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Endoluminal calprotectin measurement in assessment of pouchitis and a new index of disease activity: a pilot study

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ABSTRACT

Pouchitis is the most common complication following proctocolectomy with ileal pouch-anal anastomosis for ulcerative colitis (UC). To provide a standardized definition of pouchitis clinical, endoscopic and histological markers were grouped and weighted in the pouch disease activity index (PDAI). However, the delay in the assessment of the final score due to the time requested for histological analysis remains the main obstacle to the index implementation in clinical practice so that the use of modified-PDAI (mPDAI) with exclusion of histologic subscore has been proposed. We tested the ability of calprotectin measurement in the pouch endoluminal content to mimic the histologic score as defined in the PDAI, the index that we adopted as gold standard for pouchitis diagnosis. Calprotectin was measured by ELISA in the pouch endoluminal content collected during endoscopy in 40 consecutive patients with J-pouch. In each patient PDAI and mPDAI were calculated and 15% of patients were erroneously classified by mPDAI. ROC analysis of calprotectin values vs. acute histological subscore ≥ 3 identified different calprotectin cut-off values with corresponding sensitivity and specificity allowing the definition and scoring of different range of calprotectin subscores. We incorporated the calprotectin score in the mPDAI obtaining a new score that shows the same specificity as PDAI for diagnosis of pouchitis and higher sensitivity when compared with mPDAI. The use of the proposed new score, once validated in a larger series of patients, might be useful in the early management of patients with symptoms of pouchitis.

Key words: Pouchitis. Calprotectin. Ulcerative colitis. Disease activity index.

INTRODUCTION

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for patients with ulcerative colitis (UC). Pouchitis, a non-specific, idiopathic inflammation of the ileal reservoir, is the most common complication following surgery for UC (1-3). The reported incidence of pouchitis varies because of the different diagnostic criteria employed (4,5). To standardize the definition and assess the severity of pouchitis, diagnosis should be made on the basis of clinical, endoscopic, and histological criteria. Pouchitis disease activity index (PDAI), as proposed by Sandborn et al., represents an objective and reproducible scoring system in which endoscopic and histological evaluation together with symptom assessment independently contribute to diagnose pouchitis (6). The role of histology in pouchitis diagnosis has been debated (7). In addition, the delay in the assessment of the final score due to the time requested for histological analysis, the high cost of this procedure as well as the need of an experienced pathologist remain the main obstacle to its implementation in clinical practice. A simplified approach to the diagnosis of pouchitis with the use of modified pouchitis disease activity index (mPDAI) that takes into account symptoms and endoscopy with exclusion of histological score has been proposed (8).

Fecal calprotectin measurement has been demonstrated to be a useful non-invasive tool in the diagnosis of inflammatory bowel disease (9-12). Recently fecal calprotectin measurement has been also investigated in acutely inflamed ileal pouches and showed to correlate closely with the Objective Pouchitis Score, the PDAI as well as with endoscopic and histological inflammatory subscores (13). Moreover, it appears that elevated faecal calprotectin levels are significant predictors of pouchitis (14). On this basis, monitoring of faecal calprotectin has been proposed in the follow up of patients with IPAA. However, it is generally accepted that laboratory findings should not replace pouch endoscopy as first-line evaluation method (15).

In the present study, we evaluated the contribution of calprotectin measurement to the diagnosis of pouchitis

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assessed with PDAI. To make the whole process more accessible to the patient and the analyst we integrated the collection of the sample for calprotectin measurement in the pouch endoscopy procedure by collection of the pouch endoluminal content.

MATERIALS AND METHODS

We carried out an observational cross-sectional study on patients who underwent restorative proctocolectomy with J-pouch IPAA for UC at the Department of General Surgery "P. Stefanini", Sapienza University, Rome. Patients with original indications to IPAA other than UC, pouch conformation other than J, or patients who were treated with antibiotic, immunomodulating agents, steroids or non-steroidal anti-inflammatory drugs in the previous 3 months, or who were treated with antiplatelet or anticoagulant medicinal products in the previous 2 weeks were excluded from the study

Patients eligible for the study underwent clinical assessment and endoscopic examination of the ileal pouch in the same day.

Clinical assessment

Patients were interviewed using a structured form, and symptom score for PDAI/mPDAI was calculated. The criterion used to define the existence of pouchitis was a PDAI ≥ 7.

Endoscopy

Pouchscopy was performed by a single experienced endoscopist. All patients underwent a pouch enema (Sorbiclis®) from 4 to 2 hours before pouchscopy. All pouchscopies were performed by flexible fine endoscope (Olympus®). Pouchscopy included macroscopic exploration of the entire pouch, afferent ileum within 15 cm and the anal canal. Mucosal appearance abnormalities, particularly the specific acute macroscopic inflammatory features for PDAI score calculation (edema, granularity, friability, loss of vascular pattern, mucus exudate, ulceration) were recorded and scored as 1 each when present. Final endoscopic score was annotated by the endoscopist, who was blinded to the clinical data.

Multiple biopsies (n = 8-12) were taken from the upper and lower pouch. Biopsies were fixed in 10% buffered formalin and send to the histopathology section.

Histology

Specimens, after processing, were scored according to Shepherd-Moskowitz classification (16) and finally scored from 0 to 6 using Sandborn's criteria for PDAI (6) by the pathologist, who was blinded to the clinical and endoscopic data. For the purpose of the present investigation a histological subscore ≥ 3 indicates the presence of histological pouchitis. Data from clinical, endoscopic and histological subscores were finally added to obtain PDAI score, while mPDAI was calculated on the basis of clinical and endoscopic subscores only.

Pouch endoluminal content sample collection and calprotectin measurement

Immediately after scope insertion, a sample of 3-5 ml of pouch endoluminal content was collected by aspiration with endoscope and retrieved in a basket trap (Endo-technik®). It was then transferred in a screw cap test tube and delivered at room temperature to the laboratory within 4 hours after collection. Each sample was centrifuged at 4,000 rpm for 10 minutes at room temperature; the clear extract supernatant was collected and transferred to an Eppendorf tube labeled with the patient's code, and immediately frozen and stored at -30 °C for subsequent calprotectin measurement. After thawing serial dilutions of each sample were performed using the dilution buffer provided in the kit, and calprotectin was measured by ELISA using Calprest Test® (Eurospital, Trieste, Italy).

Statistical analysis

Statistical analysis was performed using the statistical package GraphPad® Prism 5 (San Diego, CA, USA). Results of numerical data are presented as mean ± standard deviation (SD) or median and range, with a 95% confidence interval (CI) when appropriate. Statistically significant differences between median values were determined using the Mann-Whitney U test. The Spearman rank test or Pearson correlation test were used to determine the degree of correlation, as appropriate. The receiver operating characteristic curve (ROC) was used to predict the sensitivity and specificity of fecal calprotectin for the prediction of PDAI- defined pouchitis and the acute histological score.

Ethical considerations

All procedures complied with the principles of the Declaration of Helsinki. Patients were provided with detailed information about the study aims and gave their written, informed consent.

RESULTS

Forty consecutive patients attending the department for routine clinical and endoscopic surveillance from September 2013 to September 2014 who underwent restorative proctocolectomy with J- pouch IPAA for UC were recruited in the study. In 6 patients the IPAA was performed at the dentate line by hand-sewn transanal suture after mucosectomy. In 34 patients stapled IPAA without mucosectomy was performed at level of transitional zone at an average distance of 0.5 cm from the dentate line. The employed technique minimizes the risk of residual rectal mucosa and development of cuffitis. All patients were in early-medium and long-term follow-up. Characteristics of patients included in the study were as follows: male/female: 20/20; age of patients: 52 (33-71) years, median (range); age of pouch: 127 (3-307) months, median (range); quality of life score (QoL) (0-87): 23 (1-62), median (range); number of patients with history of pouchitis: 20 (episodic: 10; antibi-

otic-sensitive recurrent pouchitis: 6; antibiotic-dependent chronic pouchitis: 2; chronic resistant pouchitis: 2). At study entry patients' median (range) values of different scores were: symptoms (0-6): 1 (0-4); endoscopic (0-6): 1 (0-6); neutrophils (0-3): 2 (0-3); acute histology (0-6): 2 (0-6); chronic histology (0-6): 4 (1-6); PDAI (0-18): 5 (1-15); mPDAI (0-12): 3 (0-10). Endoluminal calprotectin value was: 167.73 (6.50-1,125.0) mg/kg, median (range).

Measurement of calprotectin in the pouch endoluminal content shows similar sensitivity and specificity in predicting PDAI-defined pouchitis as calprotectin measurement in feces.

We judged PDAI as gold standard for pouchitis evaluation. Accordingly, we started our analysis by assessing the correlation between PDAI and calprotectin levels measured in the pouch endoluminal content. As shown in figure 1, the two variables were significantly correlated confirming previous data obtained with the measurement of fecal calprotectin (13). Moreover, ROC analysis for the calprotectin measurement in the pouch endoluminal content at two different cut-off values (66.2 and 37.6 mg/kg) showed values of sensitivity (0.85 [0.57-0.98]; 0.92 [0.63-0.99] [95% confidence interval, CI], respectively) and specificity (0.38 [0.20-0.59]; 0.19 [0.06-0.39] [95% CI], respectively) at predicting PDAI-defined pouchitis comparable to the values previously reported for fecal calprotectin (13). Similarly, area under curve (AUC) shows a good diagnostic accuracy of the test (AUC = 0.832 [0.68-0.97] [95% CI]). Thus, measurement of calprotectin in the pouch endoluminal content, at least in this setting, gives results comparable to calprotectin measurement in feces.

Pouch endoluminal calprotectin measurement efficiently complements mPDAI in predicting PDAI defined pouchitis.

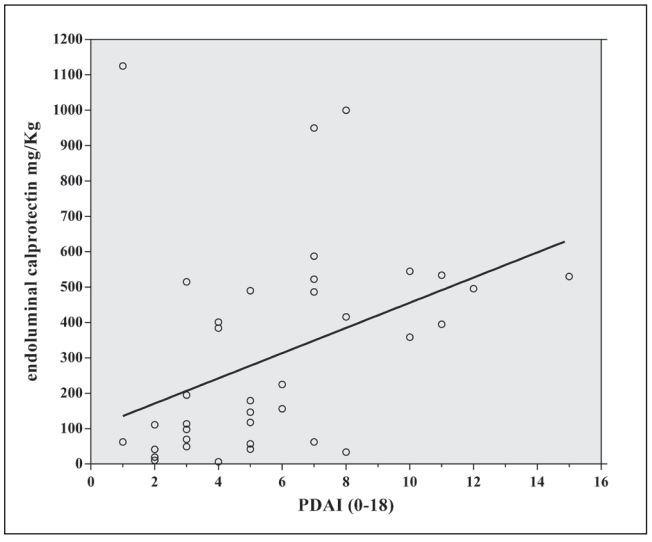

Fig. 1. Correlation between the pouchitis disease activity index (PDAI) score and the levels of pouch endoluminal calprotectin. Spearman r = 0.55, p = 0.0002, 95% CI: 0.28 to 0.74.

We then tested the ability of mPDAI in diagnosing pouchitis assessed with PDAI in our study's population. We observed that while using PDAI 65% (26/40) and 35% (14/40) of our patients were defined as without or with pouchitis, respectively, using mPDAI the frequency was 80% (32/40) and 20% (8/40), respectively. In particular, 7 of the 14 patients (50%) as having pouchitis using PDAI would miss the diagnosis using mPDAI. This observation suggests that histological assessment of acute inflammation was not redundant, at least in our study's population. Therefore, we evaluated the contribution of histology to PDAI. We observed a high correlation (Spearman r = 0.82, $p < 0.0001$, 95% CI: 0.67-0.90) between the acute histologic subscore and PDAI as well as a relatively high dependence of PDAI on acute histologic subscore ($r^2 = 0.6408$, $p < 0.0001$, 95% CI: 0.31 to 0.51, by Pearson correlation). This feature might well explain the differences observed in the present study between PDAI and mPDAI in the assessment of the presence of pouchitis. Since calprotectin is a protein abundantly expressed in neutrophils (17), we investigated the ability of calprotectin measurement to reflect the histological subscore. We evaluated the differences of calprotectin concentration between patients with acute histological subscore ≥ 3 (n = 19) and patients with acute histological subscore < 3 (n = 21) as defined in PDAI. As shown in figure 2, we observed a significant difference in calprotectin concentration between the two groups of patients (486 [34-1,000], 70 [6.5-1,125] mg/kg, median [range], respectively; p = 0.0004, by Mann-Whitney test), suggesting that calprotectin values may reflect the presence of histological inflammation. We performed a ROC analysis for the ability of calprotectin measurement to diagnose acute histological subscore ≥ 3. On the basis of the obtained results we fixed different range of calprotectin values corresponding to different values of sensitivity and specificity for the presence of inflammation to which we attributed a corresponding score ranging from 0-3. Specifically, we attributed score 0 to endoluminal calprotectin values < 37.625 that show sensitivity (%) 1 and 1specificity ranging from 1 to 0.762; score 1 to values ≥ 37.625 and < 66.200 that show sensitivity (%) 0.947 and 1-specificity ranging from 0.762 to 0.524; score 2 to values ≥ 66.200 and < 291.650 that show sensitivity (%) ranging from 0.895 to 0.737 and 1-specificity ranging from 0.524 to 0.190; score 3 to values ≥ 291.650 that show sensitivity (%) 0.737 and 1-specificity 0.143.

We combined the calprotectin subscore in the mPDAI index obtaining the new Cal-mPDAI score detailed in table I.

As shown in table II, the new Cal-mPDAI score shows values ranging from 0-15 with the illustrated frequency distribution of each value in our patients' study group. As also shown in table II, values ≥ 7 identify only patients with pouchitis, suggesting value ≥ 7 as the cut-off value of the new score. The new score correlates with PDAI and shows a high dependency on it ($r^2 = 0.792$, by Pearson correlation) (Fig. 3).

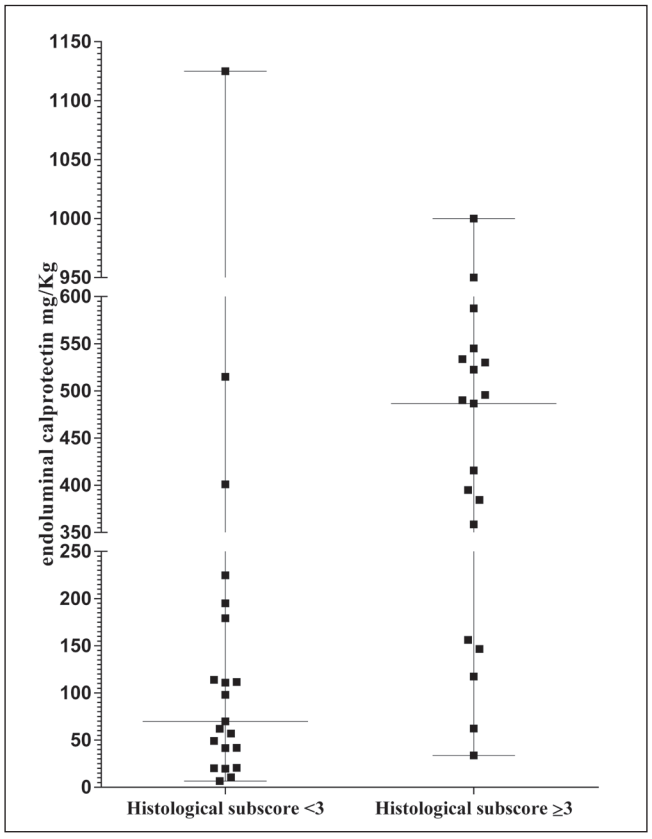

Fig. 2. Scatter plot showing distribution of the pouch endoluminal calprotectin values in patients with (subscore ≥ 3) and without (subscore < 3) histologically acute inflamed pouch.

We finally compared sensitivity, specificity, positive and negative predictive value of mPDAI and Cal-mPDAI in pouchitis diagnosis assessed with PDAI. As shown in table III, it appears that both sensitivity and specificity for pouchitis diagnosis were increased in Cal-mPDAI when compared with mPDAI, reaching the same specificity of PDAI. As a consequence, negative predictive value and positive predictive value of Cal-mPDAI values for diagnosis of pouchitis were both increased when compared with the values observed for mPDAI.

DISCUSSION

This is a pilot study with a small number of patients enrolled according to very strict admission criteria to obtain a homogeneous population. Diagnosis of pouchitis was defined using PDAI, which was considered as gold standard for the diagnosis. Our results demonstrated that the new score (Cal-mPDAI) resulting from the combination of pouch endoluminal calprotectin measurement with the mPDAI is associated with an increased sensitivity and specificity for the diagnosis of pouchitis when compared with mPDAI-defined pouchitis. In particular, it is demonstrated that the new score shows the same specificity as

Table I. The new Cal-mPDAI score range (0-15) and the subscores of each variable

Criteria	*Score*
Clinical	
Postoperative stool frequency	
Usual stool frequency	0
1-2 stools/day more than postoperative usual	1
3 or more stools/day than postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramp	
None	0
Occasional	1
Usual	2
Fever (temperature > 100 °F)	
Absent	0
Present	1
Endoscopic	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucosal exudate	1
Ulcerations	1
Endoluminal calprotectin values (mg/kg)	
< 37.6	0
≥ 37.6 < 66.2	1
≥ 66.2 < 291.6	2
≥ 291.6	3

PDAI, which was assessed to be 96.2%, when compared to the physician and surgeon's concomitant independent diagnosis of pouchitis (18). Since no symptoms and signs are unique to pouchitis, it is generally accepted that concurrent evaluation of endoscopic and histological findings brings to a more precise diagnosis that can be standardized by the utilization of score system based indices (19). To date the PDAI score represents the more widely used index for the assessment of pouchitis. However, the delay in calculating PDAI score related to the histology component represents a major obstacle for its application in daily practice. To overcome this limitation, the use of the mPDAI instead of PDAI has been proposed as an acceptable compromise between the diagnostic accuracy and the inconvenient delay of diagnosis itself. Nevertheless, as shown in the present study, at least in our patients, this approach results in a significant proportion of patients with pouchitis missing the diagnosis showing that the exclusion of histological assessment from the score is inappropriate. In patients with IPAA, faecal calprotectin was shown to correlate with the histologic subscore (13),

Table II. Distribution of patients assessed with the new score (Cal-mPDAI) according to the presence or the absence of PDAI-defined pouchitis

Cal-mPDAI score	*PDAI-defined pouchitis n = patients*		*Total n = patients (40)*
	No (26)	*Yes (14)*	
0	2	0	2
1	0	0	0
2	3	0	3
3	7	1	8
4	7	1	8
5	4	0	4
6	3	3	6
7	0	2	2
8	0	3	3
9	0	1	1
10	0	0	0
11	0	2	2
12	0	0	0
13	0	1	1
14	0	0	0
15	0	0	0

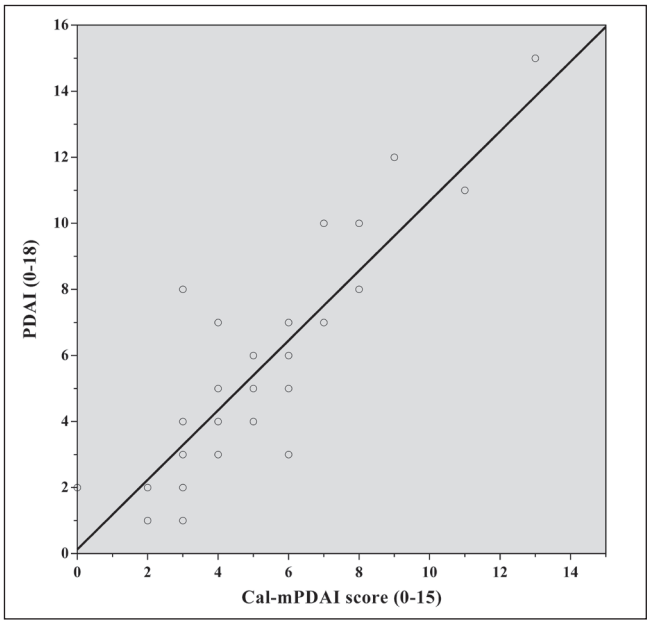

Fig. 3. Correlation between the Cal-mPDAI score and PDAI. Spearman r = 0.88, p < 0.0001.

suggesting that measurement of calprotectin may represent a potentially useful substitute for histology in the assessment of pouchitis. Clearly, histological evaluation remains mandatory to identify special features, such as granulomas, viral inclusion bodies, pyloric gland metaplasia and dysplasia (20).

In the present study, we observed that calprotectin measurement in pouch endoluminal fluid shows similar sensitivity and specificity in predicting pouchitis to the previously reported calprotectin measurement in feces (13), allowing us to use calprotectin values for further analysis. We observed a significant difference in the mean values of calprotectin in patients with histological subscore ≥ 3 when compared with patients with subscore < 3. On the basis of different values of sensitivity and specificity associated with histological subscore ≥ 3, we associated different range of calprotectin values with a subscore. The addition of this subscore to the mPDAI score resulted in a new score (Cal-mPDAI) showing specificity comparable to PDAI for the diagnosis of pouchitis and increased sensitivity when compared to mPDAI. The advantage of this approach is inherent to the fact that calprotectin measurement could be done during the same endoscopic session using the newly developed ELISA tests, which are easier and faster to be performed when compared to previous tests (21). Moreover, calprotectin measurement may reflect the global inflammatory activity of the pouch, possibly overcoming the disadvantage of histological examination related to the patchy expression of inflammation.

The main limitations of the present pilot study is the small number of patients enrolled according to very strict admission criteria to obtain an homogeneous population and that the new score still misdiagnosed 36% of patients with pouchitis. However this proportion is lower than the one observed using mPDAI score.

In conclusion, the present pilot study supports the idea that pouch endoluminal calprotectin measurement might improve the assessment of pouchitis activity made by mPDAI, resulting in a new index that, once validated in larger and less selected series of patients (i.e., including also other inflammatory conditions of the pouch), may be helpful in the management of patients with symptoms of suspected pouchitis.

Table III. Comparison between Cal-mPDAI and mPDAI in PDAI-defined pouchitis

Score	*Range*	*Pouchitis cut-off*	*SE (95% CI)*	*SP (95% CI)*	*PVP (95% CI)*	*PVN (95% CI)*
Cal-mPDAI	0-15	≥ 7	64 (0.49-0.79)	100 (1)	100 (1)	83 (0.72-0.94)
mPDAI	0-12	≥ 5	50 (0.35-0.65)	96 (0.36-1.56)	87 (0.77-0.87)	78 (0.65-0.91)

SE: Sensitivity; SP: Specificity; PVP: Predictive value of positive test; PVN: Predictive value of negative test; CI: Confidence interval.

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