bacterial overgrowth in the gastrointestinal tract and alterations in the colonic microenvironment of the bacterial flora[14,15]. All these factors may contribute to colonic carcinogenesis.

As the serological test is not able to discriminate current from past infections, one study suggests that such a distinction may be crucial because only current *H. pylori* infection would induce humoral and cellular immune responses that provoke or perpetuate chronic inflammatory processes in the gastrointestinal tract with potential oncogenic sequelae including metastases and mortality. The authors also suggest that *H. pylori* eradication might inhibit the development or delay progression of CRC and recommend large-scale studies[16].

H. pylori virulence factors expressed by different bacterial strains can modulate gastric adenocarcinoma risk[17]. To assess whether such effects may also exist in the CRC, three recent studies were carried out in Europe[18,19] and USA[20] using *H. pylori* multiplex serology, a recently developed technique able to quantify seroreactivity immune response to several different *H. pylori* proteins. Fernández de Larrea-Baz N et al., in Spain, in a case-control study, analyzed 1488 CRC cases and 2495 controls and found that neither *H. pylori* seropositivity, nor seropositivity to the virulence factor cagA is associated with higher CRC risk[18]. The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort measured antibody responses to 13 *H. pylori* proteins in pre-diagnostic serum samples from 485 CRC cases and 485 matched controls[19]. Specifically, *Helicobacter* cysteine-rich protein C (HcpC) (OR: 1.66, 95%CI: 1.19–2.30) and vacA (OR: 1.34, 95%CI: 0.99–1.82), were associated with an increased risk of developing CRC. In the study from USA, serum samples were analyzed from 4063 incident cases of CRC and 4063 controls and found serologic responses to *H. pylori* vacA associated with increased risk of CRC risk, particularly for African Americans (OR: 1.45, 95%CI: 1.08–1.95)[20]. These two studies suggest that antibody responses to different *H. pylori* virulence factors, mainly vacA and cagA, were significantly associated with increased risk of developing CRC and the association could vary by race/ethnicity. Further studies are needed to investigate causality among this association and the underlying biological mechanisms involved (see below).

The sequence infection by *H. pylori* → chronic gastritis → glandular atrophy → intestinal metaplasia → dysplasia → gastric cancer constitutes a set of associated alterations that are very frequently observed in the development of gastric cancer since *H. pylori* is recognized as a class I carcinogen[21]. The risk of gastric adenocarcinoma increases significantly with premalignant progression[22,23]. To investigate the relationship between gastric mucosa histological changes induced by the bacterium and colonic neoplasms, Sonnenberg and Genta conducted a large study including 156,000 patients undergoing both colonoscopy e gastroscopy, with histological assessment of the gastric mucosa and the colon[24]. *H. pylori* status was performed by a polyclonal anti-*H. pylori* immunohistochemical stain. Compared with normal gastric mucosa, *H. pylori* gastritis occurred more frequently among patients with hyperplastic polyps, adenomatous polyps, advanced adenomas, villous adenomas, or adenomas with high-grade dysplasia, and adenocarcinomas. Other gastric conditions etiologically associated with *H. pylori*, such as intestinal metaplasia, adenoma, lymphoma, and adenocarcinoma, were also significantly associated with an increased risk of colonic neoplasm. Similar results were also observed in a Chinese study involving 233 patients[25]. To explore whether *H. pylori* atrophic gastritis, a pre-malignant condition, plays some role in the relation between *H. pylori* infection and advanced colonic neoplasms, Lee JY et al. in a cross-sectional study investigated the relationship between the presence of serum anti-*H. pylori* IgG antibodies, atrophic gastritis endoscopically-diagnosed, and advanced colonic neoplasms in 6,351 consecutive asymptomatic subjects who underwent a screening colonoscopy[26]. A total of 316 (5.0%) participants had advanced colonic neoplasm. The results showed that advanced colonic neoplasms occurred more frequently in *H. pylori*-infected patients with atrophic gastritis than without atrophic gastritis (7.3% vs 4.4%, $P<0.001$). A large recent study also investigated the association of gastric *H. pylori* presence with the risk of colorectal polyps and CRC[27]. The results confirmed that patients with *H. pylori* infection were 2.19 and 3.05 times more likely to develop colorectal polyps and CRC, respectively, than those without *H. pylori* active infection. Additionally, they found that the incidence of *H. pylori* infection coexisting with atrophic gastritis or intestinal metaplasia was higher in patients with colorectal polyps and CRC than in the control group. These findings reproduced Sonnenberg & Genta's study which showed that the *H. pylori*-positive gastritis and intestinal metaplasia, a more easily recognizable pre-malignant lesion, increased risk for colonic neoplasms while *H. pylori*-negative gastritis did not[24].

Potential mechanisms for causality

H. pylori is a pathogen restricted to primates (natural infection restricted to humans and monkeys) and, in humans, binds exclusively to the surface of the mucus-secreting cells of the stomach. In vivo, only 2–20% of the bacterial population exhibits adherence to the epithelial surface, the rest of the microorganisms remaining protected in the gastric mucus layer[28]. This specific tissue tropism is partly explained by the existence of specific adhesin molecules on the bacterial surface that acts as ligands in gastric epithelium receptors. The challenge is to account any action at a distance from this unique bacteria on colonic neoplasms risks. *H. pylori* direct and/or indirect effects on colorectal carcinogenesis have been considered trying to explain causality[28].

Regarding direct effects, studies evaluating the presence of *H. pylori* in the colorectal neoplastic epithelium are still scarce. Three pilot studies, two using immunohistochemical methods and a PCR technique identified *H. pylori* between 22–27% in samples of polyps or CRC fragments[29-31]. Greek authors, using immunohistochemical staining technique to identify *H. pylori* in 50 patients with CRC, 25 patients with colorectal adenomas and 10 controls described a significantly higher prevalence of *H. pylori* in the adenoma (68%) and CRC (84%) groups when compared to the control group (30%)[32]. As almost everything present in the stomach can be found in the stools, *H. pylori* is eliminated via this route and, although difficult, has been cultured in the stools since 1992[33]. Although *H. pylori* or its DNA have already been identified in the colonic epithelium, it is important to note that *H. pylori* infects only the gastric type mucosa and, in vivo, it has never been described adhered to the colonic epithelium. Bacterial adherence to a cell can trigger a cascade of events where adhesins can act as biological effector molecules[34]. One study showed that some components of the cell wall of *H. pylori* itself can be carcinogenic to the colorectal epithelial cell lining[30]. Studies are still needed to assess whether *H. pylori* acting directly on the colonic epithelium is capable of causing carcinogenic effects on the colon and rectum as observed in the gastric mucosa.

The mechanisms by which *H. pylori* virulence factors are involved in increasing the risk of developing colorectal neoplasms remain unclear. One of the major *H. pylori* virulent factors is the multifunctional vacA toxin. Considered a gastric cancer virulence factor, it has been speculated that it would be able to exert, outside the stomach, its effects on cellular vacuolation, cellular permeability, interference with cellular pathways, in addition to immunomodulatory and pro-inflammatory properties[35,36]. One recent suggestion is that vacA forms chloride channels that become inserted into the cell and mitochondrial membranes thereby reducing the membrane potential and mitochondrial energy production, interfering on cell proliferation control. Therefore, it would be biologically plausible that the vacA toxin of *H. pylori* could increase the risk of colon cancer, by chronically altering ionic equilibrium enterocytes exposed to the toxin[37]. Regarding the virulent factor cagA, which requires direct contact between bacteria and host cells, a Japanese study[38] suggested that exosomes containing cagA were detectable in the blood of cagA-positive *H. pylori*-infected individuals and could facilitate the development of multiple extragastric diseases. Because cagA is a bacterial oncoprotein, exosome-mediated cagA delivery may also be involved in the development of neoplasias outside the stomach and further studies are required in this area.

Indirect mechanisms have been also hypothesized involving *H. pylori* infection and increased risk of colorectal neoplasms such as gastrin-17 levels and *H. pylori* and microbiota interactions. Gastrin-17 belongs to a subgroup of gastrin composed of 17 amino acids, being produced by G cells of the gastric antrum and indicative of the glandular integrity of the antral mucosa. Their levels are closely related to the stomach's intraluminal pH, that is, they are reduced in acidic medium and abnormally high in case the patient has hypo or achlorhydria[39]. The rationale for exploring the association between gastrin and colorectal neoplasms is the putaitve role of the hormone in epithelial cell growth and to prevent apoptosis[40]. Experimental, in vitro, and human studies have shown discrepant results about the gastrin role to stimulate the growth of normal colonic epithelium and colorectal neoplasms[41-45]. A large nested case-control study of CRC and gastrin evaluated gastrin levels in subjects before cancer development[40]. The results support the hypothesis that, for a subset of CRC patients, hypergastrinemia may play a small role in tumor development, accounting for 8.6% of the CRC cases. However, these results have not been reproduced by other studies[46-49]. In addition to *H. pylori* (the most common cause of hypergastrinemia), three other conditions can increase gastrin expression: use of proton pump inhibitor (PPI), autoimmune gastritis, and Zollinger-Ellison syndrome (gastrinoma). A meta-analysis performed in 2012 found no association between PPI use and the risk of CRC[50]. A recent nationwide cohort study performed in Taiwan involving 45,382 elegible PPI users suggests that PPIs use might increase the risk of CRC in a dose-dependent manner[51] while another USA recent nested case-control study in a large community-based integrated healthcare setting involving 18,595 CRC patients suggests that PPI use for at least two years was not associated with CRC risk (OR: 1.05, 95%CI: 0.99–1.12)[52]. A systematic review with meta-analysis was performed to evaluate the incidence of cancer (other than gastric cancer) in pernicious anemia (PA), a late sequel of autoimmune gastritis where hypergastrinemia is secondary to damaged oxyntic mucosa and impaired gastric secretion[53]. It was found that PA patients had a lower RR (0.14, 95%CI: 0.01–0.19) for CRC compared to the general population. Likewise, the long-term hypergastrinemia secondary to Zollinger-Ellison syndrome has shown no effect on colonic adenomas or CRC development[54]. Some other studies included investigations related to an eventual autocrine production of gastrin by colorectal neoplasms[55], other forms of gastrin serum determinations and not only amidated forms[56], the real status of *H. pylori* infection[57], and previous information about PPI use and surgeries in the patients[48], but even though, more studies to clarify the conflicting studies of hypergastrinemia and colonic neoplasms in humans are needed.

H. pylori relation to the intestinal microbiota has also been investigated. CRC results from a combination of inherited and acquired mutations in the colon's epithelial cells and associated with different factors including the intestinal microbiota[58]. Increasing evidence shows that specific bacteria and bacterial dysbiosis can potentiate the initiation or progression of CRC. The speculated mechanisms involve damaging DNA, activating oncogenic signaling pathways, producing tumor-promoting metabolites such as secondary bile acids, and suppressing antitumor immunity[59,60]. *H. pylori* infection seems to span beyond gastric microbiota and affects downstream gastrointestinal microbiota[15]. Experimental studies with atrophic gastritis patients demonstrate that acid secretion reduction induces colorectal microbiota changes, intestinal bacterial overgrowth, and may favor carcinogenesis[61-63]. The enhanced

production of secondary bile acids by colonic bacterial overgrowth can also increase the risk for CRC, especially proximal colon cancer[26,64]. It is still unclear whether there are specific microbes that are particularly pathogenic and directly cause colorectal carcinogenesis, or whether the process requires specific interactions between host tissues and microbes[58].

Association or causation?

As the main human cancer-associated bacteria, studies continue to search for other *Helicobacter* species with oncogenic potential[65] as well as for other non-gastric tumors where it could have a causal role, such as CRC and adenomas (TABLE 1). The Bradford Hill criteria[66] (FIGURE 2), described in 1965 to establish a causal relationship between an agent and a disease, are still useful tools in establishing causation and also in proposing other necessary researches to confirm a potentially causal association[67] and will be briefly discussed here.

- ✓ Strength of association
- ✓ Consistency of observation
- ✓ Specificity
- ✓ Temporality
- ✓ Biological gradient
- ✓ Biological plausibility
- ✓ Coherence
- ✓ Experiment
- ✓ Analogy

FIGURE 2. Bradford-Hill causality criteria[66].

Strength and consistency: the positive association demonstrated between *H. pylori* and colorectal neoplasms is evidenced mainly by case-control studies and some prospective studies. Such observation has been consistently confirmed in different regions of the world, with some predominance in Asian countries. However, the OR found always ranges below 2, considered a weak association to the assessment of potentially causal relationships[8-11]. Specificity: needless to point out that *H. pylori* and colorectal neoplasm association is not specific given the well-known relationship between *H. pylori* and gastroduodenal diseases. However, this is not uncommon in the infectious diseases field where an individual may harbor a microorganism as an asymptomatic carrier while others, in the presence of genetic or environmental factors, will develop associated diseases. In other words, the presence of *H. pylori* could be necessary, but not sufficient for the development of colorectal neoplasms. Temporality: the concept that an agent´s exposure must precede disease´s onset, seems to be observed in the association, since infection by *H. pylori* uses to be acquired in childhood, before 10 years of age, in most patients[17]. The positivity of the association also observed in prospective studies excludes the possibility of reverse causality[8,40]. Biological gradient: the presence of a dose-response effect traditionally supports a causal relationship between an agent and the effect. Some studies suggest that patients infected with *H. pylori* strains expressing gastric cancer virulence factors such as cagA and vacA, or presenting premalignant gastric lesions have higher odds of association with CRC[19,20,27,57]. Biological plausibility and coherence: these criteria seek to analyze whether the relationship between the agent and the disease is consistent with current knowledge concerning the etiology and mechanism of the disease. Although the pathophysiological mechanisms underlying the association between *H. pylori* and colonic neoplasms remain unclear, important progress has been recently observed in the direct and/or indirect potential actions of *H. pylori* infection, or even associated with intestinal dysbiosis, in colonic carcinogenesis process[4,24,26,61]. Experiment: considered by Hill[67] as the strongest support for causal inference, the intervention through which can be demonstrated that disease´s risk declines after a treatment or exposition cessation, still lacks definite proof in studies about the association between *H. pylori* and colorectal neoplasms. To assess the development of colorectal adenoma, a recent retrospective study followed 615 patients for nine years with no history of colorectal adenoma or cancer at baseline[68]. Patients underwent upper digestive endoscopy and colonoscopy and were classified into three groups: individuals with no *H. pylori* infection, successful *H. pylori* eradication, and persistent *H. pylori* infection. During follow-up, the incidence rates of colorectal adenoma progression in participants uninfected with *H. pylori* were similar to the eradication group while the risk seen in the persistent infection group was 3-fold higher (HR: 3.04, 95%CI: 1.905.86. Despite retrospective study limitations, the results might support a causal relationship. Analogy: the situation where for analogous exposures and outcomes an effect has already been shown, would be sometimes acceptable to "judge by analogy". The tools diversity available today allowing the search for specific analogies such as the pattern of CRC progression, common risk factors, confounders and disease mechanisms, the modern value of the analogy seems more relevant in proposing and testing mechanistic hypotheses that confirm a causal inference[67].

CONCLUSION

Although the strength of the positive association between *H. pylori* infection and colonic neoplasms is considered weak from an epidemiological point of view, new mechanisms have been postulated trying to explain how a bacterium acting far from its ecological niche − the gastric milieu – could directly or indirectly interfere in colonic carcinogenesis, either through its virulence factors and/or metabolites or by promoting intestinal dysbiosis. A preliminary study suggests that the colorectal adenoma ratio might decrease after successful eradication of *H. pylori*. Several further studies are still necessary to establish a causal relationship in a disease with complex multifactorial etiology as colorectal neoplasms. Well-designed studies to better understand the changing incidence of colorectal cancer, the prevalence of *H. pylori* infection, and ethnic and environmental aspects involved in CRC are warranted. Prospective confirmatory studies to establish the role of *H. pylori* eradication in the process of carcinogenic transformation of the colonic epithelium may define its eventual role in the treatment and prevention of colonic neoplasms.

Authors' contribution

Coelho LGV contributed to the conception, design, and writing the paper; Coelho MCF contributed for reviewing the literature and writing the paper.

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RESUMO – *Helicobacter pylori* é o principal agente etiológico dos tumores malignos causados por doenças infecciosas. Constitui fator importante, às vezes dominante, na patogênese de um amplo espectro de doenças como gastrite aguda e crônica, úlceras gástricas e duodenais, carcinoma gástrico e linfoma. Estudos epidemiológicos e experimentais sugerem que a infecção crônica por *H. pylori* pode estar relacionada a diferentes doenças extragástricas, incluindo neoplasias colorretais. Esta concisa revisão tem como objetivo explorar a associação da infecção por *H. pylori* com câncer colorretal e adenoma, incluindo os recentes achados epidemiológicos, os métodos de diagnóstico empregados para detectar *H. pylori* e seus fatores de virulência com os mecanismos potencialmente envolvidos nesta relação. Além disso, procura-se estabelecer a integração dos dados atuais na busca de inferência causal com o emprego dos critérios de causalidade de Bradford-Hill. A associação epidemiológica positiva entre infecção por *H. pylori* e neoplasias do cólon embora classificada como fraca – porém global – do ponto de vista epidemiológico, quando associada a mecanismos recentemente postulados para explicar essa interação, incluindo disbiose intestinal, deverá estimular a realização de investigações futuras. Estudos prospectivos confirmatórios para estabelecer o papel da erradicação do *H. pylori* no processo de transformação carcinogênica do epitélio do cólon são aguardados para definir seu eventual papel no tratamento e prevenção de neoplasias do cólon.

DESCRITORES – Infecções por *Helicobacter*. Neoplasias colorretais. Adenoma. Fatores epidemiológicos.

REFERENCES

1. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8:e180-e190.
2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1:1311-5.
3. Gravina AG, Zagari RM, De Mussis C, Romano L, Loguercio C, Romano M. Helicobacter pylori and extragastric diseases. A review. World J Gastroenterol. 2018;24:3204-21.
4. Butt J, Epplein M. Helicobacter pylori and colorectal cancer – A bacterium going abroad? PLoSPathog. 15:e1007861.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
6. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg. 2009;22:191-7.
7. Lucas C, Barnich N, Nguyen HTT. Microbiota, inflammation, and colorectal cancer. Int J Mol Sci. 2017;18 pii: E1310.
8. Wu Q, Yang ZP, Xu P, Gao LC, Fan DM. Association between Helicobacter pylori infection and the risk of colorectal neoplasia: a systematic review and meta-analysis. Colorectal Dis. 2013;15:e352–64.
9. Zhao Y, Wang X, Wang Y. Helicobacter pylori infection and colorectal carcinoma risk: A meta-analysis. J Can Res Ther. 2016;12:15-8.
10. Yang F, Xu YL, Zhu RF. Helicobacter pylori infection and the risk of colorectal carcinoma: a systematic review and meta-analysis. Minerva Med. 2019;110:464-70.
11. Choi DS, Seo SI, Shin WG, Park CH. Risk for Colorectal Neoplasia in Patients With Helicobacter pylori Infection: A Systematic Review and Meta-analysis. Clin Transl Gastroenterol. 2020;11:e00127.
12. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20:280-322.
13. Cover TL. Helicobacter pylori Diversity and Gastric Cancer Risk. MBio 2016;7:e01869–15.
14. Coelho LG, Marinho JR, Genta R, Ribeiro LT, Passos MCF, Zaterka S, et al. IV Brazilian Consensus Conference on Helicobacter pylori infection. Arq Gastroenterol. 2018;55:97-121.
15. Kanno T, Matsuki T, Oka M, Utsunomiya H, Inada K, Magari H, et al. Gastric acid reduction leads to an alteration in lower intestinal microflora. Biochem Biophys Res Commun. 2009;381:666-70.
16. Kountouras J, Kapetanakis N, Polyzos AS, Katsinelos P, Gavalas E, Tzivras D, et al. Active Helicobacter pylori Infection Is a Risk Factor for Colorectal Mucosa: Early and Advanced Colonic Neoplasm Sequence. Gut Liver. 2017;5:733-4.
17. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med. 2002;347:1175-86.
18. Fernández de Larrea-Baz N, Michel A, Romero B, Perez-Gomez B, Moreno V, Martin V, et al. Helicobacter pylori Antibody Reactivities and Colorectal Cancer Risk in a Case-control Study in Spain. Front Microbiol. 2017;8:888.
19. Butt J, Jenab M, Pawlita M, Tjønneland A, Cecilie K, Boutron-Ruault MC, et al. Antibody responses to Helicobacter pylori and risk of developing colorectal cancer in a European cohort. Cancer Epidemiol Biomarkers. 2020;29: 1475-81.
20. Butt J, Varga MG, Blot WJ, Teras L, Visvanathan K, Marchand LL, et al. Serological response to Helicobacter pylori proteins associate with risk of colorectal cancer among diverse populations in the United States. Gastroenterology. 2019;156:175-186.e2.
21. No authors listed. Schistosomes, liver flukes, and Helicobacter pylori. IARC. Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1-241.
22. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process – First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res. 1992;52:6735-40.
23. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, et al. Progression of chronic atrophic gastritis associated with Helicobacter pylori infection increases risk of gastric cancer. Int J Cancer. 2004;109:138-43.
24. Sonnenberg A, Genta RM. Helicobacter pylori is a risk factor for colonic neoplasms. Am J Gastroenterol. 2013;108:208-15.
25. Qing Y, Wang M, Lin YM, Wu D, Zhu JY, Gao L, et al. Correlation between Helicobacter pylori-associated gastric diseases and colorectal neoplasia. World J Gastroenterol. 2016;22:4576-84.
26. Lee JY, Park HW, Choi JY, Lee JS, Koo JE, Chung EJ, et al. Helicobacter pylori Infection with Atrophic Gastritis Is an Independent Risk Factor for Advanced Colonic Neoplasm. Gut Liver. 2016;10:902-9.
27. Wang M, Kong WJ, Zhang JZ, Lu JJ, Hui WJ, Liu WD, et al. Association of Helicobacter pylori infection with colorectal polyps and malignancy in China. World J Gastrointest Oncol. 2020;12:582-91.
28. Hessey SJ, Spencer, Wyatt JI, Sobala G, Rathbone BJ, Axon ATR, et al. Bacterial adhesion and disease activity in Helicobacter associated chronic gastritis. Gut. 1990;31:134-8.
29. Jones M, Helliwell P, Pritchard C, Tharakan J, Mathew J. Helicobacter pylori in colorectal neoplasms: is there an aetiological relationship? World J Surg Oncol. 2007;5:51.
30. Soylu A, Ozkara S, Alis H, Dolay K, Kalayci M, Yasar N, et al. Immunohistochemical testing for Helicobacter pylori existence in neoplasms of the colon. BMC Gastroenterol. 2008;8:35.
31. Grahn N, Hmani-Aifa M, Fransén K, Söderkvist P, Monstein H. Molecular identification of Helicobacter DNA present in human colorectal adenocarcinomas by 16S rDNA PCR amplification and pyrosequencing analysis. J Med Microbiol. 2005;54:1031-5.
32. Kountouras J, Kapetanakis N, Zavos C, Romiopoulos I, Polyzos AS, Tsiaousi E, et al. Impact of Helicobacter pylori infection on colon oncogenesis. Am J Gastroenterol. 2013;108:625-6.
33. Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of Helicobacter pylori from human faeces. Lancet 1992;340:1194-5.
34. Clyne M, Drum B. Adherence of Helicobacter pylori to primary human gastrointestinal cells. Infect Immun. 1993;61:4051-7.
35. McClain MS, Beckett AC, Cover TL. Helicobacter pylori vacuolating toxin and gastric cancer. Toxins (Basel) 2017;9.
36. Chauhan N, Tay ACY, Marshall BJ, Jain U. Helicobacter pylori VacA, a distinct toxin exerts diverse functionalities in numerous cells: An overview. Helicobacter. 2019;24:e12544.

37. Ponzetto A, Figura N. Colon Cancer Risk and VacA Toxin of Helicobacter pylori. Gastroenterology. 2019;156:2356.
38. Shimoda A, Ueda K, Nishiumi S, Murata-Kamiya N, Mukai SA, Sawada SI, et al. Exosomes as nanocarriers for systemic delivery of the Helicobacter pylori virulence factor CagA. Sci Rep. 2016;6:18346.
39. di Mario F, Cavallaro LG. Non-invasive tests in gastric diseases. Dig Liv Dis. 2008;40:523-30.
40. Thorburn CM, Friedman GD, Dickinson CJ, et al. Gastrin and colorectal cancer: a prospective study. Gastroenterology. 1998;115:275-80.
41. Chu M, Rehfeld JF, Borch K. Effects of gastric fundectomy and antrectomy on the colonic mucosa in the hamster. Digestion. 1992;53:28-34.
42. Sirinek KR, Levine BA, Moyer MP. Pentagastrin stimulates in vitro growth of normal and malignant human colon epithelial cells. Am J Surg. 1985;149:35-9.
43. Creutzfeldt W, Lamberts R. Is hypergastrinemia dangerous to man? Scand J Gastroenterol. 1991;26(Suppl 180):179-91.
44. Penman ID, El-Omar E, Ardill JES, McGregor JR, Galloway DJ, O'Dwyer PJ. Plasma gastrin concentrations are normal in patients with colorectal neoplasia and unaltered following tumor resection. Gastroenterology. 1994;100:1264-70.
45. Chueca E, Lanas A, Piazuelo E. Role of gastrin-peptides in Barrett's and colorectal carcinogenesis. World J Gastroenterol. 2012;18:6560-70.
46. Machida-Montani A, Sasazuki S, Inoue M, Natsukawa N, Shaura K, Koizumi Y, et al. Atrophic gastritis, Helicobacter pylori, and colorectal cancer risk: a case-control study. Helicobacter. 2007;12:328-32.
47. Kikendall JW, Glass AR, Sobin LH, Bowen PE. Serum gastrin is not higher in subjects with colonic neoplasia. Am J Gastroenterol. 1992;87:1394-7.
48. Selgrad M, Bornschein J, Kandulski A, Hille C, Weigt J, Roessne A, et al. Helicobacter pylori but not gastrin is associated with the development of colonic neoplasms. Int J Cancer. 2014;135:1127-31.
49. Robertson DJ, Sandler RS, Ahnen DJ, Greenberg ER, Mott LA, Cole BF, et al. Gastrin, Helicobacter pylori, and Colorectal Adenomas. Clin Gastroenterol Hepatol. 2009;7:163-7.
50. Ahn JS, Park SM, Eom CS, Kim S, Myung SK. Use of Proton Pump Inhibitor and Risk of Colorectal Cancer: A Meta-analysis of Observational Studies. Korean J Fam Med 2012;33:272-9.
51. Lei W-Y, Wang JH, Yi CH, Hung JS, Wong MW, Bair MJ, et al. Association between use of proton pump inhibitors and colorectal cancer: A nationwide population-based study. Clin Res Hepatol Gastroenterol. (2020). Available from: https://doi.org/10.1016/j.clinre.2020.02.017.
52. Lee JK, Merchant SA, Schneider JL, Jensen CD, Fireman BH, Quesenberry CP, et al. Proton Pump Inhibitor Use and Risk of Gastric, Colorectal, Liver, and Pancreatic Cancers in a Community-Based Population. Am J Gastroenterol. 2020;115:706-15.
53. Lahner E, Capasso M, Carabotti M, Annibale B. Incidence of cancer (other than gastric cancer) in pernicious anaemia: A systematic review with meta-analysis. Dig Liver Dis. 2018;50:780-6.
54. Orbuch M, Venzon DJ, Lubensky IA, Weber HC, Gibril F, Jensen RT. Prolonged hypergastrinemia does not increase the frequency of colonic neoplasia in patients with Zollinger-Ellison syndrome. Dig Dis Sci. 1996;41:604-13.
55. Singh P, Dai B, Wu H, Owlia A. Role of autocrine and endocrine gastrin-like peptides in colonic carcinogenesis. Curr Opin Gastroenterol. 2000;16:68-77.
56. Watson SA, Michaeli D, Grimes S, Morris TM, Robinson G, Varro A, et al. Gastroimmune raises antibodies that neutralize amidated and glycine-extended gastrin-17 and inhibit the growth of colon cancer. Cancer Res. 1996;56: 880-5.
57. Kawahara Y, Kodama M, Mizukami K, Saito T, Hirashita Y, Sonoda A, et al. Endoscopic gastric mucosal atrophy as a predictor of colorectal polyps: a large scale case control study. J Clin Biochem Nutr. 2019;65:153-9.
58. Abreu MT, Peek Jr RM. Gastrointestinal Malignancy and the Microbiome. Gastroenterology. 2014;146:1534-46.
59. Garrett WS. The gut microbiota and colon cancer. Science 2019;364:1133-5.
60. Mima K, Ogino S, Nakagawa S, Sawayamaa H, Kinoshitaa K, Krashima R, et al. The role of intestinal bacteria in the development and progression of gastrointestinal tract neoplasms. Surg Oncol. 2017:368-76.
61. Dash NR, Khoder G, Nada AM, Al Bataineh MT. Exploring the impact of Helicobacter pylori on gut microbiome composition. PLoS One. 2019;14: e0218274.
62. Husebye E. The pathogenesis of gastrointestinal bacterial overgrowth. Chemotherapy. 2005;51 (Suppl 1):1-22.
63. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS One. 2011;6:e16393.
64. Giovannucci E, Colditz GA, Stampfer MJ. A meta-analysis of cholecystectomy and risk of colorectal cancer. Gastroenterology. 1993;105:130-41.
65. de Martel C, Plummer M, Parsonnet J, Van Doorn LJ, Franceschi S. Helicobacter species in cancers of the gallbladder and extrahepatic biliary tract. Br J Cancer. 2009;100:194-9.
66. Hill AB. The environment and disease: association or causation? Proc R Soc Med. 1965;58:295-300.
67. Fedak KM, Bernal A, Capshaw ZA, Gross S. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol. 2015;12:14.
68. Hu KC, Wu MS, Chu CH, Wang HY, Lin SC, Liu CC, et al. Decreased Colorectal Adenoma Risk After Helicobacter pylori Eradication: A Retrospective Cohort Study. Clin Infect Dis. 2019;68:2105-13.

REVIEW

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Interventions for the treatment of irritable bowel syndrome: a review of Cochrane systematic reviews

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ABSTRACT – **Background** – Irritable bowel syndrome (IBS) is a complex gastrointestinal disorder, whose understanding is relatively uncertain, and the treatment guidance decision still represents a challenge. **Objective** – To identify and critically appraise systematic reviews (SRs) published in the Cochrane Database of SRs (CDSR) on the effects of interventions (pharmacological and non-pharmacological) for the treatment of IBS. **Methods** – The search was conducted at the Cochrane Library in May 2020. The methodological quality of the SRs was evaluated by the AMSTAR-2 tool. **Results** – Eight SRs with moderate to high quality were included, which addressed the treatments: (a) pharmacological: volume agents, antispasmodics, antidepressants and tegaserod; and (b) non-pharmacological: homeopathy, acupuncture, phytotherapy, biofeedback, psychological interventions and hypnotherapy. The results were favorable to antispasmodic drugs and antidepressants regarding the improvement of clinical symptoms. There was no difference between volume agents or tegaserod when compared to placebo. Acupuncture and homeopathy showed a little improvement in symptoms compared to placebo, but the certainty of this evidence was considered low to very low. Psychological interventions seem to improve the overall assessment of the patient and relief symptoms such as abdominal pain. However, there was no long-term follow-up of these patients. The results of the other treatments were considered uncertain due to the high risk of bias. **Conclusion** – Considering the low quality of the studies included in the SRs, pharmacological treatment with antispasmodics and antidepressants seems to be beneficial for patients with IBS. Among non-pharmacological interventions, psychological interventions seem to be beneficial. However, further clinical trials are recommended with greater methodological rigor to prove these findings.

HEADINGS – Irritable bowel syndrome. Systematic review. Evidence-based medicine.

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder, characterized by abdominal pain, swelling, constipation and changes in bowel habit. Symptoms vary among individuals and, for this reason, both diagnosis and treatment represent a challenge[1-3]. Other common symptoms in patients with IBS include urinary frequency, chronic fatigue, sleep, and mood disorders, and may lead to the patient being mistakenly referred to other clinical specialties[4,5].

It is estimated that the prevalence of IBS in the general population ranges from 1 to 45%, depending on the country and its socioeconomic differences[6]. In Europe and North America, the prevalence of IBS was estimated between 10 and 15%; in China 15.9%. Data from South America are very scarce to reach a real conclusion, however, Uruguay had an overall prevalence of 10.9% and Venezuela 16.8%[1]. The direct and indirect costs associated with IBS were estimated at more than one billion dollars a year in the United States[7], and 6 to 8 billion euros per year in Europe[8], which makes IBS a public health problem.

The physiological course of the disease is not yet fully known, since there is a wide variety of related mechanisms[1,2]. Psychological factors, changes in the connection of the central nervous system with the intestine, endocrine imbalance, visceral hypersensitivity, gastrointestinal infections and allergies and previous food intolerances are some of the criteria frequently analyzed. IBS occurs mainly between 15 and 65 years of age, and is more prevalent among women[3,4,6].

Diagnosis is difficult, mainly because it is a syndrome with signs and symptoms common to various pathologies. Diagnostic criteria are based on Rome IV criteria, used for functional gastrointestinal disorders. According to these criteria, IBS is diagnosed based on recurrent abdominal pain related to defecation or associated with a change in the frequency or shape of feces[1,4,6].

Currently, the treatment used for IBS varies according to clinical presentation, that is, there is no standard measure to be performed, both because of the difficulty in clarifying the etiopathogenesis and the heterogeneity of symptoms. However, the treatment covers non-pharmacological and pharmacological measures, including lifestyle changes, nutritional and behavioral guidelines, acupuncture, phytotherapy, possibly associated with medications such as antispasmodics, antidepressants, among others[5,6].

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The importance of treatment for patients with IBS is due to the decrease in the impact on quality of life that symptoms can cause, impairing interpersonal and social relationships, productivity at work and routine activities. Therefore, the relevance of identifying and summarizing in a single document the studies of higher level of evidence on all possible therapeutic measures for the clinical recommendation in the treatment of IBS, contributing to the standardization of an adequate treatment with prognosis of improvement in the quality of life of these patients. Thus, the objective of this review was to identify and critically appraise the systematic reviews (SRs) published in the Cochrane Database of SRs (CDSR) on the effects of interventions (pharmacological and non-pharmacological) for the treatment of IBS.

METHODS

This review followed the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions[9] and PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[10].

Criteria for inclusion of studies

We included all systematic reviews (SRs) published by Cochrane on any pharmacological and non-pharmacological treatment for adult patients diagnosed with irritable bowel syndrome (IBS) were included. SRs that included mixed population, e.g. children and adults, or individuals with IBS and chronic constipation, were included only if the data were presented separately. All outcomes analyzed by the SRs were presented, including clinical and laboratory outcomes.

Search strategy

The search strategy was performed in the Cochrane Database of Systematic Reviews – CDSR (via Wiley) (on May 26, 2020) (FIGURE 1). There was no restriction on the date of publication. SRs in the protocol phase or marked as "withdrawn" from the Cochrane Library were not included.

Search strategy	
Cochrane Database of Systematic Reviews (CDSR) (via Wiley)	MeSH descriptor: [Irritable Bowel Syndrome] explode all trees [in Cochrane Reviews]

FIGURE 1. Search strategy.

Selection of studies and data extraction

The SRs identified in the search were selected by two independent reviewers (ACLS and MMBR), using the Rayyan online platform[11]. The reviewers analyzed the titles and abstracts, and the SRs with eligibility potential were evaluated by reading the full text. In case of divergence, a third reviewer (ALCM or DM) decided to include or exclude the review. Two independent reviewers (EFBCB and MESC) extracted the data from the SRs using a previously prepared form containing information about the characteristics of the participants, interventions analyzed, comparison groups, outcomes, and results.

Methodological quality assessment

The methodological quality of the SRs was evaluated by two reviewers independently (ACLS and ALCM), using the AMSTAR-2 tool (Assessing the Methodological Quality of Systematic Reviews)[12]. This tool involves 16 items: (1) research question and inclusion criteria according to PICO (population, intervention, comparator and outcomes); (2) prior planning of the SR (protocol registration); (3) justification for the selection of the study design for inclusion; (4) comprehensive literature search strategy; (5) study selection in duplicate; (6) extraction of data in duplicate; (7) list of excluded studies; (8) details on the characteristics of the included studies; (9) methods used to assess the risk of bias in the included studies; (10) reporting of the funding sources of the included studies; (11) methods used to combine the results (meta-analysis); (12) assessment of the impact of the risk of bias in meta-analyses; (13) account for risk of bias in the interpretation and discussion of the results; (14) explanation of heterogeneity; (15) investigation of publication bias; and (16) report of conflict of interest for conducting the review. Each item is classified as: completely suitable ("yes"); partially adequate ("partially yes"), inadequate ("no") or not applicable. The domains 2, 4, 7, 9, 11, 13 and 15 were considered critical. The overall assessment of the quality of SRs takes into account the amount of critical flaws, and can be classified as critically low (More than one critical flaw), low (one critical flaw), moderate (more than one non-critical weakness) and high (one non-critical weakness). The evaluation was performed through the checklist available on the AMSTAR-2 website (http://amstar.ca/Amstar_Checklist.php).

Summary of data

The results of the included SRs were summarized narratively, considering the methodological quality evaluated by the AMSTAR 2 tool. Since each SRs included in this review evaluated a different intervention, there was no duplication of primary studies.

RESULTS

The search resulted in 19 systematic reviews (SRs), and 10 were excluded because they did not meet the inclusion criteria. Nine SRs were analyzed in full text and one was excluded for evaluating only individuals with constipation. Thus, eight SRs[13-20] were included.

Characteristics of systematic reviews included

The eight SRs were published from 2007 to 2019, included only randomized clinical trials (RCTs) on pharmacological and non-pharmacological treatment for patients diagnosed with irritable bowel syndrome (IBS). Most RCTs presented an unclear risk of bias due to methodological limitations. The samples of the RCTs were predominantly of women, aged between 21 and 60 years and who had a minimum duration of symptoms between 3 and 6 months.

Methodological quality of systematic reviews included

Based on AMSTAR-2 tool assessment, most of the SRs were considered high quality (87.5%), and only one was classified as moderate quality due to the absence of meta-analysis and investigation of publication bias. Three SRs (37.5%) did not report the sources of funding for the included studies. The other items were presented appropriately. TABLE 1 showed the details of the evaluation.

TABLE 1. Methodological quality assessment of the included systematic reviews with the AMSTAR-2 tool.

AMSTAR-2	Systematic reviews included							
	Ruepert 2011[13]	Evans 2007[14]	Manheimer 2012[15]	Peckham 2019[16]	Goldenberg 2019[17]	Liu 2006[18]	Zijdenbos 2009[19]	Webb 2007[20]
1. Search question (PICO)	Y	Y	Y	Y	Y	Y	Y	Y
2. Study planning (protocol)	Y	Y	Y	Y	Y	Y	Y	Y
3. Justification for the selection of the study design	Y	Y	Y	Y	Y	Y	Y	Y
4. Search strategies	Y	Y	Y	Y	Y	Y	Y	Y
5. Study selection in duplicate	Y	Y	Y	Y	Y	Y	Y	Y
6. Data extraction in duplicate	Y	Y	Y	Y	Y	Y	Y	Y
7. Report of excluded studies	Y	Y	Y	Y	Y	Y	Y	Y
8. Characteristics of the studies included	Y	Y	Y	Y	Y	Y	Y	Y
9. Risk of bias assessment	Y	Y	Y	Y	Y	Y	Y	Y
10. Reporting of the sources of funding for the studies	N	Y	Y	Y	Y	Y	N	N
11. Appropriate statistical methods for meta-analysis	Y	Y	Y	Y	Y	NA	Y	Y
12. Assessment of the impact of the risk of bias in meta-analyses	Y	Y	Y	Y	Y	NA	Y	Y
13. Risk of bias in interpretation and results	Y	Y	Y	Y	Y	Y	Y	Y
14. Discussion and explanation of heterogeneity	Y	Y	Y	Y	Y	Y	Y	Y
15. Investigation of publication bias	Y	Y	Y	Y	Y	NA	Y	Y
16. Report of conflict of interest of the authors of the review	Y	Y	Y	Y	Y	Y	Y	Y
Total (quality)	High	High	High	High	High	Moderate	High	High

PICO: population, intervention, comparator and outcomes; Y: yes; N: no; NA: not apply. Evaluated by the http://amstar.ca/Amstar_Checklist.php.

Effects of interventions

Pharmacological treatment

• *Bulking agents, antispasmodics, antidepressants*

One SR[13] included 56 RCTs (3,725 patients), which evaluated the following drugs compared to placebo: bulking agents (12 RCTs), antispasmodics (29 RCTs) and antidepressants (15 RCTs). The duration of the treatments ranged from 1 week to 6 months. In general, the risk of bias was classified between unclear and low. There were limitations mainly in the description of the methods used for randomization and allocation concealment (selection bias).

There was no beneficial effect of volume agents in relation to placebo for any of the outcomes analyzed, as well as between types of volume agents (soluble *versus* insoluble fibers). Overall, antispasmodics showed improvement in all outcomes. Data from individual studies showed statistically significant benefit for: cimetropium/dicyclomine, peppermint oil, pinaverium and trimebutin compared to placebo. As for antidepressants, there was also significant improvement in symptoms, especially for Selective serotonin reuptake inhibitor and tricyclic antidepressants.

• *Tegaserod*

One SR[14] included 10 RCTs, with 8,598 patients diagnosed with predominant IBS-constipation. The overall risk of bias was unclear for most of the RCTs. Treatment with tegaserod (4 and 12 mg) was compared to placebo and lasted from 4 to 12 weeks.

Both tegaserod doses (4 and 12 mg) showed no difference compared to placebo for the improvement of abdominal pain. In the overall evaluation of the individual, tegaserod was higher than placebo after 4 weeks of treatment, with both doses. However, this improvement was not clinically significant. Regarding symptoms, the placebo group showed a significant reduction in diarrhea episodes compared to tegaserod. There was no difference between the groups for episodes of headache and nausea. The effects of tegaserod on gastrointestinal symptoms, such as swelling, stool consistency and exertion, were not consistent in the studies.

The estimates of the effect of meta-analyses for pharmacological treatments compared to placebo are detailed in TABLE 2.

Non-pharmacological treatments

• *Acupuncture*

This SR[15] included a total of 17 RCTs, totaling 1806 participants, who compared acupuncture with placebo *(sham),* other active treatments or no treatment, in addition to acupuncture analysis as an adjunct to another treatment. In general, the risk of bias of the included RCTs was classified as low, except for five RCTs, which did not perform the randomization process adequately and the blinding of the participants and personnel. The certainty of the body of evidence, evaluated by the GRADE approach, was considered moderate due to the small sample size.

The results of the meta-analyses showed no benefits of acupuncture compared to placebo for both the improvement of symptom severity (standardized mean difference [SMD] -0.11, confidence interval [CI] 95% -0.35 to 0.13; 4 RCTs; 281 patients), and for quality of life (SMD -0.03; 95%CI -0.27 to 0.22; 3 RCTs; 253 patients), after 3 to 10 weeks of treatment. There was improvement in symptoms in favor of acupuncture compared to pharmacological treatment (pinaverium bromide, sulfasalazine, trimebutine maleate), and no treatment (relative risk [RR] 2.11; 95%CI 1.18 to 3.79; 2

TABLE 2. Results of meta-analyses of pharmacological interventions compared to placebo for irritable bowel syndrome.

Interventions compared to placebo	Outcomes [95% CI]		
	Abdominal pain	Overall assessment	Symptom improvement
Bulking agents	There was no difference SMD 0.03 [-0.34 to 0.40] 4 RCTs, n=186	There was no difference RR 1.10 [0.91 to 1.33] 11 RCTs, n=565	IBS score: there was no difference SMD -0.00 [-0.43 to 0.43] 3 RCTs, n=126
Antispasmodic	Improvement in favor of intervention RR 1.32 [1.12 to 1.55] 13 RCTs, n=1392	Improvement in favor of intervention RR 1.49 [1.25 to 1.77] 22 RCTs, n=1983	IBS score: improvement in favor of intervention RR 1.86 [1.26 to 2.76] 4 RCTs, n=586
Antidepressants	Improvement in favor of intervention RR 1.49 [1.05 to 2.12] 8 RCTs, n=517	Improvement in favor of intervention RR 1.57 [1.23 to 2.0] 11 RCTs, n=750	IBS score: improvement in favor of intervention RR 1.99 [1.32 to 2.99] 3 RCTs, n=159
Tegaserode 4 mg	There was no difference RR 1.10 [0.82 to 1.49] 3 RCTs, n=1675	Improvement in favor of intervention RR 1.15 [1.02 to 1.31] 3 RCTs, n=1675	Bowel habits: improvement in favor of intervention RR 1.21 [1.02 to 1.43] 3 RCTs, n=1675
Tegaserode 12 mg	There was no difference RR 1.16 [0.89 to 1.51] 3 RCTs, n=1675	Improvement in favor of intervention RR 1.19 [1.09 to 1.29] 4 RCTs, n=3194	Bowel habits: there was no difference RR 1.10 [0.93 to 1.31] 3 RCTs, n=1675

RCTs: randomized clinical trials; n: number of participants; SMD: Standardized mean difference; RR: relative risk; 95% CI: 95% confidence interval; IBS score: Symptom score for Irritable Bowel Syndrome.

RCTs, 181 patients). However, the certainty of these evidence was considered low by the GRADE approach due to methodological limitations, small sample size and a wide confidence interval with imprecision of the results. There was no difference between acupuncture and probiotics, psychotherapy, as well as associated with other treatments of traditional Chinese medicine.

• *Homeopathy*

This SR[16] included four RCTs (307 participants), two compared homeopathic treatment to placebo, and two to conventional treatment for patients with IBS-constipation. The risk of bias of RCTs was classified as unclear to low for most studies, and two did not blind the participants and personnel and had a high risk of bias for this domain. The certainty of the evidence was classified as very low by the GRADE approach due to methodological limitations, small sample size and the short follow-up period (2 weeks). Only one meta-analysis was conducted and showed very low quality evidence in favor of homeopathy compared to placebo for the overall improvement of IBS symptoms (RR 1.61; 95%CI 1.18 to 2.18; 2 RCTs, 129 participants). Individual data from the included RCTs showed no difference between homeopathy and conventional treatment. None of the studies reported abdominal pain, stool frequency, stool consistency or adverse events.

• *Biofeedback*

Biofeedback has been proposed as a therapy that can assist the individual in learning conscious control over sympathetic-vagal balance and symptom management. This SR[17] included a total of eight RCTs, totaling 300 participants diagnosed with IBS. The risk of bias assessment varied between unclear and high.

Evidence of very low certainty has shown that the clinical benefits of biofeedback alone or associated with usual treatment, compared to the usual treatment alone or placebo are uncertain. A meta-analysis with data from three RCTs showed a benefit in favor of thermal biofeedback associated with cognitive behavioral therapy in the overall improvement of symptoms. However, the very low quality of evidence represents uncertainty about this result (mean difference [MD] 30.34; 95%CI 8.47 to 52.21; 3 RCTs, 101 participants). Only one RCT evaluated the quality of life of patients and reported no difference between treatment with biofeedback and cognitive behavioral therapy (RR 1.10; 95%CI 0.72 to 1.69; 2 RCTs, 80 participants). There were no reports of adverse events resulting from the intervention.

• *Phytotherapy*

This SR[18] included 75 RCTs, involving 7,957 participants. Seventy-one different herbal medicines were tested in the included studies, and compared with: placebo, conventional pharmacological therapy or no treatment. The methodological quality of three double-blind, placebo-controlled studies was high, but the quality of the others was mostly low.

Since it was not possible to perform meta-analysis due to heterogeneity among the included RCTs, data from individual studies showed some benefit in reducing the symptoms of IBS reported by the patient in favor of herbal formulas (herbal compounds, standard Chinese formula, Tibetan formula Padma lax, ayurvedic preparation), compared to placebo. However, there was no difference between the groups in the improvement of abdominal pain and relief of constipation. Compared with conventional therapy, individual data from 65 RCTs testing 51 different herbal medicines showed little benefit in improving symptoms, but most studies showed no differences between groups. No adverse events related to herbal medicines have been reported.

Given the low methodological quality of the included studies, these results should be interpreted with caution and studies with greater rigor are necessary to support the findings of this SR.

• *Psychological interventions*

This SR[19] included 25 RCTs, totaling 1,858 participants, comparing psychological interventions associated with conventional treatment or placebo, regarding symptom relief, improvement of abdominal pain and quality of life. The risk of bias was classified from unclear to high. The follow-up ranged from 2 to 3 months. Main findings:

• Group psychological interventions versus conventional treatment or waiting list: significant improvement in favor of intervention in the overall assessment (RR 2.02; 95%CI 1.13 to 3.62; 2 RCTs, 254 participants), in the symptom score (RR 0.62; 95%CI 0.45 to 0.79; 8 RCTs, 593 participants) and in the relief of abdominal pain (SMD 0.26; 95%CI 0.07 to 0.45; 10 RCTs, 727 participants), after 3 months of treatment. There was no difference between the groups in improving quality of life, as well as in comparison with placebo.

• Cognitive behavioral therapy versus conventional treatment: significant improvement in favor of intervention in symptom improvement (MD 0.58; 95%CI 0.36 to 0.79; 5 RCTs, 395 participants) after 3 months. There was no difference between the groups in abdominal pain and quality of life.

• Interpersonal psychotherapy and stress relaxation techniques were shown to be beneficial when compared to conventional treatment.

However, since the included studies did not evaluate the long-term results, there is no evidence that the effects of treatment will continue after the end of the treatments. The results of this SR should be analyzed with caution, as the clinically significant effect was not found for most outcomes. The studies showed substantial heterogeneity and small sample size.

• Hypnotherapy

This SR[20] consisted of four RCTs including a total of 147 patients. Only one study compared hypnotherapy to an alternative therapy (psychotherapy and placebo pill), two studies compared hypnotherapy with waiting list control groups, and one study compared hypnotherapy to conventional medical treatment. The therapeutic effect of hypnosis was higher than that observed in the waiting list group, and when associated with conventional treatment, it was better than the conventional treatment alone. However, the data were not grouped in meta-analysis due to the heterogeneity among the included studies, which also have low methodological quality and small sample which led to high risk of bias. The quality of the studies was inadequate to allow any conclusion on the efficacy of hypnotherapy for IBS. Clinical trials with greater methodological rigor are still needed.

DISCUSSION

The present study mapped and summarized the evidence of Cochrane systematic reviews (SRs) on the different interventions for the treatment of irritable bowel syndrome (IBS) in adults. Cochrane's methodological rigor for the development and conduct of SRs is well established and recognized worldwide as the highest level of scientific evidence to support health decision-making. For this reason, we chose to include only SRs Cochrane, who, according to the AMSTAR-2 tool assessment, presented sufficient methodological quality to provide reliable information to the health professional and guide clinical practice.

The clinical variability of the characteristics and subtypes of IBS makes it difficult to decide on the best choices for the treatment of these patients. Of the eight SRs included, two analyzed the effects of pharmacological treatment for IBS. When comparing different classes of drugs with placebo, the results of meta-analyses showed significant improvement in symptoms and overall evaluation of patients with antispasmodics, antidepressants, and tegaserod. On the other hand, volume agents (soluble and insoluble fibers) showed no difference in any of the outcomes analyzed, and there was no improvement in abdominal pain and discomfort with the use of tegaserod.

Although both SRs were published more than 5 years without updating, a recent comprehensive review of literature[21] on pharmacological treatments for IBS, published in 2020, showed similar results and corroborates these findings, that is, new studies were not sufficient to modify the results.

It is noteworthy that, among the SRs included on pharmacological treatment for IBS, only one evaluated safety through the rate of adverse events resulting from the intervention, and no difference was observed between tegaserod and placebo in episodes of nausea and headache.

Regarding non-pharmacological treatments, acupuncture has shown no benefit compared to placebo or other treatments such as probiotics, psychotherapy or other types of traditional Chinese medicine treatments. There was improvement in favor of acupuncture when compared to medications, but these results should be interpreted with caution because the certainty of the evidence was classified as low by the GRADE approach, due to methodological limitations in the studies analyzed. On the other hand, the evidence for homeopathy, despite showing some benefit in favor of the intervention, was classified as very low certainty, which represents in the GRADE approach an entire uncertainty in these findings. The same interpretation of the results should be considered for treatments with biofeedback, herbal medicines, and hypnosis. Finally, psychological interventions seem to be beneficial for patients with IBS, although the clinical significance of these results is debatable, as there was substantial heterogeneity between the studies, a fact that may compromise the external validity of the evidence.

Quality of life, despite being an important indicator of clinical improvement, was not evaluated by most of the primary studies included in the SR analyzed. Both quality of life and patient satisfaction are outcomes frequently reported by patients, and for this reason can be influenced by individual variables such as symptom severity, age, gender, socioeconomic status, among others. A systematic review published in 2020 showed that psychological interventions seem to improve the quality of life of patients with IBS. However, the authors reported the need to standardize the evaluation of this outcome in future studies[4].

Most of the RCTs included in the SRs presented small sample size and methodological limitations related to randomization, blinding and lack in the description of losses during the study. These limitations can negatively influence the results and increase the risk of bias. The identification of the true effect of the interventions was also challenged by variations between the intervention and control groups, related to the different doses and treatment regimens, and especially the absence of long-term follow-up. Thus, most of the SRs included presented as implications for future research the conduction of further RCTs, strictly following the Consort statement[22].

This review had as main limitation the restriction to the interventions analyzed by the Cochrane SRs, thus excluding some interventions that were not studied in these SRs, for example, nutritional diets, exercises, probiotics, some classes of medications, as well as other alternative therapies. It is important to highlight that, given the low certainty of evidence, therapeutic choice should be based on an effective doctor-patient relationship, and the combination of pharmacological and non-pharmacological interventions seems to be an alternative to be considered individually.

CONCLUSION

Considering the low quality of primary studies analyzed in the included SRs, pharmacological treatment with antispasmodics and antidepressants seems to be beneficial for patients with IBS in relation to the improvement of clinical symptoms. Among non-pharmacological interventions, psychological interventions seem to be beneficial. The results of the other treatments were considered uncertain due to the high risk of bias. All the included SRs recommended as implications for research new clinical trials with greater methodological rigor to prove these findings. Adverse events and quality of life are fundamental outcomes and need to be evaluated in future studies, as well as the clinical effects of long-term interventions.

Authors' contribution

Scaciota ACL: conceptualization, resources, data collection, writing-original draft. Matos D: conceptualization, methodology, writing–review and editing. Rosa MMB: resources, data collection, writing-original draft. Colovati MES: conceptualization, methodology, writing–review and editing. Bellotto EFBC: data collection, formal analysis; writing–review and editing. Martimbianco ALC: conceptualization, methodology, data collection, project administration, writing–review and editing.

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RESUMO – **Contexto** – A síndrome do intestino irritável (SII) é um distúrbio gastrointestinal complexo, cujo entendimento é relativamente incerto e a decisão de orientação do tratamento ainda representa um desafio. **Objetivo** – Identificar e avaliar criticamente as revisões sistemáticas (RSs) publicadas na base de dados de RSs Cochrane (CDSR) sobre os efeitos das intervenções (farmacológicas e não farmacológicas) para o tratamento da SII. **Métodos** – A busca foi realizada na Biblioteca Cochrane em maio de 2020. A qualidade metodológica das RSs foi avaliada pela ferramenta AMSTAR-2. **Resultados** – Foram incluídas oito RSs com qualidade moderada a alta, as quais abordaram os tratamentos: (a) farmacológico – agentes de volume, antiespasmódicos, antidepressivos e o tegaserod; e (b) não farmacológico – homeopatia, acupuntura, fitoterapia, *biofeedback*, intervenções psicológicas e hipnoterapia. Os resultados foram favoráveis aos medicamentos antiespasmódicos e antidepressivos em relação à melhora dos sintomas clínicos. Não houve diferença entre os agentes de volume ou tegaserod quando comparados ao placebo. Acupuntura e homeopatia apresentaram pequena melhora dos sintomas em comparação ao placebo, porém a qualidade da evidência foi considerada baixa a muito baixa. As intervenções psicológicas parecem melhorar a avaliação global do paciente e alívio de sintomas como dor abdominal. Contudo, não houve acompanhamento desses pacientes a longo prazo. Os resultados dos demais tratamentos foram considerados incertos devido ao alto risco de viés. **Conclusão** – Considerando a baixa qualidade dos estudos incluídos nas RSs, o tratamento farmacológico com antiespasmódicos e antidepressivos parece ser benéfico para os pacientes com SII. Entre os não-farmacológicos, as intervenções psicológicas parecem obter benefícios. Entretanto, novos ensaios clínicos são recomendados com maior rigor metodológico para comprovar estes achados.

DESCRITORES – Síndrome do intestino irritável. Revisão sistemática. Medicina baseada em evidências.

REFERENCES

1. World Gastroenterology Organisation Global Guidelines. Irritable Bowel Syndrome: a Global Perspective. September 2015. [Internet]. [Accessed 2019 August 08]. Available from: https://www.worldgastroenterology.org/UserFiles/file/guidelines/irritable-bowel-syndrome-english-2015.pdf
2. Ooi AL, Correa D, Pak SC. Probiotics, Prebiotics, and Low FODMAP Diet for Irritable Bowel Syndrome - What Is the Current Evidence? Complement Ther Med. 2019;43:73-80.
3. Ford AC, Lacy BE, Talley NJ. Irritable Bowel Syndrome. N Engl J Med. 2017;376:2566-78.
4. Cassar GE, Youssef GJ, Knowles S, Moulding R, Austin DW. Health-Related Quality of Life in Irritable Bowel Syndrome: A Systematic Review and Meta-analysis. Gastroenterol Nurs. 2020;43:E102-E22.
5. Ribeiro LM, Alves NG, Silva-Fonseca VA, Nemer ASA. Influence of individual response to stress and psychiatric comorbidity in irritable bowel syndrome. Rev Psiq Clín. 2011;38:77-83.
6. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10:712-21.
7. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States part I: overall and upper gastrointestinal diseases. Gastroenterology. 2009;136:376-86.
8. Flacco ME, Manzoli L, De Giorgio R, Gasbarrini A, Cicchetti A, Bravi F, et al. Costs of irritable bowel syndrome in European countries with universal healthcare coverage: a meta-analysis. Eur Rev Med Pharmacol Sci. 2019;23:2986-3000.
9. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019.
10. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS med. Public Library of Science; 2009;6(7):e1000097.
11. Mourad Ouzzani, Hossam Hammady, Zbys Fedorowicz, and Ahmed Elmagarmid. Rayyan — a web and mobile app for systematic reviews. Systematic Reviews. 2016;5:210.
12. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ. 2017;358:j4008.
13. Ruepert L, Quartero AO, de Wit NJ, van der Heijden GJ, Rubin G, Muris JWM. Bulking agents, antispasmodics and antidepressants for the treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2011, Issue 8. Art. No.: CD003460.
14. Evans BW, Clark WK, Moore DJ, Whorwell PJ. Tegaserod for the treatment of irritable bowel syndrome and chronic constipation. Cochrane Database of Systematic Reviews 2007, Issue 4. Art. No.: CD003960.
15. Manheimer E, Cheng K, Wieland LS, Min LS, Shen X, Berman BM, Lao L. Acupuncture for treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2012, Issue 5. Art. No.: CD005111.

16. Peckham EJ, Cooper K, Roberts ER, Agrawal A, Brabyn S, Tew G. Homeopathy for treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2019, Issue 9. Art. No.: CD009710.
17. Goldenberg JZ, Brignall M, Hamilton M, Beardsley J, Batson RD, Hawrelak J, Lichtenstein B, Johnston BC. Biofeedback for treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2019, Issue 11. Art. No.: CD012530.
18. Liu JP, Yang M, Liu Y, Wei ML, Grimsgaard S. Herbal medicines for treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2006, Issue 1. Art. No.: CD004116.
19. Zijdenbos IL, de Wit NJ, van der Heijden GJ, Rubin G, Quartero AO. Psychological treatments for the management of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2009, Issue 1. Art. No.: CD006442.
20. Webb AN, Kukuruzovic R, Catto-Smith AG, Sawyer SM. Hypnotherapy for treatment of irritable bowel syndrome. Cochrane Database of Systematic Reviews 2007, Issue 4. Art. No.: CD005110.
21. Chen M, Tang T-C, Qin D, Yue L, Zheng H. Pharmacologic treatments for irritable bowel syndrome: an umbrella systematic review. J Gastrointestin Liver Dis. 2020;29:199-209.
22. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol. 2010;63:834-40.

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Robotic anatomical resection of liver segment 4 with glissonian approach and selective hepatic artery clamping

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Minimally invasive surgery has increased in the last decade, including liver procedures. The laparoscopic technique is considered safe and effective. However, this technique is still used in selected patients and in specialized centers with greater surgical volume[1,2]. Conventional laparoscopy has some limitations, including two-dimensional view, unstable camera platform, and the use of rigid instruments with a limited degree of motion, which makes complex liver resection more difficult. Robotic system provides a good opportunity to perform even difficult procedures in the minimally invasive context but still restricted to few trained surgeons. The usefulness of the robotic platform in liver resection is still debated. However, patients who need complex liver procedures are good candidates for robotic approach. Procedures that require excellent accuracy and dexterity are the best candidates for robotic surgery[3-5]. This video shows a robotic resection of liver segment 4 using the glissonian approach and selected hepatic artery clamping in a patient with a single breast liver metastasis (FIGURES 1-2). We present the case of a 79-year-old woman with previous history of a right mastectomy 10 years before and laparoscopic cholecystectomy. During follow up, an increase in tumor markers was observed that raised a suspicion of recurrence. PET-CT disclosed a 3-cm single liver metastasis in the segment 4. Multidisciplinary team decided for resection of liver segment 4 (E-VIDEO*). Robotic approach was proposed, and consent was obtained. The patient was placed in a supine position and 30º reverse Trendelenburg position. Robotic surgery was performed using the da Vinci Xi robotic platform (Intuitive Surgical Inc., Sunnyvale, CA). This technique used five trocars. A pneumoperitoneum was created using an open technique in the infra-umbilical port. The pneumoperitoneum was established at 14 mmHg. The remaining trocars were inserted under direct vision. 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,

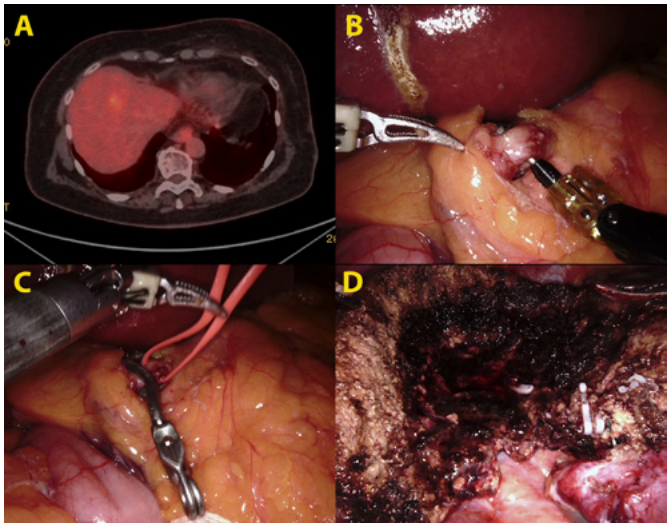

FIGURE 1. Robotic anatomic resection of liver segment 4.
A. PET-CT shows a single liver metastasis. B. Intraoperative view of dissection of common hepatic artery. C. Intraoperative view of selective hepatic artery clamping. D. Intraoperative view after completion of S4 resection.

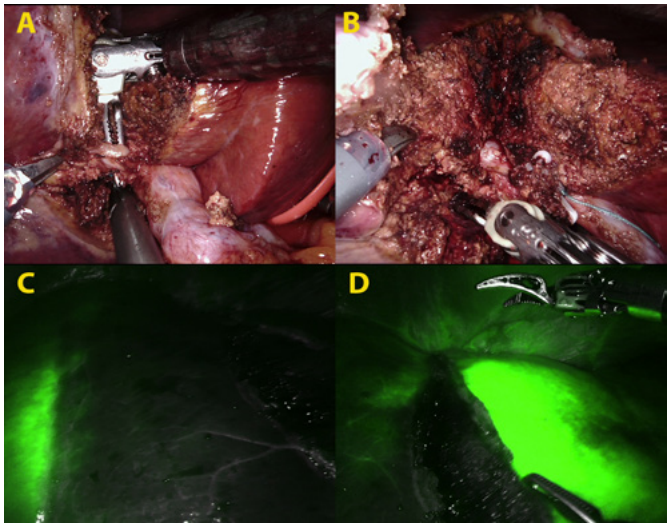

FIGURE 2. Robotic anatomic resection of liver segment 4.
A. Intraoperative view: pedicle from segment 4B is encircled. B. Intraoperative view: pedicle from segment 4A is encircled. C. Fluorescent image after injection of Indocyanine green shows S4 ischemic and perfused right liver. D. Fluorescent image after injection of Indocyanine green shows S4 ischemic and perfused segments 2 and 3.

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***E-VIDEO: https://www.youtube.com/watch?v=NwJCbsHSFag**

suction, clipping, and changes the robotic instruments. After docking the robotic system, adhesions from previous surgery are divided and liver ultrasound is used to locate the tumor and to stablish the surgical margins. Common hepatic artery is dissected, and selectively clamped[6]. Liver parenchyma is divided right from the insertion of the round ligament and glissonian pedicles from segments 4B and 4A are subsequently identified, ligated and divided, resulting in ischemic delineation of the entire segment 4. Indocyanine green fluorescent imaging was used to check ischemic area. The liver is then transected with robotic bipolar forceps under continuous saline irrigation and scissors. Ultrasound is constantly used to check surgical margins and relationship with major hepatic veins, specially the middle hepatic vein which is found within the posterior area of resection and ligated. Surgical specimen is removed inside a plastic retrieval bag through extension of the infra-umbilical incision and anatomical resection of liver segment 4 is completed. Operative time was 205 minutes, with minimal bleeding, and no need for blood transfusion. There was no need of intensive care unit and she was discharged on the 4th postoperative day. She is asymptomatic 15 months after the procedure. Robotic anatomical liver resection is feasible and safe. Glissonian approach allows for a precise anatomical resection[1] and selective hepatic artery clamping is useful to reduce bleeding and liver ischemia. Long-term results, cost-benefit analysis as well learning curve studies are necessary[5,7]. This video shows main steps (E-VIDEO*) necessary to perform this complex operation.

Authors' contribution

Machado MA, Lobo Filho M and Makdissi FF carried out the operative procedure. Ardengh AO and Bruno H Mattos edited the video. Ardengh AO, Lobo Filho M and Makdissi FF supervised and commented on the manuscript. All authors discussed the results and contributed to the final manuscript.

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Machado MAC, Ardengh AO, Lobo Filho MM, Mattos BH, Makdissi FF. Ressecção anatômica do segmento 4 do fígado por via robótica com acesso glissoniano e oclusão seletiva da artéria hepática. Arq Gastroenterol. 2021;58(1):127-8.

REFERENCES

1. Machado MA, Surjan RC, Basseres T, Schadde E, Costa FP, Makdissi FF. The laparoscopic Glissonian approach is safe and efficient when compared with standard laparoscopic liver resection: Results of an observational study over 7 years. Surgery. 2016;160:643-51.
2. 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.
3. Machado MAC, Surjan RC, Makdissi F. Robotic ALPPS. Ann Surg Oncol. 2020;27:1174-9.
4. Machado MA, Mattos BV, Lobo Filho MM, Makdissi F. Robotic Resection of Hilar Cholangiocarcinoma. Ann Surg Oncol. 2020;27:4166-70. doi:10.1245/s10434-020-08514-6.
5. 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.
6. Nomi T, Fuks D, Agrawal A, Govindasamy M, Araki K, Gayet B. Modified Pringle maneuver for laparoscopic liver resection. Ann Surg Oncol. 2015;22:852.
7. Troisi RI, Pegoraro F, Giglio MC, Rompianesi G, Berardi G, Tomassini F, et al. Robotic approach to the liver: Open surgery in a closed abdomen or laparoscopic surgery with technical constraints? Surg Oncol. 2020;33:239-48.

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