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Original article

Low predictive value of histopathological scoring system for complications development in children with Crohn's disease

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abstract

Objectives: In pediatric Crohn's disease (PCD), the benefit of microscopy in disease activity assessment and prediction of clinical outcome is, due to the focality and transmurality of the inflammation, disputable. We investigated whether histopathological scoring system predicts complications in pediatric CD and correlates with endoscopical and clinical scores.

Methods: We performed a retrospective study on 63 patients. Endoscopy in the time of diagnosis was evaluated using the Simple Endoscopic Score (SES) and histopathology with the Global Histology Activity Score, both in its original version (GHAS) and its modification (modGHAS). Pediatric Crohn's Disease Activity Index (PCDAI) was also calculated. The patients were grouped according to the presence or absence of defined complications (intraabdominal abscess or fistula, perianal fistulating disease or stricture impenetrable for endoscope or with prestenotic dilatation) during one year of follow-up, or the necessity to initiate anti-TNF treatment for persisting or relapsing active disease in the same time period. Associations were tested with Cox regression analysis.

Results: SES was higher in patients with complications. However, in case of GHAS, modGHAS and PCDAI we did not find any significant association with complicated course of disease. SES above 16 points was revealed as an independent risk factor for complications development in PCD, in contrary to GHAS, modGHAS and PCDAI. We demonstrated only a weak correlation between GHAS, modGHAS and SES and no correlation between the histopathological scoring systems and PCDAI.

Conclusions: In conclusion, the histopathological scoring system cannot be recommended as a reliable predictor of development of complications in children with CD.

1. Introduction

According to the recommendations of the European Crohn's and Colitis Organisation [1], the European Society of Pathology [1] and the Porto criteria (as defined by the working group of pediatric inflammatory bowel diseases experts from ESPGHAN) [2], the endoscopic assessment of the intestine with multiple segmental biopsies represents an obligatory procedure in the diagnosis of adult and pediatric inflammatory bowel diseases (IBD) [1–5]. It is also considered to be the most reliable method for the assessment of the luminal inflammation activity along with the extension of bowel involvement, and plays a crucial role in consecutive management and surveillance of patients with IBD [3].

On the other hand, the benefit of histopathological analysis in the assessment of the inflammation activity in IBD and for the prediction of prognosis remains disputable [1]. Especially in the case of Crohn's disease (CD) with its discontinual and transmural character of inflammation, there is often poor correlation between the microscopic and endoscopic findings [6–8]. Thus, histopathological scoring systems, validated or established for pediatric patients, are lacking.

The purpose of the present study was to examine whether microscopical assessment of the intensity of inflammation in pediatric patients with CD has a predictive value for the complications development and whether the histopathological scoring system correlates with endoscopical and clinical scores in children.

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Table 1
Simple Endoscopic Score (SES).

Endoscopical Changes	Score			
	0	1	2	3
Size of ulcers (cm)	None	Aphthous ulcers (diameter 0.1–0.5)	Large ulcers (diameter 0.5–2) 10–	Very large ulcers (diameter >2)
Ulcerated surface	None	<10%	30%	>30%
Affected surface	Unaffected segment	<50%	50–75%	>75%
Presence of narrowings	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Final score is a sum of all variables for the 5 bowel segments. Values are given to each variable for all the examined bowel segments.

Table 2
Global Histology Activity Score (GHAS).

Histopathological Changes	Grading
Epithelial damage	0 = normal; 1 = focal; 2 = extensive
Architectural changes	0 = normal; 1 = moderate; 2 = severe
Mononuclear cells in lamina propria	0 = normal; 1 = moderate increase; 2 = severe increase
Neutrophils in lamina propria	0 = normal; 1 = moderate increase; 2 = severe increase
Neutrophils in epithelium	1 = surface epithelium; 2 = cryptitis; 3 = crypt abscess
Erosion or ulceration	0 = no; 1 = yes
Granuloma	0 = no; 1 = yes
Number of segmental biopsy specimens affected	1 = <1/3; 2 = 1/3–2/3; 3 = >2/3

Each variable is scored independently. The total score is the sum of all individual scores.

2. Material and methods

2.1. Subjects

All pediatric patients diagnosed with CD at their first endoscopic assessment at our centre between the years 2009 and 2015 were included in this retrospective study. The senior pediatric gastroenterologists reevaluated patients' clinical data and endoscopical findings. Archive histopathological slides were assessed by specialized pathologists. The endoscopic findings were objectified using the Simple Endoscopic Score (SES) (Table 1), which was chosen for its simplicity and good correlation with clinical scoring systems [9]. In addition, the Pediatric Crohn's Disease Activity Index (PCDAI), a validated and one of the most commonly used clinical scoring systems for pediatric CD with good correlation with other clinical scores, was calculated retrospectively [10–14]. The microscopical intensity of the inflammation was assessed using the Global Histology Activity Score (GHAS), both in its original version [15] (Table 2) and in its modification (modGHAS) [16], which does not include the evaluation of the number of involved bowel segments. The medical records of each patient were reviewed in detail.

The patients were grouped according to the presence or absence of defined complications during one year of follow up period from the point of diagnosis, or the necessity to initiate anti-TNF treatment for persisting or relapsing active disease in the same time period. The time to complication was also recorded. The defined complications included stricture impenetrable with endoscope or with prestenotic dilatation, intra-abdominal fistula or abscess and fistulating perianal involvement. Initiating of anti-TNF treatment wasn't regarded as a complication in it's own record, but was implicated in the outcomes as an indication of severe clinical course of the disease. Patients who presented with any of the complications at the time of diagnosis, as well as the patients with previous diagnosis of Inflammatory Bowel Disease Unclassified (IBD-U) or patients who had previously underwent treatment for IBD and thus could have modified histopathological features of the disease were excluded from the study. Patients with anti-TNF treatment at the time of diagnosis as a part of top down therapy also were not included. Out of 105 patients who entered the study, 63 fulfilled the inclusion criteria. The details of the inclusion process are shown in a flowchart (Fig. 1). Clinical characteristics of the patients are given in Table 3.

2.2. Statistical analysis

We used statistical software R-project (R Core Team, version 3.2.3) for data analysis [17]. The differences between the subgroups of patients in the GHAS, modGHAS, SES and PCDAI scores were tested by Mann-Whitney test. After that, ROC curves were used to find the most significant cut-off value for each scoring system, which classified our patients into high-risk and low-risk groups for complications development (using the software package "survivalROC") [18]. Based on the optimal area under the curve, the cut-off value of 15 points was established for SES, 35 points for PCDAI and 9 points for GHAS and modGHAS. Then, a Kaplan-Meier survival curve was constructed and analyzed (using the software package "rms") [19]. To test, whether the histopathological score is an independent predictor for the defined complications, univariate and multivariate Cox proportional hazards regression analyses were used (using the software package "survival") [20]. The modGHAS (as a cathegorical value), PCDAI (as a numerical value), SES (as a cathegorical value), presence of epitheloid granulomas, male gender, upper GI involvement and age at diagnosis were used as the predictors in the final constructed model. The period of time to the complication development was used as the outcome. Probability (P) values of <0.05 were considered significant. A 95% confidence interval was used.

3. Results

The defined complications developed in 19 of the 63 patients (30.2%) who fulfilled the inclusion criteria (Fig. 1). We did not find any statistically significant difference in the GHAS (Fig. 2A), modGHAS (Fig. 2B) and PCDAI (Fig. 2C) scores in the subgroups of children with and without complications. On the other hand, we showed a significant difference in case of SES (Fig. 2D).

By use of the ROC curve and the survival analysis, the patients with SES score 16 points and more developed complications significantly more frequently (Fig. 3A); however, no significant differences were observed in patients grouped by the established cut offs of histopathological scoring systems and PCDAI (Fig. 3B–D). SES score higher than 15 points also turned out to be an indepen-

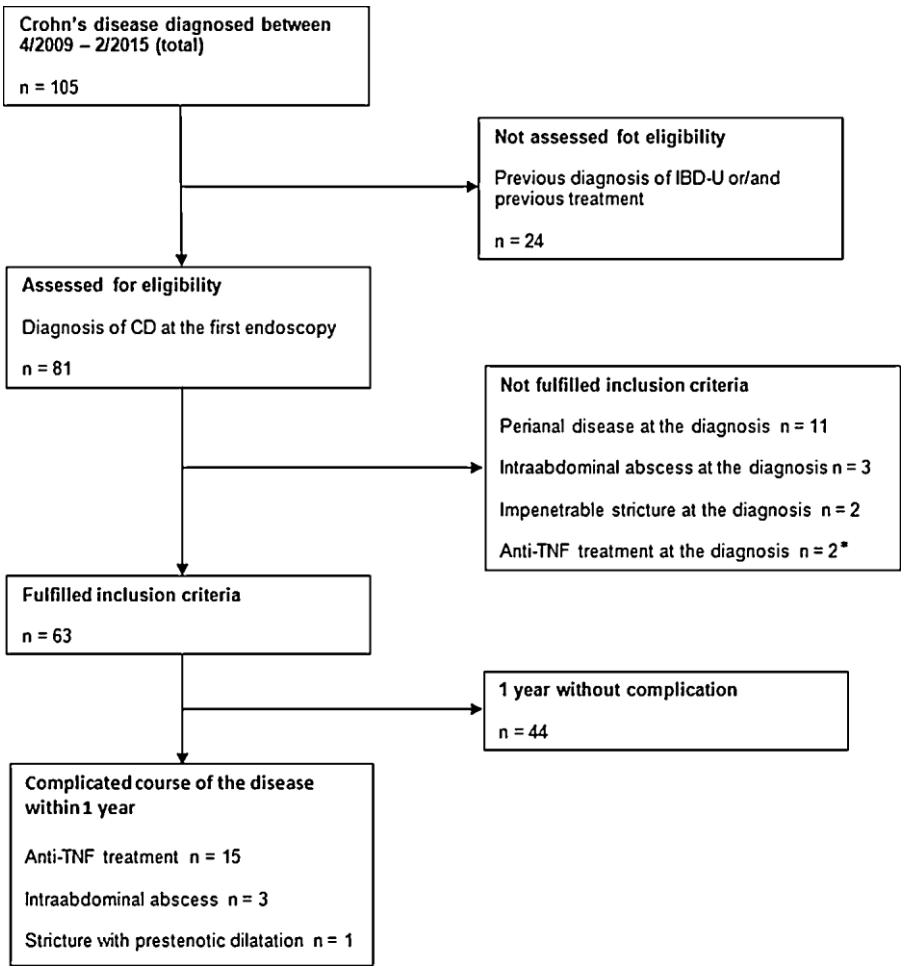

Fig. 1. Flowchart of Included and Excluded Patients.
IBD-U = Inflammatory Bowel Disease Unclassified.
CD = Crohn's Disease.
TNF = Tumor Necrosis Factor.
*anti-TNF treatment in terms of top-down strategy.

Table 3
Demographic and clinical characteristics of patients.

	All patients (n = 63)	With complications (n = 19)	Without complications (n = 44)
Sex (M/F)	36/27	8/11	28/16
Age at diagnosis, year, median (range)	12 (11–15)	12 (11–14)	12.5 (11–15)
Localization (%)			
L1 (term. ileum)	12 (19.05%)	2 (10.53%)	10 (22.73%)
L2 (colon)	13 (20.6%)	1 (5.26%)	12 (27.27%)
L3 (ileocolon)	38 (60.32%)	16 (84.21%)	22 (50%)
Upper GI (%)	41 (65.08%)	13 (68.42%)	28 (63.64%)
SES, median (range)	17 (12.5–23.5)	22 (16.5–28)	15 (12–22)
GHAS, median (range)	10 (11–12)	11 (10.5–12.5)	11 (10–12)
modGHAS, median	9 (7–9)	9 (8–10)	9 (7–9)
(range)	35 (26–43)	38 (28–40)	34 (25–45)
PCDAI, median (range)			

GI = Gastrointestinal Involvement; SES = Simple Endoscopic Score; PCDAI = Pediatric Crohn's Disease Activity Index; GHAS = Global Histology Activity Score; mod-GHAS = Modified Global Histology Activity Score; M/F = Male/Female.

dent risk factor for complications development according to Cox regression analysis (Table 4).

We demonstrated only a weak correlation between histopathological and endoscopical scoring systems (r = 0.48, p = 0.0001 for GHAS and r = 0.45, p = 0.0002 for modGHAS) and no correlation between the histopathological scoring systems and PCDAI (r = 0.15, p = 0.24 for both GHAS and modGHAS).

4. Discussion

The effort to objectify the intensity and activity of the inflammation in IBD dates back to the sixties of the past century when Truelove & Richards presented their histopathological scoring system for UC, which was also the first histopathological scoring system for IBD [6]. Later, a number of other scoring systems were

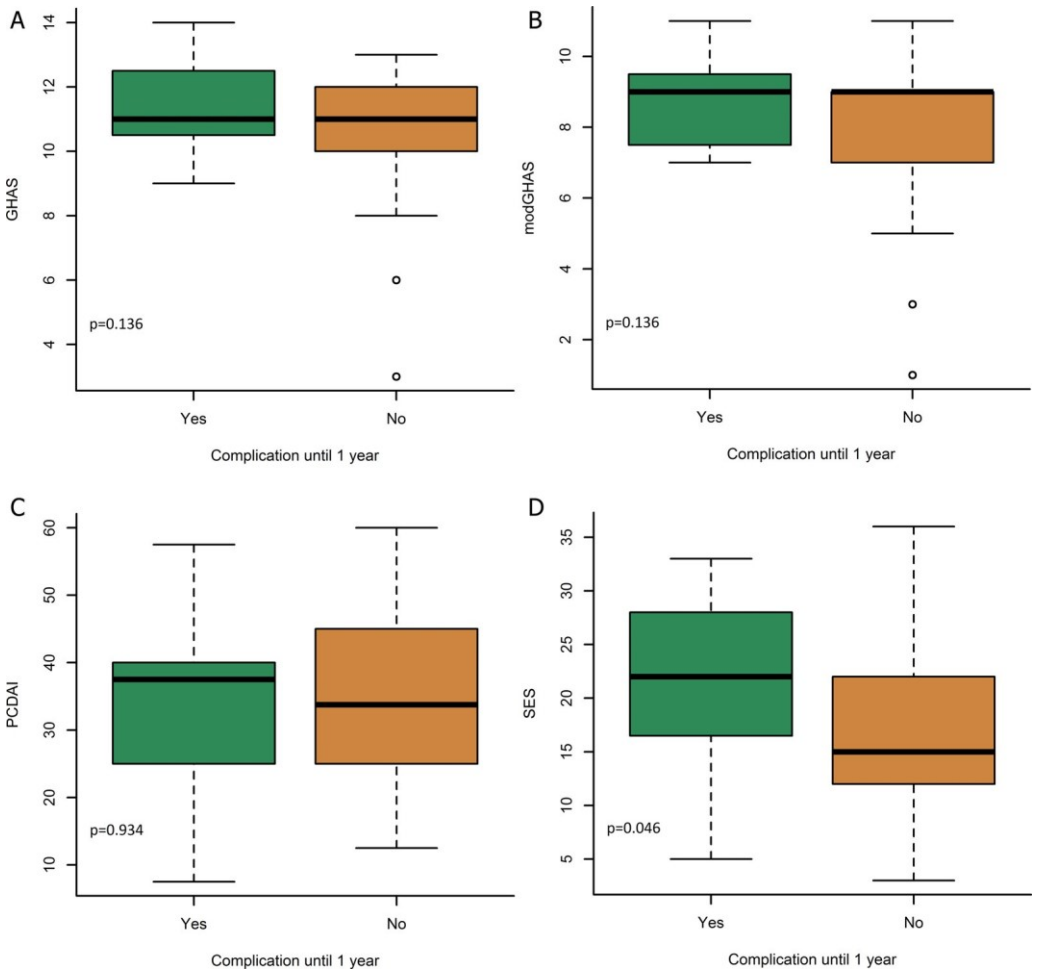

Fig. 2. A: Box graphs comparing Global Histology Activity Scores of patients with and without complications. B: Box graphs comparing Modified Global Histology Activity Scores of patients with and without complications. C: Box graphs comparing Pediatric Crohn's Disease Activity Indexes of patients with and without complications. D: Box graphs comparing Simple Endoscopic Scores of patients with and without complications.

Table 4
Multivariate analysis of clinical, endoscopical and microscopical factors byl Cox proportional hazards modeling.

	Risk Ratio (CI)	P
SES	**3.20 (1.04–4.91)**	**0.04**
modGHAS	1.14 (0.46–2.84)	0.78
PCDAI	0.99 (0.96–1.03)	0.70
Age at diagnosis	0.95 (0.85–1.05)	0.29
Male gender	0.86 (0.43–1.74)	0.68
Granulomas in biopsy	1.04 (0.53–2.02)	0.91
Upper GI involvement	0.84 (0.43–1.64)	0.62

SES = Simple Endoscopic Score; modGHAS = Modified Global Histology Activity Score; PCDAI = Pediatric Crohn's Disease Activity Index.
Bold value highlights a statisticaly signifinant result.

introduced, predominantly for UC, some of which are validated and remain widely used in the clinical practice [6,21,22]. As previously mentioned, scoring systems for CD are not validated and limited in the number. A classification system from 1998, established by D'Haens et al. called the Global Histological Activity Score in subsequent studies, has been the most widely used one [15,23–25]. However, these scoring systems show only a weak correlation with endoscopy in patients with UC, CD and IBD-U [23,26–28] and none of them was established for pediatric population.

Histopathological assessment should provide valid information about current state of the disease intensity and its activity. But it may also serve as a helpful tool for the consecutive management of patients and as a predictor of their clinical course. In the case of UC, some studies showed a better prediction of microscopy for a clinical relapse compared to endoscopical findings [21,29,30]. On the other hand, the prognostic value of microscopy in CD remains unclear. The study of Baars et al. [31] failed to demonstrate any association between the intensity of mucosal inflammation seen on microscopy and clinical relapse, development of a stricture or the need of surgery.

The significance of microscopical evaluation of inflammation intensity and activity for prediction of disease outcome in pediatric IBD has not been studied until now. According to the recent studies [28,32], incorporation of microscopy into the Paris classification (which classifies pediatric IBD according to clinical characteristics and endoscopical findings) [33] significantly changed the assessment of the extent of inflammation in children with UC, CD, as well as IBD-U. However, these studies did not clarify whether this increase in disease extent actually does reflect the real clinical presentation of the disease and whether it provides any predictive value in terms of future clinical course.

In our study, we did not find any value of the used histopathological scoring system for prediction of complication development, neither in the original version nor in its modification. The modGHAS was used on the basis of the premise, that the intensity of the inflammation in the most affected bowel segment has a higher impact on the development of complications than the overall extent of the disease. The results of our study also showed a weak corre-

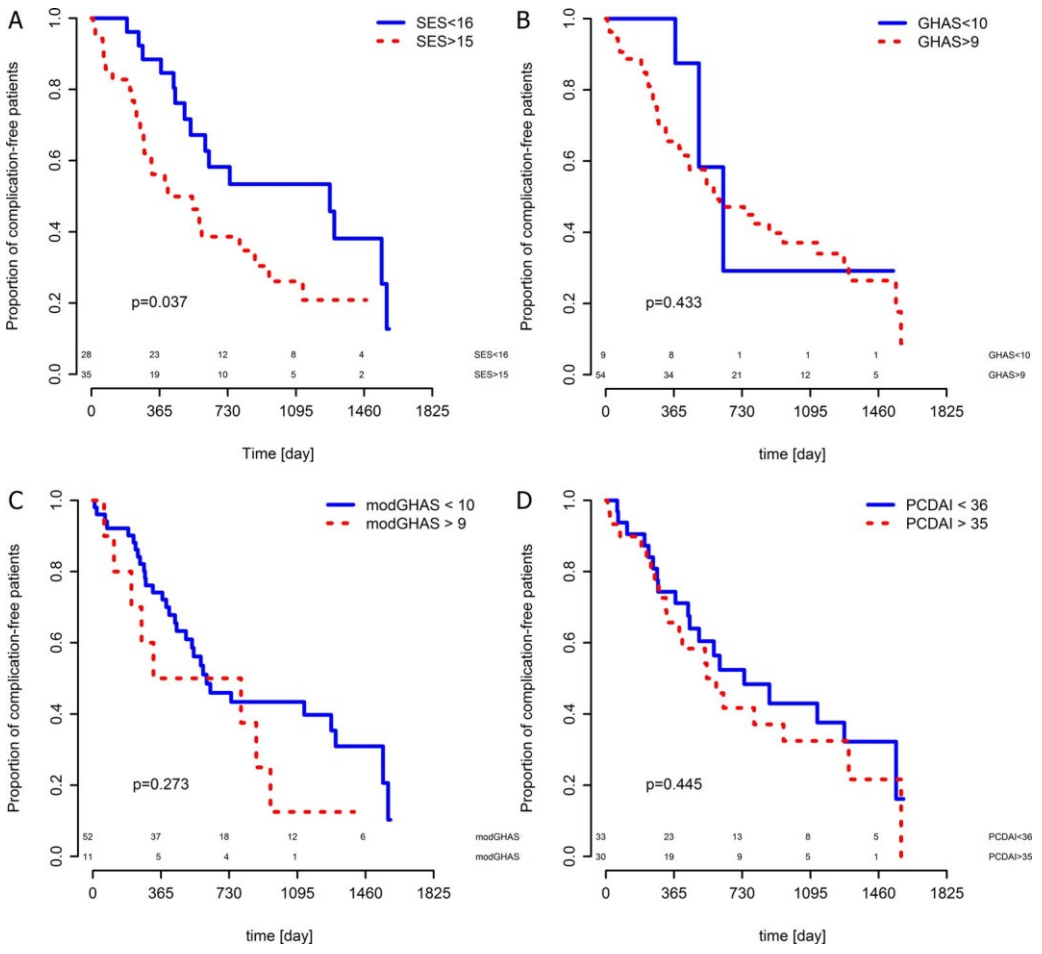

Fig. 3. A: A Kaplan-Meier curves of complication free survival for pediatric patients with Simple Endoscopic Score lower or equal to 15 versus patients with score 16 and higher. B: A Kaplan-Meier curves of complication free survival for pediatric patients with Global Histology Activity Score lower or equal to 9 versus patients with score 10 and higher. C: A Kaplan-Meier curves of complication free survival for pediatric patients with Modified Global Histology Activity Score lower or equal to 9 versus patients with score 10 and higher. D: A Kaplan-Meier curves of complication free survival for pediatric patients with Pediatric Crohn's Disease Activity Index lower or equal to 35 versus patients with score 36 and higher.

lation between the histopathological scores and the endoscopical findings and no correlation between the histopathology and PCDAI. We believe that this is due to the focality of the inflammation both on the microscopic and macroscopic levels with a possible sampling error of bioptical specimens, which makes its valid evaluation based on small bioptical samples difficult. Also, due to the transmural spreading of the inflammation, the histopathological finding in the mucosa may fail to correlate with the involvement of deeper parts of the bowel. Thus, the endoscopy, providing a better view of the entire mucosal surface of the bowel, has a better predictive value for further development of the disease, and, as supported by our data, also gives more valid information about the actual intensity of the involvement. We demonstrated, that the severity of the endoscopic findings predicted the development of complications as patients with the Simple Endoscopic Score >16 had a more than three times higher risk of complications, irrespective of microscopical picture.

There is a possible limitation of the study in its restrospective nature. A few missing archive microscopical slides required new sections from archive paraffin blocks, that may lead to a partial cutting off the material. Moreover, the retrospective assessment of PCDAI from the electronical documentation could have a limited value. A magnetic resonance imagiing, as a part of the complex assessment of pediatric CD, could be another predictor of complicated course of the disease, but further studies are required for this subject-matter.

5. Conclusions

In conclusion, the histopathological scoring system cannot be recommended as a reliable predictor of development of complications in pediatric patients with CD.

Conflicts of interest and source of funding

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Ethical considerations

Legal representatives of the patients signed an informed consent form for inclusion into the study. The study was approved by the Ethics Committee of Motol University Hospital.

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References

[1] F. Magro, C. Langner, A. Driessen, A. Ensari, K. Geboes, G.J. Mantzaris, et al., European consensus on the histopathology of inflammatory bowel disease, J. Crohns Colitis 7 (2013) 827–851.

[2] A. Levine, S. Koletzko, D. Turner, J.C. Escher, S. Cucchiara, L. de Ridder, et al., ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents, J. Pediatr. Gastroenterol. Nutr. 58 (6) (2014) 795–806.

[3] V. Annese, M. Daperno, M.D. Rutter, A. Amiot, P. Bossuyt, J. East, et al., European evidence based consensus for endoscopy in inflammatory bowel disease, J. Crohns Colitis 7 (2013) 982–1018.

[4] F. Goutorbe, M. Goutte, R. Minet-Quinard, A.L. Boucher, B. Pereira, G. Bommelaer, et al., Endoscopic factors influencing fecal calprotectin value in Crohn's disease, J. Crohns Colitis 9 (2015) 1113–1119.

[5] C.I. de Bie, S. Buderus, B.K. Sandhu, L. de Ridder, A. Paerregaard, G. Veres, et al., Diagnostic workup of paediatric patients with inflammatory bowel disease in Europe: results of a 5-year audit of the EUROKIDS registry, J. Pediatr. Gastroenterol. Nutr. 54 (2012) 374–380.

[6] R.V. Bryant, S. Winer, S.P. Travis, R.H. Riddell, Systematic review: histological remission in inflammatory bowel disease. Is complete remission the new treatment paradigm? An IOIBD initiative, J. Crohns Colitis 8 (2014) 1582–1597.

[7] S. Nicholls, P. Domizio, C.B. Williams, A. Dawnay, C.P. Braegger, T.T. MacDonald, et al., Cyclosporin as initial treatment for Crohn's disease, Arch. Dis. Child. 71 (1994) 243–247.

[8] E.J. Breese, C.A. Michie, S.W. Nicholls, C.B. Williams, P. Domizio, J.A. Walker-Smith, et al., The effect of treatment on lymphokine-secreting cells in the intestinal mucosa of children with Crohn's disease, Aliment. Pharmacol. Ther. 9 (1995) 547–552.

[9] M. Daperno, G. D'Haens, G. van Assche, F. Baert, P. Bulois, V. Maunoury, et al., Development and validation of a new simplified endoscopic activity score for Crohn's disease: the SES-CD, Gastrointest. Endosc. 60 (2004) 505–512.

[10] J.S. Hyams, G.D. Ferry, F.S. Mandel, J.D. Gryboski, P.M. Kibort, B.S. Kirschner, et al., Development and validation of a pediatric Crohn's disease activity index, J. Pediatr. Gastroenterol. Nutr. 12 (1991) 439–447.

[11] S.T. Leach, L. Nahidi, S. Tilakaratne, A.S. Day, D.A. Lemberg, Development and assessment of modified pediatric crohn disease activity inde, J. Pediatr. Gastroenterol. Nutr. 51 (2010) 232–236.

[12] J. Hyams, J. Markowitz, A. Otley, J. Rosh, D. Mack, A. Bousvaros, et al., Evaluation of the pediatric Crohn disease activity index: a prospective multicenter experience, J. Pediatr. Gastroenterol. Nutr. 41 (2005) 416–421.

[13] M.A. Shepanski, J.E. Markowitz, P. Mamula, L.B. Hurd, R.N. Baldassano, Is an abbreviated pediatric Crohn's disease activity index better than the original? J. Pediatr. Gastroenterol. Nutr. 39 (2004) 68–72.

[14] D. Turner, A.M. Griffiths, T.D. Walters, T. Seah, J. Markowitz, M. Pfefferkorn, et al., Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions, Inflamm. Bowel Dis. 18 (2012) 55–62.

[15] G.R. D'Haens, K. Geboes, M. Peeters, F. Baert, F. Penninckx, P. Rutgeerts, Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum, Gastroenterology 114 (1998) 262–267.

[16] D. Laharie, A. Reffet, G. Belleannée, E. Chabrun, C. Subtil, S. Razaire, et al., Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study with azathioprine and infliximab, Aliment. Pharmacol. Ther. 33 (2011) 714–721.

[17] R. Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2015, Availible at: https://www.R-project.org.

[18] Patrick J. Heagerty, Packaging by Paramita Saha-Chaudhuri [The Comprehensive R Archive Network]. SurvivalROC: Time-dependent ROC curve estimation from censored survival data. R package version 1.0.3. January 13, 2013, Availible at: https://cran.r-project.org/web/packages/survivalROC/index.html.

[19] Frank E. Harrell Jr., [The Comprehensive R. Archive Network]. rms: Regression Modeling Strategies. R package version 4. 4-2. April 4, 2016. Availible at: https://cran.r-project.org/web/packages/rms/index.html.

[20] Terry M. Therneau, Thomas Lumley, [The Comprehensive R. Archive Network]. .A Package for Survival Analysis in S .version 2.38. February 7, 2015. Availible at: https://cran.r-project.org/web/packages/survival/index.html.

[21] S.A. Riley, V. Mani, M.J. Goodman, S. Dutt, M.E. Herd, Microscopic activity in ulcerative colitis: what does it mean, Gut 32 (1991) 174–178.

[22] K. Geboes, R. Riddell, A. Ost, B. Jensfelt, T. Persson, R. Löfberg, A reproducible grading scale for histological assessment of inflammation in ulcerative colitis, Gut 47 (2000) 404–409.

[23] K. Geboes, P. Rutgeers, G. Opdenakker, A. Olson, K. Patel, C.L. Wagner, Endoscopic and histological evidence of persistent mucosal healing and correlation with clinical improvement following sustained infliximab treatment for Crohn's disease, Curr. Med. Res. Opin. 21 (2005) 1741–1754.

[24] G. D'Haens, S. Van Deventer, R. Van Hogezand, D. Chalmers, C. Kothe, F. Baert, et al., Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial, Gastroenterology 116 (1999) 1029–1034.

[25] A. Mojtahed, R. Khanna, W.J. Sandborn, G.R. D'Haens, B.G. Feagan, L.M. Shackelton, et al., Assessment of histologic disease activity in Crohn's disease. A systematic review, Inflamm. Bowel Dis. 20 (2014) 2092–2103.

[26] C.G. Kleer, H.D. Appelman, Ulcerative colitis—patterns of involvement in colorectal biopsies and changes with time, Am. J. Surg. Pathol. 22 (1998) 983–989.

[27] P. Gomez, C. du Boulay, C.L. Smith, G. Holdstock, Relationship between disease activity indices a colonoscopic findings in patients with colonic inflammatory bowel diseases, Gut 27 (1986) 92–95.

[28] J.J. Ashton, T. Coelho, S. Ennis, B. Vadgama, A. Batra, N.A. Afzal, et al., Endoscopic versus histological disease extent at presentation of paediatric inflammatory bowel disease, J. Pediatr. Gastroenterol. Nutr. 62 (2016) 246–251.

[29] S. Azad, N. Sood, A. Sood, Biological and histological parameters as predictors of relapse in ulcerative colitis: a prospective study, Saudi J. Gastroenterol. 17 (2011) 194–198.

[30] T. Zenlea, E.U. Yee, L. Rosenberg, M. Boyle, K.S. Nanda, J.L. Wolf, et al., Histology grade is independently associated with relapse risk in patients with ulcerative colitis in clinical remission: a prospective study, Am. J. Gastroenterol. 111 (2016) 685–690.

[31] J.E. Baars, V.J. Nuij, B. Oldenburg, E.J. Kuipers, C.J. van der Woude, Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation, Inflamm. Bowel Dis. 18 (2012) 1634–1640.

[32] M.A. Fernandez, S.G. Verstraete, E.A. Garnett, M.B. Heyman, Addition of histology to the Paris classification of pediatric Crohn disease alters classification of disease location, J. Pediatr. Gastroenterol. Nutr. 62 (2016) 242–245.

[33] A. Levine, A. Griffiths, J. Markowitz, D.C. Wilson, D. Turner, R.K. Russell, et al., Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification, Inflamm. Bowel. Dis. 17 (2010) 1314–1321.

PŘÍLOHA II

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ORIGINAL ARTICLE

Immunohistochemical Assessment of CD30+ Lymphocytes in the Intestinal Mucosa Facilitates Diagnosis of Pediatric Ulcerative Colitis

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Abstract

Background Diagnosis of pediatric inflammatory bowel diseases (IBD) remains challenging. We aimed at the value of immunohistochemical assessment of CD30+ lymphocytes in the intestinal mucosa in differential diagnosis between pediatric Crohn's disease (CD) and ulcerative colitis (UC) and its utility as a predictor of future differentiation in patients with IBD unclassified (IBDU).

Methods Seventy-four treatment naive pediatric patients with IBD (33 CD, 30 UC and 11 IBDU) were enrolled into the study. Biopsy samples from six different regions (terminal ileum, cecum, ascending colon, transverse colon, descending colon and rectum) were immunohistochemically stained with anti-CD30 antibody, and the number of positive cells per one high power field was quantified.

Results Significant differences between CD and UC were found when compared total counts of CD30+ cells in median numbers, mean values and maximal numbers and also for separate counts in terminal ileum, transverse colon, descending colon and rectum. The most profound difference between CD and UC was shown for total median values of CD30+ cells and for the values in rectal localization. The difference was independent on the intensity of inflammation. A cutoff value of 2.5 CD30+ cells with sensitivity 83% and specificity 90% was found for the rectum. There was no difference between patients with CD and IBDU, but a marked difference between UC and IBDU patients was revealed.

Conclusion Histopathological assessment of biopsy with rectal CD30+ count is reliable and simple method that could help in differential diagnosis among IBD subtypes in children with IBD.

Keywords Inflammatory bowel disease · Immunohistochemistry · CD30

Introduction

Until recently, the diagnosis of inflammatory bowel disease (IBD) was based on proof of the chronic inflammation of the gastrointestinal tract with typical distribution and exclusion of other causes of inflammation, mainly on clinical grounds [1]. This approach, apparently straightforward and simple, has become complicated and establishing diagnosis of IBD turned into a multidisciplinary process heavily supported by laboratory, radiological, endoscopic and histopathological data [2, 3]. Atypical presentations of IBD have been stressed, and various other diseases manifesting with IBD-like morphology were highlighted [4–6]. Moreover, none of the established criteria is 100% specific for IBD, let alone for Crohn's disease (CD) or ulcerative colitis (UC) [7, 8]. Situation in pediatric population is even more challenging, given the fact that pediatric onset IBD often presents with nonspecific clinical features, it lacks characteristic morphological signs of chronicity and harbors many atypical phenotypes in all three main forms of IBD (CD, UC and IBD unclassified) [1, 9–13]. Porto criteria from 2005 [14] and their revised version from 2014 [1] established diagnostic criteria, integrating the up to date evidence from all diagnostic fields. However, there is up to 30% of pediatric IBD that remain

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further unclassified, in contrast with adult population, where the proportion of such cases remains only 10–15% [15]. This has led to increased demand for new invasive and noninvasive markers that would increase the certainty of the correct diagnosis, since the exact subclassification of pediatric IBD has crucial therapeutic consequences. According the actual recommendations, children with newly diagnosed CD should be treated primarily with exclusive enteral nutrition [16], while treatment of UC should be initiated by corticosteroids or 5-aminosalicylic acid according to severity of the disease [12].

IBD is immune-mediated disease that involves a complex interplay of host genetics, disrupted mucosal integrity and environmental influences [17]. Some T helper lymphocyte subsets in IBD express antigen CD30 at their cytoplasmic membranes. According to a recent study [18], patients with UC show higher amount of CD30+ cells in gut mucosa than those with CD. The aim of our study was to find out, whether the immunohistochemical assessment of CD30 in the intestinal mucosa could facilitate differential diagnosis between pediatric CD and UC an if it could serve as an early predictor of future reclassification into CD and UC in patients with IBD unclassified (IBDU).

Materials and Methods

Between November 2012 and January 2017, all 171 newly diagnosed pediatric patients with IBD were retrospectively screened in our referral center for eligibility to enter this study. First 30 consecutive treatment naïve patients primarily diagnosed as CD and UC and all patients with the primary diagnosis of IBDU were reevaluated by pediatric gastroenterologists, and the diagnosis at the time of the first endoscopic assessment was confirmed or modified following the actual revised Porto criteria for the diagnosis of pediatric IBD. Patients that had already been initiated on IBD treatment, as well as those patients with missing data for the revision of the diagnosis, were excluded from the study. After this revision, 29 patients with CD, 31 with UC and 14 with IBDU entered the study. During the minimum of 18-month follow-up, one patient from UC group developed perianal fistulizing disease and was reclassified to CD. Among 14 patients with IBDU, three cases were reclassified to CD due to the manifestation of the CD traits (all three patients manifested perianal disease). In the end, 33 children with CD, 30 with UC and 11 with IBDU (Fig. 1) were included in the study. Clinical characteristics of the patients are given in Table 1.

Archive histopathological slides stained with hematoxylin and eosin were reviewed by a pathologist blinded to clinical data. To evaluate microscopical findings, six bowel segments from each case of CD, UC and IBDU were analyzed (terminal ileum, cecum, ascending colon, transverse colon, descending colon and rectum). Intensity and activity of the inflammation was objectified using a histopathological scoring system. Given the diversity in scoring systems that are only partially validated and none of them is designed for pediatric population [19], as well as the absence of any scoring system for patients with IBDU, more universal modification of the established systems was used in our study. This modified system evaluates the intensity of chronic and active inflammation and the presence of mucosal defects (Table 2). A similar version of this system has been already used in our previous study [20]. Additional assessed morphological variables included the intensity of the eosinophilic inflammation (graded as none, mild and severe), the presence of the basal plasmocytosis, recently described basal plasmocytosis associated with eosinophilia [21] and the presence of lymphatic follicles. Given the fact that CD30+ cells show predilection to lymphoid tissue, as declared in previous studies [18], the assessment of the follicles was fundamental. Especially in terminal ileum, where physiological presence of the lymphoid tissue (Peyer's patches) can falsely overestimate number of CD30+ cells.

Then, immunohistochemical staining of CD30 antigen was performed on all archive biopsy samples from patients with CD, UC and IBDU. 1-μm tissue sections were deparaffinized, and the anti-CD30 primary antibody (Cell Marque, at a dilution of 1:50) was used. Detection was performed by the PolyDet Dab chromogen (Dako REAL) with phosphate-buffered saline solution. CD30 expression was assessed by counting the highest number of positive cells per one hpf (400×) in the most affected region of each bowel segment.

Ethical Considerations

Legal representatives of the patients signed an informed consent form for inclusion into the study. The study was approved by the Ethics Committee of Motol University Hospital.

Statistical Analysis

We used statistical software R-project (R Core Team, version 3.4.1) for data analysis. Differences among the subgroups of the patients with CD and UC in the median values, maximal numbers and mean values of CD30+ cells calculated from all bowel segments, as well as numbers of positive cells in separate bowel segments, were tested by Welch two sample *t* test. For constructing model of the discrimination between CD and UC, the definitive diagnosis of CD or UC was used. Then, the definitive diagnosis including reclassification of IBDU to CD served as a template to test differences between CD and IBDU, as well as UC and IBDU. After that, the receiver operating characteristics (ROC) curves were used to

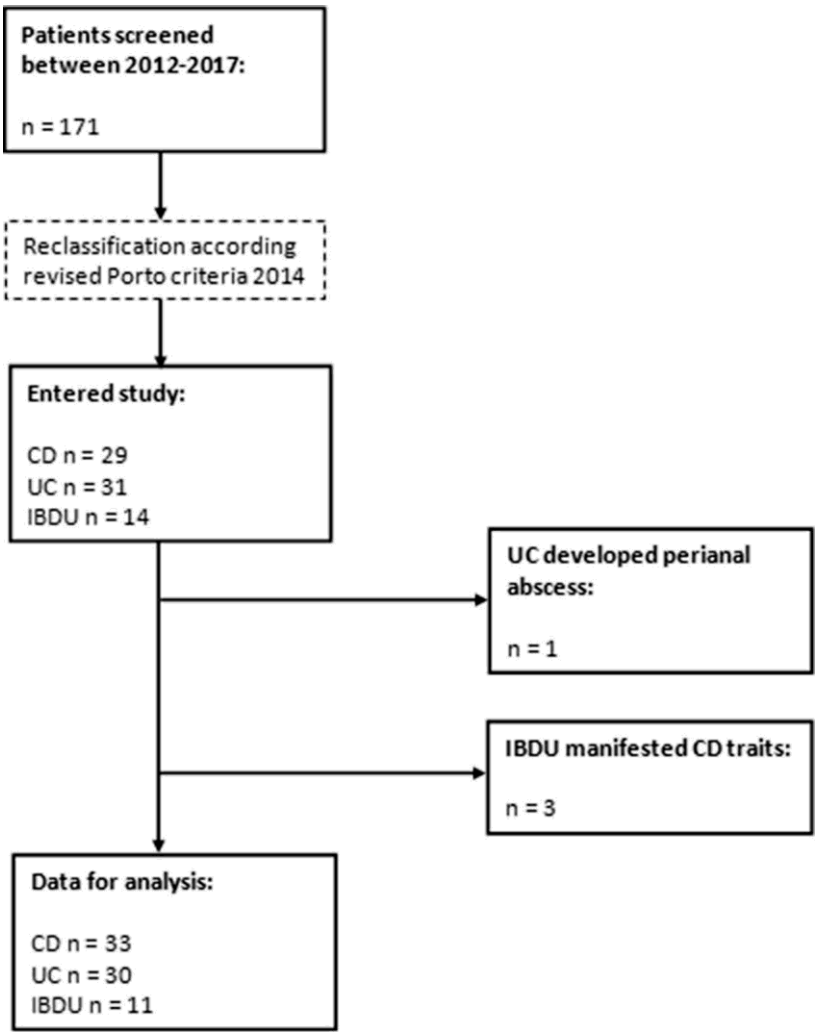

Fig. 1 Flowchart of included and excluded patients. *CD* Crohn's disease, *UC* ulcerative colitis, *IBDU* inflammatory bowel disease unclassified

find the most significant cutoff values for the discrimination between CD and UC (using the software package "pROC"). To compare individual ROC curves, DeLong's test was used. To test, whether the difference in numbers of CD30+ cells in terminal ileum is independent on the presence of lymphatic follicles, a generalized linear regression model was constructed. The same model was applied to find out, if the difference in numbers of CD30+ cells in rectum is dependent on the intensity of histological and endoscopic inflammation. Probability (p) values of < 0.05 were considered significant. A 95% confidence interval was used.

Results

Statistically significant differences among patients with CD and UC in median values, maximal numbers and mean values of CD30+ cells were found (Fig. 2a, b). Also, there was a difference between CD and UC in each bowel segment except for cecum and ascending colon. The most significant results were shown for the median values ($p < 0.001$), see Fig. 3a, and for the values in rectal localization ($p < 0.001$). The box plots of CD30 counts in all bowel segments are given in Fig. 3b–g. Based on the analysis of area under curve, a cutoff value of 4.25 positive cells per 1 HPF was established for median values, with sensitivity 93% and specificity 83%, Fig. 4a. For the values in rectum, the optimal cutoff (2.5 positive cells per 1 HPF) with sensitivity 83% and specificity 90% was found (Fig. 4b). Using Delong's test, the total median values turned out to be more reliable discriminating factor between CD and UC than the values in the most affected bowel segment, but there was no difference between the median values and the values in rectum. Among the patients with CD, 16 of them had microscopical inflammation in rectum, and 13 of them endoscopical signs of rectal involvement. According to the regression analysis, the association between the number of CD30+ cells and diagnosis was independent on the intensity of endoscopic or microscopic inflammation ($p < 0.0001$). The basal plasmocytosis was found in 15 cases of UC, five cases of IBDU,

Table 1 Demographic and clinical characteristics of patients

	CD	UC	IBDU
Number of patients	33	30	31
Sex, male (%)	20 (0.61)	14 (0.47)	7 (0.64)
Age at diagnosis, years, median (IQR)	13.1 (11.5–16.2)	11.8 (6.7–15.5)	9.7 (3–12.6)
L1 (%)	10 (0.3)		
L2 (%)	3 (0.09)		
L3 (%)	19 (0.58)		
L4 (%)	21 (0.64)		
B1 (%)	29 (0.88)		
B2 (%)	3 (0.09)		
B3 (%)	1 (0.03)		
p (%)	8 (0.24)		
G1 (%)	4 (0.12)		
E1 (%)		4 (0.13)	
E2 (%)		2 (0.07)	
E3 (%)		2 (0.07)	
E4 (%)		22 (0.73)	
S0 (%)		28 (0.99)	
S1 (%)		2 (0.07)	

L = Localization of the disease (L1—ileal, L2—colonic, L3—ileocolonic); B = Behavior (B1—non-stricturing, non-penetrating, B2—stricturing, B3—penetrating, p—perianal); G1 = Growth delay; E = Extension (E1—proctitis, E2—left side colitis, E3—extensive, E4—pancolitis); S = Severity (S0—never had acute severe colitis, S1—at least one acute severe colitis)

Table 2 Modified histopathological scoring system

Grade	Histopathological change
0	No inflammation
1	Mononuclear cells only
2	Neutrophils in lamina propria
3	Neutrophils in epithelium (superficial epithelium, cryptitis, crypt pseudoabscess)
4	Mucosal defect (erosion or ulceration)

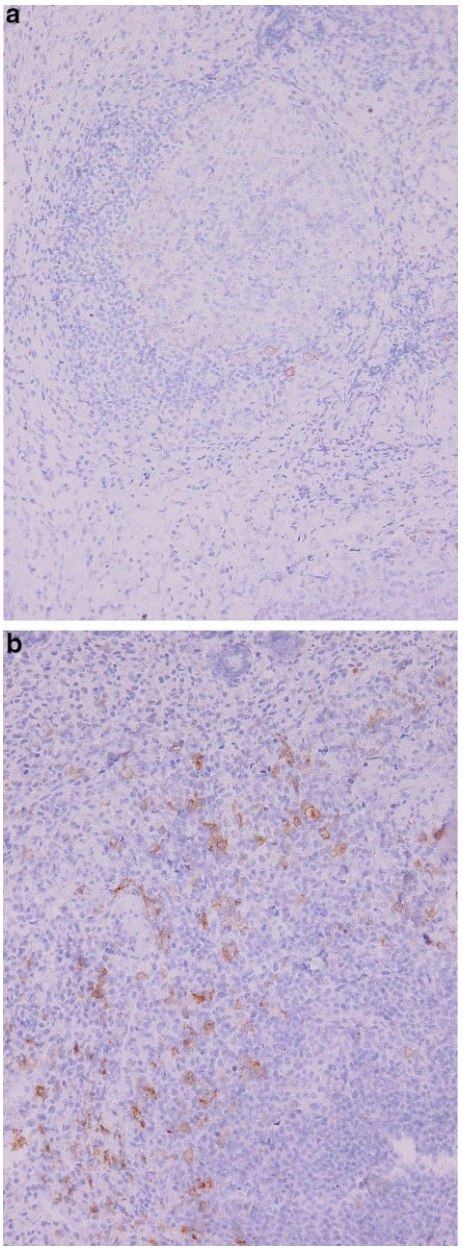

Fig. 2 Photomicrograph showing CD30+ cells by immunohistochemistry (×400). Membrane staining of CD30+ lymphocytes in Crohn's disease (**a**) and ulcerative colitis (**b**) in rectal samples

but only in one case of CD. Also the presence of any eosinophilia (both mild and severe) in rectum was significantly more frequented in patients with UC than CD ($p < 0.0001$). A lymphatic tissue in terminal ileum was not the confounding factor, since the significant difference between CD30+ cells in the ileum was independent on the presence of lymphatic follicles.

Based on the given results, a diagnostic model using calculated cutoff in rectal biopsy was constructed and applied on patients with IBDU. Using the final diagnosis for the median values and the values in rectal localization, there was no difference in CD30+ cells between patients with CD and IBDU ($p = 0.08$), but there was a marked difference between the UC and the IBDU ($p = 0.007$), see Fig. 5. Three patients with IBDU developed CD traits, and two of them had low numbers of CD30+ cells (below the established cutoff). Among the patients without CD traits, there were cases with both low and high numbers (Fig. 5).

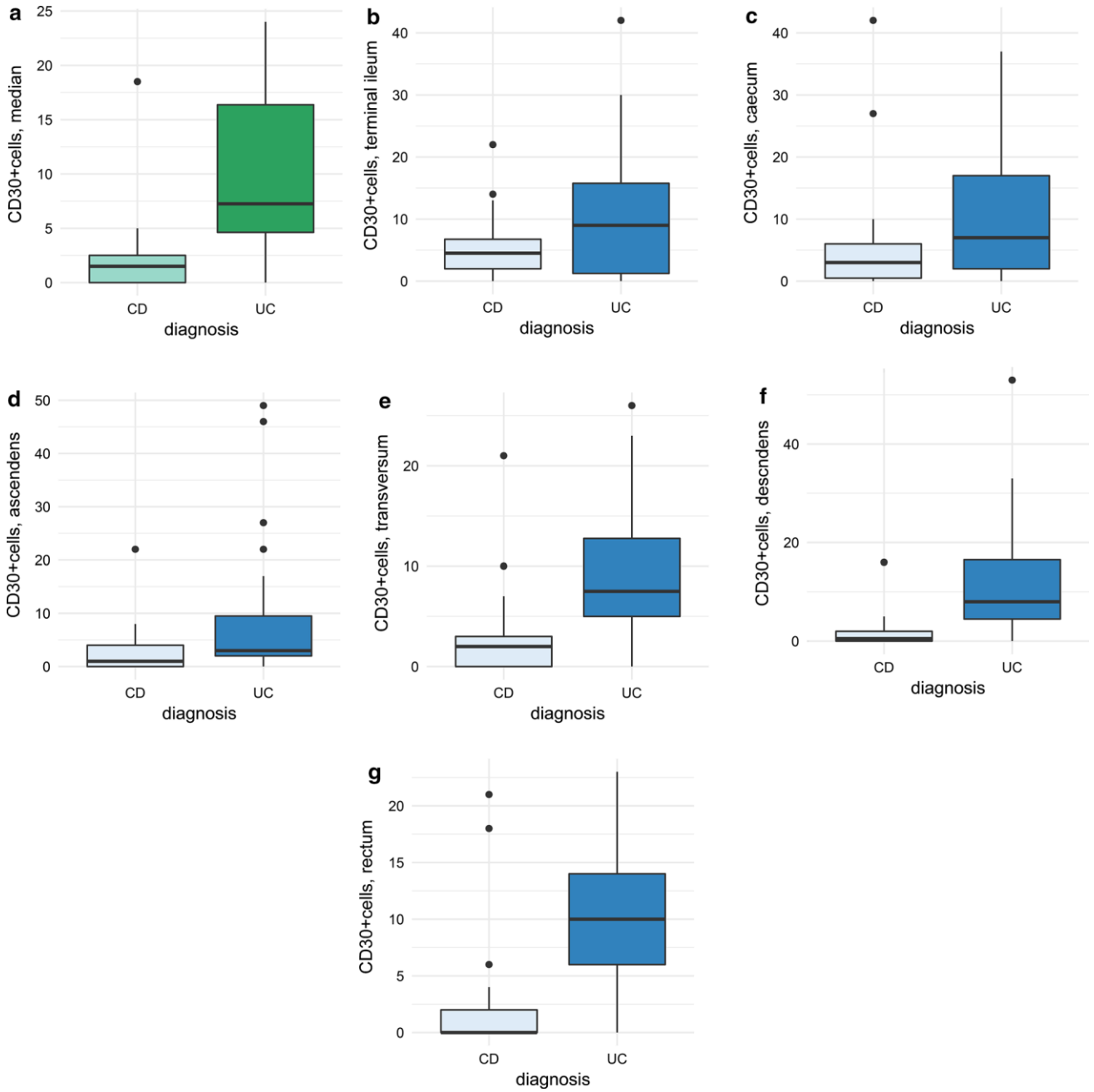

Fig. 3 **a** Box graphs comparing medians values of CD30+ cells, calculated from all bowel segments, in Crohn's disease and ulcerative colitis. **b** Box graphs comparing numbers of CD30+ cells in terminal ileum in patients with Crohn's disease and ulcerative colitis. **c** Box graphs comparing numbers of CD30+ cells in cecum in patients with Crohn's disease and ulcerative colitis. **d** Box graphs comparing numbers of CD30+ cells in ascending colon in patients with Crohn's disease and ulcerative colitis. **e** Box graphs comparing numbers of CD30+ cells in transverse colon in patients with Crohn's disease and ulcerative colitis. **f** Box graphs comparing numbers of CD30+ cells in descending colon in patients with Crohn's disease and ulcerative colitis. **g** Box graphs comparing rectal numbers of CD30+ cells in patients with Crohn's disease and ulcerative colitis

Discussion

The pathophysiological mechanisms of IBD are not fully understood, but are characterized by the immunological imbalance of the intestinal mucosa, associated with cells of the both innate and adaptive immune system. The major role in triggering the repetitive intestinal inflammatory damage in IBD is T cell subpopulations, mainly T helper 1 (Th1), Th17 and regulatory T cells. Th1 lymphocytes are stimulated by certain cytokines, especially interleukin (IL) 12 and

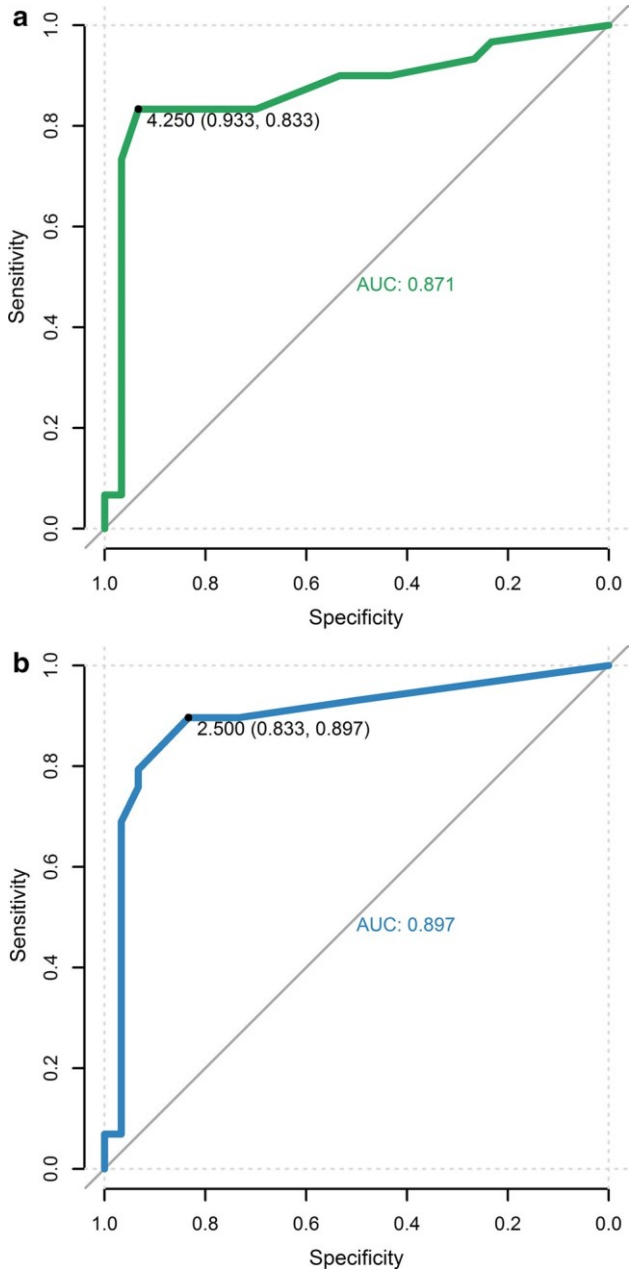

Fig. 4 **a** Receiver operating characteristics curve to determine the CD30 cutoff for ulcerative colitis diagnosis in median. **b** Receiver operating characteristics curve to determine the CD30 cutoff for ulcerative colitis diagnosis in rectal localization

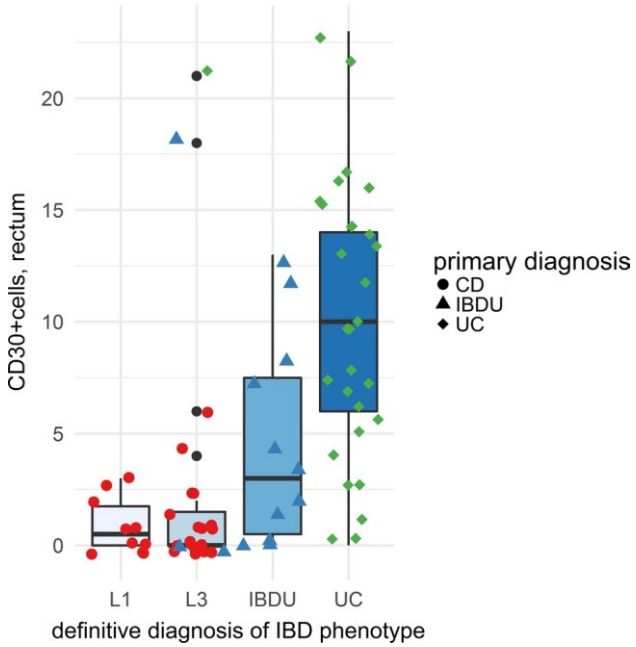

Fig. 5 Box graphs comparing numbers of CD30+ cells in rectum for Crohn's disease, ulcerative colitis and inflammatory bowel disease unclassified, also with highlighted changes in diagnoses after the revision, according the revised Porto criteria

18, inducing production of interferon gamma (IFN-γ). This pathway is more prominent in CD, but increased levels of IFN-γ can be demonstrated also in patients with UC. Th2 lymphocytes play a role in immunopathogenesis of both diseases as well, with oversecretion of IL5, IL10 and IL13. In both CD and UC, there is also a strong influence of Th-17-related cytokines [22].

The role of CD30 antigen in IBD pathogenesis still remains unexplained. It represents a protein with uncertain function, member of tumor necrosis family, firstly described in association with certain malignances as Hodgkin's lymphoma, anaplastic large cell lymphoma or embryonal carcinoma. It was also discovered in normal human tissues, especially some portions of resting and activated T lymphocytes [23]. To the best of our knowledge, only one study [18] has examined the diagnostic utility of CD30 detection in colonic biopsies so far. The authors assessed counts of CD30+ cells in the most inflamed segment of bowel mucosa in adult patients with IBD and demonstrated a significant difference between CD and UC. Our goal was to ascertain, whether we can find a significant difference in numbers of CD30+ cells also in pediatric IBD population and if this difference is independent on localization and the intensity as well as the activity of inflammation. Our study demonstrated a significant difference between CD and UC in median values, mean values and maximal values calculated from all bowel segments. A difference in values from all separate bowel segments was observed as well, but for the values obtained in cecum and ascending colon they were not statistically significant. The most significant difference was found for the median values of CD30+ cell counts and the values obtained in rectal localization. Given the fact that counting of median values would require staining of all bioptic samples for CD30, it seems more practical and economically efficient to assess the CD30+ cell counts in rectum only. Both these variables were proved to be better

discriminating factors than the counts from the most affected bowel segment, as declared in the previously study [18]. The limiting factor of this method could be the fact, that less than half of the CD patients had either macroscopic or microscopic rectal involvement. However, the difference of rectal CD30+ cell counts was independent on the intensity and the activity of the inflammation (both histological and endoscopic); hence, this method is applicable also for the patients with mild rectal involvement only. Neither rectal sparing in UC nor absence of the rectal inflammation in CD should affect the clinical utility of such testing. Interestingly, both patients with UC and rectal sparing had high numbers of CD30+ cells (mean 13.5). Our results are supported by findings of Giacomelli et al. [24], who illustrated elevated levels of serum soluble CD30 antigen in patients with UC. Serum level of CD30 could therefore serve as a simple non-invasive marker of the disease activity and monitoring of the treatment response. Sun et al. [25] even propose a possible therapeutic usefulness of soluble CD30 in case murine IBD models.

Our study demonstrated that other morphological variables can also be independently supportive for the UC diagnosis. The eosinophilia in the rectum was associated with UC. Also the basal plasmocytosis was present in more cases of UC in contrast with CD. These microscopical signs along with rectal localization and the assessment of CD30+ cells may represent reliable and simple methods for the diagnosis of UC.

Eventual benefit of CD30+ cells evaluation is even more important for the patients with IBDU. This subtype of IBD was traditionally regarded as a temporary "immature" form of IBD with the expectation of further differentiation. Even the Porto criteria for IBDU are based rather on the practical clinical experience and do not encompass eventual biological markers or genetic background. However, a recent study by Cleyen et al. [26] indicates that IBDU may represent an independent subtype of IBD with its own characteristic genetic profile. Keeping with this finding, we consider IBDU to be a definite diagnosis in our study, without further shift to CD or UC during the follow-up. We did not find any difference between IBDU and CD, but a marked difference between IBDU and UC was shown. Therefore, CD30+ cell counts should be regarded more likely as a supportive biomarker of UC rather than discriminating factor between CD and UC. These findings diverge from current therapeutic recommendations, where the treatment of IBDU and UC is actually the same [12]. But the difference in CD30+ cell counts may indicate that the biology of those diseases is different and that IBDU stands closer to CD.

On the other hand, if IBDU truly represents an undifferentiated form of IBD with the capacity for further maturation, CD30+ cell counts could be beneficial even in this case. Children with IBDU, that manifest CD traits later, should be reclassified to CD. However, current state of the art in IBD diagnostics does not have a capacity to select IBDU patients that are progressing to UC and re-diagnose them. In other words, children with IBDU will not be able to be changed to UC in the future. Given the fact that our IBDU group contained patients with both high and low numbers of CD30+ cells, their assessment could serve as a marker of possible maturation to UC. Patients with diagnosis of IBDU and high numbers of rectal CD30+ cells that are refractory to current treatment could profit from the switch to the UC therapy. And, as stated above, the serum CD30 could prove useful too, as the progressive elevation of serum levels of CD30 could represent a sign of the progression to UC. However, this hypothesis is based on the limited number of the patients in our IBDU group. Further studies, preferably multicenter, with greater numbers of IBDU patients and their prospective observation are required to confirm such premise.

Conclusion

In conclusion, routine histopathological assessment of biopsy samples with rectal CD30+ cell count represents a supportive biomarker that could improve the differential diagnosis among subtypes of IBD in treatment naïve children with IBD.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

References

1. Levine A, Koletzko S, Turner D, et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr*. 2014;58:795–806.
2. Magro F, Gionchetto P, Eliakim R, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis*. 2017;11:649–670.
3. Gomollón F, Dignass A, Annese V, et al. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis*. 2017;11:3–25.

4. James SD, Wise PE, Zuluaga-Toro T. Identification of pathologic features associated with "ulcerative colitis-like" Crohn's disease. *World J Gastroenterol.*. 2014;20:13139–13145.
5. Odze RD, Goldblum JR. *Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas*. 3rd ed. New York: Elsevier; 2015:300.
6. Russo P, Ruchelli ED, Piccoli DA. Pathology of Pediatric Gastrointestinal and Liver Disease. 2nd ed. Springer; 2014:155–189, 233, 238.
7. Geboes K. What histologic features best differentiate Crohn's disease from ulcerative colitis? *Inflamm Bowel Dis*. 2008;14:5168–5169.
8. Magro F, Langner C, Driessen A, et al. European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis*. 2013;7:827–851.
9. Van Limbergen J, Russell RK, Drummond HE, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. *Gastroenterology*. 2008;135:1114–1122.
10. Levine A, de Bie CL, Turner D, et al. Atypical disease phenotypes in paediatric ulcerative colitis: 5-year analyses of the EUROKIDS registry. *Inflamm Bowel Dis*. 2013;19:370–377.
11. Bousvaros A, Antonioli DA, Colletti RB, et al. Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. *J Pediatr Gastroenterol Nutr*. 2007;44:653–674.
12. Turner D, Levine A, Escher JC, et al. Joint ECCO and ESPGHAN evidence-based consensus guidelines on the management of pediatric ulcerative colitis. *J Pediatr Gastroenterol Nutr*. 2012;55:340–361.
13. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification of inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis*. 2011;17:1314–1321.
14. IBD Working Group of the European Society for. Paediatric Gastroenterology Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis—the Porto criteria. *J Pediatr Gastroenterol Nutr*. 2005;41:1–7.
15. Cuffari C. Diagnostic considerations in pediatric inflammatory bowel disease management. *Gastroenterol Hepatol*. 2009;11:775–783.
16. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis*. 2014;8:1179–1207.
17. Dave M, Papadakis KA, Faubion WA Jr. Immunology of inflammatory bowel disease and molecular targets for biologics. *Gastroenterol Clin N Am*. 2014;43:405–424.
18. Flores C, Francesconi CF, Meurer L. Quantitative assessment of CD30+ lymphocytes and eosinophils for the histopathological differential diagnosis of inflammatory bowel disease. *J Crohns Colitis*.. 2015;9:763–768.
19. Bryant RV, Winer S, Travis SP, et al. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. *J Crohns Colitis*. 2014;8:1582–1597.
20. Fabian O, Hradsky O, Potuznikova K, et al. Low predictive value of histopathological scoring system for complications development in children with Crohn's disease. *Pathol Res Pract*. 2017;213:353–358.
21. Canavese G, Villanacci V, Antonelli E. Eosinophilia—associated basal plasmacytosis: an early and sensitive histologic feature of inflammatory bowel disease. *APMIS*. 2017;125:179–183.
22. Giuffrida P, Corazza GR, Di Sabatino A. Old and new lymphocyte players in inflammatory bowel disease. *Dig Dis Sci*. 2018;63:277–288. https://doi.org/10.1007/s10620-017-4892-4.
23. Falini B, Pileri S, Pizzolo G, et al. CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis receptor superfamily as a tool for diagnosis and immunotherapy. *Blood*. 1995;85:1–14.
24. Giacomelli R, Passacantando A, Parzanese I, et al. Serum levels of soluble CD30 are increased in ulcerative colitis (UC) but not in Crohn's disease (CD). *Clin Exp Immunol*. 1998;111:532–535.
25. Sun X, Yamada H, Shibata K, et al. CD30 ligand is a target for a novel biological therapy against colitis associated with Th17 responses. *J Immunol*. 2010;185:7671–7680.
26. Cleynen I, Boucher G, Jostins L, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet*. 2016;387:156–167.

PŘÍLOHA III

Journal Pre-proof

Limited clinical significance of tissue calprotectin levels in bowel mucosa for the prediction of complicated course of the disease in children with ulcerative colitis

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Limited clinical significance of tissue calprotectin levels in bowel mucosa for the prediction of complicated course of the disease in children with ulcerative colitis.

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Abstract

Background

Fecal calprotectin (F-CPT) represents one of the most widely used biomarkers for intestinal inflammation. However, the levels may be false negative or false positive in some situations.

Aims

To evaluate the usefulness of immunohistochemical (IHC) detection of tissue calprotectin (T-CPT) in bowel mucosa in children with ulcerative colitis (UC). We focused at correlation of T-CPT with levels of F-CPT and endoscopic and microscopic disease activity at the time of diagnosis and tested whether T-CPT could serve as predictor of complicated course of the disease.

Methods

Forty-nine children with newly diagnosed UC between 6/2010-1/2018 entered the study. Endoscopic activity was objectified using the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), clinical activity by Pediatric Ulcerative Colitis Activity Index (PUCAI) and microscopic activity by Geboes and Nancy score. The IHC staining for CPT antigen was performed on bioptic samples from 6 bowel segments and the number of CPT+ cells were counted per 1HPF. During the minimal follow-up of 12 months we searched for presence of complications. As outcome for Cox regression model we used composite endpoints: A) Acute Severe Colitis, colectomy, anti-TNF treatment; B) systemic corticotherapy; C) systemic 5-aminosalicylic acid therapy.

Results

Neither levels of T-CPT nor values of UCEIS, Geboes or Nancy score predicted the given complications. We found F-CPT levels (HR 2.42 and 2.52) and PUCAI>40 points (HR 2.98) as predictors of time to endpoints B and C. Good correlation was found between T-CPT levels and Geboes score (k=0.65) and Nancy score (k=0.62) and modest with F-CPT (k=0.44), UCEIS (k=0.38) and PUCAI (k=0.42).

Conclusions

T-CPT correlated well with microscopic scores. F-CPT and PUCAI appear to be better predictors of unfavorable outcome in patients with UC.

Keywords

Calprotectin; immunohistochemistry; ulcerative colitis; outcome

Introduction

Ulcerative colitis (UC) is a chronic systemic inflammatory disorder with predominant involvement of the gastrointestinal tract [1]. Periodic monitoring evaluating the disease intensity and activity is essential for optimizing the treatment strategy. Even though the disease activity may be assessed by clinical scores, namely Pediatric Ulcerative Colitis Activity Index (PUCAI) [2], the endoscopy is still considered the gold standard for the assessment of the intestinal inflammation and mucosal healing, especially at the time of diagnosis [3,4]. However, this procedure is invasive, time-consuming and burdening, especially for pediatric population, where the general anesthesia is usually required. Therefore, reliable non-

invasive markers for monitoring a disease activity are required. Fecal calprotectin (F-CPT) represents one of the most widely used biomarkers for intestinal inflammation with high sensitivity for both adult and pediatric population [5,6]. However, the F-CPT cannot be used to localize the focus of the disease activity and its levels may be false negative or false positive in some situations [7-9].

In our work, we focused at the immunohistochemical assessment of the tissue CPT (T-CPT) in the bowel mucosa of the children with UC. The aim of this work was: 1) to evaluate, whether the immunohistochemical assessment of the T-CPT may serve as an independent predictor of the complicated course of the disease, 2) to establish, whether the numbers of CPT positive cells in the bowel mucosa correlate with the levels of F-CPT at the time of diagnosis, and 3) to correlate the CPT+ cell counts with the microscopic, endoscopic and clinical activity of the disease.

Materials and Methods

All children (n=130) with newly diagnosed UC in period between June 2010 and January 2018 were screened for eligibility to enter this retrospective cohort study. Patients were retrospectively re-evaluated by expert pediatric gastroenterologists and the diagnosis at the time of the first endoscopy was confirmed or modified according to revised Porto criteria for the diagnosis of pediatric inflammatory bowel diseases (IBD) [10]. Patients that had already been initiated on UC treatment at the time of biopsy as well as those with missing clinical data, endoscopic documentation or archive bioptic material for the revision of the diagnosis were excluded from the study. After the revision, 49 children with UC entered the study. Eight patients had to be subsequently excluded from the statistical analysis due to insufficient clinical data at the end of the time period of follow-up (Fig. 1). Clinical

characteristics of the patients at the end of the minimal follow-up are given in the Table 1. The endoscopic findings at the time of the diagnosis were objectified using the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) [11]. This score was chosen for its good reproducibility among the endoscopists and its good correlation with clinical, laboratory and histopathological markers of activity in UC [12,13]. The clinical severity of the disease at the time of the diagnosis was assessed by the PUCAI.

The archive histopathological slides from the bioptic samples obtained at the time of diagnosis stained with hematoxylin and eosin were reviewed by senior pediatric gastrointestinal pathologist blinded to clinical data. To evaluate microscopic findings, six bowel segments from each patient were analyzed (terminal ileum, cecum, ascending colon, transverse colon, descending colon and rectum). The samples were taken from the most affected areas of each segment. Intensity and activity of the inflammation was objectified using the Geboes and Nancy score, two widely used validated histopathological scoring systems for UC with good correlation with endoscopic findings and suitable interobserver reliability [12,14,15].

After that, the immunohistochemical staining of CPT antigen was performed on all archive biopsy samples. One micrometer thin tissue sections were deparaffinized and the anti-CPT primary antibody (Invitrogen, at a dilution of 1:1000) was used. Detection was performed by the PolyDet Dab chromogen (Dako REAL) with phosphate-buffered saline solution. CPT expression was assessed by counting the highest number of positive cells per one high power field (400x magnification) in the most affected region of each microscopic slide. The positive cells were counted separately for lamina propria and the epithelium (Fig. 2, 3 and 4).

During the minimum 12 months of prospective follow-up, we searched for the presence of following composite endpoints, presented as 3 separate substudies based on their

severity: A) development of the Acute Severe Colitis (ASC) defined as the PUCAI > 65, necessity of colectomy or initiation of the Infliximab (IFX) treatment; B) first initiation of systemic corticosteroid (CS) therapy; C) first initiation of systemic 5-aminosalicylic acid (5ASA) treatment. The time to endpoint was chosen as outcome. Patients manifesting with ASC at the time of the diagnosis were not included in the study, since the ileocolonoscopy is not recommended in the setting of ASC [16,17] and these patients therefore did not fulfill the inclusion criteria.

Ethical Considerations

Legal representatives of the patients signed an informed consent form for inclusion into the study. The study was approved by the Ethics Committee of Motol University Hospital on 28.03.2018.

Statistical analysis

Statistical software R-project (R Core Team, version 3.4.4) was used for data analysis. For all scoring systems included in the study, the mean values, medians and maximal values from all bowel segments were calculated and used in the subsequent analysis. The CPT+ cell counts were assessed separately for lamina propria and epithelium and in total sum for the whole mucosa as well. To test, whether the levels of T-CPT and F-CPT and values of UCEIS, PUCAI and both histopathological scores at the time of diagnosis are independent predictors of the complicated course of the disease, a univariate Cox proportional hazards regression analysis (using the software package "survival") was used. Since the PUCAI > 40 (moderate disease activity) appeared to be one of the predictors (see Results section), a receiver operating curves (ROC) were constructed (using the software package "pROC") to find the

cut-off values for both T-CPT and F-CPT, that would be able to select patients on this level of disease activity. Individual ROC curves were compared using the DeLong's test. To verify the correlation between F-CPT and T-CPT levels and values of the histopathological, endoscopic and clinical scores, the Pearson's product-moment correlation was performed. Probability (p) values of < 0.05 were considered significant. A 95% confidence interval was used.

Results

T-CPT as a predictor of the complicated course of the disease

Neither levels of the T-CPT, nor the values of UCEIS, Geboes or Nancy score predicted any complication during minimum 12 months of follow-up since diagnosis. However, the levels of the F-CPT in logarithmic scale and moderate disease activity (defined as PUCAI > 40) were independently associated with the B and C endpoints (initiation of the systemic CS and/or 5ASA therapy) (Table 2). Based on the analysis of the area under curve, no suitable cut-offs for T-CPT or F-CPT levels that could be linked to moderate disease activity at the time of diagnosis was found (AUC=0.691 for F-CPT and 0.678 for T-CPT). DeLong's test showed no significant difference between those curves.

Association of T-CPT and F-CPT with microscopic, endoscopic and clinical activity scores

A good level of correlation was found between the median values of T-CPT and Geboes score ($k=0.65$, $p<0.001$) and median values of T-CPT and Nancy score ($k=0.62$, $p<0.001$). There was a weak correlation between T-CPT and UCEIS ($k=0.38$, $p=0.02$), PUCAI ($k=0.42$, $p=0.01$) and F-CPT ($k=0.44$, $p=0.01$).

We found a weak correlation between F-CPT and Geboes score (k=0.39, p=0.025), Nancy score (k=0.38, p=0.03) and UCEIS (k=0.36, p=0.039). There was no significant association between F-CPT and PUCAI (k=0.36, p=0.06).

Discussion

To the best of our knowledge, this is the first study exploring the contribution of the immunohistochemical assessment of T-CPT in children with IBD. CPT is a member of the S100 family and contributes to approximately 60% of the protein content in the cytosol of neutrophils [6]. Any active inflammatory process in the bowel mucosa results in leakage of CPT into the lumen and subsequently in the stool, where it can be detected and may serve as a non-invasive marker of the presence of active inflammation in the gut [18]. It appears to be a reliable marker for distinguishing inflammatory bowel disease from irritable bowel syndrome, which may share some common clinical symptoms as abdominal pain, bloating or diarrhea [6]. However, F-CPT yields some significant limitations. It represents a general marker of the active inflammation and is not disease specific, has no informative value in regards to the exact localization of the inflammation in the gut and may be false positive or false negative in some instances. Up to one quarter of healthy adult individuals show abnormal levels of the F-CPT according to some studies (probably due to presence of sporadic large bowel adenomas) [8]. On the other hand, levels of F-CPT can be decreased or even normalized iatrogenicaly by a bowel preparation procedure before colonoscopy [9]. Finally, there are published cases of patients with IBS and high levels of F-CPT, probably due to the inflammatory reaction to bowel dysbiosis [7]. There seems to be an intrapersonal day-to-day variability in F-CPT levels for both healthy persons and patients with IBD as well [19,20]. Moreover, the physiological levels of F-CPT are higher in children with the

maximum in the neonatal age and then declines till the adulthood [21]. A metanalysis from Degraeuwe et al. [22] reported a 17% rate of false negative results in children with IBD when using the standard cut-off 50ug/g.

Therefore, we aimed at the immunohistochemical assessment of the T-CPT in the bowel mucosa. We speculated that the direct visualization of the CPT+ inflammatory cells could more accurately reflect the actual level of the mucosal disease activity. Of course, the microscopic activity of the inflammation can be adequately assessed on the basis of the presence of neutrophils in the standard hematoxylin and eosin staining. However, in pediatric IBD, there is a lack of data about the predictive value of microscopy for complications development and its correlation with endoscopic or clinical scores of the activity. A study of Ashton et al. [23] demonstrated more extensive disease on the microscopic level compared to the endoscopic appearance in pediatric IBD. However, the study did not clarify, whether this increase in disease extent actually does reflect the real clinical presentation of the disease and whether it provides any predictive value in terms of future clinical course. The T-CPT staining may be beneficial especially for patients with no microscopic signs of inflammation activity in routine haematoxylin and eosin stain. In this setting, the direct visualization of CPT+ cells may assess the presence of subtle signs of the active inflammation more precisely. Therefore, we investigated, whether the T-CPT could improve the exactness of the histopathological assessment of the inflammation activity and better predict the complicated course of the disease. To this day, there are almost no studies regarding this topic. Only two studies engaged in IBD included the T-CPT in their data analysis and both of them were performed on the cohorts of adult patients. Guirgis et al. [24] found a good correlation of the low T-CPT with clinical, endoscopic and microscopic remission in adult patients with UC. On the other hand, elevated T-CPT (median value >5

cells/HPF) was associated with adverse clinical outcome. A work from Fukunaga et al. [3] focused at the correlation of the F-CPT with the serum CPT and fecal hemoglobin. The immunohistochemical assessment of the T-CPT was a secondary outcome only and the authors showed that the patients with IBD have higher counts of the CPT+ cells in the bowel mucosa compared to healthy individuals. No study ever examined the T-CPT as a possible prognostic marker or correlated it with other means of the assessment of the disease activity in children with IBD. In our work, we demonstrated a good correlation between T-CPT and both Geboes and Nancy histopathological scores. The T-CPT thus probably truly reflects the actual microscopic activity of the inflammation. However, we failed to link the levels of the T-CPT and the values of the Geboes and Nancy histopathological scoring systems with the development of the complications.

Regarding the F-CPT and its correlation with the endoscopic findings, the vast majority of the information stem from adult cohorts and the data for pediatric population are sparse. In the study of Fagerberg et al. [E], F-CPT correlated significantly with the endoscopic findings in the cohort of 39 pediatric patients with IBD. Ricciuto A et al. [25] showed that levels of F-CPT were associated with UCEIS values in children with IBD and primary sclerosing cholangitis. Our work confirms a significant, but only a weak correlation between F-CPT and UCEIS. For the clinical scores of the disease activity there is a considerable deal of children in clinical remission (defined as PUCAI < 10) with elevated F-CPT [26]. Our findings are in keeping with these observations, since we failed to prove any correlation between F-CPT and PUCAI. In contrast to previous findings [27], our work showed only a weak correlation of F-CPT with microscopic activity of the disease, neither in Geboes nor Nancy score.

The fundamental question is the ability of the F-CPT to predict the clinical outcome. According to several studies [28,29], level of the F-CPT adequately reflects the actual disease

activity with respect to sustained remission or relapse rate. However, there is a lack of data about the F-CPT at the time of diagnosis and its predictive value for the development of subsequent complications. In our study, the levels of the F-CPT at the first endoscopy predicted the necessity of the initiation of the systemic CS and 5ASA therapy. Besides, the moderate clinical disease activity (PUCAI > 40) was the predictor of those complications as well. These findings are in contrast to previous studies, where PUCAI values at the time of diagnosis were not associated with the necessity of immunomodulatory therapy or 5ASA treatment during 1 year of follow-up [30,31].

There are several possible limitations of the study. Only three patients with previous local 5ASA therapy were subsequently initiated with the systemic 5ASA treatment. Therefore, the association of the outcome C with the moderate disease activity and F-CPT levels, although significant, needs to be confirmed by subsequent studies. Another limitation may be the retrospective design of the study. The immunohistochemical staining of anti-CPT required new sections from archive paraffin blocks, which may lead to partial cutting of the material. Moreover, the retrospective assessment of the PUCAI from the electronic documentation could have a limited value. Finally, a large amount of the patients was not enrolled in the study due to strict inclusion criteria. However, since the included sub-cohort shared similar baseline characteristics with the excluded patients, the non-intentional selection bias is unlikely.

Conclusions

This is the first study aiming at the immunohistochemical assessment of the T-CPT in pediatric population generally. Despite its good correlation with the histopathological indexes of the disease activity, T-CPT failed to predict the complicated course of the disease.

Therefore, the usefulness of the histopathological assessment of the inflammation activity in the prediction of the complications in children with UC, whether in conventional staining or in specialized methods, remains uncertain. On the other hand, F-CPT levels at the time of diagnosis together with moderate disease activity based on the PCUAI score appeared to be independent predictors of the initiation of systemic CS and 5ASA therapy.

Funding

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Conflict of interest

The authors have no conflict of interest.

References

1. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J. Clin. Invest. 117 (2007) 514-521.

2. Turner D, Otley AR, Mack D et al. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. Gastroenterology. 133 (2007) 423-432.

3. Fukunaga S, Kuwaki K, Mitsuyama K et al. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int. J. Mol. Med. 41 (2018) 107-118.

4. Turner D, Levine A, Escher JC et al. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. J. Pediatr. Gastroenterol. Nutr. 55 (2012) 340-361.

5. Fagerberg UL, Lööf L, Lindholm J, Hansson LO, Finkel Y. Fecal calprotectin: a quantitative marker of colonic inflammation in children with inflammatory bowel disease. J. Pediatr. Gastroenterol. Nutr. 45 (2007) 414-420.

6. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin. Exp. Gastroenterol. 9 (2016) 21-29.

7. D'Angelo F, Felley C, Frossard JL. Calprotectin in Daily Practice: Where Do We Stand in 2017? Digestion. 95 (2017) 293-301.

8. Poullis A, Foster R, Shetty A, Fagerhol MK, Mendall MA. Bowel inflammation as measured by fecal calprotectin: a link between lifestyle factors and colorectal cancer risk. Cancer. Epidemiol. Biomarkers. Prev. 13 (2004) 279-284.

9. Kolho KL, Alfthan H, Hämäläinen E. Effect of bowel cleansing for colonoscopy on fecal calprotectin levels in pediatric patients. J. Pediatr. Gastroenterol. Nutr. 55 (2012) 751-753.

10. Levine A, Koletzko S, Turner D et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J. Pediatr. Gastroenterol. Nutr. 58 (2014) 795-806.

11. Travis SP, Schnell D, Krzeski P et al. Reliability and initial validation of the ulcerative colitis endoscopic index of severity. Gastroenterology. 145 (2013) 987-995.

12. Irani NR, Wang LM, Collins GS, Keshav S, Travis SPL. Correlation Between Endoscopic and Histological Activity in Ulcerative Colitis Using Validated Indices. J. Crohns. Colitis. 12 (2018) 1151-1157.

13. Xie T, Zhang T, Ding C et al. Ulcerative Colitis Endoscopic Index of Severity (UCEIS) versus Mayo Endoscopic Score (MES) in guiding the need for colectomy in patients with acute severe colitis. Gastroenterol. Rep. (Oxf) 6 (2018) 38-44.

14. Geboes K, Riddell R, Ost A, Jensfelt B, Persson T, Löfberg R. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut. 47 (2000) 404-409.

15. Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C et al. Development and validation of the Nancy histological index for UC. Gut. 66 (2017) 43-49.

16. Turner D, Ruemmele FM, Orlanski-Meyer E 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. 67 (2018) 257-291.

17. Turner D, Ruemmele FM, Orlanski-Meyer E et al. Management of Paediatric Ulcerative Colitis, Part 2: Acute Severe Colitis-An Evidence-based Consensus Guideline From the European Crohn's and Colitis Organization and the European Society of Paediatric Gastroenterology, Hepatology and Nutrition. J. Pediatr. Gastroenterol. Nutr. 67 (2018) 292-310.

18. Røseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion. 58 (1997) 176-180.

19. Lasson A, Stotzer PO, Öhman L, Isaksson S, Sapnara M, Strid H. The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. J. Crohns. Colitis. 9 (2015) 26-32.

20. Calafat M, Cabre E, Manosa M, Lobaton T, Marin L, Domenech E. High within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling? Inflamm. Bowel. Dis. 21 (2015) 1072-1076.

21. Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. J. Pediatr. Gastroenterol. Nutr. 34 (2002) 323-324.

22. Degraeuwe PL, Beld MP, Ashorn M et al. Faecal calprotectin in suspected paediatric inflammatory bowel disease. J. Pediatr. Gastroenterol. Nutr. 60 (2015) 339-346.

23. Ashton JJ, Coelho T, Ennis S et al. Endoscopic Versus Histological Disease Extent at Presentation of Paediatric Inflammatory Bowel Disease. J. Pediatr. Gastroenterol. Nutr. 62 (2016) 246-51.

24. Guirgis M, Wendt E, Wang LM et al. Beyond Histological Remission: Intramucosal Calprotectin as a Potential Predictor of Outcomes in Ulcerative Colitis. J. Crohns. Colitis. 11 (2017) 460-467.

25. Ricciuto A, Fish J, Carman N et al. Symptoms Do Not Correlate With Findings From Colonoscopy in Children With Inflammatory Bowel Disease and Primary Sclerosing Cholangitis. Clin. Gastroenterol. Hepatol. 16 (2018) 1098-1105.

26. Sipponen T, Kolho KL. Faecal calprotectin in children with clinically quiescent inflammatory bowel disease. Scand. J. Gastroenterol. 45 (2010) 872-877.

27. Hradsky O, Ohem J, Mitrova K et al. Fecal calprotectin levels in children is more tightly associated with histological than with macroscopic endoscopy findings. Clin. Lab. 60 (2014) 1993-2000.

28. Mooiweer E, Severs M, Schipper ME et al. Low fecal calprotectin predicts sustained clinical remission in inflammatory bowel disease patients: a plea for deep remission. J. Crohns. Colitis. 9 (2015) 50-55.

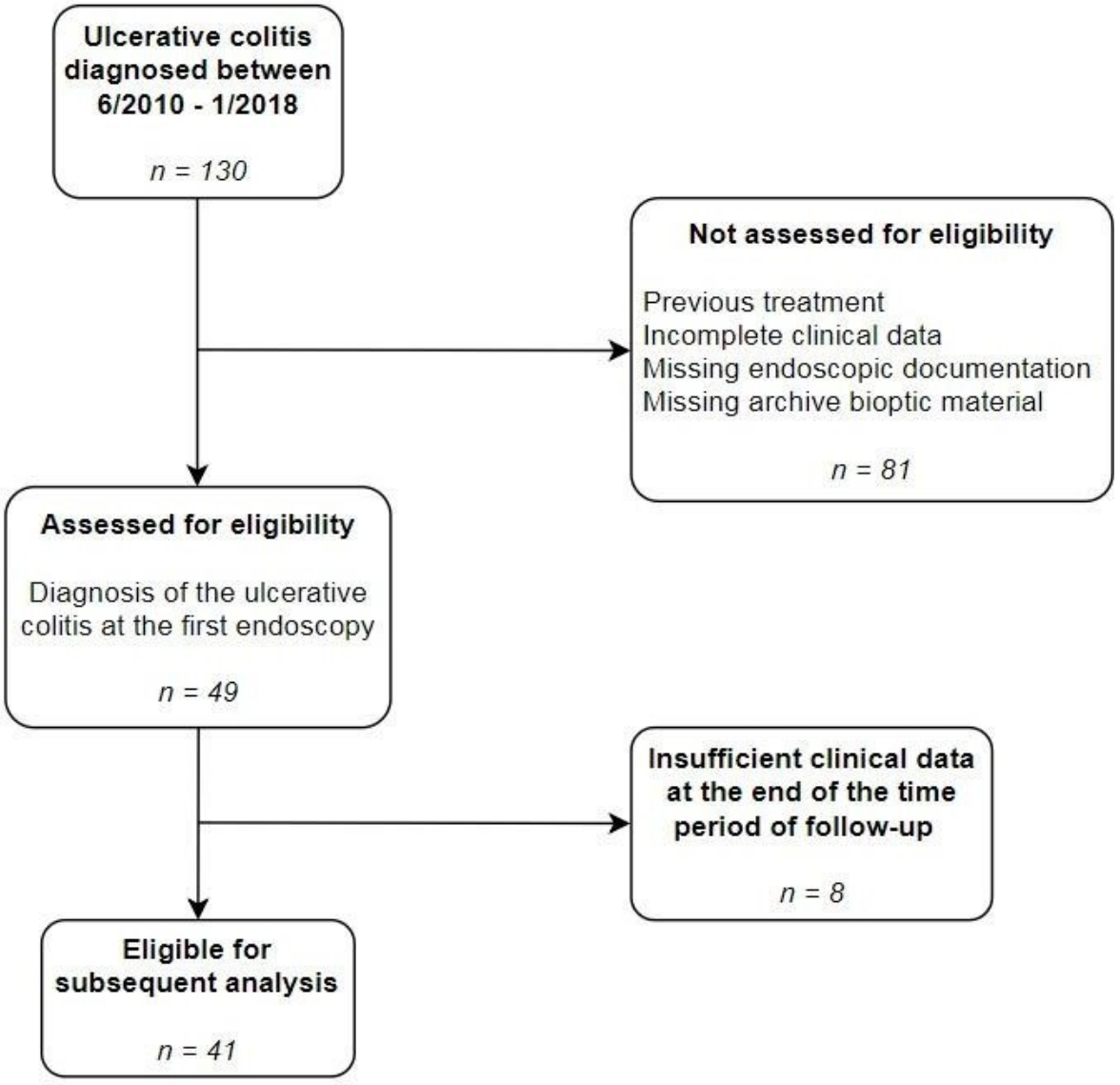
29. Theede K, Holck S, Ibsen P, Kallemose T, Nordgaard-Lassen I, Nielsen AM. Fecal Calprotectin Predicts Relapse and Histological Mucosal Healing in Ulcerative Colitis. Inflamm. Bowel. Dis. 22 (2016) 1042-1048.

30. Zeisler B, Lerer T, Markowitz J et al. Outcome following aminosalicylate therapy in children newly diagnosed as having ulcerative colitis. J. Pediatr. Gastroenterol. Nutr. 56 (2013) 12-18.

31. Mossop H, Davies P, Murphy MS. Predicting the Need for Azathioprine at First Presentation in Children with Inflammatory Bowel Disease. J. Pediatr. Gastroenterol. Nutr. 47 (2008) 123-129.

Figure legends

1. Flowchart of included and excluded patients.

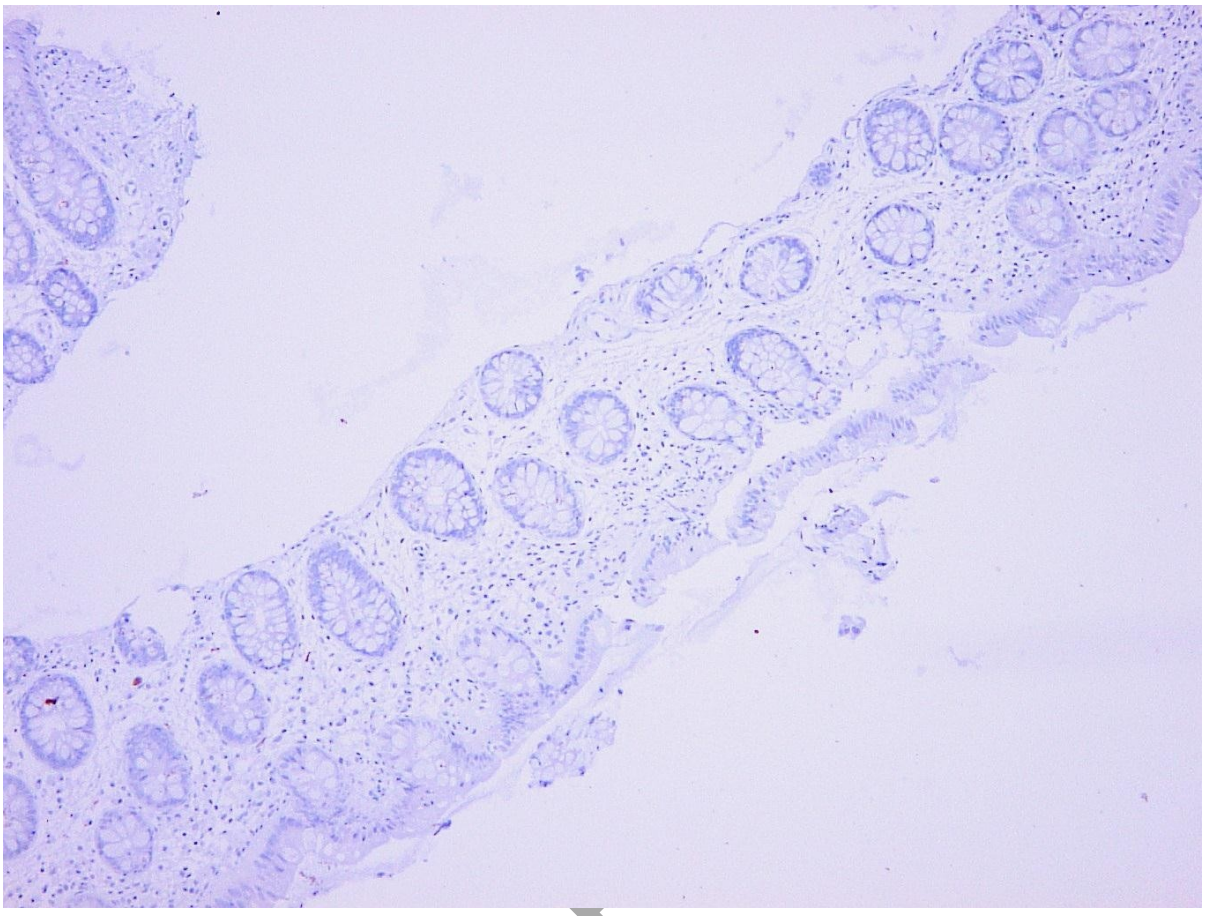


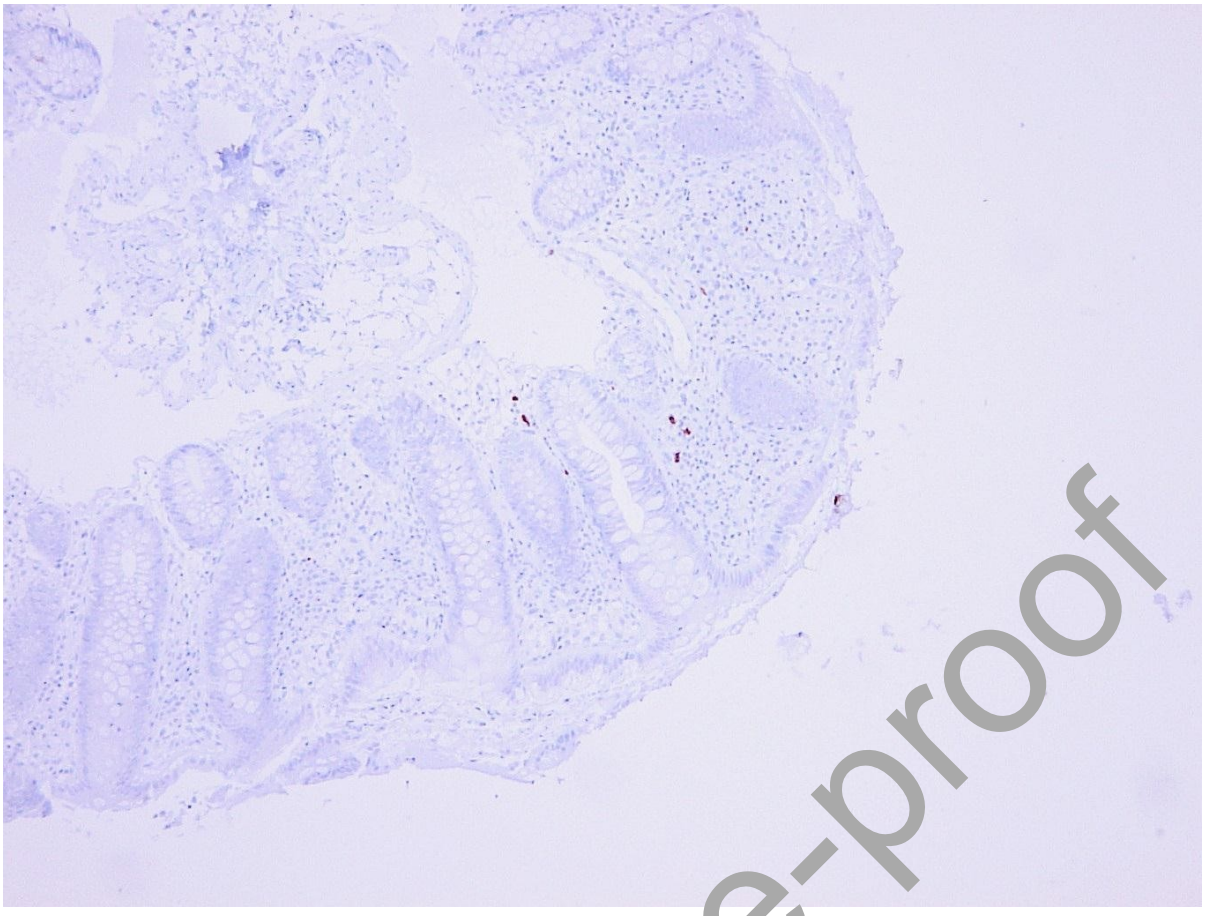
2. Photomicrographs showing calprotectin (CPT) positive cells by immunohistochemistry (×400):

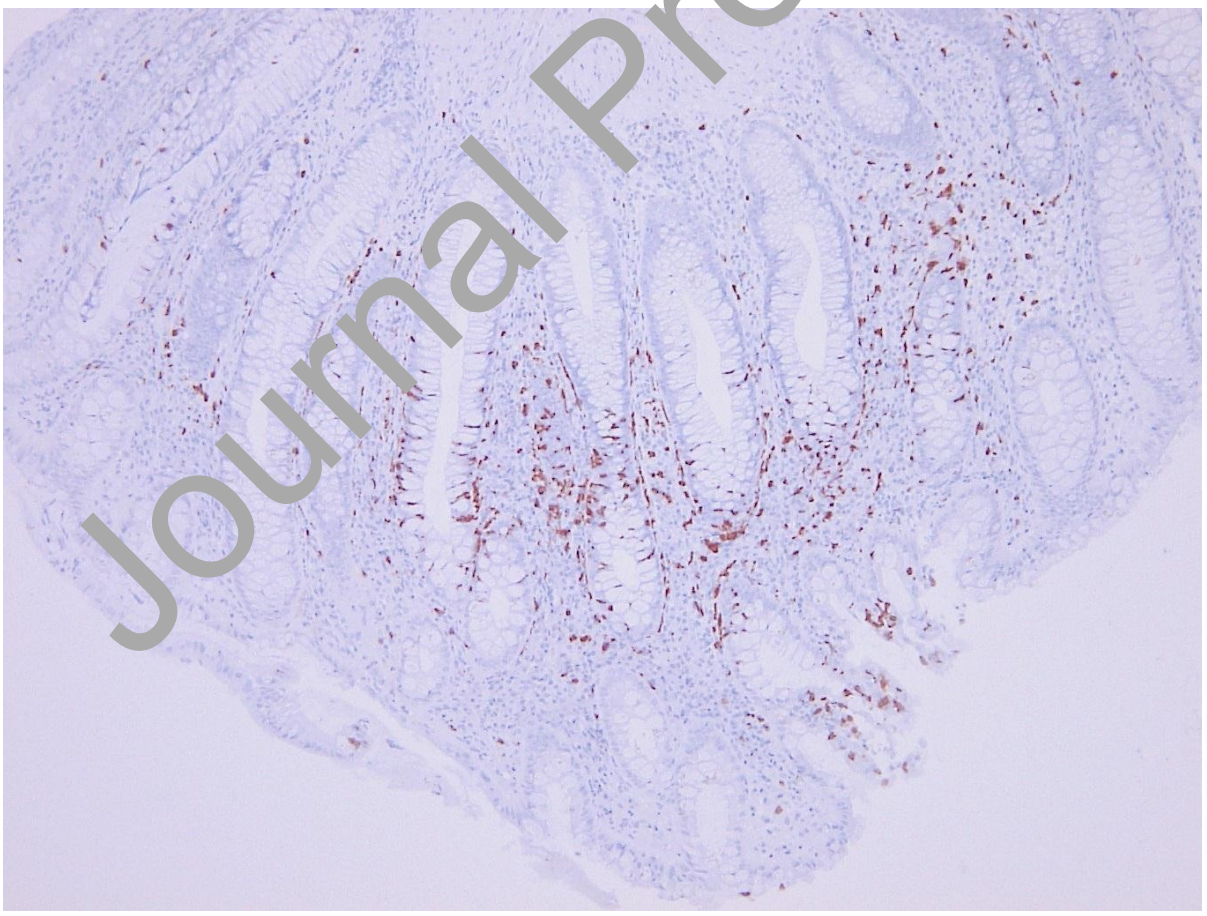
A. Bioptic sample of colonic mucosa devoid of any inflammation. No CPT+ cells are present.

B. Bioptic sample of colonic mucosa with mild chronic inflammation. Scarce CPT+ cells in the lamina propria.

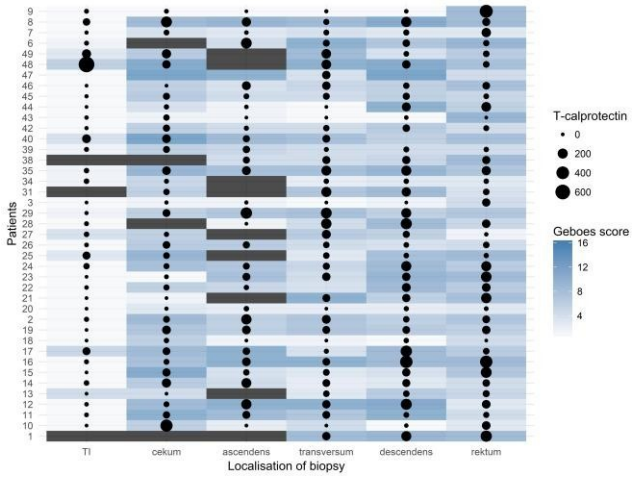
C. Bioptic sample of colonic mucosa with marked chronic inflammation. Numerous CPT+ cells in the lamina propria and the epithelium.







3. Heat map reflecting the distribution of the Geboes histopathological score values and numbers of the CPT+ cells in all bowel segments.



4. Heat map reflecting the distribution of the Nancy histopathological score values and numbers of the CPT+ cells in all bowel segments.

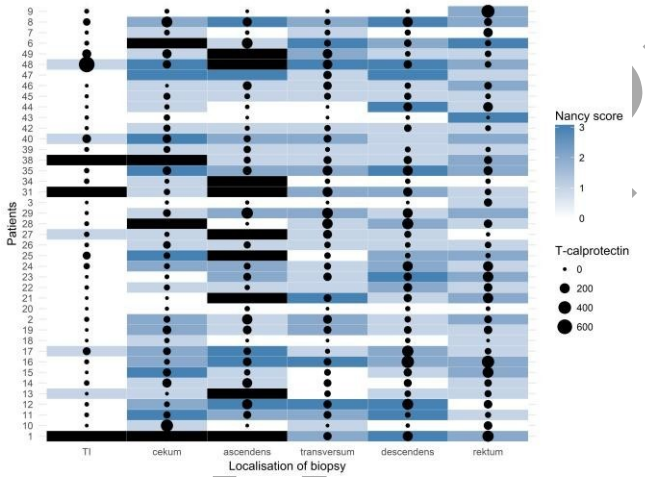


Table 1: Demographic and clinical characteristics of the patients with UC at the end of the minimal follow-up

Number of the patients	41
Sex, male, n (%)	23 (56.10)
Age at diagnosis, years, median (IQR)	12 (7-15)
Acute Severe colitis, n (%)	2 (4.88)
Anti-TNF, n (%)	11 (26.83)
Colectomy, n (%)	0 (0)
Systemic CS, n (%)	12 (29.27)
Systemic 5ASA, n (%)	3 (7.32)

UC = ulcerative colitis; IQR = interquartile range; anti-TNF = anti-tumor necrosis factor therapy; CS = corticotherapy; 5ASA = 5-aminosalicylic acid treatment

Table 2: Univariate Cox proportional hazards regression analysis of PUCAI > 40 and T-CPT as the predictors of complicated course of the disease

		Risk Ratio (CI)	P
PUCAI > 40	Endpoint A	2.237 (0.539-9.291)	0.268
	Endpoint B	**2.98 (1.011-8.787)**	**0.048**
	Endpoint C	**2.98 (1.011-8.787)**	**0.048**

		Risk Ratio (CI)	P
log(T-CPT)	Endpoint A	2.447 (0.873-6.859)	0.089
	Endpoint B	**2.422 (1.042-5.631)**	**0.04**
	Endpoint C	**2.517 (1.115-5.681)**	**0.026**

T-CPT = tissue calprotectin; CI = confidence interval; PUCAI = Pediatric Ulcerative Colitis Activity Index

Bold value highlights a statistically significant result.

PŘÍLOHA IV

Histopatologické hodnocení intenzity a aktivity zánětu u zánětlivých střevních onemocnění: Důležitý doplněk endoskopie nebo marná snaha?

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SOUHRN

Prohlubováníznalostína polizánětlivých střevních onemocněnís sebou nesezměnu terapeutických cílů. Zatímcodříve bylosnahou gastroenterologů zmírnění pacientových symptomů, dnes je diskutována zejména otázka slizničního hojení a dosažení endoskopické, případně i mikroskopické remise. Do popředí se tak dostává nutnost objektivního posouzení mikroskopické intenzity a aktivity zánětu, k čemuž mohou sloužit histopatologické skórovací indexy. Jejich reálný přínos je však dosud nejasný.

Cílem tohoto přehledového článku je podat informaci o dostupných histopatologických skórovacích indexech pro ulcerózní kolitidu (UC) a Crohnovu chorobu (CD) a zamyslet se nad jejich přínosem a limitacemi. Systematickou literární rešerší v databázích OVID SP MEDLINE, OVID EMBASE a The Cochrane library bylo nalezeno 19 skórovacích indexů pro UC a 4 pro CD. Naprostá většina z nich však není validována a jejich přínos stran predikce klinického průběhu onemocnění je sporný. Endoskopie tak stále zůstává zlatým standardem hodnocení intenzity a aktivity zánětlivého střevního onemocnění.

Klíčová slova: Zánětlivé střevní onemocnění – Crohnova choroba – ulcerózní kolitida – histopatologie – skórovací index

Histopathological assessment of the intensity and activity of the inflammation in inflammatory bowel diseases: An important addition to the endoscopy, or a pointless effort?

SUMMARY

Expanding amount of knowledge about inflammatory bowel diseases has changed current therapeutic goals. In the past times, the main effort of the gastroenterologists was to alleviate patients' symptoms. But nowadays, one of the hot topics is a mucosal healing and achieving the endoscopic, eventually even microscopic remission. Therefore, the objective assessment of the microscopic intensity and activity of the inflammation starts to assume its importance and histopathological scoring systems can represent an useful tool. However, their actual contribution is ill-defined.

Theaim of this reviewistoinform about availablehistopathological scoring systems for ulcerativecolitis(UC) and Crohn's disease (CD) and discuss theirbenefits and limitations. A systematic literature search in databases OVID SP MEDLINE, OVID EMBASE a The Cochrane library found 19 scoring indexes for UC and 4 for CD were found. The vast majority of them are not validated and their benefit for prediction of the clinical outcome is controversial. Endoscopy still represents a gold standard in the assessment of the extent of the bowel inflammation.

Keywords: Inflammatory bowel disease – Crohn's disease – ulcerative colitis – histopathology – scoring index

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Když v roce 1932 Burill Bernard Crohn publikoval článek s názvem „Regional ileitis: A pathologic and clinical entity" (1), nepřímo tak definoval novou skupinu onemocnění, známou jako zánětlivá střevní onemocnění (IBD). Crohnova choroba (CD) a ulcerózní kolitida (UC) se tak na následujících téměř sto let staly jedním z hlavních témat gastroenterologů a gastrointestinálních patologů. V průběhu doby se však pohled na danou problematiku zásadně změnil, a to s ohledem na definice, klasifikace, diagnostiku i léčbu. IBD již dávno nejsou „pouhými záněty střev", v dnešní době je chápeme jako chronická systémová zánětlivá onemocnění s predilekcí k trávicímu traktu, jejichž etiologie je neznámá a patogeneze přinejmenším nejasná (2-4). Nové práce na poli IBD jsou publikovány téměř na denním pořádku a prohlubující se znalosti o této skupině chorob s sebou přinášejí i zásadní změny ve strategii léčby včetně vytyčení nových terapeutických cílů. Zatímco dříve bylo hlavní snahou gastroenterologů zmírnění pacientových symptomů, v dnešní době je diskutováno zejména tzv. slizniční hojení (5-11), neboli dosažení endoskopické remise, kterou definuje absence endoskopicky viditelných známek zánětu (2,5). Negativní endoskopický nález však zdaleka nemusí znamenat negativní nález mikroskopický (12-14). Dle dostupných studií až 37 % pacientů s CD v klinické a endoskopické remisi vykazuje mikroskopické zámky přetrvávajícího zánětu (15,16) a u pacientů s UC údaje kolísají od 16 % až po 100 % pacientů (17). Do popředí tak vstupuje otázka objektivizace mikroskopické intenzity a aktivity zánětu pomocí histopatologických skórovacích indexů.

Účelem tohoto přehledového článku je poskytnout informaci o dostupných histopatologických skórovacích indexech pro CD a UC a zamyslet se nad aktuálním stavem mikroskopického hodnocení aktivity zánětu IBD s jeho benefity i limitacemi.

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METODIKA

Byl proveden bibliografický průzkum od data prvních uveřejněných publikací až do 10. 10. 2018 v následujících databázích: OVID SP MEDLINE, OVID EMBASE a The Cochrane library. Použitá klíčová slova zahrnovala „inflammatory bowel disease“; „inflammatory bowel diseases“;„ulcerativecolitis“;„colitis“;„crohn“; „crohn's“; „mucosal healing“; „histologic healing“; „histologic remission“; „histopathological remission“; „microscopic remission“; „scoring system“; „scoring index“; „histological scoring“; „histopathological scoring“;„pathologicalscoring“;„activity assessment“. Vpřípadě, ženěkterýzdřívějších dostupnýchpřehledových článků citoval publikaci, která touto metodikou nebyla nalezena, byla tato publikace přidána ručně.

VÝSLEDKY

Celkově bylo nalezeno 2842 publikací. Z průzkumu byly vyloučeny studie v jiném než anglickém a českém jazyce, kazuistická sdělení, abstrakta a studie na zvířatech. Dále byly vyřazeny práce, v nichž autoři ke svému výzkumu využívali skórovací indexy původně etablované jinými autory, nebo indexy určená pro jiná onemocnění. Po uplatnění vyřazovacích kritérií bylo nalezeno 19 skórovacích indexů pro UC a 4 pro CD (tab. 1) (13,16,18-38).

Histopatologické skórovací indexy pro ulcerózní kolitidu

Již v 60. letech minuléhostoletí Truelove& Richardsprezentovali jejich histopatologický skórovací systém pro UC (18), který je zároveň prvním doloženým histopatologickým skórovacím indexem pro IBD obecně. Jedním z nejpoužívanějších indexů se pak stalo Geboesovo skóre z roku 2000 (29). Hodnotí 7 morfologických znaků, mezi které patří 1) porucha architektoniky sliznice, 2) přítomnost neutrofilů v lamina propria, 3) eosinofily v lamina propria, 4) přítomnost neutrofilů v povrchovém epitelu, 5) kryptitida, 6) destrukce krypt a 7) slizniční defekty (eroze aulcerace). Každá zdílčíchproměnných jedálesubklasifikována na základě svojí tíže. Ačkoliv se takové skóre na první pohled může zdát příliš komplikované a v rutinní praxi obtížně použitelné (např. dělení rozsahu kryptitidy do 5 %, do 50 % a nad 50 % krypt ve vzorku), vykazuje překvapivě vysokouinterpersonální

Tabulka 1. Histopatologické skórovací systémy pro IBD.

IBD	Autor, rok	Charakteristika
UC	Truelove & Richards, 1956 (18)	Třístupňová škála: 1) bez zánětu; 2) mírný až střední zánět; 3) intenzivní zánět.
	Matts et al., 1961 (19)	Pětistupňová škála: 1) bez zánětu; 2) - 4) různá míra zánětlivé celulizace; 5) eroze, ulcerace nebo nekrózy sliznice.
	Watts et al., 1966 (20)	Čtyřstupňová škála: 0) bez zánětu, až po 3) intenzivní zánět.
	Korelitz et al., 1976 (16)	Kromě standardních mikroskopických znaků přidávají i rozpočet zánětlivých buněk.
	Powell-Tuck et al., 1982 (21)	Třístupňová škála: 1) bez zánětu; 2) mírný zánět; 3) střední až intenzivní zánět.
	Keren et al., 1984 (22)	Aktivní vs. inaktivní zánět.
	Friedman et al., 1986 (23)	Čtyřstupňová škála: 0) bez zánětu; 1) zánět v LP; 2) destrukce krypt; 3) ulcerace.
	Gomes et al., 1986 (24)	Pětistupňová škála: 0) bez zánětu, až po 4) intenzivní zánět s ulceracemi.
	Saverymuttu et al., 1986 (25)	Hodnotí 4 mikroskopické znaky: 1) poškození enterocytů; 2) abnormality krypt; 3) zánět v LP; 4) akutní zánět v LP. Každá proměnná hodnocena stupni 1-3.
	Floren et al., 1987 (26)	Šestistupňová škála: 0) bez zánětu, až po 5) intenzivní zánět a ulcerace.
	Riley et al., 1991 (13)	Hodnotí 6 mikroskopických znaků, každý klasifikován na čtyřstupňové škále intenzity.
	Hanauer et al., 1993 (27)	Čtyřstupňová škála: 0) bez zánětu, až po 3) high-grade aktivní IBD (na základě kombinace mikroskopických a endoskopických znaků).
	Sandborn et al., 1993 (28)	Čtyřstupňová škála: 0) inaktivní chronická kolitida, až po 3) výrazně aktivní chronická kolitida.
	Geboes et al., 2000 (29)	7 mikroskopických znaků s různými podstupni. Celkový rozsah skóre je od 0 po 5.4.
	Rutter et al., 2004 (30)	Pětistupňová škála: 0) bez zánětu, až po 4) intenzivní aktivní zánět.
	Baars et al., 2012 (31)	Čtyřstupňová škála: 0) absence aktivity zánětu, až po 4) závažná aktivita zánětu (početné pseudoabscesy).
	Nancy Index (Marchal-Bressenot et al.), 2015 (36)	Pětistupňová škála: 0) mírný neaktivní zánět nebo bez zánětu; 1) střední nebo intenzivní neaktivní zánět; 2) mírný aktivní zánět; 3) střední nebo intenzivní aktivní zánět; 4) závažné aktivní onemocnění (ulcerace).
	Robarts' s Histopathological Index (Mosli et al.), 2015 (37)	Čtyřstupňová škála: 1) chronický zánět; 2) neutrofily v LP; 3) neutrofily v epitelu; 4) eroze/ulcerace.
	Simplified Geboes Score (Jauregui-Amezaga et al.), 2016 (38)	7 mikroskopických znaků s různými podstupni. Celkový rozsah skóre je od 0 po 4.4.
CD	D'Haens et al., 1998 (32) *Později nazýván CGHAS a IGHAS*	Hodnotí 8 mikroskopických znaků na 16 stupňové škále.
	Nicholls et al., 1994 (33) *Etablován pro kontrolní biopsie u pacientů na léčbě*	Čtyřstupňová škála: 1) horší; 2) beze změny; 3) zlepšení; 4) vymizení zánětu
	Breese et al., 1995 (34)	5 mikroskopických znaků hodnocení na 4 stupňové škále.
	Baars et al., 2012 (35)	Pětistupňová škála: 0) neaktivní onemocnění, až po 4) intenzivní zánět (početné pseudoabscesy).

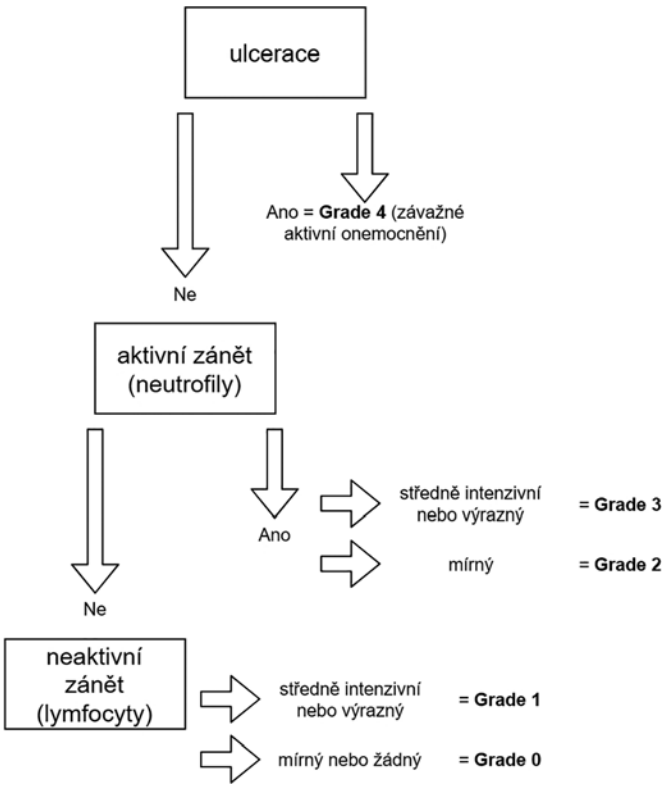
IBD = zánětlivá střevní onemocnění; UC = ulcerózní kolitida; CD = Crohnova choroba; LP = lamina propria; CGHAS = Colonic Global Histologic Activity Score; IGHAS = Ileal Global Histologic Activity Score.

shodu (kappa 0.70) (29). V roce 2017 bylo publikováno modifikované a zjednodušené Geboesovo skóre (38), do kterého byla nově zakomponována přítomnost bazální plazmocytózy.

Jak již bylo zmíněno v úvodu, značná část pacientů s UC v endoskopické remisi nečekaně vykazuje mikroskopické známky přetrvávajícího zánětu. Jelikož je UC charakterizována kontinuálnímarovnoměrněrozloženýmzánětem, dala by sepředpokládat vysoká míra shody mezi mikroskopickým a endoskopickým nálezem, a tudíž i histopatologickými a endoskopickými skórovacími indexy. Bohužel, studiena toto téma vykazují přinejmenším rozporuplné výsledky (21,24,39-41). Lemmens B et al. (40) se pokusili o korelaci Mayo Endoscopic Score (42) s dvěma pravděpodobně nejčastěji používanými histopatologickými indexy pro UC - Riley Score (13) a již výše komentované Geboesovo skóre (29). Zjištění, že pod Mayo 1, čili mírnou endoskopickou aktivitou, se skrývaly všechny histologické stupně aktivity zánětu, hovoří za vše. Na základě dostupných studií lze shrnout, že nejlepší korelace endoskopie a mikroskopie bývá u intenzivních floridních zánětů aunormálních nálezů, zatímco minimálníshoda panuje u mírného neaktivního zánětu (40).

Poněkud optimističtější výsledky u pacientů s UC přináší výzkum na téma histopatologie jako prognostického faktoru. Již od začátku panoval názor, že kombinace endoskopie a mikroskopie bude mít lepší výpovědní hodnotu stran klinického průběhu onemocnění než endoskopie samotná (43), což bylo v průběhudalšíchletdoloženouřadoudalšíchstudií(13,30,31,43-50). Například ve studii Riley et al. (13) mikroskopická intenzita zánětu predikovala u endoskopicky negativních pacientů klinický relaps v průběhu následujících 12 měsíců akonkrétně Geboesovo skóre se taktéž ukazuje být prediktorem klinického relapsu u pacientů s UC v klinické a endoskopické remisi (44,49).

V nedávné době byly etablovány dva nové histopatologické indexy pro UC - Nancy Index (36) a Robarts‘ Histopatholo-



Obr. 1. Nancy Indexpro histopatologické hodnocení intenzity a aktivity zánětu u ulcerózníkolitidy.

Tabulka 2. Global Histology Activity Score (GHAS). Používá se separátně pro terminální ileum a tlusté střevo.

Histopatologická změna	Grading
Poškození epitelu	0 = žádné; 1 = fokální; 2 = extenzivní
Porucha architektoniky	0 = žádná; 1 = mírná; 2 = závažná
Lymfoplazmocytární zánět v LP	0 = žádný; 1 = mírný; 2 = intenzivní
Neutrofily v LP	0 = žádné; 1 = mírně; 2 = intenzivně
Neutrofily v epitelu	1 = povrchový epitel; 2 = kryptitida; 3 = kryptový pseudoabsces
Eroze nebo ulcerace	0 = ne; 1 = ano
Granulom	0 = ne; 1 = ano
Počet postižených segmentů střeva	1 = <1/3; 2 = 1/3 – 2/3; 3 = >2/3

Každá proměnná je hodnocena nezávisle
Výsledné skóre je součet všech individuálních proměnných (max. = 16)
LP = lamina propria

gy Index (37). První jmenovaný používá pětistupňovou škálu hodnocení na základě přítomnosti ulcerací a intenzity aktivního a chronického zánětu (obr. 1). Robarts‘ Histopathology Index pak využívá čtyřstupňový grading (1 - chronický zánět, 2 - přítomnost neutrofilů v lamina propria, 3 - neutrofily v epitelu a 4 - přítomnost slizničních defektů). Jde o první skutečně validovaná skóre pro UC, která navíc vykazují shodu s endoskopickým nálezem. Ve studii od Irani NR et al. (51) byla prokázána dobrá korelace obou indexů s Ulcerative Colitis Endoscopic Index of Severity (52) i mezi oběma histopatologickými indexy navzájem. Stran jejich případného prediktivního významu zatím chybí dostatek dostupných informací.

Histopatologické skórovací indexy pro Crohnovu chorobu

Vzhledem k diskontinuálnímu charakteru zánětu jemikroskopické hodnocení intenzity a aktivity zánětu u Crohnovy choroby náročnější a dostupné skórovací indexy jsou limitované počtem i významem. Jediný plošněji používaný index pochází od D‘Haense et al. z roku 1998, který se v praxi ujal pod názvem Global Histologic Activity Score (GHAS) aječastoužívanýseparátněpro terminální ileum a tlusté střevo (jako tzv. Colonic a Ileal GHAS) (32,53,54). Tentoindexhodnotí 1) poškozeníepitelu, 2) poruchu architektoniky sliznice, 3) chronický zánět v lamina propria, 4) neutrofilyv laminapropria, 5) neutrofilyv epitelu, 6) přítomnost slizničních defektů a 7) přítomnost epiteloidních granulomů, k čemuž následně přičítá počet postižených segmentů střeva (tab. 2). Nenívšak validovaný ajedoložená jeho špatná korelace s endoskopickými indexy (24,54).

Prognostický význam histopatologie je v případě CD sporný. V jediné nalezenéstudiinatototéma Baars et al. (31) nebylaprokázána asociace histopatologického nálezu s klinickým relapsem onemocnění, rozvojem striktury či nutností chirurgického zákroku.

Histopatologické skórovací indexy pro pediatrická IBD

Histopatologický skórovací index primárně etablovaný pro dětská IBD nebyl doposud vytvořen (55) a mikroskopické hodnocení intenzity a aktivity zánětu tak u dětí s IBD zůstává čistě subjektivní. Nejasný je i přínos histopatologie stran predikce klinického průběhu onemocnění. Podle recentních studií (56,57) zakomponování mikroskopie do Pařížské klasifikace (58) (která dětská IBD fenotypicky klasifikuje na základě klinických charakteristik a endoskopického nálezu) výrazně zvýšilo hodnocený rozsah zánětlivého postižení u dětí s CD, UC i neklasifikovanou IBD (IBDU). Z daných studií však nevyplývá, nakolik toto odráží

klinickou prezentaci choroby a zda mikroskopie přinesla reálnou přidanou hodnotu stran predikce následného klinického průběhu.

Tuto otázku se naše supina pokusila zodpovědět v roce 2017 ve studii s názvem *„Low predictive value of histopathological scoring system for complications development in children with Crohn's disease."* (59). Provedli jsme retrospektivní analýzu 63 dětí s nově diagnostikovanou CD. Zpětně byly zhodnoceny vstupní etážové biopsie před zahájením terapie a histopatologická intenzita a aktivita zánětu byla objektivizována za použití výše zmíněného Global Histology Activity Score (32). Následně byla hledána asociace s endoskopickou aktivitou zánětu stanovenou pomocí široce užívaného endoskopického indexu Simple Endoscopic Score for Crohn's Disease (SES-CD) (60), a dále s Pediatric Crohn's Disease Activity Index (PCDAI), kterýpředstavuje jedno z nejpoužívanějších klinických skóre aktivity pediatrické CD (61). Zároveň jsme zjišťovali, zda lze na základě histopatologie predikovat rozvoj komplikací v průběhu následujícího roku. Bohužel, mikroskopická aktivita zánětu, stanovená za pomocí GHAS, se neukázala být prediktorem komplikovaného průběhu onemocnění (na rozdíl od SES-CD) a nekorelovala s aktivitou zánětu, stanovenou za pomocí SES a PCDAI.

DISKUZE

Vsoučasnédobějeznámoněkolikdesítekskórovacíchindexů, z nichž jen minimum je validovaných, vykazují slabou korelaci s endoskopickým nálezem a jejich význam stran predikce klinického průběhu onemocnění je limitovaný (17,62,63). V mnoha případech šlo o jednoduchá skóre o několika proměnných, které byly sestaveny skupinami autorů pro potřeby jejich vlastních studií a nebyly určeny primárně pro běžnou rutinní bioptickou praxi. Již samotný fakt, že jsme v pravidelných intervalech konfrontováni se stále novými indexy, nepřímo naznačuje, že doposud stále selháváme ve snaze nalézt jeden ideální, který by výše zmíněné náležitosti splňoval. I z těchto důvodů zatím dosažení histologické remise není považováno za primární terapeutický cíl (5,64,65). Příčiny je možné nalézat v průběhu celého multidisciplinárního diagnostického procesu, od vlastních biologických charakteristik onemocnění přes techniku endoskopického vyšetření, bioptický odběr vzorků, zpracování materiálu až po samotné histopatologické hodnocení a jeho interpretaci:

Kolísání rozsahu zánětu

Vlastní biologická povaha CD se svým kolísavým charakterem zánětu na makroskopické i mikroskopické úrovni značně limituje množství dostupných histologických indexů i jejich využitelnost v praxi. Endoskopické vyšetření, které dokáže přehlédnout celý povrch střevní sliznice, má v takto ložiskově změněném terénu lepší vypovídající hodnotu než bioptické vzorky milimetrových rozměrů. V případě léčené IBD je však situace ještě složitější. Všeobecně známý fakt, že dlouhodobě léčená UC může taktéž vykazovat diskontinuální charakter zánětu (66,67), tak značně limituje přínos mikroskopie i v tomto případě.

Postižení tenkéhostřeva ahorního gastrointestinálního traktu

CD i UC jsou systémová zánětlivá onemocnění se schopností postihnout jakoukoliv oblast trávicí trubice (2-4). Na celkové závažnosti onemocnění se tak může podílet i postižení horního gastrointestinálního traktu, jehož hodnocení zatím není součástí žádného dostupného indexu, endoskopického ani mikroskopického. U dospělých pacientů, na rozdíl od pediatrické IBD, není horní endoskopie ani součástí oficiálních doporučení pro diagnostiku IBD (3,68).

Endoskopické vyšetření dokáže postihnout pouze proximální (duodenum a někdy proximální část jejuna) a distální (terminální ileum) úsek tenkého střeva, avšak právě přítomnost zánětu v tenkém střevě může být pro další klinický průběh choroby určující. Proto byly v posledních letech etablovány indexy aktivity zánětu tenkého střeva pro kapslovou endoskopii. Mezi nejpoužívanější patří Lewis Score (69) a Capsule Endoscopy Crohn's Disease Activity Index (70), které doporučují i European Crohn's and Colitis Organisation a European Society of Gastrointestinal Endoscopy.

Nemožnost posouzení transmurality zánětu

Crohnova choroba, a v některých případech i ulcerózní kolitida, mohou vykazovat zánětlivé postižení hlubších struktur střevní stěny. Histopatologické i endoskopické vyšetření však podává informaci pouze o luminální aktivitě zánětu. Transmuralita zánětu je jedním z definujících prvků CD a spolupodílí se na celkovém klinickém stavu pacienta a rozvoji případných komplikací. Například záchyt myenterické plexitidy v resekčních okrajích predikuje časný relaps onemocnění po ileocékální resekci (71). Z tohoto důvodu se v posledních letech objevuje snahaovytvořenívalidníhoindexu transmurálníaktivityzánětu. Jedním z nich je tzv. Lémann Index (72), který však představuje klinické skóre, nikoliv histopatologické, a hodnotí kumulativní postiženístřevnístěnyna základěpřítomnostistriktur, penetrujícího postižení (přítomnost píštělí a abdominálních abscesů), předchozích chirurgických výkonů a perianálního postižení. V některých případech je dokonce bioptický odběr natolik povrchový, že nezachytí ani bazální partie lamina propria mucosae a znemožní tak posouzení například bazální plazmocytózy, která je součástí některých skórovacích indexů, jako je kupříkladu již zmíněné modifikované Geboesovo skóre (38).

Počet odebraných vzorků

Na základě oficiálních doporučení European Society of Pathology a European Crohn's and Colitis Organisation (2-4) by měl bioptický odběrv dobědiagnózy zahrnovat minimálnědva vzorkyzpřinejmenším pětietážístřeva, mezikterýmibynemělo chybětterminálníileumarektum. Doposud všakneexistuje oficiální stanovisko stran minimálního počtu vzorků v rámci kontrolních endoskopií (4). Vběžné gastroenterologické praxi mnohdy stále přetrvává tendence nebioptovat endoskopicky negativní úseky střeva, a naopak provádět bohatý samplingv intenzivně zánětlivých změněných úsecích, čímž dochází k falešnému podhodnocení, respektive nadhodnocení mikroskopické intenzity a aktivity zánětu v závislosti na endoskopickém obrazu. Tato diskrepance se může negativně odrazit i na výpovědní hodnotě některých samotných skórovacích indexů jako například GHAS (32), který do hodnocení započítává ipočet postižených segmentů střeva.

Histopatologické skóre u vstupních endoskopií

Jedním z hlavních důvodů odběru biopsie v rámci kontrolních endoskopií je zjistit stav mikroskopické intenzity a aktivity zánětu a tím získat informaci o účinnosti dosavadní terapie. Pro klinického lékaře a jeho následný rozhodovací proces je však stěžejní i mikroskopická intenzita zánětu v době diagnózy. Nicméně, patolog představuje pouze jednu z diagnostických modalit, definitivní diagnóza IBD je klinická diagnóza, kterou stanovuje příslušný klinický lékař až po kompletaci výsledků všech provedených vyšetření (2-4,68). Patolog tak ve fázi histopatologického hodnocení vzorků ze vstupní etážové biopsie neví, jaká bude výsledná diagnóza. Zejména pokud nemá k dispozici validní klinické informace, může se jeho diagnóza omezit na „chronickou nespecifickou kolitidu" (respektive enterokolitidu), přičemž pacient bude z klinického pohledu vykazovat přesvědčivé známky

CD s recidivujícími perianálnímiabscesy, sonografickýmztluštěním kliček tenkého střeva, mezenteriální lymfadenopatií a pozitivitouprotilátekproti Saccharomyces cerevisiae. Naopak, nález floridní chronické terminální ileitidy a kolitidy s poruchou architektoniky sliznice a záchytem drobných rozvolněných epiteloidních granulomů může patologa bez validní klinické korelace mylně svést na scestí diagnózy CD, ačkoliv ve skutečnosti půjde o malé dítě se septickými stavy nejasné etiologie a následně geneticky potvrzenou diagnózou chronické granulomatózní choroby. Vzhledem k faktu, že se histopatologické skórovací indexy liší pro CD a UC, je jejich použití v době vstupní etážové biopsie možné až zpětně, po stanovení definitivní klinické diagnózy. Celkový problém podtrhuje i absence jakéhokoliv skórovacího indexu pro IBDU, přičemž tato diagnóza tvoří 10-15 % dospělých IBD a cca 5-7 % dětských IBD a mnohdy se stane diagnózou definitivní (73-76).

Hodnocení slizničních defektů

Pro většinu histopatologických indexů je přítomnost erozí a/nebo ulcerací známkou maximální intenzity zánětu a jsou hodnoceny nejvyššími stupni ve svých škálách. Příkladem je výše zmíněný Nancy Index (36), pro který jakákoliv přítomnost ulcerace automaticky znamená nejvyšší grade bez ohledu na charakter zánětu v okolní sliznici. Z hlediska endoskopie a potažmo i celkového klinického stavu pacienta je však zcela zásadní, jestli mikroskopicky popsaný defekt sliznice pochází z jedné drobné aftózní léze či se jedná o střevo s extenzivně ulcerovaným povrchem ve většině svého rozsahu. Jiné indexy jako např. Geboesovo skóre (29) naopak rozlišují mezi erozemi a ulceracemi. Standardní endoskopický odběr vzorku ze slizničních defektů by však měl zastihnout okraj defektu s přechodem do zachovalé sliznice. Co se tak v mikroskopu jeví jako mělká eroze, může být ve skutečnosti okrajový odběr z hlubšího defektu.

ZÁVĚR

Ačkoliv má histopatologie nezastupitelnou úlohu v diagnostice IBD, její přínos stran hodnocení intenzity a aktivity zánětu je stále omezený. Jak již bylo zmíněno, příčinou je samotná biologická povaha onemocnění, diskrepance s endoskopií i technické aspekty odběru vzorků. Zůstává však otázkou, zda tyto nedostatky odrážejí skutečné limitace mikroskopie azda nejsou spíše vyvolány uměle. Z analýzy předchozích prací totiž vyplývá, že současná představa ideálního histopatologického skórovacího systému je validovaný index s dobrou interpersonální shodou, který koreluje s endoskopickým nálezem a zároveň slouží jako prediktor klinického průběhu onemocnění. Zjevná snaha o co nejlepší korelaci mikroskopie a endoskopie, což zároveň představuje i jeden z hlavních indikátorů validity, však popírá samotnou smysluplnost takového indexu, protože devalvuje mikroskopii nametodu, kterámůže býtmaximálně stejně dobrá jako endoskopie. Diskrepance mezi endoskopií a histopatologií je tak naopak žádoucí. Histopatologický index by neměl endoskopický nález potvrzovat, nýbrž zpřesňovat a představovat tak přidanou hodnotu k celkové objektivizaci tíže zánětlivého onemocnění. Jedině tak může být případný skórovací index využíván v rutinní bioptické praxi a nesloužit pouze jako nástroj objektivizace dat pro potřeby studií. Další výzkum by se tak měl zaměřit spíše napřínos histopatologie jakožto prediktoru klinického průběhu onemocnění.

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Autor práce prohlašuje, že v souvislosti s tématem, vznikem a publikací tohoto článku není ve střetu zájmů a vznik ani publikace článku nebyly podpořeny žádnou farmaceutickou firmou.

LITERATURA

1. **Crohn BB, Ginzburg L, Oppenheimer GD.** Regional Ileitis: A Pathologic and Clinical Entity. *JAMA* 1932; 99(16): 1323-1329.
2. **Magro F, Gionchetti P, Eliakim R, et al.** Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J Crohns Colitis* 2017; 11(6):649-670.
3. **Gomollón F, Dignass A, Annese V, et al.** 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management. *J Crohns Colitis* 2017; 11(1): 3-25.
4. **Magro F, Langner C, Driessen A, et al.** European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis* 2013; 7(10): 827-851.
5. **Travis SP, Higgins PD, Orchard T, et al.** Review article: defining remission in ulcerative colitis. *Aliment Pharmacol Ther* 2011; 34(2): 113-124.
6. **Daperno M, Castiglione F, de Ridder L, et al.** Results of the 2nd part Scientific Workshop of the ECCO. II: Measures and markers of prediction to achieve, detect, and monitor intestinal healing in inflammatory bowel disease. *J Crohns Colitis* 2011; 5(5): 484-498.
7. **Peyrin-Biroulet L, Ferrante M, Magro F, et al.** Results from the 2nd Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel disease. *J Crohns Colitis* 2011; 5(5): 477-483.
8. **Stange EF, Travis SP, Vermeire S, et al.** European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut* 2006; 55(Suppl 1): i1-i15.
9. **Reinisch W, Van Assche G, Befrits R, et al.** Recommendations forthetreatment of ulcerative colitis with infliximab: a gastroenterology expert group consensus. *J Crohns Colitis* 2012; 6(2): 248-258.
10. **Ardizzone S, Cassinotti A, Duca P, et al.** Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol* 2011; 9(6): 483-189.
11. **Froslie KF, Jahnsen J, Moum BA, et al.** Mucosal healing in inflammatory bowel disease: results from a Norwegian population based cohort. *Gastroenterology* 2007; 133(2): 412-422.
12. **Korelitz BI.** Mucosal healing as an index of colitis activity: back to histological healing for future indices. *Inflamm Bowel Dis* 2010; 16(9): 1628-1630.
13. **Riley SA, Mani V, Goodman MJ, et al.** Microscopic activity in ulcerative colitis: what does it mean? *Gut* 1991; 32(2): 174-178.
14. **Rosenberg L, Nanda KS, Zenlea T, et al.** Histologic markers of inflammation in patients with ulcerative colitis in clinical remission. *Clin Gastroenterol Hepatol* 2013; 11(8):991-996.
15. **Molander P, Sipponen T, Kemppainen H, et al.** Achievement of deep remission during scheduled maintenance therapy with TNFalpha-blocking agents in IBD. *J Crohns Colitis* 2013; 7(9): 730-735.
16. **Korelitz BI, Sommers SC.** Response to drug therapy in Crohn'sdisease: evaluation byrectal biopsy and mucosal cell counts. *J Clin Gastroenterol* 1984; 6(2): 123-127.
17. **Bryant RV, Winer S, Travis SP, Riddell RH.** Systematic review: histologicalremission ininflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. *J Crohns Colitis* 2014; 8(12): 1582-1597.
18. **Truelove SC, Richards WC.** Biopsy studies in ulcerative colitis. *Br Med J* 1956; 1(4979): 1315-1318.
19. **Matts SG.** The value of rectal biopsy in the diagnosis of ulcerative colitis. *Q J Med* 1961; 30: 393-407.
20. **Watts JM, Thompson H, Goligher JC.** Sigmoidoscopy and cytology in the detection of microscopic disease of the rectal mucosa in ulcerative colitis. *Gut* 1966; 7(3): 288-294.
21. **Powell-Tuck J, Day DW, Buckell NA, et al.** Correlations between defined sigmoidoscopic appearances and other measures of disease

activity in ulcerative colitis. *Dig Dis Sci* 1982; 27(6): 533-537.
22. **Keren DF, Appelman HD, Dobbins III WO, et al.** Correlation of histopathologic evidence of disease activity with the presence of immunoglobulin-containing cells in the colons of patients with inflammatory bowel disease. *Hum Pathol* 1984; 15(8): 757-763.
23. **Friedman LS, Richter JM, Kirkham SE, et al.** 5-Aminosalicylic acid enemas in refractory distal ulcerative colitis: a randomized, controlled trial. *Am JGastroenterol* 1986; 81(6): 412-418.
24. **Gomes P, du Boulay C, Smith CL, et al.** Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut* 1986; 27(1): 92-95.
25. **Saverymuttu SH, Camilleri M, Rees H, et al.** Indium 111-granulocyte scanning in the assessment of disease extent and disease activity in inflammatory bowel disease. A comparison with colonoscopy, histology, and fecal indium 111-granulocyte excretion. *Gastroenterology* 1986; 90(5 Pt 1): 1121-1128.
26. **Floren CH, Benoni C, Willen R.** Histologic and colonoscopic assessment of disease extension in ulcerative colitis. *ScandJGastroenterol* 1987; 22(4): 459-462.
27. **Hanauer S, Schwartz J, Robinson M, et al.** Mesalamine capsules for treatment of active ulcerative colitis: results of a controlled trial. Pentasa Study Group. *Am J Gastroenterol* 1993; 88(8): 1188-1197.
28. **Sandborn WJ, Tremaine WJ, Schroeder KW, et al.** Cyclosporine enemas for treatment-resistant, mildly to moderately active, left-sided ulcerative colitis. *Am J Gastroenterol* 1993; 88(5): 640-645.
29. **Geboes K, Riddell R, Ost A, et al.** A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. *Gut* 2000; 47(3): 404-409.
30. **Rutter M, Saunders B, Wilkinson K, et al.** Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 2004; 126(2): 451-459.
31. **Baars JE, Nuij VJ, Oldenburg B, et al.** Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis* 2012; 18(9): 1634-1640.
32. **D'Haens GR, Geboes K, Peeters M, et al.** Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998; 114(2): 262-267.
33. **Nicholls S, Domizio P, Williams CB, et al.** Cyclosporin as initial treatment for Crohn's disease. *Arch Dis Child* 1994; 71(3): 243-247.
34. **Breese EJ, Michie CA, Nicholls SW, et al.** The effect of treatment on lymphokine-secreting cells in the intestinal mucosa of children with Crohn's disease. *Aliment Pharmacol Ther* 1995; 9(5): 547-552.
35. **Baars JE, Nuij VJ, Oldenburg B, et al.** Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis* 2012; 18(9): 1634-1640.
36. **Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C, et al.** Development and validation of the Nancy histological index for UC. *Gut* 2017; 66(1): 43-49.
37. **Mosli MH, Feagan BG, Zou G, et al.** Development and validation of a histological index for UC. *Gut* 2017; 66(1): 50-58.
38. **Jauregui-Amezaga A, Geerits A, Das Y, et al.** A Simplified Geboes Score for Ulcerative Colitis. *J Crohns Colitis* 2017; 11(3): 305-313.
39. **Bessho R, Kanai T, Hosoe N, et al.** Correlation between endocytoscopy and conventional histopathology in microstructural features of ulcerative colitis. *J Gastroenterol* 2011; 46(10): 1197-1202.
40. **Lemmens B, Arijs I, Van Assche G, et al.** Correlation between the endoscopic and histologic score in assessing the activity of ulcerative colitis. *Inflamm Bowel Dis* 2013; 19(6): 1194-1201.
41. **Fluxa D, Simian D, Flores L, et al.** Clinical, endoscopic and histological correlation and measures of association in ulcerative colitis. *J Dig Dis* 2017; 18(11): 634-641.
42. **Schroeder KW, Tremaine WJ, Ilstrup DM.** Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; 317(26): 1625-1629.
43. **Wright R, Truelove SR.** Serial rectal biopsy in ulcerative colitis during the course of a controlled therapeutic trial of various diets. *Am J Dig Dis* 1966; 11(11): 847-857.
44. **Azad S, Sood N, Sood A.** Biological and histological parameters as predictors of relapse in ulcerative colitis: a prospective study. *Saudi J Gastroenterol* 2011; 17(3): 194-198.
45. **Zenlea T, Yee EU, Rosenberg L, et al.** Histology Grade Is Independently Associated With Relapse Risk in Patients With Ulcerative Colitis in Clinical Remission: A Prospective Study. *Am J Gastroenterol* 2016; 111(5): 685-690.
46. **Bitton A, Peppercorn MA, Antonioli DA, et al.** Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterology* 2001; 120(1): 13-20.
47. **Hefti MM, Chessin DB, Harpaz NH, et al.** Severity of inflammation as a predictor of colectomy in patients with chronic ulcerative colitis. *Dis Colon Rectum* 2009; 52(2): 193-197.
48. **Burger DC, Thomas SJ, Walsh AJ, et al.** Depth of remission may not predict outcome of UC over 2 years. *J Crohns Colitis* 2011; 5(S3): S4-5.
49. **Bessissow T, Lemmens B, Ferrante M, et al.** Prognostic value of serologic and histologic markers on clinical relapse in ulcerative colitis patients with mucosal healing. *Am J Gastroenterol* 2012; 107(11): 1684-1692.
50. **Gupta RB, Harpaz N, Itzkowitz S, et al.** Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007; 133(4): 1099-1105.
51. **Irani NR, Wang LM, Collins GS.** Correlation between Endoscopic and Histological Activity in Ulcerative Colitis using Validated Indices. *J Crohns Colitis* 2018; *in press*.
52. **Travis SP, Schnell D, Krzeski P, et al.** Reliability and initial validation of the ulcerative colitis endoscopic index of severity. *Gastroenterology* 2013; 145(5): 987-995.
53. **D'Haens G, Van Deventer S, Van Hogezand R, et al.** Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. *Gastroenterology* 1999; 116(5): 1029-1034.
54. **Geboes K, Rutgeerts P, Opdenakker G, et al.** Endoscopic and histologic evidence of persistent mucosal healing and correlation with clinical improvement following sustained infliximab treatment for Crohn's disease. *Curr Med Res Opin* 2005; 21(11): 1741-1754.
55. **Noble A, Turner D**. Clinical indices for pediatric inflammatory bowel disease research. *In*: **Mamula P, Markowitz JE, Baldassano RN**, eds. Pediatric Inflammatory Bowel Disease (1st ed). New York, NY: Springer; 2008: 507-530.
56. **Ashton JJ, Coelho T, Ennis S, Vadgama B, Batra A, Afzal NA, Beattie RM.** Endoscopic Versus Histological Disease Extent at Presentation of Paediatric Inflammatory Bowel Disease. *J Pediatr Gastroenterol Nutr* 2016; 62(2): 246-251.
57. **Fernandes MA, Verstraete SG, Garnett EA, Heyman MB.** Addition of Histology to the Paris Classification of Pediatric Crohn Disease Alters Classification of Disease Location. *JPediatr Gastroenterol Nutr* 2016; 62(2):242-245.
58. **Levine A, Griffiths A, Markowitz J, et al.** Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; 17(6): 1314-1321.
59. **Fabián O, Hradský O, Potužníková K, et al.** Low predictive value of histopathological scoring system for complications development in children with Crohn's disease. *Pathol Res Pract* 2017; 213(4): 353-358.
60. **Daperno M, D'Haens G, Van Assche G, et al.** Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004; 60(4): 505-512.
61. **Hyams JS, Ferry GD, Mandel FS, et al.** Development and validation of a pediatric Crohn's disease activity index. *J PediatrGastroenterol Nutr* 1991; 12(4):439-447.
62. **Mosli MH, Parker CE, Nelson SA, et al.** Histologic scoring indices for evaluation of disease activity in ulcerative colitis. *Cochrane Database Syst Rev* 2017; 5: CD011256.
63. **Novak G, Parker CE, Pai RK, et al.** Histologic scoring indices for evaluation of disease activity in Crohn's disease. *Cochrane Database Syst Rev* 2017; 7: CD012351.
64. **D'Haens G, Sandborn WJ, Feagan BG, et al.** A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007; 132(2): 763-786.
65. **D'Haens GR, Fedorak R, Lemann M,et al.** Endpoints for clinical trials evaluating disease modification and structural damage in adults with Crohn's disease. *Inflamm Bowel Dis* 2009; 15(10): 1599-1604.
66. **Peyrin-Biroulet L, Bressenot A, Kampman W.** Histologic remission: the ultimate therapeutic goal in ulcerative colitis? *Clin Gastroenterol Hepatol* 2014; 12(6): 929-934.
67. **Villanacci V, Antonelli E, Geboes K, et al.** Histological healing in inflammatory bowel disease: a still unfulfilled promise. *World J Gastroenterol* 2013; 19(7): 968-978.
68. **Levine A, Koletzko S, Turner D, et al.** ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014; 58(6): 795-806.
69. **Gralnek IM, Defranchis R, Seidman E,**

Leighton JA, Legnani P, Lewis BS. Development of a capsule endoscopy scoring index for small bowel mucosal inflammatory change. *Aliment Pharmacol Ther* 2008; 27(2): 146-154.

70. **Gal E, Geller A, Fraser G, Levi Z, Niv Y.** Assessment and validation of the new capsule endoscopy Crohn's disease activity index (CECDAI). *Dig Dis Sci* 2008; 53(7): 1933-1937.
71. **Ferrante M, de Hertogh G, Hlavaty T, et al.** The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence. *Gastroenterology* 2006; 130(6): 1595-1606.
72. **Pariente B, Mary JY, Danese S, et al.** Development of the Lémann index to assess digestive tract damage in patients with Crohn's disease. *Gastroenterology* 2015; 148(1): 52-63.
73. **Fabián O, Hradský O, Dršková T, Mikuš F, Zámečník J, Bronský J.** Immunohistochemical Assessment of CD30+ Lymphocytes in the Intestinal Mucosa Facilitates Diagnosis of Pediatric Ulcerative Colitis. *Dig Dis Sci* 2018; 63(7): 1811-1818.
74. **Cuffari C.** Diagnostic considerations in pediatric inflammatory bowel diseasemanagement. *Gastroenterol Hepatol* 2009; 11: 775-783.
75. **Winter DA, Karolewska-Bochenek K, Lazowska-Przeorek I, et al.** Pediatric IBD-unclassified Is Less Common than Previously Reported; Results of an 8-Year Audit of the EUROKIDS Registry. *Inflamm Bowel Dis* 2015; 21(9): 2145-2153.
76. **Martin-de-Carpi J, Rodriguez A, Ramos E, et al.** The complete picture of changing pediatric inflammatory bowel disease incidence in Spain in 25 years (1985-2009): the EXPERIENCE registry. *J Crohn Colitis* 2014; 8(8): 763-769.

PŘÍLOHA V

Predictive value of tissue calprotectin for disease recurrence after ileocecal resection in pediatric Crohn´s disease

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Abstract

Background: Detection of possible predictive factors of endoscopic recurrence after ileocecal resection in Crohn´s disease could be very beneficial for individual adjustment of postoperative therapy.

Aim: The aim of this study was to verify, whether immunohistochemical detection of calprotectin in resection margins is useful in diagnostics of endoscopic recurrence.

Methods: In this study we included pediatric patients with Crohn's disease who underwent ileocecal resection, regardless of pre-operative or post-operative therapy (N=48). We collected laboratory, clinical, surgical, endoscopic and histopathological data at the time of surgery and at 6 months after surgery. The immunohistochemical staining of calprotectin antigen was performed on all paraffin blocks from the resection margins.

Results: Out of 48 patients 52 % had endoscopic recurrence in anastomosis (defined by Rutgeerts score) within 6 months after surgery. Number of cells positive on calprotectin in proximal resection margin was negatively associated with recurrence ($p=0.008$), as was the elevated level of total calprotectin (from both resection margins). There was no correlation of calprotectin in distal resection margin and endoscopic recurrence. Faecal calprotectin over 100 ug/g ($p=0.0005$) and high CRP ($p<0.001$) at 6 months after ileocecal resection and presence of peritonitis ($p=0.048$) were associated with endoscopic recurrence.

Conclusion: Approximately half of the patients develop endoscopic recurrence within 6 months after ileocecal resection. Predictive value of tissue calprotectin is questionable, as it is negatively associated with endoscopic recurrence. There are other potentially useful predictors, such as CRP and fecal calprotectin at 6 months after resection and presence of peritonitis.

Keywords: relapse, endoscopy, immunohistochemistry, surgery, prediction

Introduction

Almost 70 % of patients with Crohn's disease (CD) undergo intestinal surgery in 10 years from diagnosis [1, 2]. Ileocecal resection (ICR) is the most performed procedure. Post-operative recurrence risk factors in adults are known from previously published studies – smoking, prior intestinal resection, penetrating disease, perianal disease and extensive resection [3, 4]. Studies performed in pediatric population focusing on endoscopic recurrence (ER) after ICR are few. Baldassano et al evaluated at first clinical recurrence and found potential risk factors – high Paediatric Crohn´s Disease Activity Index (PCDAI), colonic disease at the time of surgery and 6-mercaptopurine treatment preoperatively [5]. Colonic disease was also described as a risk factor by Pacilli et al [6]. Based on our previously published data, low serum level of albumin at the time of surgery is a potential predictor of ER at 6 months after ICR [7].

Fecal calprotectin (F-CPT) is usually used for disease course monitoring, also post-operatively. Previously performed studies have proven a good correlation between relapse and F-CPT concentration in adults [8] as well as in children [9]. Immunohistochemical detection of calprotectin (CPT) in tissues is not a standard method for monitoring or predicting disease behavior or its prognosis. Fukunaga et al presented higher levels of CPT-positive cells in colonic mucosa in patients with inflammatory bowel disease (IBD) than in control group [10]. Lower intramucosal CPT was detected in UC patients in remission when compared to active disease [11, 12]. High CPT concentration was also associated with acute inflammation in patients after appendectomy [13].

Histopathological features have been investigated as potential predictors of disease relapse in many previously published studies. Active disease and myenteric plexitis were found to be potential predictors [14-16], on the other hand chronic inflammation and granulomas in resection margin were not [17]. A meta-analysis from Simmillis et al focusing on granulomas

as a potential predictor shows higher recurrence rate in patients with histopathologically confirmed granulomas, however considering a heterogeneity of studies, it is not possible to use this finding as a main predictor [18]. Li et al found that higher count of S100-positive enteric glial cells is associated with endoscopic and clinical recurrence [19]. Study from Diederen et al presented microscopically positive resection margins (histopathological inflammation) as one of risk factors for surgical recurrence in a group of pediatric patients after ICR [20].

The primary aim of this study was to verify, whether immunohistochemical detection of CPT in resection margins is useful as a predictor of endoscopic recurrence after ICR. Secondarily, we aimed to identify number of patients with relapse at 6th month after ICR in colon or UGI and to reveal other potential predictors of ER, such as albumin, CRP or F-CPT.

Materials and methods

Patients

In this study we included pediatric patients with CD, who underwent ICR, regardless of pre-operative or post-operative therapy.

Inclusion criteria were: age of 0 – 18 years (at the time of surgery), diagnosis of CD (according to Porto criteria or revised Porto criteria)[21] , history of performed ICR with primary anastomosis (could be combined with other surgery – evacuation of abscess; jejunal, ileal or colonic resection; strictureplasty; fistulectomy; right hemicolectomy; surgical treatment of bowel perforation), endoscopy performed 6 month after ICR or earlier (in the

case of CD relapse), available bioptic tissue from surgery and endoscopic control, informed consent signed.

Exclusion criteria were: change of therapy before endoscopy (substantial changes – e.g. initiation of biological therapy, switch between biologics), ileostomy, missing histopathological samples or substantial part of laboratory data, refusal of patient/parents to participate in this study.

Between December 2008 and September 2018, 63 patients with CD underwent ICR performed by a team of pediatric surgeons, who closely cooperates with our department. Four patients were excluded, because they needed ileostomy and therefore had second surgery with anastomosis done later. Eleven patients had no endoscopy during the determined interval. Forty-eight patients fulfilled inclusion criteria and were included in the study. Patients´ characteristics are given in table 1.

Data and samples

Laboratory results/parameters were collected from hospital electronic records. Surgical (other procedure combined with ICR, length of resection) and clinical data (therapy, elective/urgent surgery, penetrating disease) before ICR were collected from hospital electronic records retrospectively.

Histopathological evaluation was divided into two parts:

The first part of the study was retrospective and all microscopic slides from proximal and distal resection margins were evaluated by senior gastrointestinal pathologist blinded to

clinical data. The assessed morphological variables included: 1) intensity of chronic inflammation; 2) intensity of active inflammation (established on the basis of neutrophilic infiltration in the lamina propria, presence of neutrophils in the epithelium and presence of erosions or ulcerations); 3) intensity of eosinophilic inflammation; 4) distortion of the mucosal architecture; 5) epithelioid granulomas; 6) basal plasmocytosis; 7) pyloric metaplasia; 8) fibrosis; 9) lymphocytic lymphangitis; 10) transmural lymphoid aggregates; 11) submucosal plexitis; 12) myenteric plexitis; 13) myenteric plexus hyperplasia; 14) obliterative vasculopathy; 15) acute fibrinous-purulent peritonitis and 16) chronic adhesive peritonitis. The assessed variables were evaluated separately for proximal and distal resection margin.

Then, the immunohistochemical staining of CPT antigen was performed on all paraffin blocks from the resection margins. Tissue sections (thickness 1 um) were deparaffinized, and the anti-CPT antibody (Invitrogen, at a dilution of 1:1000) was used. Detection was performed by the PolyDet Dab chromogen (Dako REAL) with phosphate-buffered saline solution. CPT expression was assessed by counting the highest number of positive cells per one high power field (400x) in the most affected region of each microscopic slide. The positive cells were counted separately for lamina propria and the epithelium. In areas with presence of erosions or ulcerations we counted CPT-positive cells from the tissue adjacent to the margin of the respective erosion/ulceration.

In the second part of the study (retrospective evaluation of prospectively collected data), the bioptic samples taken during the endoscopic control in the 6th month after the ICR were evaluated. During the duration of the study, according to standard protocol all patients in our center undergo regular endoscopic evaluation 6 months after ICR (or earlier in case of flare). The histopathological slides from the six bowel segments (neoterminal ileum, colon close to the anastomosis, ascending colon, transverse colon, descending colon and rectum) were

examined. The intensity and activity of the inflammation were assessed using the modified Global Histology Activity Score. This scoring system evaluates the intensity of chronic and acute inflammation, presence of epithelial damage and mucosal defects, presence of epithelioid granulomas and distortion of the mucosal architecture. This scoring system was described in detail in our previous publication and has an advantage in the possibility to evaluate each bowel segment separately [22].

Endoscopic data, actual clinical and laboratory data were collected at the time of endoscopy, which was performed between 4th and 7th month after ICR (mean 6th month). Findings in anastomosis were described using Rutgeerts score [23], in upper gastrointestinal tract (UGI) and colon using modified simple endoscopic score (mSES) [24]. In UGI we assessed esophagus, gastric body, gastric antrum and duodenum and scored from 0 to 3 points for size of ulcers, ulcerated surface, affected surface and presence of narrowing. Colon was divided in 4 parts (right colon, transverse colon, left colon and rectum), scoring was the same as in UGI. ER was defined as Rutgeerts score equal or more than i2 and/or mSES in UGI or colon more than 3.

Primary and secondary outcomes

As a primary outcome we have chosen identification of predictive factors of disease relapse in anastomosis (Rutgeerts score equal or more than i2). As a main potential predictive factor we appointed a positivity of CPT in resection margins of resected bowel. Secondary outcomes were number of patients with relapse at 6th month after ICR in colon or UGI, evaluation of other predictors of disease recurrence, assessment of role of laboratory results and histopathological findings at the time of surgery and influence of the therapy.

Ethical considerations

Legal representatives of the patients signed an informed consent form for inclusion into the study. The study was approved by the Ethics Committee of Motol University Hospital.

Statistical analysis

Statistical software R-project (R Core Team, version 3.6.0) was used for data analysis. Continuous variables were described as median and inter quartile range (IQR). Categorical variables were described as absolute frequencies and percentages. Values of F-CPT were analyzed on a logarithmic scale. Univariate association with categorical outcome was assessed using linear logistic regression model. For testing of association between two linear predictors, we used linear regression models. Adjusted models were constructed with multiple logistic regression. The selection of predictors for multiple logistic regression models were based on clinical decision only. Probability (p) values of <0.05 were considered significant. A 95% confidence interval was used. Figures were constructed using R package "ggplot2".

Results

Rate of endoscopic recurrence (ER)

Out of 48 patients 52 % had ER in anastomosis within 6 months after ICR (12 patients had Rutgeerts score i2, 5 had i3 and 8 had i4). Among patients in remission based on Rutgeerts score (N=23), 1 had relapse in colon and 4 in UGI.

Tissue CPT in resection margins

Number of cells positive on CPT in proximal resection margin was negatively associated with ER defined by Rutgeerts score (OR=0.969 95 % CI 0.936 – 0.996, p=0.008) – figure 1, as was the elevated level of total CPT (from both resection margins) (OR=0.993, 95 % CI 0.972 - 0.9997, p=0.034). If we use model adjusted on patients treated with biological therapy after resection, this correlation is also confirmed (OR= 0.971, 95 % CI 0.938 – 0.997, p=0.008, OR=0.993, 95 % CI 0.973 – 0.999, p=0.021, respectively). There was no correlation of CPT in distal resection margin and ER.

Laboratory results and clinical data at the time of surgery

We did not find any association between age at the time of ICR or sex and primary outcome. Low serum concentration of albumin (OR=0.871, 95 % CI 0.736 – 1.009, p=0.066) and elevation of CRP (OR=1.02, 95 % CI 0.999 – 1.045, p=0.060) were found to be borderline associated with ER, in adjusted model on biological therapy after ICR, the results were significant for albumin (OR=0.851, 95 % CI 0.713 – 0.992, p=0.039), but not for CRP (OR=1.02, 95 % CI 0.999 – 1.045, p=0.075). F-CPT (samples from 58 % patients were available) was not significantly associated with ER (OR=1.0, 95 % CI 0.9996 – 1.0006, p=0.637) regardless of post-operative therapy. F-CPT cut-off value for remission was set at 100 ug/g.

Laboratory results at the time of endoscopic examination

Out of 48 patients 75 % submitted sample of stool for F-CPT evaluation at 6th month after ICR. Levels of log F-CPT were in association with ER (OR=2.19, 95 % CI 1.28 – 4.28, p = 0.003) - figure 2a, in model adjusted for biological therapy even more significantly (OR=233, 95 % CI 1.37 – 4.79, p = 0.002). F-CPT over 100 ug/g at 6 months was strongly positively associated with ER (OR=15, 95 % CI 3.1 – 96.6, p = 0.0005). Three patients had ER despite having F-CPT below 100 ug/g.

The CRP at 6 months was tested in all patients. Elevated values of CRP were associated with ER (OR=2.21, 95 % CI 1.353 – 4.385, p<0.001 - figure 2b, resp. OR = 2.53, 95 % CI 1.414 – 6.047 (after using adjusted model for biological therapy)). Albumin levels (examined in 94 % of patients) were not in association with ER in group of all patients, but if we again use adjusting for biologics, it was statistically significant (OR=0.759, 95 % CI 0.5797 – 0.943, p=0.011).

Histopathology

Only a presence of the peritonitis (chronic or florid) was found to be associated with ER (OR=4.15, 95 % CI 1.015 – 21.599, p=0.048), also borderline in model adjusted for biological therapy (OR=4.08, 95 % CI 0.988 – 21.390, p=0.052). Other markers as plexitis (submucosal or myenteritic), active inflammation, intensity of inflammation or chronic inflammation were not in association with ER.

Discussion

Studies on rates of ER after ICR in children are scarce. Our previous study [7] , focused on patients treated postoperatively only with AZA, found ER in 38 % of them at 6 months. Other

pediatric study by Bobanga et al described ER in 87 %, but mean follow-up was longer than 6 months, endoscopy was not performed in all patients and postoperative therapy differed between patients [25]. In a small study from Hukkinen et al ER was found in 51% of patients in long time follow-up (mean 38 months), 88 % of these patients had immunosupressive therapy [9]. More detailed data are available in adult population - in randomized controlled POCER study published by De Cruz et al, ER rate after 6 months was 45 % in high-risk patients treated with AZA and 21 % in patients treated with adalimumab (ADA) [3]. Kotze at al described early ER (within 1 year from the time of the surgery, defined as Rutgeerts score more than i2) in patients treated with biologics. In ADA group it was 24 % and in infliximab (IFX) group it was 27 %. [26]. In other study from Auzolle et al, ER from 6th to 12th month after ICR was found in 47 % of patients [27]. Thus, ER described in the present study (52 %) is comparable to previously published data both from pediatric and adult population. It is important to remind here, that each study uses slightly different definition of ER and moreover, Rutgeerts score is not validated for children, but there is no better scoring system available at the moment. Based on relapse rate we can assume, that it is necessary to observe these patients closely, repeat laboratory tests as CRP and F-CPT regularly and as recommended by European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) - endoscopy should be performed in 6 – 9 months after surgery [28].

Immunohistochemical detection of CPT in intestinal tissue is not commonly used method to evaluate intensity of inflammation or predict disease recurrence. There are no studies in patients after ICR, neither in adults nor children. Study by Fukunaga et al proved higher level of CPT-positive cells in colonic mucosa in IBD patients than in controls [10]. Also Liu et al found higher concentration of CPT in colonic mucosa in patients with active UC than in healthy patients or those with inactive UC [12]. Results of our study are not sufficient enough to recommend measurement of tissue CPT for prediction of ER after ICR. Contrary to our

expectations, we found negative association between tissue CPT at proximal resection margin and risk of ER. However, we would expect higher tissue CPT to be associated with higher risk - in analogy to higher Rutgeerts score, which is considered as an important predictive factor in adults. We do not have any pathophysiological explanation for our findings that can be due to relatively small sample size in our pilot study (with potential influence of few outlier high values in individual patients). Our IBD surgeons stick to standard resection procedure according to current guidelines and perform resection in substantial distance from macroscopically inflamed area. However, differences in microscopic inflammatory intensity in resection margins that are not macroscopically visible, cannot be excluded. Because model adjusted on biological therapy brought the same results, it seems, that postoperative treatment is not potential confounder of these findings and thus it is not likely that biological therapy used in more difficult cases (with higher tissue CPT) would lead to better post-surgical prophylaxis of ER. It is also questionable, if counting the highest number of tissue CPT positive cells in the most affected area is the optimal way of evaluation of the histopathological sample. However, there is no such study published evaluating different histological approaches of tissue CPT measurement and further research on bigger sample size is necessary to either prove or contradict our findings.

F-CPT is commonly used marker for monitoring postoperative disease recurrence and behavior [29]. Pediatric study performed in CD patients after surgery by Hukkinen et al, found F-CPT levels of 139 ug/g as threshold for ER (beyond 6 months) [9]. In 2018 Tham et al published systematic review on using F-CPT as marker for ER after ICR. As the best threshold authors considered F-CPT values of 150 ug/g with 70 % (95% CI 59–81 %) sensitivity and 69 % (95% CI 61–77 %) specificity[30]. These findings were also confirmed in our study, there was an association between F-CPT at the time of endoscopy and ER, despite the fact we did not have samples from all patients. As recommended by ESPGHAN

guidelines, F-CPT could be successfully used for timing of endoscopy after ICR (threshold >100 ug/g) [28]. However, using this threshold in our study population, we would miss 3 patients with ER.

Also, CRP is a helpful marker of inflammatory activity, but it is not specific for IBD. Wright et al or De Cruz et al in POCER study found that CRP is not as accurate as F-CPT in monitoring adult patients after ICR [3, 29]. This is also supported by above-mentioned ESPGHAN guidelines [28]. In our study, CRP at the time of surgery is not associated (although borderline) with ER, but at 6 months after ICR the association is significant. CRP reflects systemic inflammation, which can persist after ICR, although F-CPT decreases (after resection of affected bowel).

It is known that low albumin level as marker of poor nutritional status is risk factor for peri- and postoperative complications, e.g. in cardiac [31], orthopedic [32] and also in bowel surgery [33, 34]. In our previous study we also found that low albumin level is potential predictor of ER in selected patients treated with AZA without biologicals after ICR [7]. In the present study we have found only borderline association at the time of surgery (statistically significant after adjusting on biological therapy). Similar situation was found at the time of endoscopy.

From histopathological features, only the presence of peritonitis (chronic or florid) was found to be associated with ER. To our knowledge, this is a newly described finding that could become a subject of future research focused on possible predictive histopathological factors. On the contrary, we did not confirm previously published data on plexitis [14-16] as a clinically useful predictor or disease recurrence.

From models adjusted on biological therapy, our data suggest that we cannot reliably use histopathological or biochemical predictors as helpful markers for decision on the best

postoperative therapy for individual patient. At the moment, there is not enough data to change current clinical practice based on postoperative therapy stratified according to presence of residual disease.

Possible weakness of our study is that the number of the patients was not high enough to show all possible relationships between evaluated predictors and outcomes. Moreover, the tissue from ICR was examined retrospectively and there are also some data missing due to partly retrospective design of the study.

On the other hand, the strength of our study is that we describe a homogenous group of consecutive patients, postoperatively treated by standardized manner based on presence of residual disease. The patients underwent standardized prospective endoscopic evaluation. Moreover, this is the first study evaluating possible value of tissue CPT in pediatric patients after ICR.

Conclusions

Approximately half of the patients develop ER within 6 months after ICR. Tissue CPT does not seem to be a valuable predictor of ER, irrespective of postoperative treatment. However, there are other potentially useful predictors, such as high F-CPT and high CRP at 6 months after ICR, low albumin (after adjustment to biological therapy) at the time of surgery as well as 6 months after ICR and presence of peritonitis. In accordance with ESPGHAN guidelines, such patients should be closely monitored and regularly evaluated endoscopically in order to tailor therapy in those who develop early ER.

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K.Z: lectures/congress fees/consultancy (outside submitted work) – Nutricia and Nestlé; O.H.: lectures/congress fees/consultancy (outside submitted work) - MSD, AbbVie, Nutricia, Nestlé, Ferring, and Falk; T.L.: lectures/congress fees/consultancy (outside submitted work) - Nutricia, Ferring and Biocodex; J.B.: lectures/congress fees/consultancy (outside submitted work) - MSD, AbbVie, Nutricia, Nestlé, Ferring, Biocodex, and Walmark; O.F., F.M., V.D., L.P., and R.S. report no conflicts of interest.

References

1. Bernell O, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. Ann Surg 2000; 231: 38-45.

2. Peyrin-Biroulet L, Harmsen WS, Tremaine WJ, Zinsmeister AR, Sandborn WJ, Loftus EV, Jr. Surgery in a population-based cohort of Crohn's disease from Olmsted County, Minnesota (1970-2004). Am J Gastroenterol 2012; 107: 1693-1701.

3. De Cruz P, Kamm MA, Hamilton AL, Ritchie KJ, Krejany EO, Gorelik A et al. Crohn's disease management after intestinal resection: a randomised trial. Lancet 2015; 385: 1406-1417.

4. Buisson A, Chevaux JB, Allen PB, Bommelaer G, Peyrin-Biroulet L. Review article: the natural history of postoperative Crohn's disease recurrence. Aliment Pharmacol Ther 2012; 35: 625-633.

5. Baldassano RN, Han PD, Jeshion WC, Berlin JA, Piccoli DA, Lautenbach E et al. Pediatric Crohn's disease: risk factors for postoperative recurrence. Am J gastroenterol 2001; 96: 2169-2176.

6. Pacilli M, Eaton S, Fell JM, Rawat D, Clarke S, Haddad MJ. Surgery in children with Crohn disease refractory to medical therapy. J Pediatr Gastr Nutr 2011; 52: 286-290.

7. Zarubova K, Hradsky O, Copova I, Rouskova B, Pos L, Skaba R et al. Endoscopic Recurrence 6 Months After Ileocecal Resection in Children With Crohn Disease Treated With Azathioprine. J Pediatr Gastr Nutr 2017; 62: 207-211.

8. Qiu Y, Mao R, Chen BL, He Y, Zeng ZR, Xue L et al. Fecal calprotectin for evaluating postoperative recurrence of Crohn's disease: a meta-analysis of prospective studies. Inflamm Bowel Dis 2015; 21: 315-322.

9. Hukkinen M, Pakarinen MP, Merras-Salmio L, Koivusalo A, Rintala R, Kolho KL. Fecal calprotectin in the prediction of postoperative recurrence of Crohn's disease in children and adolescents. J Pediatr Surg 2016; 51: 1467-1472.

10. Fukunaga S, Kuwaki K, Mitsuyama K, Takedatsu H, Yoshioka S, Yamasaki H et al. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int J Mol Med 2018; 41: 107-118.

11. Guirgis M, Wendt E, Wang LM, Walsh A, Burger D, Bryant RV et al. Beyond Histological Remission: Intramucosal Calprotectin as a Potential Predictor of Outcomes in Ulcerative Colitis. J Crohns Colitis 2017; 11: 460-470.

12. Liu WB, Lu YM, Jin Z, Yang XL. [Expression of calprotectin in colon mucosa and fecal of patients with ulcerative colitis]. Beijing Da Xue Xue Bao Yi Xue Ban 2005; 37:179-182.

13. Ambe PC, Godde D, Bonicke L, Papadakis M, Storkel S, Zirngibl H. Calprotectin could be a potential biomarker for acute appendicitis. J Transl Med 2016; 14: 107.

14. Misteli H, Koh CE, Wang LM, Mortensen NJ, George B, Guy R. Myenteric plexitis at the proximal resection margin is a predictive marker for surgical recurrence of ileocaecal Crohn's disease. Colorectal Dis 2015; 17: 304-310.

15. Lemmens B, de Buck van Overstraeten A, Arijs I, Sagaert X, Van Assche G, Vermeire S et al. Submucosal Plexitis as a Predictive Factor for Postoperative Endoscopic Recurrence in Patients with Crohn's Disease Undergoing a Resection with Ileocolonic Anastomosis: Results from a Prospective Single-centre Study. J Crohns Colitis 2017; 11: 212-220.

16. Ferrante M, de Hertogh G, Hlavaty T, D'Haens G, Penninckx F, D'Hoore A et al. The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence. Gastroenterology 2016; 130: 1595-1606.

17. Bressenot A, Peyrin-Biroulet L. Histologic features predicting postoperative Crohn's disease recurrence. Inflam Bowel Dis 2015; 21: 468-475.

18. Simillis C, Jacovides M, Reese GE, Yamamoto T, Tekkis PP. Meta-analysis of the role of granulomas in the recurrence of Crohn disease. Dis Colon Rectum 2010; 53: 177-185.

19. Li Y, Ge Y, Zhu W, Gong J, Cao L, Guo Z et al. Increased enteric glial cells in proximal margin of resection is associated with postoperative recurrence of Crohn's disease. Journal of gastroenterology and hepatology 2018; 33: 638-44.

20. Diederen K, de Ridder L, van Rheenen P, Wolters VM, Mearin ML, Damen GM et al. Complications and Disease Recurrence After Primary Ileocecal Resection in Pediatric Crohn's Disease: A Multicenter Cohort Analysis. Inflam Bowel Dis 2017; 23: 272-282.

21. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J Pediatr Gastr Nutr 2014; 58: 795-806.

22. Fabian O, Hradsky O, Potuznikova K, Kalfusova A, Krskova L, Hornofova L et al. Low predictive value of histopathological scoring system for complications development in children with Crohn's disease. Pathol Res Pract 2017; 213: 353-358.

23. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. Gastroenterology 1990; 99: 956-963.

24. Ledder O, Church P, Cytter-Kuint R, Martinez-Leon M, Sladek M, Coppenrath E et al. A Simple Endoscopic Score Modified for the Upper Gastrointestinal tract in Crohn's Disease (UGI-SES-CD): a report from the ImageKids study. J Crohns Colitis 2018; 12:

25. Bobanga ID, Bai S, Swanson MA, Champagne BJ, Reynolds HJ, Delaney CP et al. Factors influencing disease recurrence after ileocolic resection in adult and pediatric onset Crohn's disease. Am J Surg 2014; 208: 591-596.

26. Kotze PG, Yamamoto T, Danese S, Suzuki Y, Teixeira FV, de Albuquerque IC 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 Crohns Colitis 2015; 9: 541-547.

27. Auzolle C, Nancey S, Tran-Minh ML, Buisson A. Male gender, active smoking and previous intestinal resection are risk factors for post-operative endoscopic recurrence in Crohn's disease: results from a prospective cohort study. Aliment Pharmacol Ther 2018; 48: 924-932.

28. Amil-Dias J, Kolacek S, Turner D, Paerregaard A, Rintala R, Afzal NA et al. Surgical Management of Crohn Disease in Children: Guidelines From the Paediatric IBD Porto Group of ESPGHAN. J Pediatr Gastr Nutr 2017; 64: 818-835.

29. Wright EK, Kamm MA, De Cruz P, Hamilton AL, Ritchie KJ, Krejany EO et al. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology 2015; 148: 938-941.e1.

30. Tham YS, Yung DE, Fay S, Yamamoto T. Fecal calprotectin for detection of postoperative endoscopic recurrence in Crohn's disease: systematic review and meta-analysis. Therap Adv Gastroenterol 2018; 11: 1756284818785571.

31. Karas PL, Goh SL, Dhital K. Is low serum albumin associated with postoperative complications in patients undergoing cardiac surgery? Interact Cardiov Th 2015; 21: 777-786.

32. Kamath AF, Nelson CL, Elkassabany N, Guo Z, Liu J. Low Albumin Is a Risk Factor for Complications after Revision Total Knee Arthroplasty. J Knee Surg 2017; 30: 269-275.

33. Galata C, Kienle P, Weiss C, Seyfried S, Reissfelder C, Hardt J. Risk factors for early postoperative complications in patients with Crohn's disease after colorectal surgery other than ileocecal resection or right hemicolectomy. Int J Colorectal Dis 2019; 34: 293-300.

34. Huang W, Tang Y, Nong L, Sun Y. Risk factors for postoperative intra-abdominal septic complications after surgery in Crohn's disease: A meta-analysis of observational studies. J Crohns Colitis 2015; 9: 293-301.

Figure legends

Figure 1. Association between log tissue CPT in proximal resection margin and ER at 6 months

Legend: HPF = high power field, CPT = calprotectin, ER = endoscopic recurrence

Figure 2a. Association between log F-CPT at 6 months and ER at 6 months

Legend: F-CPT = faecal calprotectin, ER = endoscopic recurrence

Figure 2b. Association between CRP at 6 months and ER at 6 months

Legend: CRP = C-reactive protein, ER = endoscopic recurrence

Figure 2a

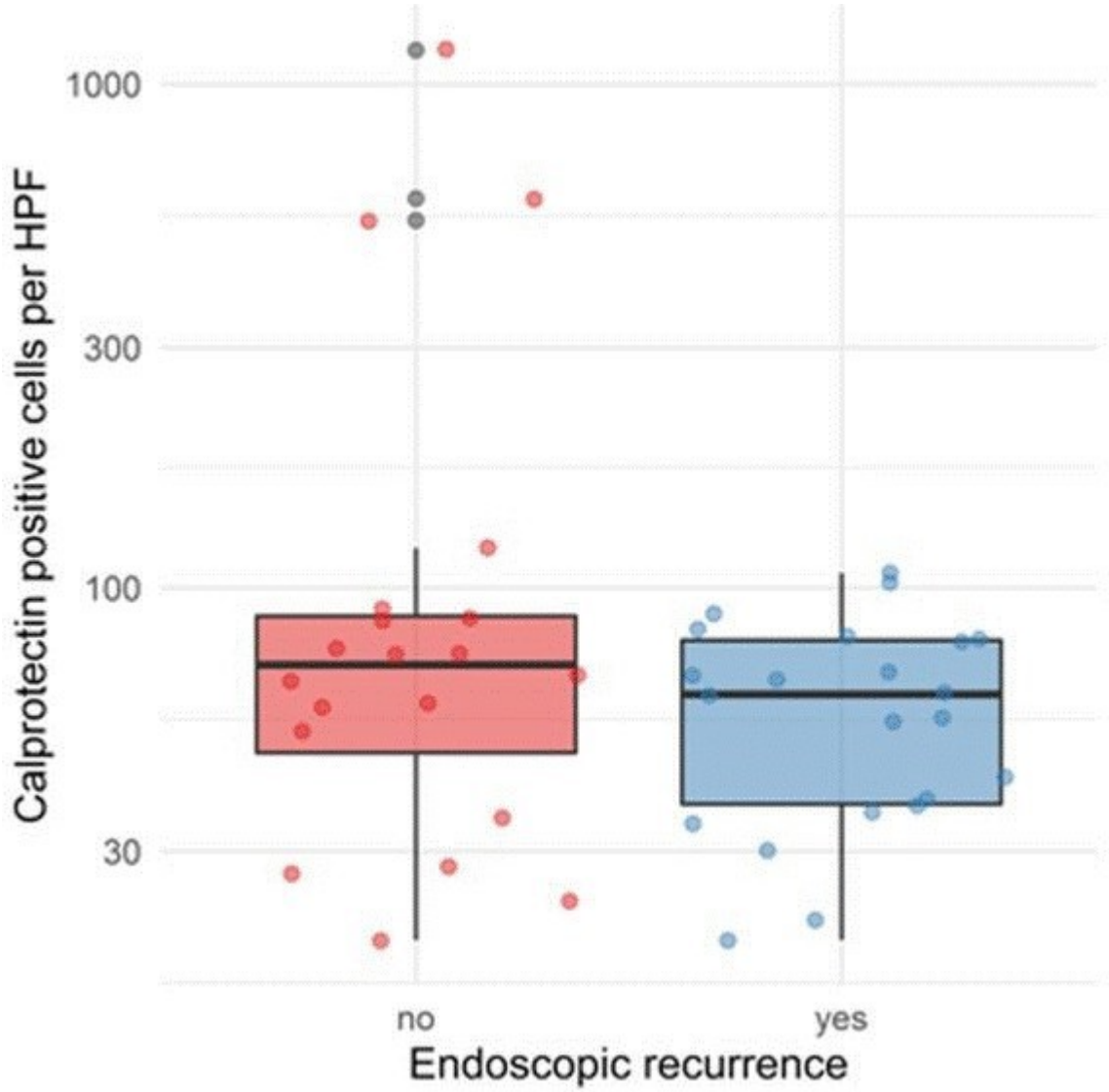
1000
300
100
30
Calprotectin positive cells per HPF
no
yes
Endoscopic recurrence

Figure 1

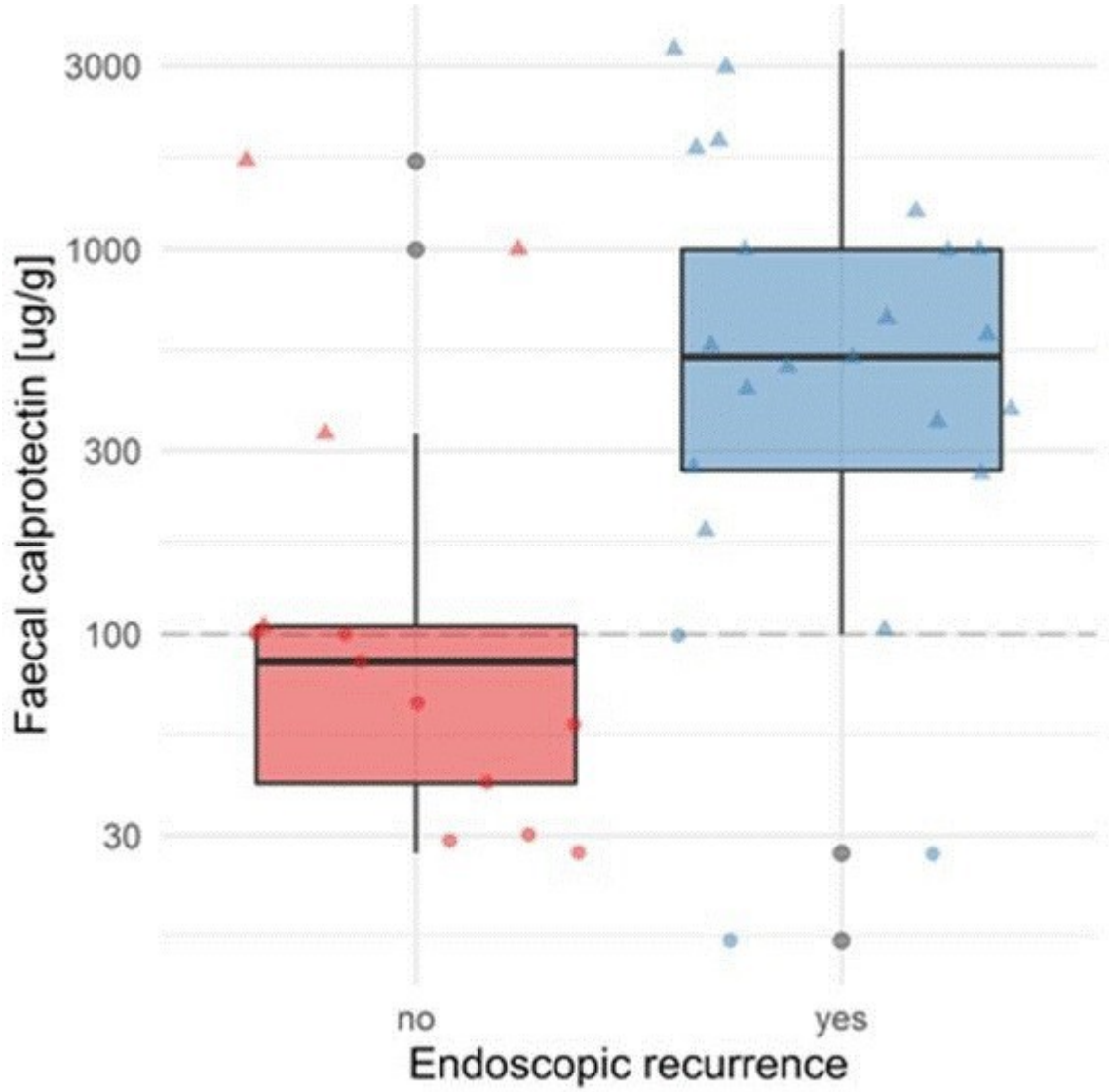
3000
1000
300
100
30
Faecal calprotectin [ug/g]
no
yes
Endoscopic recurrence

Table 1: Patients´ characteristics before surgery

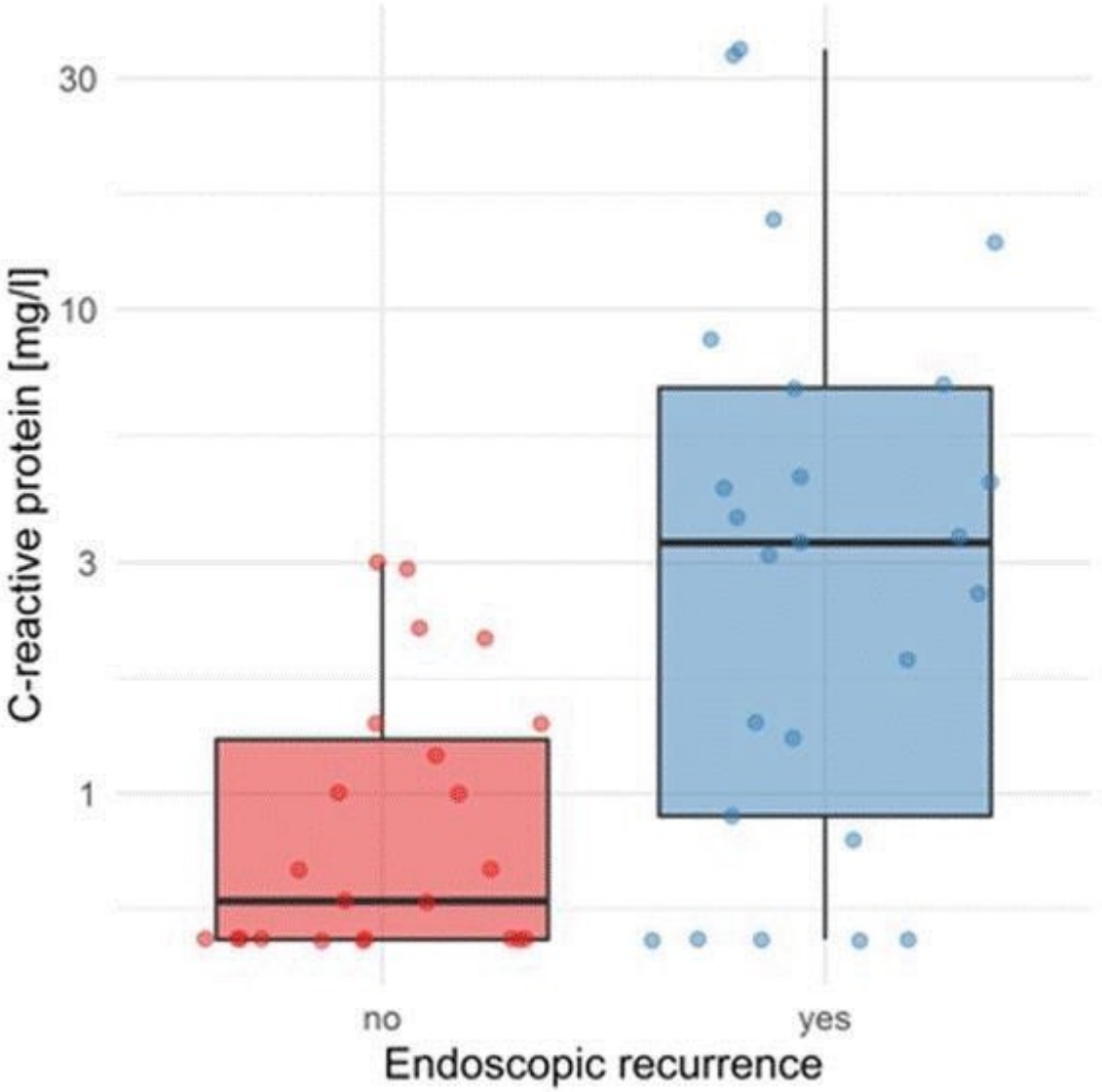

Figure 2b

Characteristics	**All**
Female	0.42
Age at the time of ICR	16 (15-17)
Ciprofloxacine at the time of ICR	0.29
Metronidazole at the time of ICR	0.38
Azathioprine at the time of ICR	0.81
Infliximab at the time of ICR	0.38
Adalimumab at the time of ICR	0.15
Exclusive enteral nutrition at the time of ICR	0.15
Corticosteroids at the time of ICR	0.25
Piperacillin/Tazobactam at the time of ICR	0.06
Length of resection	22 (16.5-28.5) NA=1
Penetrating disease	0.35
Elective surgery	0.62

ICR = ileocaecal resection; NA = not available