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Do standardization and quantification of histopathological criteria improve the diagnosis of inflammatory bowel disease?

Padronização e quantificação de critérios histopatológicos melhoram o diagnóstico da doença intestinal inflamatória?

Rosimeri Kuhl Svoboda Baldin¹; José Ederaldo Queiroz Telles²; Renato Araújo Bonardi³; Heda Maria Barska dos Santos Amarante⁴; Antônio Baldin Júnior⁵

ABSTRACT

Introduction: Inflammatory bowel disease comprises two major categories: Crohn's disease and ulcerative rectocolitis, both with different clinical and histological aspects, causing sometimes significant morbidity. **Objectives:** Choose and apply standardized and quantified histopathological diagnosis method, and compare the results and quality index with the original diagnosis. **Materials and methods:** 43 histological colonoscopic biopsies of 37 patients were re-evaluated by standardized system. **Results and discussion:** The original diagnoses were more inconclusive (23.3%) than those standardized (2.3%). The agreement with gold standard (clinical, colonoscopic, and radiological diagnosis) was higher on standardized diagnoses (95.3%) than in original (74.4%), especially in relation to Crohn's disease, which percentages were 92.3% and 46.1%, respectively. The quality index was calculated in conclusive diagnosis of each method. For ulcerative rectocolitis, both methods showed sensitivity and negative predictive value of 100%; otherwise the original diagnosis demonstrated specificity of 85.7%, positive predictive value of 96.3% and accuracy of 97.0%, and the standardized diagnosis 92.3%, 96.7% and 97.6%, respectively. For Crohn's disease, there is specificity and positive predictive value of 100% in both methods; the original diagnosis showed sensitivity of 85.7%, negative predictive value of 96.3% and accuracy of 97%, while for the standardized diagnoses 92.3%, 96.7%, and 97.6%, respectively. **Conclusion:** The standardized diagnosis presented a higher percentage of correct and conclusive diagnoses than those presented in the original diagnosis, especially for Crohn's disease, as well as equal or slightly higher values in some quality index.

Key words: ulcerative rectocolitis; Crohn's disease; biopsy; pathology.

INTRODUCTION

Inflammatory bowel disease (IBD) is the generic term for a specific group of chronic inflammatory disorders of unknown origin affecting the gastrointestinal tract, with outbreaks of acute exacerbation⁽¹⁰⁾. For not presenting specific pathognomonic signs the diagnosis is made by the correlation of clinical symptoms

with various signals detected on endoscopic, radiological and histopathological findings, which are add together to a more specific conclusion. Colonoscopy is essential to make possible the diagnosis and evaluate the extent, severity and distribution of IBD. Information obtained at colonoscopy, associated histopathological criteria, providing data for classify IBD in one of its two major subgroups: ulcerative rectocolitis (UC) and Crohn's disease (CD).

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About 10%-20% of cases are designated as indeterminate IBD, which diagnosis cannot be defined as UC or CD⁽⁸⁾. The specific diagnosis is important to evaluate surgical treatment as well as long-term follow up since the UC and DC medical management is different as regards the evolution of the disease and the indication for surgery. The relative risk of colorectal cancer is highest in patients with IBD than in the general population^(5,7).

OBJECTIVES

Choose and apply standardized and quantified histopathological diagnosis method, and compare the results and quality index with the original diagnosis.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee at Hospital de Clínicas-Universidade Federal do Paraná (UFPR), under protocol number: 718.137/2003-09, and was recorded in Research Database System at UFPR (BANPESQ) under code number: 2003005726. Retrospective study was performed on IBD of 255 outpatient registers at Hospital de Clínicas at UFPR, in July 2004. There were 130 patients diagnosed with CD and 125 UC. We found 445 histopathological examinations, colonic endoscopic biopsies of these patients in files of Department of Pathology. Among these, only 43 examinations of 37 patients had all the inclusion criteria (some had made more than one exam that met the inclusion criteria). Each test consisted of a series of 5-8 slides, and each slide assembly (relative to one exam) there was 9-39 fragments of bowel mucosa. All these material totaled 264 slides and 846 fragments. For sample selection we established the following criteria: 1) patients with active colitis at the time of biopsy; 2) clinical diagnosis established; 3) clinical segment of at least 12 months; 4) at least five different samples of the colon, one of which necessarily rectum. The material was previously set in aqueous 10% formalin and submitted for processing. The capsules were placed in an automatic tissue processor, which held dehydration, diaphanization, impregnation, and embedment in paraffin. The specimens were included in paraffin for holding samples microtome cuts of 4-5 mm. The material was stained with hematoxylin-eosin (HE) and examined under light microscopy^(2,19,22). For histopathological evaluation, the method developed by Tanaka *et al.*⁽²⁵⁾ was chosen. It was reproduced in **Table 1** and **2** just like elaborated by the authors. The data in these tables defined criteria H1 to H9, which were marked as present (1) or absent (0). From these values were made the

calculations to IBD and CD, which scores were used to classify the cases into the categories described in Table 1 and 2. The original histopathological diagnoses of 37 patients included in this study (43 exams) were classified as UC or CD when these hypotheses were suggested or indicated compatibility with them, and classified as inconclusive when these possibilities were not expected.

TABLE 1 – Simple criteria for differentiate IBD and other forms of colitis (non IBD)

Category	Definition	SCORE IBD (S_{IBD}) = $2H_1 + 3H_2 + 3H_3 + 2H_4 - 4$
Definite IBD	$S_{IBD} \geq 2$	H ₁ : Cryptic atrophy
Probable IBD	$S_{IBD} = 1$	H ₂ : Cryptic distortion
Unknown	$S_{IBD} = 0$	H ₃ : Basal plasmacytosis with intense MMNII
Probable non-IBD	$S_{IBD} = -1$	H ₄ : Paneth cells metaplasia distal to the right angle of the colon
Definite non-IBD	$S_{IBD} \leq -2$	

Source: Tanaka *et al.*⁽²⁵⁾.

IBD: inflammatory bowel disease; MMNII: monomorfonuclear inflammatory infiltrate.

TABLE 2 – Simple criteria for differentiate CD and UC

Category	Definition	Score CD (S_{CD}) = $2H_5 + 3H_6 + 2H_7 + 3H_8 - 3H_9 - 5$
Definite CD	$1.0 \leq S_{CD}$ or EG	H ₅ : Segmental distribution of atrophy and crypt distortion
Probable CD	$0.3 \leq S_{CD} < 1.0$	H ₆ : Segmental distribution of mucin depletion
Undeterminate	$-0.3 < S_{CD} < 0.3$	H ₇ : Preservation of mucin at the edges of ulcers or crypts surrounded by neutrophils
Probable UC	$-1.0 < S_{CD} \leq 0.3$	H ₈ : Division of NB* with MMNII focal by NB* with MMNII
Definite UC	$S_{CD} \leq -1.0$	H ₉ : Division of NB* with cryptic atrophy and severe MMNII by NB* with severe MMNII

Source: Tanaka *et al.*⁽²⁵⁾.

CD: Crohn's disease; UC: ulcerative colitis; EG: epithelioid granuloma; NB*: number of biopsies; MMNII: monomorfonuclear inflammatory infiltrate.

RESULTS

We selected 253 patients – 107 men (42%) and 146 women (58%) – from IBD outpatient, classified into 129 UC (51.0%) and 124 CD (49.0%), which had a total of 445 subjected to histopathological colon biopsies examination, with an average of 1.7 per patient. After surveying the results of 445 biopsies, only 43 (9.6%) met the inclusion criteria for the present study. **Table 3** shows clinical data and diagnosis based on gold standard, diagnosis reported during request, the histopathological original diagnosis (OD) and reviewed standardized diagnoses (RSD), if granulomas are present.

TABLE 3 – Main clinical and histopathological data from analyzed exams (43 exams of 37 patients)

Exam	Age	Gender	Follow (years)	Gold standard clinical diagnosis	Hypothesis on request exam	OD	RSD	Presence of granuloma in OD	Presence of granuloma in RSD
1	15	M	6	CD	CD	Inconclusive	CD	No	No
2	27	F	8	UC	UC	Inconclusive	UC	No	No
3	26	F	5	UC	UC	UC	UC	No	No
4	26	F	5	UC	UC	UC	UC	No	No
5	22	M	3	CD	CD	Inconclusive	CD	Yes	Yes
6	19	F	6	UC	UC	UC	UC	No	No
7	30	F	9	UC	UC	UC	UC	No	No
8	30	F	2	UC	UC	UC	UC	No	No
9	41	M	1	CD	CD	Inconclusive	CD	No	Yes
10	45	F	21	UC	UC	UC	UC	No	No
11	20	F	3	UC	UC	UC	UC	No	No
12	27	M	1	UC	UC	UC	UC	No	No
13	25	F	2	UC	UC	UC	UC	No	No
14	24	M	1	UC	UC	UC	UC	No	No
15	32	M	17	UC	UC	UC	UC	No	No
16	36	F	11	UC	UC	UC	UC	No	No
17	31	F	4	UC	UC	UC	UC	No	No
18	47	M	14	UC	UC	UC	UC	No	No
19	50	F	13	UC	UC	Inconclusive	UC	No	No
20	29	M	5	UC	UC	UC	UC	No	No
21	30	F	6	UC	UC	UC	UC	No	No
22	41	F	5	CD	CD	CD	CD	Yes	Yes
23	50	F	13	UC	UC	Inconclusive	UC	No	No
24	15	M	6	CD	None	CD	CD	Yes	Yes
25	37	F	8	CD	CD	Inconclusive	UC	No	No
26	36	M	7	CD	CD	Inconclusive	CD	No	No
27	41	F	11	UC	UC	UC	UC	No	No
28	14	M	5	CD	CD	CD	CD	Yes	Yes
29	17	F	5	UC	UC	UC	UC	No	No
30	15	M	5	CD	CD	UC	CD	No	Yes
31	27	F	7	UC	UC	UC	UC	No	No
32	23	M	8	CD	CD	Inconclusive	CD	No	Yes
33	22	F	5	UC	UC	UC	UC	No	No
34	37	M	9	UC	UC	UC	Inconclusive	No	No
35	20	M	9	CD	CD	CD	CD	No	Yes
36	36	F	6	CD	CD	CD	CD	Yes	Yes
37	57	F	4	CD	CD	CD	CD	Yes	Yes
38	23	F	8	UC	UC	UC	UC	No	No
39	15	F	13	UC	UC	Inconclusive	UC	No	No
40	23	M	5	UC	UC	UC	UC	No	No
41	43	M	14	UC	UC	UC	UC	No	No
42	42	M	10	UC	CD	UC	UC	No	No
43	44	M	14	UC	UC	UC	UC	No	No
Average	25	-	6	-	-	-	-	-	-
Total	-	-	-	-	-	-	-	6 (46.1%)	10 (76.9%)

OD: original diagnosis; RSD: reviewed standardized diagnoses; M: male; F: female; CD: Crohn's disease; UC: ulcerative colitis.

All RSD were classified as definite for one out of the two diseases (CD or UC), except for case 34 that was classified as inconclusive. These 43 exams were discriminated in 30 (69.8%) UC diagnoses, and 13 (30.2%) CD diagnoses. 37 patients presented 43 exams, which were categorized in 25 (67.6%) UC and 12 (32.4%) CD (there was more than one examination for the same patient included in the study). From these 37 patients, 16 (43.2%) were male, and 21 (56.8%) female. From 25 patients with UC, 8 (32.0%) were male, and 17 (68.0%) female. From the 12 individuals with CD, 8 (66.7%) were male, and 4 (33.3%) female. Patients were diagnosed with IBD at 4-57 years of age, mean 25 years \pm 10.1. The comparison between OD and RSD regarding the percentage of exams with conclusive and inconclusive diagnoses is presented in **Table 4**. To compare OD and RSD results in percentages of assertiveness and conclusiveness of diagnosis, binomial test was applied, since each sample was evaluated by two diagnostic methods (OD and RSD), determining the result classified into two categories. Values of $p < 0.05$ were considered statistically significant. The binomial test results indicated rejection of the null hypothesis at a significance level of 5% ($p = 0.0117$), this allows us to affirm that there is a statistically significant difference between OD and RSD in relation to the percentage of exams with inconclusive diagnoses. Table 4 shows that the percentage of tests considered inconclusive by OD (23.3%) is higher than the well regarded by RSD (2.3%). The assessment of the proportion of cases with correct diagnosis by OD and RSD for each disease (UC and CD) is presented in **Table 5**. For UC, the test result indicated the non-rejection of the null hypothesis at a significance level of 5% ($p = 0.1250$). Thus, it can be stated that there is no statistically significant difference between OD and RSD in relation to the percentage of samples with correct diagnoses for UC. For CD, the test result indicated rejection of the null hypothesis at a significance level of 5% ($p = 0.0313$). Thus, it can be stated that there is a statistically significant difference between OD and RSD in relation to the percentage of samples with correct diagnoses for CD. Table 5 shows that the percentage of correct cases by RSD (92.3%) is higher than the percentage for OD (46.1%). The results of the quality rates for the conclusive diagnoses obtained by OD and RSD are shown in **Table 6**.

TABLE 4 – Distribution of diagnosis as conclusive or inconclusive ($n = 43$ exams)

Diagnosis	OD	RSD
Conclusive	33 (76.7%)	42 (97.7%)
Inconclusive	10 (23.3%)	1 (2.3%)
Total (exams)	43	43

OD: original diagnosis; RSD: reviewed standardized diagnosis.

TABLE 5 – Comparison of OD and RSD in relation to the percentage of cases with correct diagnosis of UC and CD

Diagnosis	Gold standard			
	UC		CD	
	OD	RSD	OD	RSD
Correct	26 (86.7%)	29 (96.6%)	6 (46.1%)	12 (92.3%)
Incorrect(*)	4 (13.3%)	1 (3.3%)	7 (53.8%)	1 (7.7%)
Total	30	30	13	13

OD: original diagnosis; RSD: reviewed standardized diagnosis; UC: ulcerative colitis; CD: Crohn's disease.

Incorrect(*): inconclusive or incorrect in relation with gold standard.

TABLE 6 – Quality indices and their estimated 95% interval of confidence obtained from OD and RSD

Quality indices	OD (%)		RSD (%)	
	UC	CD	UC	CD
Sensibility	100	85.7 (59.8, 100)	100	92.3 (77.8, 100)
Specificity	85.7 (59.8, 100)	100	92.3 (77.8, 100)	100
Positive predictive value	96.3 (89.2, 100)	100	96.7 (90.2, 100)	100
Negative predictive value	100	96.3 (89.2, 100)	100	96.7 (90.2, 100)
Accuracy	97.0 (91.1, 100)	97.0 (91.1, 100)	97.6 (93.0, 100)	97.6 (93.0, 100)

OD: original diagnosis; RSD: reviewed standardized diagnosis; UC: ulcerative colitis; CD: Crohn's disease.

DISCUSSION

Inconclusive diagnoses such as “chronic inflammation” and “nonspecific inflammation” are of limited value for both the physician and the pathologist^(11, 28), and despite the clinicopathological correlation be the goal of histopathology, it is not always possible because the information is not available, or clinical data provided contradict the histopathological findings. In these cases, the pathologist needs to extract as much information as possible from the biopsy. For an appropriate evaluation of biopsy in IBD, the pathologist should be informed about the duration of symptoms and what kind of treatment was given to the patient because the treatment can produce patchiness or discontinuity of mucosal inflammation in UC making a differential diagnosis more difficult⁽¹⁰⁾. Another study refers that the treatment of UC can lead to partial cure, which results in focal lesions simulating distribution of CD⁽²⁹⁾. This fact has already been reported⁽¹⁴⁾ highlighting the focal distribution of the lesions in the UC treated. The treatment can produce a variety of changes including normalization of the mucosa. Some authors emphasize that the

information about therapeutics should be given to the pathologist avoid misdiagnosis^(17, 18, 27). Other authors state that only the epithelioid granulomas without ruptured crypts and or chronic active ileitis are features highly suggestive of CD on mucosal biopsy analysis and that some UC exams may show discontinuous disease, no rectal disease, inflammation in ileum, extracolonic involvement, granulomatous inflammation, aphthous ulcers or mural inflammation. In these cases, the differential diagnose with CD can be almost impossible⁽³⁰⁾. In the appropriate clinical presentation the presence of granuloma inflammation in gastrointestinal biopsy specimens confirms the diagnosis of CD^(16, 32), and when the granuloma is not found, the diagnoses of CD should be suggested emphasizing the necessity of correlation with others clinical, endoscopic and imaging features⁽⁹⁾. In a study the evaluation of mucosal biopsy in patients with CD demonstrated increased numbers of macrophages and microgranulomas with the help of the technique of immunohistochemistry for CD68+⁽³¹⁾. Another difficulty is the presence of granulomas related with crypts ruptures. To distinguish between epithelioid granuloma and a granulomatous lesion by rupture of crypt, cuts in multiple tissue levels may be necessary to find the presence of neutrophils, lymphocytes, and foamy macrophages that are absents in the former⁽³⁰⁾.

In the present study, the histopathological diagnosis was correct and conclusive in OD at 74.4% of patients, and in 97.7% RSD, demonstrating that the last one increased correlation with the gold standard for diagnosis. This percentage was also higher than those found by several studies^(6, 15, 20, 21, 27, 28) in which the correlation was 94%, 80%, 75%, 72%, 73%, and 73% respectively. Our hypothesis that the standardized histopathological diagnosis increases the correlation with the gold standard in IBD was supported on two other studies^(3, 13). There is several studies on determining the histopathological criteria more discriminately, and on how better applying them to the diagnosis of IBD. In some studies, the criteria are repeated, consolidating its importance in the diagnosis of IBD, as occurred, for example, with the alteration in cryptic architecture, which is present in seven reviewed studies^(6, 13, 15, 21, 23, 24, 27). The authors of the study that was used as a model for the standardization review of cases evaluated⁽²⁴⁾ observed that basal plasmacytosis (**Figure 1**) associated with severe inflammatory infiltrate is more discriminative than plasmacytosis considered individually. They also observed that the diffuse crypt atrophy (**Figure 2B**) is characteristic of UC, whereas segmental atrophy is related with CD. **Figure 2A** shows a comparison between a normal mucosa and a mucosa with crypt atrophy. However, they did not consider cryptic abscesses and cryptitis discriminative for the differential diagnosis between UC and CD, contradicting what other authors have stated^{(13, 15, 21,}

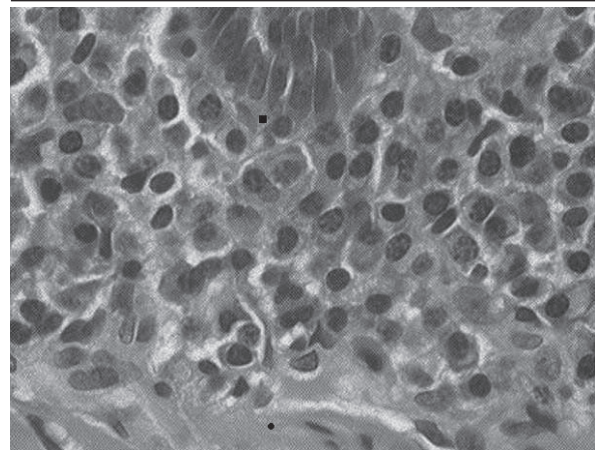


FIGURE 1 – Basal plasmacytosis: presence of numerous plasma cells between the crypt base (●) and muscularis mucosa (■) (HE, lens 400×)
HE: hematoxylin-eosin.

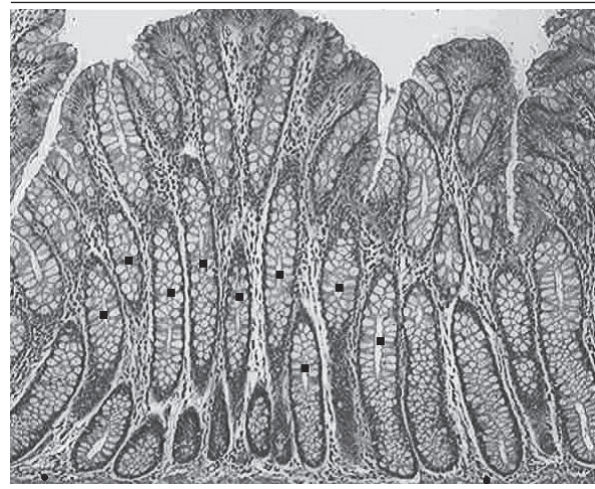


FIGURE 2A – Normal colonic mucosa with crypts (■) tubular, parallel, straight, and juxtaposed to the muscularis mucosa (●) (HE, lens 40×)
HE: hematoxylin-eosin.

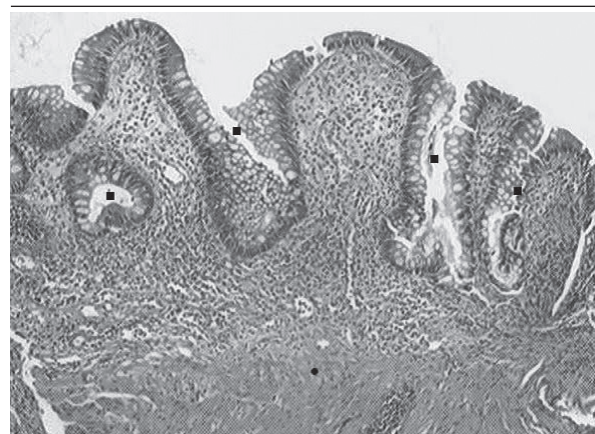


FIGURE 2B – Crypt atrophy characterized by tortuosity, scarcity and shortening of the crypts (■) which basis is far from muscularis mucosa (●) (HE, lens 40×)
HE: hematoxylin-eosin.

^{27, 28)}. Paneth metaplasia (**Figure 3**) was not analyzed in some studies^(13, 20, 21), but was analyzed and considered important in six other studies^(6, 24-28). In the studies reviewed, the histopathological findings were evaluated taking into account different criteria. The study we have chosen as model for standardized review, assessed 70 criteria (the largest number of criteria among the studies reviewed), which were tested and selected by statistical calculations⁽²⁴⁾. The series of three studies of these authors⁽²⁴⁻²⁶⁾ consisted of 431, 726, and 60 cases respectively. Together, it is the largest study that used the same criteria for IBD biopsies histopathological evaluation. In the present study, only one case of CD (number 25 in Table 3) was diagnosed as UC by RSD. In this case, we observed that rectal mucosa was compromised, there was diffuse distribution of atrophy and crypt distortion (**Figure 4**) (criterion H5), segmental distribution of mucin depletion (criterion H6) and preservation of

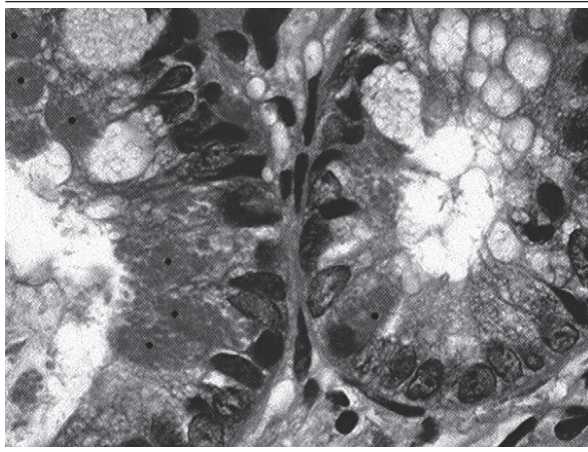


FIGURE 3 – Paneth cell metaplasia: presence of Paneth cells (●) lined crypts in samples after colon right angle (HE, lens 400×)
HE: hematoxylin-eosin.

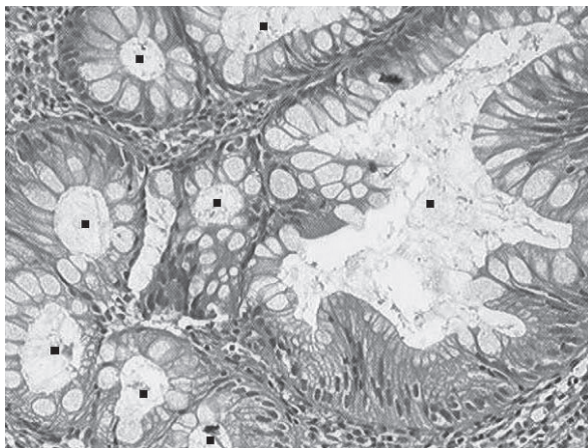


FIGURE 4 – Crypt distortion: branching, dilatation, and tortuous crypts (■) (HE, lens 100×)
HE: hematoxylin-eosin.

mucin in areas of acute inflammation (criterion H7). A conflict was observed in the histopathological criteria in this case: the diffuse and continuous distribution of cryptic changes from the rectum is characteristic of UC according to some authors observations^(23, 24). Another unusual feature found in this CD case was the commitment of the rectum, which is usually spared in CD⁽¹²⁾. The case inconclusive by RSD (number 34 in Table 3) was defined by the gold standard as UC, but it presented: segmental distribution of crypt architectural changes (criterion H5), segmental distribution of depletion of mucus (criterion H6), and focal inflammation, which are histopathological characteristics of CD^(23, 24). On this exam request there was no information about the patient being in treatment at the time of biopsies, even though it has endoscopic appearance of UC, but with segmental distribution. In biopsies of children with UC, architectural distortion of crypts occurs in 32.1%, while it happens in 57.9% of adults⁽²⁹⁾. The authors correlate this difference with the shorter duration of disease before biopsy in pediatric cases. There is a contradiction between the observations of some studies^(23, 29) about the time for the appearance of architectural changes promoted by IBD. A study argues⁽²³⁾ that they are precocious and can be detected in biopsies from patients with seven days of onset of disease activity, and moreover the architectural changes are the most important in the diagnosis of IBD histopathology, once it differ in each type of IBD: diffuse for UC, and focal for CD. These authors also note that these changes are not exclusive to IBD as they can be found in intestinal amebiasis and shigellosis cases, depending on the strain and duration of infection (greater than one week). The quality scores obtained by OD and RSD in the present research were not consistent with one study⁽⁴⁾. Here RSD presented specificity, positive predictive value, and accuracy for UC slightly larger than OD. RSD also showed sensitivity, negative predictive value, and accuracy for CD slightly larger than OD. In conclusive cases the specificity for UC and sensitivity to CD (both 92.3%) were higher in RSD than in OD (both 85.7%), agreeing with the results of several studies^(1, 3, 13, 24-27) that consider the standardization of histopathological criteria a necessary instrument for improving the quality indices of histopathological diagnosis.

CONCLUSION

RSD diagnosis had a higher percentage of correct and conclusive diagnoses than those presented in OD, especially statistically significant difference for CD exams. RSD's conclusive cases obtained slightly higher specificity rates, positive predictive value, and accuracy for UC; slightly higher sensitivity rates, negative predictive value, and accuracy for CD, when compared to OD.

RESUMO

Introdução: Duas são as formas de manifestação da doença intestinal inflamatória: doença de Crohn e retocolite ulcerativa, ambas com evolução clínica, tratamento e aspectos histopatológicos diferentes, causando, por vezes, significativa morbidade. **Objetivos:** Escolher e aplicar método padronizado e quantificado de diagnóstico histopatológico e comparar os resultados e os índices de qualidade, com os dos diagnósticos originais. **Materiais e métodos:** Foram reavaliadas histologicamente 43 biópsias colonoscópicas seriadas de 37 pacientes por sistema padronizado. **Resultado e discussão:** Os diagnósticos originais foram mais inconclusivos (23,3%) do que os padronizados (2,3%). A concordância com o padrão-ouro (diagnóstico clínico, colonoscópico e radiológico) foi maior nos diagnósticos padronizados (95,3%) do que nos originais (74,4%), principalmente em relação à doença de Crohn, cujos percentuais foram de 92,3% e 46,1%, respectivamente. Para retocolite ulcerativa, ambos os métodos apresentaram sensibilidade e valor preditivo negativo de 100%; já nos diagnósticos originais, foram verificados especificidade de 85,7%, valor preditivo positivo de 96,3% e acurácia de 97%, e nos diagnósticos padronizados, 92,3%, 96,7% e 97,6%, respectivamente. Para doença de Crohn, verificaram-se especificidade e valor preditivo positivo de 100% nos dois métodos; nos diagnósticos originais, sensibilidade de 85,7%, valor preditivo negativo de 96,3% e acurácia de 97%, e nos diagnósticos padronizados, 92,3%, 96,7% e 97,6%, respectivamente. **Conclusão:** O diagnóstico padronizado apresentou maior percentual de diagnósticos corretos e conclusivos do que os apresentados no diagnóstico original, principalmente para doença de Crohn, assim como valores iguais ou ligeiramente maiores em alguns índices de qualidade.

Unitermos: retocolite ulcerativa; doença de Crohn; biópsia; patologia.

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