Discriminating IBD from IBS: Comparison of the Test Performance of Fecal Markers, Blood Leukocytes, CRP, and IBD Antibodies

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Background: Symptoms of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) can overlap. We aimed to determine the accuracy of fecal markers, C-reactive protein (CRP), blood leukocytes, and antibody panels for discriminating IBD from IBS and to define a "best test."

Methods: We prospectively included 64 patients with IBD (36 Crohn's disease [CD], 28 ulcerative colitis [UC]), 30 with IBS, and 42 healthy controls. Besides CRP and blood leukocytes, blinded fecal samples were measured for calprotectin (PhiCal Test, enzyme-linked immunosorbent assay [ELISA]), lactoferrin (IBD-SCAN, ELISA), Hexagon-OBTI (immunochromatographic test for detection of human hemoglobin), and LEUKO-TEST (lactoferrin latex-agglutination test). Blinded serum samples were measured for the antibodies ASCA (ELISA) and pANCA (immunofluorescence).

Results: Overall accuracy of tests for discriminating IBD from IBS: IBD-SCAN 90%, PhiCal Test 89%, LEUKO-TEST 78%, Hexagon-OBTI 74%, CRP 73%, blood leukocytes 63%, CD antibodies (ASCA+/pANCA− or ASCA+/pANCA+) 55%, UC antibodies (pANCA+/ASCA−) 49%. ASCA and pANCA had an accuracy of 78% for detecting CD and 75% for detecting UC, respectively. The overall accuracy of IBD-SCAN and PhiCal Test combined with ASCA/pANCA for discriminating IBD from IBS was 92% and 91%, respectively.

Conclusions: The PhiCal Test and IBD-SCAN are highly accurate for discriminating IBD from IBS. There is only marginal additional diagnostic accuracy when the PhiCal Test and IBD-SCAN are combined with ASCA and pANCA. ASCA and pANCA have a high specificity for IBD.

Discriminating irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD), especially with mild disease activity, is a common clinical challenge. Both conditions share a symptom complex with abdominal pain and altered bowel habits; furthermore, IBS-like symptoms are frequently reported in patients before the diagnosis of IBD.[1] The diagnostic value of the Manning criteria for diagnosing IBS is limited because of moderate sensitivity.[2] If IBS is suspected, accepted diagnostic procedure includes at least a sigmoidoscopy in younger patients, whereas a colonoscopy is recommended for patients over the age of 50.[3] In order to avoid invasive investigations, several noninvasive markers have been evaluated for their capacity to distinguish between functional and "organic," especially inflammatory gastrointestinal disease.

In the past few years, different neutrophil-derived proteins in feces have been studied, including fecal lactoferrin and calprotectin.[4] Calprotectin represents 60% of cytosolic proteins in granulocytes; the amount of calprotectin in feces is therefore proportional to the neutrophil migration to the gastrointestinal mucosa. Fecal calprotectin is stable against degradation for up to 1 week at room temperature.[5] Lactoferrin, an 80-kDa iron-binding glycoprotein, is a major component of the secondary granules of polymorphonuclear neutrophils. Leukocyte infiltration of the mucosa in intestinal inflammation results in an increase in the concentration of lactoferrin in feces.[6] Fecal calprotectin as well as lactoferrin have been shown to possess a good negative predictive value in excluding inflammation of the gut.[7,8]

The serologic panel for IBD is rapidly expanding. So far, antineutrophil cytoplasmatic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* mannan antibodies (ASCA) are the most widely studied markers.[9] ASCA, occurring mainly in Crohn's disease (CD) patients, recognizes carbohydrate

epitopes in phosphopeptidomannan, which is a 200-kDa glycoprotein in the cell wall; the major epitope was identified as mannotetraose.[10,11] The ANCAs predominantly found in ulcerative colitis (UC) present as atypical pANCA, characterized by a broad inhomogeneous rim-like staining of the nuclear periphery.[12] So far, many single markers in feces or serum have been evaluated for distinguishing inflammatory from functional colonic disease; a single best test or a combined best test has not yet been defined.

The aims of our study were to determine the accuracy of 4 fecal markers (Hexagon-OBTI, LEUKO-TEST, PhiCal Test, and IBD-SCAN), C-reactive protein (CRP), blood leukocytes, and serum antibodies (ASCA, pANCA) in a patient group with IBD, IBS, and healthy controls. We hypothesized that quantitative fecal leukocyte markers are superior to blood leukocytes and CRP for discriminating IBD from IBS and that the accuracy of fecal markers is improved when combined with IBD antibodies.

In this study we wanted to answer the following question as the primary endpoint: What is the accuracy of fecal markers, CRP, blood leukocytes, and IBD antibodies for discriminating IBD from IBS? As secondary endpoints we wanted to answer the following questions: Is the test performance of fecal leukocyte markers improved when combined with IBD antibodies? How do fecal lactoferrin and calprotectin correlate with endoscopically assessed scores of disease activity in CD and UC, respectively? What is the accuracy of ASCA and pANCA to discriminate between CD and UC?

MATERIALS AND METHODS

Subjects

Outpatients and inpatients from the Departments of Gastroenterology of the University Hospital Bern and Kantonsspital Lucerne were prospectively enrolled between April 2005 and October 2006. The reasons for admission were workup of abdominal pain, altered bowel habit, and/or anorectal bleeding (IBS patients did not present with anorectal bleeding). Written informed consent was obtained at least 24 hours before colonoscopy. The study was conducted with approval from the local ethics committees.

After instruction by the study doctor, patients were provided with a fecal specimen collection set consisting of 3 fecal tubes (tubes for 1 mL, order number 55478, Sarstedt, Nümbrecht, Germany) and a Hexagon-OBTI test. Collection of fecal specimens for the tubes and the Hexagon-OBTI was performed by the patients themselves. The fecal specimen set from the outpatients was sent by urgent mail from Monday to Thursday so that no specimens arrived in the laboratory on weekends. For inpatients the fecal collection set was prepared by a trained nurse and then sent to the laboratory.

Inclusion criteria included complete colonoscopy with intubation of terminal ileum including biopsies (at least 2

biopsies of terminal ileum and 3 colonic biopsies), informed consent, age 18–80 years, fecal samples delivered from 3 to 1 days before colonoscopy (bowel preparation was not started until the fecal specimen was delivered), and after the evaluation, an established diagnosis of bowel disease. For understandable reasons, the persons in the healthy control group had no endoscopic workup.

Exclusion criteria were incomplete ileocolonoscopy, microscopic colitis, infectious ileocolitis, colorectal cancer, colorectal polyps, unclear diagnosis (e.g., indeterminate colitis), urinary incontinence (risk of contamination of fecal samples), inability to collect fecal samples, infection with HIV and/or hepatitis B or C, history of colorectal or small bowel surgery, regular intake of aspirin and/or an NSAID ($\geq$2 tablets/week). The 2 participating gastroenterologists (both board certified) who established the diagnosis were not informed about the results of fecal markers and IBD antibodies to prevent bias.

A diagnosis of colonic disease was prospectively established based on clinical history and examination, laboratory findings (hematogram, electrolytes, CRP, ASAT, ALAT, GGT, AP, bilirubin, lipase, creatinin, glucose), abdominal ultrasonography, and ileocolonoscopy including biopsies also of endoscopically normal regions. Additionally, all IBS patients had an endoscopy of the upper gastrointestinal (GI) tract with normal histologic findings. The diagnosis was verified in a second outpatient visit 4–6 weeks after the first diagnosis; there were no changes in the primary diagnostic assessment. Patients were allocated to 3 groups based on the above-mentioned findings: IBS, IBD (CD and UC), and healthy controls.

For the diagnosis of IBS the following criteria were applied: exclusion of infectious diseases (fecal microbiology, fecal test for *Giardia lamblia* antigen), of celiac disease (deep duodenal biopsy, anti-tissue-transglutaminase IgA, anti-gliadin-IgA/IgG, total IgA), of chronic IBD (endoscopy, histology), of diverticulosis or diverticulitis (endoscopy and/or CT scan), of microscopic enterocolitis (histology), or of ischemic and medication-induced colitis. All IBS patients fulfilled the Rome II criteria; there were no alarm symptoms such as anemia or weight loss, the endoscopic and histologic workup was normal, and all patients had a normal transabdominal sonography. The decision to perform a systematic examination of the jejunum and proximal ileum was left to the judgment of the treating gastroenterologist. In total, 11 IBS patients (37%) had a small bowel examination, 9 (30%) by hydro-CT scan, and 2 (7%) by an MR enteroclysis. The small bowel examination in these 11 patients was normal and did not change anyone's diagnosis.

The IBD group included patients with CD or UC. The diagnosis was established based on symptoms and clinical examination, endoscopic findings, histologic analysis, radiologic workup, and laboratory tests (exclusion of infectious

enterocolitis). The controls were healthy persons from the clinical and laboratory staff willing to provide blood and fecal samples. All healthy controls were free of symptoms and had a normal clinical examination and abdominal ultrasonography. Except for birth control pills in some women, these people were not on any regular medication.

Classification of the Severity of Disease

The clinical activity of CD patients was measured according to the Crohn's Disease Activity Index (CDAI)[13] and the endoscopic activity was assessed using the Simplified Endoscopic Activity Score for Crohn's Disease (SES-CD).[14] The clinical activity in UC patients was evaluated using the Mayo Score.[15] As the endoscopic section of this score is not validated in isolation, we assessed the endoscopic severity according to the endoscopic part of the Rachmilewitz Score.[16]

Fecal Assays

The following test kits were used: PhiCal Test, IBD-SCAN, LEUKO-TEST, and Hexagon-OBTI. The laboratory technicians (P.S. and M.T.) performing the analyses were blinded to the patient diagnosis and the study hypothesis. All fecal samples were processed within 48 hours after collection. The assays were performed according to the test instructions. Enzyme-linked immunosorbent assay (ELISA) plates were read on a Spectra mini reader (TECAN) at an OD of 450 nm.

The PhiCal Test was purchased from Medical Instrument (Solothurn, Switzerland, Art-No. 006), delivered by CALPRO AS (Oslo, Norway). This sandwich ELISA measures quantitative calprotectin. Fecal specimens were diluted at 1:2500.

The IBD-SCAN was provided by Labo-Life (Pully, Switzerland), delivered by TechLab (Blacksburg, VA). This ELISA measures quantitative lactoferrin. Dilution of fecal specimens was 1:400.

The LEUKO-TEST was obtained from Labo-Life, an agglutination test delivered by TechLab that detects elevated values of lactoferrin indicating increased fecal leukocytes. Sample reactions were recorded as negative (no visible agglutination) or positive (visible agglutination).

The Hexagon-OBTI was obtained from Human Gesellschaft für Biochemica und Diagnostica (Wiesbaden, Germany). The test is specific for human hemoglobin, which reacts with a murine monoclonal antihuman-hemoglobin antibody. For further details on test performance data see www.human.de/data/gb/vr/1t-obti.pdf or www.human-de.com/data/gb/vr/1t-obti.pdf.

IBD Antibodies, CRP, and Blood Leukocytes

We measured ASCA and pANCA. The CD markers were defined as ASCA+/pANCA− or ASCA+/pANCA+, and the UC markers as pANCA+/ASCA−. The laboratory technician (B.S.) was blinded to the patient diagnosis and the study hypothesis. ASCA ELISA testing was done as described elsewhere.[17] A patient was considered ASCA+ when positive for ASCA IgG, IgA, or both. pANCA testing was done on cytospins of neutrophils as described elsewhere.[18] Blood leukocytes (normal range 2.6–7.8 Giga/L) as well as CRP (upper limit of normal <5 mg/L) were determined as routine laboratory values. Platelet counts were included in this setting but not analyzed; a sedimentation rate was not determined.

Statistical Analysis

Data were listed on an Excel sheet (Microsoft Excel 2003) and statistical analysis was performed with a statistical package program (STATA v. 9.0, College Station, TX). The results of numeric data are presented as mean ± standard deviation (SD) and range. Fisher's exact test (2-sided) or the chi-square test were used to explore associations of categorical data in 2 independent groups. The Wilcoxon rank sum test was used to explore associations of numeric data in 2 independent groups. $P < 0.05$ was considered statistically significant and a Bonferroni adjustment was performed where appropriate. The test characteristics are given as sensitivity, specificity, positive and negative predictive value (SENS, SPEC, PPV, NPV), and overall accuracy. The overall accuracy is calculated by addition of the true-positive and true-negative test results divided by all tests $(a+d)/(a+b+c+d)$ and admits the comparative evaluation of the various tests.

RESULTS

Patient Characteristics

A total of 187 patients were asked for inclusion and 136 (73%) participated. The reasons for exclusion were: 18 (9%) declining participation, 12 (6%) polyps, 5 (3%) incomplete ileocolonoscopy, 4 (2%) noncompliance with fecal sampling, 4 (2%) intake of a nonsteroidal antiinflammatory drug (NSAID) and/or aspirin, 3 (2%) ileocecal resection, 2 (1%) colorectal cancer, 2 (1%) urinary incontinence, and 1 (1%) indeterminate colitis. Demographic details of the included patients are shown in Table 1. Clinical activity: 23 CD patients (64%) had a CDAI up to 150 and 13 (36%) had a CDAI >150. In the UC patients none were in clinical remission (defined as ≤2 points on a 3-day basis). Fifteen patients had mild disease (3–5 points), 11 moderate (6–10 points), and 2 severe disease (11–12 points).

Detection Limits and ROC Analysis of Fecal Tests, CRP, and Blood Leukocytes

The cutoffs provided by the manufacturer or laboratory were as follows: PhiCal Test 50 μg/mL feces, IBD-SCAN 7 μg/mL feces, LEUKO-TEST range 13–60 μg/mL feces, Hexagon-OBTI 0.05 mg Hb/mg feces, CRP 5 mg/L, and

TABLE 1. Patient Characteristics

	Crohn's Disease	Ulcerative Colitis	IBS	HC
Number of patients	36	28	30	42
Female	19	13	22	28
Age: Mean $\pm$ SD, range	41 $\pm$ 18 (20–78)	45 $\pm$ 14 (23–72)	40 $\pm$ 19 (20–79)	37 $\pm$ 9 (23–52)
Time between symptoms and diagnosis (yrs)	1.3 $\pm$ 0.9	0.6 $\pm$ 0.4	2.7 $\pm$ 2	—
Location of disease	Ileocolonic 19	Proctitis 8	—	—
	Colon 7	Proctosigmoiditis 11		
	Small bowel 10	Extensive 4		
		Pancolitis 5		
Disease behavior	Inflammatory 33			
	Stenotic 1			
	Penetrating 2			
Clinical Disease activity			—	—
CDAI	162 $\pm$ 116 (12–434)			
Mayo Score		6 $\pm$ 2 (3–11)		
Endoscopic activity			—	—
SES-CD	19 $\pm$ 11 (3–41)			
Rachmilewitz Score		7 $\pm$ 2 (3–12)		
Smoking status	6 (14%)	4 (8%)	5 (17%)	3 (7%)

HC, healthy controls; IBS, irritable bowel syndrome; SD, standard deviation; SES-CD, simplified endoscopic score for Crohn's disease.

blood leukocytes 7.8G/L. The range for the LEUKO-TEST is not published; the data were delivered on request by the manufacturer.

The area under the curve of the receiver operating characteristics (ROC) in discriminating IBD from our healthy controls were as follows: PhiCal Test 0.947, IBD-SCAN 0.92, LEUKO-TEST 0.896, Hexagon-OBTI 0.836, CRP 0.836, and blood leukocytes 0.717.

Frequency of IBD Antibodies

ASCA were found in 19 (53%) CD patients and in 1 (3%) IBS patient. UC patients and healthy controls were negative for ASCA. pANCA were found in 13 (46%) of UC patients and 2 (6%) CD patients (being also ASCA+), whereas IBS patients and healthy controls were negative.

Test Characteristics of Quantitative Tests in Feces and Serum in the 3 Patient Groups

The test characteristics of the quantitative assays for fecal calprotectin, lactoferrin, CRP, and blood leukocytes are shown in Table 2, comparing the healthy controls, IBS, and IBD groups. Fecal calprotectin and lactoferrin were significantly elevated in IBD patients compared to healthy controls ($P < 0.0001$). Fecal calprotectin and lactoferrin as well as CRP and blood leukocytes in IBS patients were found in the range of healthy controls.

Test Characteristics of Serum and Fecal Markers

The test performance (given by sensitivity/specificity/ positive and negative predictive value in percent) of single serum and fecal markers are shown in Table 3. In summary, the best test performance for discriminating IBD from IBS was measured with the PhiCal Test (83/100/100/74) and IBD-SCAN (87/96/98/77), followed by LEUKO-TEST (70/96/97/60), Hexagon-OBTI (62/96/97/55), CRP (64/92/94/55), and blood leukocytes (51/88/90/46). CD markers (36/96/95/41) as well as UC markers (25/100/100/38) had both a low sensitivity and negative predictive value but a high specificity and positive predictive value for the presence of IBD.

Test Performance of PhiCal Test and IBD-SCAN Combined with IBD Antibodies

We were interested whether the combination of fecal markers and IBD antibodies would increase the test accuracy in the discrimination of IBD from IBS. The test performance of the best fecal markers, notably the PhiCal Test and IBD-SCAN, combined with CD- and UC-specific antibody panels for discrimination of IBD from IBS is shown in Table 4. In summary, the PhiCal Test combined with CD markers led to an improved sensitivity (93%) and NPV (92%) for discriminating CD from IBS; however, the specificity (96%) and PPV (97%) were slightly reduced (from 100% if the PhiCal Test was used as a single marker) because of 1 ASCA+ IBS patient. The PhiCal Test combined with UC markers led to improved sensitivity (91%) and NPV (93%) for discriminat-

TABLE 2. Test Characteristics of Fecal Calprotectin, Lactoferrin, CRP, and Blood Leukocytes

		HC	IBS	IBD	HC vs. IBS HC vs. IBD IBS vs. IBD
Calprotectin μg/mL (PhiCal-Test)	Mean ± SD	18.9 ± 2.8	21.4 ± 6.9	287.4 ± 223.1	$P = 0.077$ $P < 0.0001$
	95%-CI	18–19.8	17.9–24.6	209.5–362.5	$P < 0.0001$
Lactoferrin μg/mL (IBD-SCAN)	Mean ± SD	2.2 ± 0.9	2.8 ± 3.9	138.7 ± 112.3	$P = 0.348$ $P < 0.0001$
	95%-CI	1.9–2.5	1.1–4.7	101–175.4	$P < 0.0001$
CRP (mg/L)	Mean ± SD	3.2 ± 0.7	3.6 ± 1.6	17.6 ± 29	$P = 0.186$ $P = 0.0018$
	95%-CI	2.9–3.4	2.8–4.3	7.8–27.8	$P = 0.0027$
Blood leukocytes (G/L)	Mean ± SD	5.3 ± 1.4	5.9 ± 1.5	7.8 ± 4.4	$P = 0.106$ $P = 0.0005$
	95%-CI	4.9–5.7	5.2–6.7	6.4–9.7	$P = 0.0012$

CRP, C-reactive protein; HC, healthy controls; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease; SD, standard deviation; CI, confidence interval.

ing UC from IBS without impairment of specificity and PPV (both 100%). IBD-SCAN combined with CD markers led to improved sensitivity (93%) and NPV (92%) for discriminating CD from IBS. IBD-SCAN combined with UC markers similarly led to an improved test performance for discriminating UC from IBS.

Overall Accuracy of Fecal Markers, IBD Antibodies, CRP, and Blood Leukocytes

The overall accuracy of the tests for discriminating IBD from IBS is presented in Table 5. In summary, the PhiCal Test and IBD-SCAN were highly accurate at distinguishing between IBD and IBS (overall accuracy 89% and 90%,

TABLE 3. Test Performance of the 4 Fecal Markers, CRP, Blood Leukocytes, and Antibody Panels

	SENS	SPEC	SENS	SPEC	SENS	SPEC
	PPV	NPV	PPV	NPV	PPV	NPV
Test	CD vs. IBS		UC vs. IBS		IBD vs. IBS	
PhiCal-Test	83%	100%	83%	100%	83%	100%
	100%	83%	100%	86%	100%	74%
IBD-SCAN	83%	96%	91%	96%	87%	96%
	96%	83%	95%	92%	98%	77%
LEUKO-TEST	67%	96%	74%	96%	70%	96%
	95%	71%	94%	80%	97%	60%
Hexagon-OBTI	57%	96%	70%	96%	62%	96%
	94%	65%	94%	77%	97%	55%
CRP	73%	92%	52%	92%	64%	92%
	92%	74%	86%	68%	94%	55%
Blood leukocytes	47%	88%	57%	88%	51%	88%
	82%	58%	81%	69%	90%	46%
CD markers	63%	96%	0%	96%	36%	96%
	95%	69%	0%	51%	95%	41%
UC markers	7%	100%	48%	100%	25%	100%
	100%	47%	100%	68%	100%	38%

CD markers: ASCA+/pANCA− or ASCA+/pANCA+; UC markers: pANCA+/ASCA−. CRP, C-reactive protein; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; CD, Crohn's disease; UC, ulcerative colitis; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease.

TABLE 4. Test Performance of PhiCal-Test and IBD-SCAN in Combination with IBD Antibodies

| Combined Tests | SENS | SPEC | SENS | SPEC | SENS | SPEC |
| | PPV | NPV | PPV | NPV | PPV | NPV |
	CD vs. IBS		UC vs. IBS		IBD vs. IBS	
PhiCal-Test and CD-markers	93%	96%	83%	96%	89%	96%
	97%	92%	95%	86%	98%	80%
PhiCal-Test and UC-markers	83%	100%	91%	100%	87%	100%
	100%	83%	100%	93%	100%	78%
IBD-SCAN and CD-markers	93%	92%	91%	92%	92%	92%
	93%	92%	91%	92%	96%	85%
IBD-SCAN and UC-markers	83%	96%	96%	96%	89%	96%
	96%	83%	96%	96%	98%	80%

CD markers: ASCA+/pANCA− or ASCA+/pANCA+; UC markers: pANCA+/ASCA−. SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; CD, Crohn's disease; UC, ulcerative colitis; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease.

respectively); these tests were superior to LEUKO-TEST (78%), Hexagon-OBTI (74%), CRP (73%), blood leukocytes (63%), and IBD antibody panels (55% for CD and 49% for UC markers). The combination of PhiCal Test or IBD-SCAN with CD and UC markers led to a slightly increased overall accuracy for discriminating IBD from IBS. The accuracy was 73% for both ASCA and pANCA to discriminate CD from UC.

Correlation of Fecal Markers with Endoscopic Activity Scores

The data for endoscopic assessment of severity in CD and UC are presented in Table 1. There were 19 CD patients with up to 20 points (simplified as minor activity) and 17 with more than 20 points (simplified as moderate to severe activity). We found lactoferrin (221.5 ± 22.8 μg/mL versus 37.9 ± 11 μg/mL, $P < 0.0001$) as well as calprotectin (433 ± 42.6 μg/mL versus 112.1 ± 31.3 μg/mL, $P < 0.0001$) significantly higher in the group with moderate to severe endoscopic activity. There were 7 UC patients with endoscopically assessed mild disease (defined as up to 4 points) and 16 with moderate to severe disease (5 to 12 points). We found calprotectin (390.0 ± 49.4 μg/mL versus 123 ± 28.6 μg/mL, $P = 0.0025$) significantly higher in the group with moderate to severe endoscopic activity; the results with lac-

TABLE 5. Overall Accuracy of the Different Tests for Discriminating CD, UC, and IBD from IBS

Single Tests	CD vs. IBS	UC vs. IBS	IBD vs. IBS
PhiCal-Test	91%	92%	89%
IBD-SCAN	89%	94%	90%
LEUKO-TEST	80%	85%	78%
Hexagon-OBTI	75%	85%	74%
CRP	82%	73%	73%
Blood leukocytes	65%	73%	63%
CD markers	78%	50%	55%
UC markers	49%	75%	49%

Combined Tests	CD vs. IBS	UC vs. IBS	IBD vs. IBS
PhiCal-Test and CD-markers	95%	90%	91%
PhiCal-Test and UC-markers	89%	93%	90%
IBD-SCAN and CD-markers	93%	92%	92%
IBD-SCAN and UC-markers	89%	96%	91%

CD markers: ASCA+/pANCA− or ASCA+/pANCA+; UC markers: pANCA+/ASCA−. CRP, C-reactive protein; CD, Crohn's disease; UC, ulcerative colitis; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease.

toferrin did not reach a statistical significance (202.3 ± 22.3 μg/mL versus 124.8 ± 37.6 μg/mL, *P* = 0.078).

DISCUSSION

In this study we evaluated CRP, blood leukocytes, 4 different fecal markers, and IBD antibodies to discriminate between IBD and IBS. Regarding the use of single tests, the PhiCal Test and IBD-SCAN had the best overall accuracy. The evaluated test characteristics are in accordance with other studies.[19–21] Fecal calprotectin and lactoferrin also correlated with the endoscopically assessed disease severity in CD and UC, which reflects that the degree of mucosal damage is directly proportional to the amount of these markers. We have already assessed this correlation in a smaller cohort of IBD patients.[22] These observations indicate that quantitative fecal leukocyte markers can be useful for monitoring disease activity in IBD.[23] The LEUKO-TEST (accuracy 78%) was designed to detect fecal leukocytes and not to measure an elevated lactoferrin with a defined cutoff.[24,25] For technical reasons, latex agglutination assays cannot be standardized within a narrow range, and this may explain the lower discriminatory power compared to PhiCal Test and IBD-SCAN.

The overall accuracy of CRP (73%) was found to be only moderate, mainly influenced by the low sensitivity (52%) in the UC group. Compared with these, the sensitivity of CRP in the CD group (73%) was much better. Our results match the findings of other groups, demonstrating that the sensitivity of CRP for detection of IBD ranges between 50% and 60% for UC and between 70% and 100% for CD.[26,27]

We evaluated ASCA and pANCA because they represent the most widely studied IBD antibodies.[9] CD- and UC-specific antibody panels had an only moderate overall accuracy for discriminating IBD from IBS; however, their specificity was remarkably high. Increased concentrations of ASCA are reported in 60%–70% of patients with CD and 0%–5% of healthy controls; the frequency of our ASCA findings are in accordance with these results.[28,29] However, the relatively low ASCA frequency actually measured is in contrast to previous results from our own laboratory and probably associated with the limited number of patients tested in this study.[30] We found pANCA to have a high specificity for IBD. The frequency found in UC patients (46%) was lower than measured by other groups, including our own (60%–80%); the frequency in CD is in good accordance with the findings of other groups.[31–33] Regarding the overall accuracy, IBD antibodies had a low diagnostic yield for discriminating IBD from IBS. However, the accuracy of ASCA/pANCA was 78% for the detection of CD and 75% for the detection of UC, respectively. The accuracy of 73% for both ASCA and pANCA indicates that these markers are useful for discriminating CD from UC. The overall accuracy of the best fecal tests, notably the PhiCal Test and IBD SCAN, were only minimally improved when combined with IBD antibod-

ies. We conclude that ASCA and pANCA have little additional value in the discrimination of IBD from IBS when combined with quantitative fecal leukocyte markers.

One limitation of our study lies in the current definition of IBS. These patients were not evaluated systematically with a small bowel examination; the decision to perform was left to the clinician's judgment. Therefore, a diagnostic uncertainty remains as to whether a small bowel CD in this particular patient group is missed. However, looking at the current clinical recommendations and also the prevalence of IBS with its socioeconomic burden, it is certainly not justified to perform a systematic small bowel examination in every IBS patient.[34]

Our results support the already established practice at several centers to use calprotectin or lactoferrin as a routine test for discriminating IBS from organic, especially inflammatory bowel disease.[35] Tibble et al[36] have shown a sensitivity of positive Rome criteria of 85% for IBS (specificity 81%), and a sensitivity of elevated calprotectin of 89% for organic disease (specificity 79%), thus providing a relatively simple and cost-effective instrument in the differentiation of organic versus functional bowel disease.

In summary, we suggest that the absence of elevated fecal leukocyte markers should be documented before diagnosing a patient with IBS. As fecal leukocyte markers are unspecific for bowel inflammation, endoscopic workup remains crucial for an exact diagnosis. Quantitative fecal leukocyte markers seem useful for monitoring bowel inflammation. The IBD antibodies ASCA and pANCA should not be primarily measured for discriminating IBD from IBS as their additional diagnostic value to fecal leukocyte markers in this issue is only marginal. However, their specificity for the presence of organic bowel disease is remarkably high. The accuracy of 73% indicates that ASCA and pANCA are useful for discriminating CD from UC.

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