Fecal Calprotectin: Validation of a laboratory marker for Intestinal Inflammation

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BACKGROUND AND AIMS:

Diagnosis and follow-up of inflammatory bowel disease often still require invasive ileocolonoscopy. This leads to a large amount of unnecessary, costly, unpleasant and possibly harmful procedures in patients who are eventually diagnosed with functional bowel disorders. To avoid invasive investigations in the differential diagnosis between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), both of which often present with a similar symptom complex, several laboratory markers have been proposed.

We evaluated two ELISA kits for the determination of fecal excretion of calprotectin, a calcium and zinc binding protein of the S-100 protein family with proapoptotic and antimicrobial abilities, making up >60% of neutrophil cytosolic protein. Large inflammatory, mainly neutrophilic infiltrates being a pathogenetic feature of IBD, fecal calprotectin has been proposed as a surrogate marker for intestinal inflammation. Determination of calprotectin concentration in feces is being said to allow discrimination between functional and organic bowel disease.[1] In patients with normal calprotectin levels in stool, ileocolonoscopy could be postponed. In addition, calprotectin is extremely resistant to bacterial degradation, being stable in stool for up to one week in stool at room temperature, which makes it a very attractive laboratory marker.

Furthermore several studies indicate that calprotectin may correlate with the endoscopic severity of intestinal lesions, suggesting it can be used as a follow-up marker in the treatment of inflammatory bowel disease.

PATIENTS and METHODS:

We determined calprotectin concentrations in stool samples of 19 patients (5 male, 14 female; mean age 34 (17-60)), consulting an outpatient Gastroenterology clinic with complaints of abdominal pain and/or diarrhea. Subjects included patients with known IBD as well as undiagnosed patients.

Calprotectin in each stool sample was determined in duplo by both kits (CALPRO Calprotectin ELISA Test – Nova Tec Immunodiagnostica GmbH; Calprotectin ELISA, Bühlman Laboratories AG). Manufacturer's instructions were strictly followed. Both methods involved an extraction procedure followed by a sandwich ELISA.

For the interpretation of test results, we formulated three calprotectin concentration intervals, which are valid for both tests:

1. < 50 µg/g = negative, not suggestive for IBD
2. 50 – 200 µg/g = gray zone, result uncertain
3. >200 µg/g = positive, suggestive for IBD

Agreement between both tests was assessed. Test results were compared with clinical diagnosis – based on ileocolonoscopy. Sensitivity was calculated for both methods. For this calculation, patients 4 and 5 were excluded, because of clinical remission, as well as patients 6 and 16, because they had no diagnosis of IBD.

Because of the low number of normal patients, specificity could not be calculated.

For the most sensitive method – the Bühlman ELISA, within-run precision was calculated for each of the result intervals, using the duplo results. Furthermore, we evaluated the agreement between calprotectin level and a Crohn's endoscopic index of severity, SES-CD [2] and between calprotectin level and the Harvey Bradshaw Index (HBI).

Patient N°	Age	Bühlman result (µg/g)	Nova Tec result (µg/g)	Diagnosis	SES-CD score	HBI
1	21	847,12	418,03	infection/CD	6	3
2	36	1015,26	1593,01	CD	/	/
3	29	541,01	140,43	CD	/	12
4	38	35,22	21,76	CD	/	13
5	32	9,65	25,82	CD	4	4
6	33	204,4	77,24	ileal lymphoid hyperplasia	0	/
7	32	846,7	1667,03	CD	17	7
8	36	1525,24	2007,79	UC	/	/
9	29	796,56	523,09	CD	16	18
10	43	715,95	277,86	CD	/	2
11	35	88,32	33,63	CD	3	9
12	25	>upper range	1990,32	CD	>10	>7
13	17	1156,65	1529,16	CD	13	4
14	60	242,81	214,73	CD	/	6
15	57	91,73	33,12	CD	/	4
16	21	10,49	16,24	no diagnosis	/	/
17	37	330,39	95,51	CD	/	5
18	30	814,44	784,12	CD	12	>2
19	38	475,93	556,49	CD	/	5

Table 1. Overview of results. CD = Crohn's disease; UC = Ulcerative Colitis; / = no results available

RESULTS:

An overview of test results is given in table 1.

We observed an acceptable agreement between both tests. Only 2 discrepancies were found (patient numbers 11 and 15; negative test result for Nova Tec, positive for Bühlman in both cases). For both patients, the Bühlman ELISA correlated best with endoscopic diagnosis, revealing the better sensitivity of the latter assay. Sensitivity for the Nova Tec and Bühlman tests were 87% and 100% respectively. The negative Nova Tec result for patient 11 can be explained by the fact that she was under therapy at the time the sample was taken. The Bühlman result for this patient was also in the gray zone

Within-run CV for the Bühlman test for the three result intervals < 50 µg/g, 50 – 200 µg/g and >200 µg/g were 9,87%, 4,26% and 3,24% respectively. These good results indicate there is no need for performing duplo testing, reducing the cost of the assay.

For both tests, there seems to be good agreement between calprotectin level and SES-CD, with high calprotectin concentrations showing high SES-CD scores. Unfortunately however, SES-CD scores were not available for every CD patient.

Agreement between calprotectin test results and HBI is less convincing, reflecting the subjective nature of this scoring system.

CONCLUSION:

Because of better sensitivity, the Bühlman test was selected for implementation in our laboratory. The better sensitivity is possibly explained by the use of a monoclonal antibody against calprotectin in the Bühlman test, while Nova Tec uses a polyclonal antibody. Furthermore, one of the patients that was not detected by the Nova Tec assay was under therapy. The excellent sensitivity of the Bühlman assay allows use of this test as a screening method for IBD. Ileocolonoscopy in patients with negative results should no longer be performed.

Within-run CV for the selected method is very good, making duplo measurements unnecessary. This reduces assay costs, both for the laboratory, as well as for the patient (calprotectin is not yet reimbursed).

Finally, good agreement between Bühlman ELISA and SES-CD, suggests that calprotectin can reliably detect inflammation and may replace ileocolonoscopy as a marker for severity in IBD. Poor agreement with HBI allows us to question the clinical usefulness of this scoring system.

REFERENCES:

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