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Diagnostic Accuracy of Point-of-Care Fecal Calprotectin and Immunochemical Occult Blood Tests for Diagnosis of Organic Bowel Disease in Primary Care: The Cost-Effectiveness of a Decision Rule for Abdominal Complaints in Primary Care (CEDAR) Study

Liselotte Kok,[1*] Sjoerd G. Elias,[1] Ben J.M. Witteman,[2] Jelle G. Goedhard,[3] Jean W.M. Muris,[4] Karel G.M. Moons,[1] and Niek J. de Wit[1]

BACKGROUND: Fecal biomarker tests that differentiate between organic bowel disease (OBD) and non-OBD in primary care patients with persistent lower-abdomen complaints could reduce the number of unnecessary referrals for endoscopy. We quantified the accuracy of fecal calprotectin and immunochemical occult blood (iFOBT) point-of-care (POC) tests and a calprotectin ELISA in primary care patients with suspected OBD.

METHODS: We performed biomarker tests on fecal samples from 386 patients with lower-abdomen complaints suggestive for OBD. Endoscopic and histological diagnosis served as reference.

RESULTS: OBD was diagnosed in 99 patients (prevalence 25.9%); 19 had adenocarcinoma, 53 adenoma, and 27 inflammatory bowel disease. Sensitivity for OBD was 0.64 (95% CI 0.54–0.72) for calprotectin POC, 0.56 (0.46–0.66) for iFOBT POC, and 0.74 (0.65–0.82) for calprotectin ELISA; specificities were 0.53 (0.48–0.59), 0.83 (0.78–0.87), and 0.47 (0.41–0.53), respectively. Negative predictive values (NPVs) were 0.81 (0.74–0.86), 0.85 (0.80–0.88), and 0.84 (0.78–0.89); positive predictive values (PPVs) varied from 0.32 (0.26–0.39) and 0.33 (0.27–0.39) (calprotectin tests) to 0.53 (0.44–0.63) (iFOBT POC). Combining the 2 POC tests improved sensitivity [0.79 (0.69–0.86)] and NPV [0.87 (0.81–0.91)] but lowered specificity [0.49 (0.44–0.55)] and PPV [0.35 (0.29–0.42)]. When adenomas ≤1 cm were considered non-OBD, the NPV of all tests improved to >0.90 [combined POC tests, 0.97 (0.93–0.99)].

CONCLUSIONS: Diagnostic accuracy of the tests alone or combined was insufficient when all adenomas were considered OBD. When only adenomas >1 cm were considered OBD, all tests could rule out OBD to a reasonable extent, particularly the combined POC tests. The tests were less useful for inclusion of OBD.

Abdominal pain, rectal bleeding, or altered defecation pattern are patient complaints frequently encountered by general practitioners (GPs).[5] Most of these complaints have a nonorganic background, and the prevalence of organic bowel disease (OBD)—including inflammatory bowel disease (IBD) and colorectal cancer (CRC)—in primary care patients is approximately 7% *(1–3)*. Distinguishing OBD from non-OBD needs to be timely, and the diagnostic workup aims at not missing OBD patients, who require subsequent work-up and referral to secondary care. Consequently, many patients are referred for secondary care endoscopy to rule out OBD. Of these referred patients, only 22% to 37% actually have OBD *(1, 4–7)*.

Endoscopy is a valuable procedure for diagnosing OBD, but is costly and carries risks, including bleeding and bowel perforation, and careful patient selection is important. Better discrimination by the GP between

[1] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; [2] Department of Gastroenterology, Gelderse Vallei Hospital, Ede, the Netherlands; [3] Department of Gastroenterology, Atrium Medical Center, Heerlen, the Netherlands; [4] Department of General Practice, Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, the Netherlands.

* Address correspondence to this author at: University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Stratenum 6.131, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. Fax +31-88-756-8099; e-mail l.kok-2@umcutrecht.nl.

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[5] Nonstandard abbreviations: GP, general practitioner; OBD, organic bowel disorders; IBD, inflammatory bowel disease; CRC, colorectal cancer; iFOBT, immunochemical fecal occult blood test; POC, point-of-care; TFT, triple feces testing; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; dOR, diagnostic odds ratio; AUC, area under the ROC curve; ICC, intraclass correlation coefficient; STARD, Standards for the Reporting of Diagnostic Accuracy.

OBD and non-OBD on the basis of patient history and physical examination is challenging because of overlap in symptoms and signs *(8–10)*. Simple, noninvasive tests to better discriminate OBD from non-OBD are thus needed. Several blood tests, such as erythrocyte sedimentation rate, C-reactive protein, hemoglobin concentration and leukocyte count, have been investigated for this purpose, but are not likely to provide enough additional diagnostic information *(1, 11–13)*.

Eligible tests to meet this need are biomarkers such as fecal calprotectin and immunochemical fecal occult blood tests (iFOBTs). Several calprotectin and iFOBTs are currently available, including various ELISA methods and, more recently, point-of-care (POC) tests. POC tests may be advantageous in clinical settings such as primary care, if a rapid turnaround time is a necessity for improving patient care and a small number of specimens need to be evaluated at any given time. Interuser variability can be substantial, however, necessitating proper training and quality assurance *(14)*.

Fecal calprotectin, a degradation product from neutrophil granulocytes from the mucosal layer of the colon, is increased when colonic inflammation is present and has been shown to have high diagnostic accuracy for discriminating OBD from non-OBD in secondary care. It is stable in feces for as long as 1 week at room temperature *(15)*. On the other hand, iFOBTs detect hemoglobin and its early degradation products, which may indicate the presence of polyps or adenocarcinoma of the colon. Sensitivity is found to be higher for the iFOBTs than for the older, guaiac-based FOBTs that detect heme. Although these biomarkers seem very promising, the diagnostic value of calprotectin in primary care has not been studied, and data for iFOBTs is very limited in this setting *(16–18)*.

We quantified the diagnostic accuracy of 3 biomarker tests (Quantum Blue® calprotectin quantitative lateral flow assay *(19)* and the EK-CAL calprotectin ELISA, both from Bühlmann Laboratories, and Clearview One Step (immunochemical) Fecal Occult Blood Test Device, from Inverness Medical Innovations) for the inclusion or exclusion of OBD in patients with persistent (i.e., >2 weeks) lower-abdomen complaints in primary care, who need colonoscopy referral.

Materials and Methods

STUDY DESIGN AND PARTICIPANTS

We used data from the CEDAR (Cost-Effectiveness of a Decision Rule for Abdominal Complaints in Primary Care) study, an ongoing, prospective, cross-sectional, diagnostic study in 170 general practices in 2 regions of the Netherlands: central (Gelderse Vallei) and south (Oostelijke Mijnstreek). from July 2009 through January 2011, primary care patients consulting their GPs for persistent lower-abdomen complaints were included. We preplanned current analyses after inclusion of 400 evaluable patients, providing a maximum margin of error of 10% for sensitivity and 6% for specificity estimates (score method, $\alpha = 5\%$, assuming 25% OBD prevalence) *(20)*. The University Medical Center Utrecht Medical Research and Ethics Committee approved the study protocol.

Patients were eligible if they were at high risk of OBD, defined as lower-abdomen complaints present for at least 2 weeks in combination with 1 or more of the following: rectal bleeding, altered defecation pattern, abdominal pain, fever, diarrhea, weight loss, sudden onset in the elderly, or findings at physical examination suggestive of OBD (palpable abdominal or rectal mass). Patients in the following categories were ineligible: <18 years old, unable to give informed consent, previously diagnosed with OBD, or positive on triple feces test (TFT), not requiring endoscopy. [TFT is a laboratory test of fecal samples from 3 consecutive days for the detection of intestinal parasites *(21)*.]

Patient recruitment was either at the GP's office (19.9%) or directly after scheduling at the endoscopy department (80.1%). When patient referral outpaced study resources, we took special care to keep study participants representative by screening every *n*th case. The presence and duration of rectal bleeding, abdominal pain, persistent diarrhea, altered defecation, fever, or weight loss with no apparent cause were assessed by patient and GP questionnaires. GPs recorded additionally the presence of pain or mass upon abdominal palpation or digital rectal examination.

CALPROTECTIN AND iFOBTs

Participants collected a fecal sample directly following their inclusion into the study, and study protocol dictated a maximum of 2 days between collection and submission of the sample, all the time keeping the sample refrigerated. The median time between sample collection and endoscopy or GP referral was 5 (10th;90th percentile 1;27) days and 18 (8;35) days, respectively.

Clinical laboratory technicians performed the ELISA and trained research nurses the POC index tests, blinded to patient data and adhering to the manufacturers' instructions. Fecal samples were processed directly in 38% of cases and initially frozen in 62% [median days in freezer 5 (1;16)]. The extracts for the calprotectin ELISA were stored in the freezer for a median of 82 (27;141) days.

The calprotectin POC test (Quantum Blue® Calprotectin quantitative lateral flow assay, Bühlmann Laboratories) is a quantitative immunoassay. In brief, 80 mg feces was homogenized for 1 min with 4 mL extraction buffer by use of a vibration mixer. The extract was diluted 1:16 and centrifuged for 5 min

(3000*g*) before 60 μL supernatant was applied onto the test cartridge. Correct lateral migration of the sample leads to formation of a control and test line, ensuring valid readout. Tests were read out after 12 min by a dedicated reader (Quantum Blue® POC Reader, Bühlmann), providing a calprotectin concentration (dynamic range 30–300 μg/g). With the same fecal extract, calprotectin concentrations were also measured by ELISA (EK-CAL Calprotectin ELISA, Bühlmann). After 5 min centrifugation (3000*g*), the supernatant of 1 mL undiluted fecal extract was transferred into a clean tube and stored at −20 °C for a maximum of 4 months until analysis. The calprotectin tests were considered positive if the concentration was higher than 50 μg/g, the manufacturer's recommended cutoff.

The iFOB POC test (Clearview One Step Fecal Occult Blood Test Device, Inverness Medical Innovations) is a rapid chromatographic immunoassay for qualitative detection of human fecal hemoglobin and its immediate degradation products. The lower detection limit as stated by the manufacturer was 6 μg hemoglobin/g feces. The specimen collection stick was randomly stabbed into the fecal sample at 3 different sites, and the manufacturer's instructions were followed for extraction of the sample from the specimen collection stick in the collection tube. Two full drops of extracted specimen were applied to the test device. Correct migration of the specimen leads to formation of only a control line in case of a negative test and a control and test line in case of a positive test. Test results were read after 5 min. Samples were successfully retested if the iFOB POC test gave inconclusive results (n = 5, 1.3%).

REFERENCE STANDARD

The reference standard was defined as the presence or absence of OBD determined at endoscopy (i.e., colonoscopy or sigmoidoscopy), as performed by experienced gastroenterologists at 1 of 2 high-volume centers, taking biopsies if required according to routine clinical practice. Furthermore, all patients for whom there was an inconclusive diagnostic reference procedure were followed for 3 months to establish a definite diagnosis. Outcome assessment was blinded to the results of the index tests.

As the primary outcome, we classified CRC, all adenomas, IBD, and diverticulitis as OBD and all other findings as non-OBD. As a secondary outcome, we considered advanced adenomas (>1 cm in size) as OBD but adenomas ≤1 cm as non-OBD, as the risk of subsequent development in carcinoma is low for these small adenomas *(22, 23)*. In addition, small adenomas are rarely symptomatic and may thus be considered incidental findings *(24–26)*.

ANALYSIS

We estimated sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios (LR+ and LR−), and diagnostic odds ratios (dOR) with their corresponding confidence intervals (95% CI) for both POC tests and the calprotectin ELISA test, separately. We used the manufacturer's cutoffs and the diagnostic outcome with small adenomas as either OBD (primary analysis) or non-OBD (secondary analysis) cases. We also report accuracy estimates combining the 2 POC tests, considering this composite test positive if 1 or both of the individual tests is positive. Median calprotectin POC and ELISA concentrations in OBD and non-OBD groups were tested for differences with the Mann–Whitney test. To test differences in sensitivity and specificity between the calprotectin and iFOB POC tests, we used the McNemar test.

We further related the quantitative calprotectin results to OBD status by estimating the area under the ROC curve (AUC). We assessed agreement between the calprotectin POC and ELISA test by use of the κ statistic after dichotomization at the manufacturer's cutoff and the intraclass correlation coefficient (ICC) for the continuous test results.

We observed Standards for the Reporting of Diagnostic Accuracy (STARD) guidelines *(27)*.

Results

STUDY POPULATION

From July 2009 through January 2011, 423 patients with abdominal complaints suggestive for OBD who were referred by a GP to 1 of the endoscopy facilities were enrolled in the study (participation rate after study contact 65.1%) (Fig. 1). Of these, 382 (90.3%) formed the study base [12 (2.8%) withdrew informed consent, 25 (5.9%) had no fecal sample, and 4 (0.9%) had no reference standard].

The median age of participants was 60 years (range 18–91), and 209 participants (54.7%) were female. The predominant presenting symptom was abdominal pain, followed by rectal bleeding and diarrhea, whereas fever and weight loss were less frequent. At physical examination, palpation elicited abdominal pain in almost half the patients, but palpable abdominal or rectal mass was found in only 13 individuals (Table 1).

OBD was present in 99 patients (prevalence 25.9%) (Table 2), the majority of whom had neoplastic disease [19 (19.2%) carcinoma and 53 (53.5%) adenoma], followed by IBD [19 (19.2%)] and diverticulitis [8 (8.1%)]. Sixteen patients had advanced adenomas. Colonoscopy was performed in 351 (91.9%) patients, sigmoidoscopy in 21 (5.5%), and other bowel examinations in 10 (2.6%) (Fig. 1). Almost all OBD was confirmed by histology (89.9%). Of the 10 OBD patients

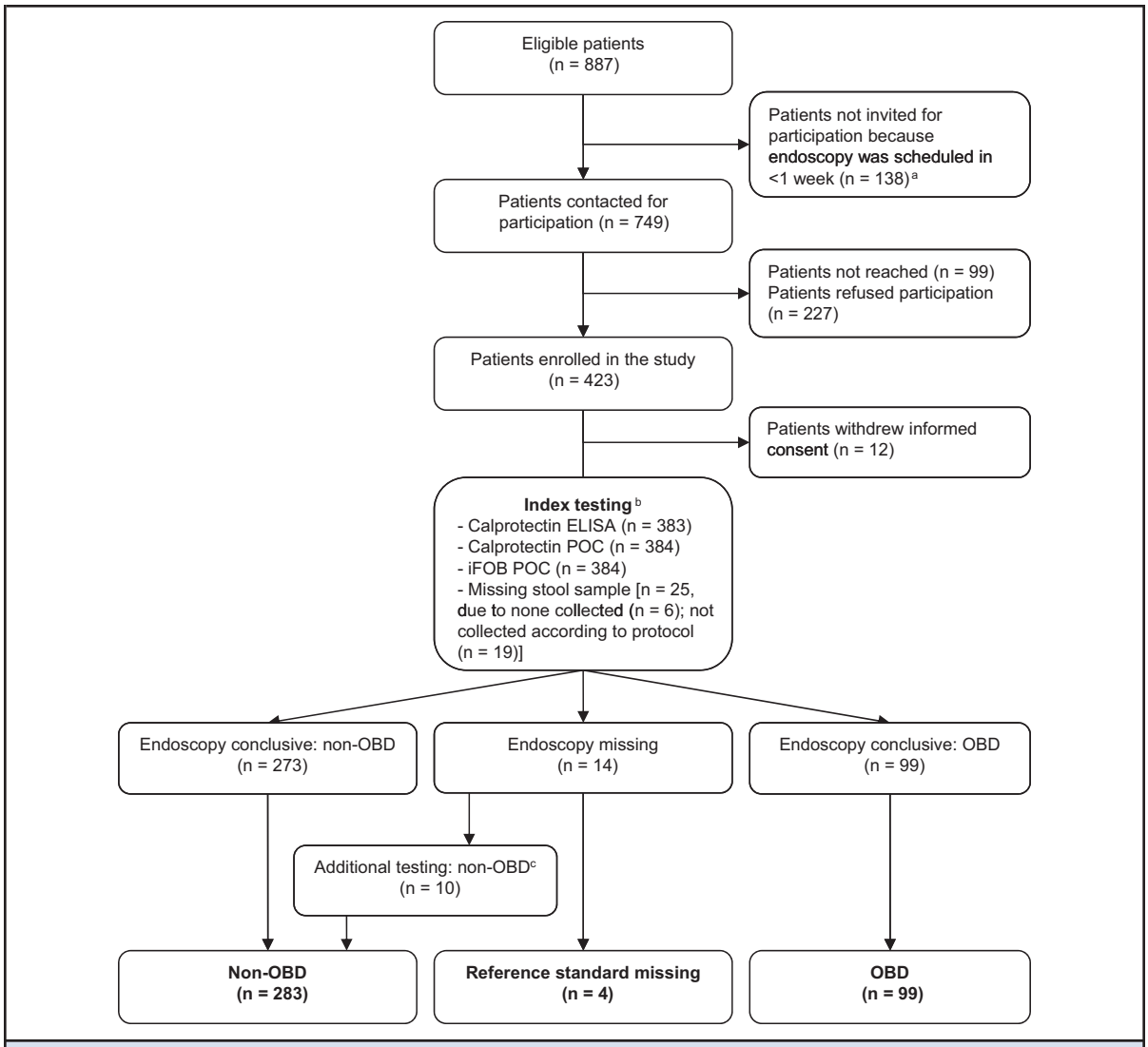

Fig. 1. Flow diagram of Dutch primary care patients with lower-abdomen complaints of >2 weeks' duration referred for secondary care endoscopy and enrolled in the study from July 2009 through January 2011.

[a] 15% of patients had endoscopy <1 week after referral (extrapolated from counts of 50% of eligible patients). [b] Calprotectin ELISA test results were missing for 3 patients, and the calprotectin POC and iFOB POC test results were missing for 2 patients. [c] Non-OBD was established by other bowel tests for 6 patients (abdominal ultrasound in 5 and barium enema in 1 patient) and by the gastroenterologist on the basis of bowel investigations performed before recruitment in the study for 4 patients.

without histological confirmation, 6 had diverticulitis and 1 radiation proctitis (both reliable colonoscopy diagnoses), 2 had adenomas (narrow-band imaging confirmed) with per-procedural biopsy loss, and 1 had proctitis diagnosed by the GP during the 3-month follow-up period. The majority of patients without OBD had no structural abnormalities [112 (39.6%)], followed by diverticulosis [47 (16.6%)], IBS [34 (12.0%)], and hemorrhoids [31 (11.0%)].

DIAGNOSTIC ACCURACY OF THE CALPROTECTIN AND OCCULT BLOOD TESTS

Of the 382 participants, logistical problems precluded calprotectin ELISA testing in 3 and iFOB POC testing in 1, and the calprotectin POC reader failed to read 2 tests despite proper test execution. These patients were excluded from the respective analyses.

Calprotectin concentrations were higher in OBD than non-OBD patients [median ELISA concentration

Table 1. Patient characteristics (n = 386).[a]

	n (%)	Missing, %
Geographic region of residency in the Netherlands		
Central (Gelderse Vallei)	257 (66.6)	
South (Oostelijke Mijnstreek)	129 (33.4)	
Median age, years (range)	60 (18–91)	
Women	211 (54.7)	
Presenting symptoms		
Rectal blood loss	141 (37.7)	3.1
Abdominal pain	267 (70.6)	2.1
Median duration of abdominal pain (range)	150 days (1 day to 30 years)	12.0
Persistent diarrhea[b]	40 (16.9)	38.6
Diarrhea	131 (37.2)	8.8
Fever	40 (11.0)	6.2
Weight loss	62 (17.1)	6.2
Bloating	195 (53.6)	5.7
Constipation	169 (46.6)	6.0
Physical examination[b]		
Pain at palpation	117 (46.8)	35.2
Palpable abdominal mass	12 (3.0)	35.2
Palpable rectal mass	1 (0.3)	13.6

[a] Data are n (%) unless noted otherwise. Symptoms reported by the participant unless noted otherwise.
[b] Symptoms reported by the GP.

102 vs 56 μg/g ($P < 0.001$) and median POC concentration 109 vs 43 μg/g ($P < 0.001$)]. Sensitivity was highest for the calprotectin ELISA test, at 0.74 (95% CI 0.65–0.82), followed by the calprotectin and iFOB POC tests (Table 3). Specificity was highest for the iFOB POC test, at 0.83 (0.78–0.87), followed by the calprotectin POC and ELISA tests. The difference in specificity between the iFOBT and the calprotectin POC test was statistically significant ($P < 0.001$), whereas the difference in sensitivity was not. NPVs were similar for all tests individually and improved slightly to 0.87 (0.81–0.91) when the 2 POC tests were combined. Evaluation of both calprotectin tests continuously showed an AUC for the ELISA of 0.66 (0.60–0.72) and for the POC of 0.65 (0.59–0.72) (Fig. 2). The agreement between the calprotectin POC and ELISA test was good [ICC 0.88 (0.85–0.90), κ 0.66 (0.59–0.73)].

When considering advanced adenomas as OBD and ≤1-cm adenomas as non-OBD (secondary end point), sensitivity estimates improved but specificity estimates did not change, PPVs were lower, and NPV estimates of all tests improved substantially to a maximum of 0.97 (0.93–0.99) for the 2 POC tests combined (Table 3). Also, the AUC for the calprotectin tests improved to 0.75 (0.67–0.82) for ELISA and 0.73 (0.66–0.81) for POC.

The calprotectin and occult blood test results subdivided according to specific diagnostic subgroups are shown in Table 4.

Discussion

This study is the first to evaluate the diagnostic accuracy of a calprotectin POC and ELISA test, with an iFOB POC test for the discrimination between OBD and non-OBD in primary care patients with lower-abdomen complaints of >2 weeks' duration who were referred by their GP for endoscopy. Overall test performance largely depended on the size of adenomas. The diagnostic accuracy of the tests alone or in combination was insufficient for clinical utility when all adenomas were considered to be OBD. When only advanced adenomas (>1 cm, more likely to bleed and be symptomatic *(24–26)*) were considered OBD, however, all tests showed improved diagnostic accuracy. Their negative predictive values, a major determinant of clinical utility in this primary care setting, were >90%. The ability to identify a large population for whom the presence of OBD

Table 2. Definitive diagnoses.[a]

Group diagnosis and specific diagnosis	n (overall %)	n (within–group %)
OBD	99 (25.9)	
Adenoma		53 (53.5)
>1 cm		16 (30.0 of adenomas)
≤1 cm		37 (70.0 of adenomas)
Adenocarcinoma		19 (19.2)
Diverticulitis		8 (8.1)
Ulcerative colitis		7 (7.1)
Colitis, other[b]		6 (6.1)
Proctitis[c]		4 (4.0)
Crohn's disease		2 (2.0)
Non–OBD	283 (74.1)	
No structural abnormality		112 (39.6)
Diverticulosis		47 (16.6)
IBS		34 (12.0)
Hemorrhoids		31 (11.0)
Hyperplastic polyps		14 (4.9)
Small polyps, no histology available		14 (4.9)
Other benign findings		14 (4.9)
Fixated sigmoid, or adhesions		6 (2.1)
Constipation		5 (1.8)
Cause outside of GI tract		4 (1.4)
Non–OBD diagnosed by gastroenterologist	2 (0.7)	
No reference standard available	4 (1.0)	

[a] Sums may not total 100% due to rounding.
[b] One patient was diagnosed with microscopic colitis, 1 with lymphocytic colitis, and 4 with nonspecific colitis.
[c] Of the 4 patients with proctitis, 1 was diagnosed with radiation proctitis.

could be ruled out to a reasonable extent was evident for each test taken individually, and even more so when the 2 POC tests were combined. However, if referral had been based only on the combined test results, some OBD cases would have been missed (predominantly adenoma cases, but also 5% of CRC and 10% of IBD cases) (Table 4).

The main strength of our study is that it was based in primary care, thus satisfying the need for studies evaluating OBD biomarkers in this setting. This is of paramount importance, as results from studies in secondary care cannot be transferred directly to primary care *(16)*. Evaluations of index and reference tests were blinded to each other, and almost all patients underwent colonoscopy or sigmoidoscopy as the reference test, with confirmatory histopathology testing as needed and 3-month follow-up if the initial colonoscopy or sigmoidoscopy was inconclusive.

Despite our considerable efforts to obtain a representative study population (e.g., participation of 170 general practices, nonselective eligibility screening procedure if referral outpaced study resources), there are 2 potential threats to the generalizability of our findings. First, approximately 15% of eligible patients could not be recruited, as endoscopy had been scheduled to occur within a week of referral. In these patients, expedited medical action was warranted, and any biomarker test results likely would not have been able to change that necessity. As such, these patients were not of interest for this study. Had these patients been included, the diagnostic accuracy estimates—in particular, sensitivity—would likely have improved owing to the inclusion of more severe cases associated with higher calprotectin and hemoglobin concentrations. Second, the overall participation rate of eligible patients was moderate (48%). However, the age and sex distributions were similar for the eligible and the participating patients, and baseline characteristics of the study population were as expected for a primary care population.

Table 3. Diagnostic accuracy of calprotectin and iFOB POC test and calprotectin ELISA test, with and without advanced adenomas classified as OBD.

	OBD, n	Non–OBD, n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR− (95% CI)	dOR (95% CI)
Including all adenomas as OBD									
Calprotectin POC			0.64 (0.54–0.72)	0.53 (0.48–0.59)	0.32 (0.26–0.39)	0.81 (0.74–0.86)	1.4 (1.1–1.7)	0.7 (0.5–0.9)	2.0 (1.3–3.2)
Positive	63	131							
Negative	36	150							
Missing	0	2							
iFOBT POC			0.56 (0.46–0.66)	0.83 (0.78–0.87)	0.53 (0.80–0.88)	0.85 (0.80–0.88)	3.3 (2.4–4.5)	0.5 (0.4–0.7)	6.3 (3.8–10.4)
Positive	55	48							
Negative	43	235							
Missing	1	0							
Calprotectin POC and/or iFOBT POC[a]			0.79 (0.69–0.86)	0.49 (0.44–0.55)	0.35 (0.29–0.42)	0.87 (0.81–0.91)	1.6 (1.3–1.8)	0.4 (0.3–0.6)	3.6 (2.1–6.1)
Positive	77	142							
Negative	21	139							
Missing	1	2							
Calprotectin ELISA			0.74 (0.65–0.82)	0.47 (0.41–0.53)	0.33 (0.27–0.39)	0.84 (0.78–0.89)	1.4 (1.2–1.6)	0.5 (0.4–0.8)	2.6 (1.5–4.3)
Positive	73	149							
Negative	25	132							
Missing	1	2							
Only including advanced (>1 cm) adenomas as OBD									
Calprotectin POC			0.76 (0.64–0.85)	0.54 (0.48–0.59)	0.24 (0.19–0.31)	0.92 (0.87–0.95)	1.6 (1.4–2.0)	0.5 (0.3–0.7)	3.6 (2.0–6.8)
Positive	47	147							
Negative	15	171							
Missing	0	2							
iFOBT POC			0.74 (0.62–0.83)	0.82 (0.78–0.86)	0.45 (0.35–0.54)	0.94 (0.91–0.96)	4.2 (3.1–5.5)	0.3 (0.2–0.5)	13.2 (7.0–25.0)
Positive	46	57							
Negative	16	262							
Missing	0	1							
Calprotectin POC and/or iFOBT POC[a]			0.92 (0.82–0.97)	0.49 (0.43–0.54)	0.26 (0.21–0.32)	0.97 (0.93–0.99)	1.8 (1.6–2.0)	0.2 (0.1–0.4)	10.9 (4.3–27.9)
≥ 1 Positive	57	162							
Both negative	5	155							
Missing	0	3							
Calprotectin ELISA			0.82 (0.71–0.90)	0.46 (0.41–0.51)	0.23 (0.18–0.28)	0.93 (0.88–0.96)	1.5 (1.3–1.8)	0.4 (0.2–0.7)	3.9 (1.9–7.7)
Positive	50	172							
Negative	11	146							
Missing	1	2							

[a] Combination of the calprotectin and iFOBT POC tests. The combination is positive if 1 of the 2 tests is positive and negative if both tests are negative.

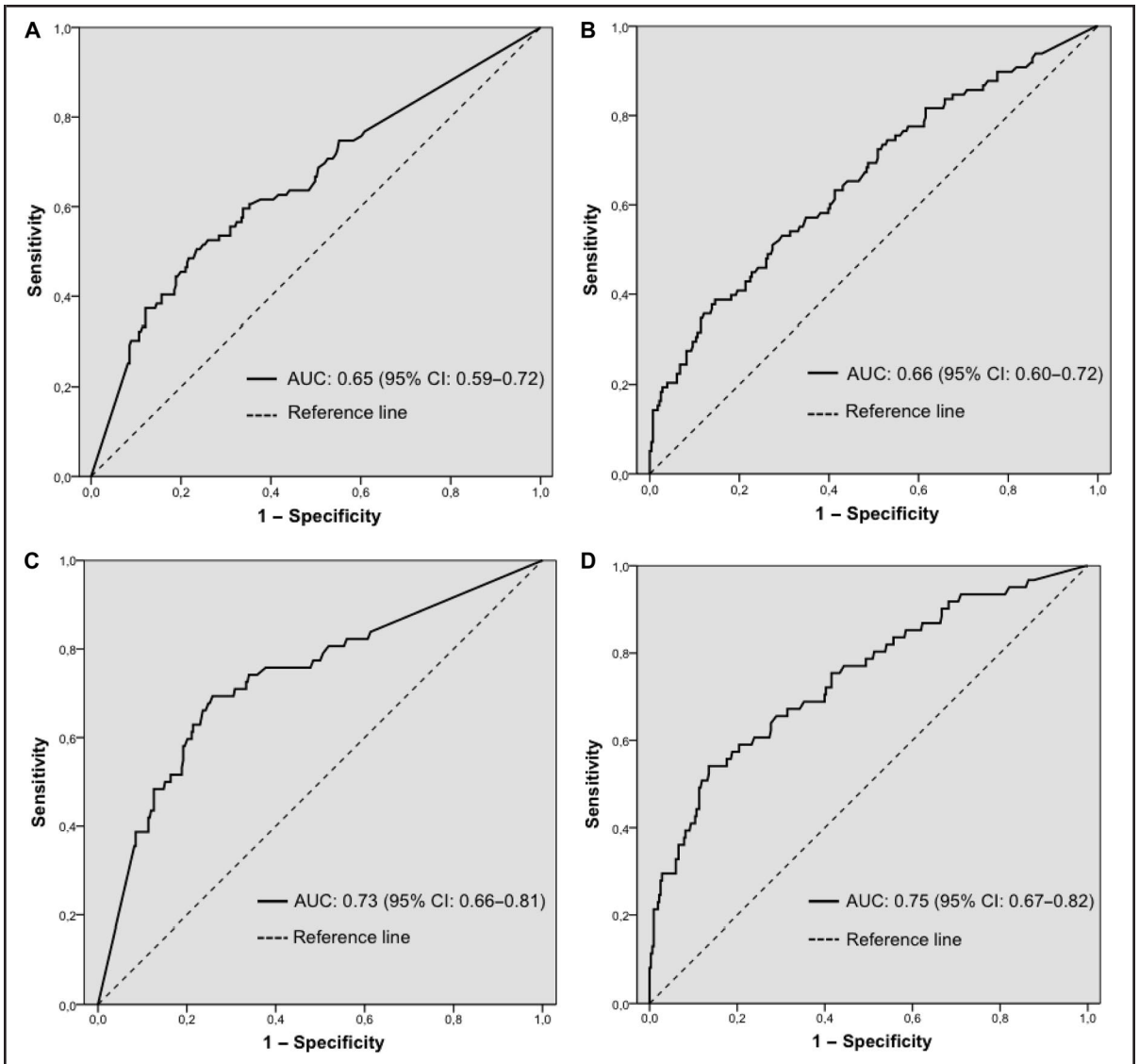

Fig. 2. ROC curves and AUCs for the calprotectin ELISA and POC test, for the 2 end points.

(A), Calprotectin ELISA test with primary end point (adenocarcinoma, IBD, diverticulitis, and all adenomas classified as OBD). (B), Calprotectin POC test with primary end point. (C), Calprotectin ELISA test with secondary end point [adenocarcinoma, IBD, diverticulitis, and only advanced (>1 cm) adenomas classified as OBD]. (D), Calprotectin POC test with secondary end point.

Two aspects of the study made the fecal sample collection and handling different from actual practice and potentially influenced the test results. Fecal sample collection took place several days after the GP visit, particularly for patients who were recruited by research nurses after endoscopy had been scheduled (80.1%); biomarker status may have changed during the time between initial presentation and fecal sampling (e.g., adenomas can stop bleeding). Reanalysis of the data for patients directly recruited by the GPs, however, yielded similar diagnostic accuracy estimates. Second, freezing and thawing may have resulted in slightly increased calprotectin concentrations owing to degradation of neutrophils and subsequent release of calprotectin *(28)*, whereas such freezing and thawing would have been unlikely to affect the iFOBT *(29)*. For efficiency, the majority of the fecal samples in our study were frozen and thawed before execution of the tests. Reanalysis of our data in patients whose samples were not frozen yielded similar diagnostic accuracy estimates for the iFOB test and slightly better estimates for the calprotectin tests.

Table 4. Percentage of positive tests per specific OBD and non–OBD diagnosis groups for the calprotectin and iFOB POC test and the calprotectin ELISA test, with median calprotectin concentrations.

		Calprotectin				iFOBT	Calprotectin POC and/or iFOBT POC[a]
		POC		ELISA		POC	POC
	n	Median, μg/g (25th;75th percentile)	>50 μg/g, n (%)	Median, μg/g (25th;75th percentile)	>50 μg/g, n (%)	Positive, n (%)	Positive, n (%)
OBD	99						
Adenoma	53	54 (30;191)	27 (51)	71 (29;122)	34 (64)	20 (38)	36 (69)
>1 cm	16	111 (30;264)	11 (68)[b]	89 (34;217)	11 (68)[b]	11 (68)[b]	16 (100)[b]
≤ 1 cm	37	42 (30;105)	16 (43)[b]	60 (24;108)	23 (62)[b]	9 (25)[b]	20 (56)[b]
Adenocarcinoma	19	215 (105;300)	15 (79)	274 (94;442)	18 (95)	16 (84)	18 (95)
IBD	19	135 (64;300)	15 (79)	201 (55;1200)	16 (84)	15 (79)	17 (90)
Diverticulitis	8	220 (57;300)	6 (75)	477 (48;1305)	7 (100)	4 (50)	6 (75)
Non–OBD[c]	283						
No structural abnormality	112	38 (30;90)	48 (43)	46 (19;101)	52 (46)	17 (15)	53 (47)
Diverticulosis[d]	53	52 (30;155)	27 (51)	63 (28;163)	32 (60)	9 (17)	29 (55)
IBS	34	40 (30;69)	15 (44)	49 (21;99)	15 (47)	4 (12)	16 (47)
Hemorrhoids	31	62 (30;109)	17 (55)	60 (18;125)	18 (58)	10 (32)	19 (61)
Other benign findings[e]	19	36 (30;107)	8 (44)	60 (26;87)	11 (58)	2 (11)	8 (44)
Hyperplastic polyp	14	53 (30;185)	8 (57)	56 (29;168)	8 (57)	4 (29)	8 (57)
Small polyps, no histology	14	32 (30;82)	5 (36)	64 (14;122)	9 (64)	2 (14)	6 (43)

[a] Combination of the calprotectin and iFOBT POC tests. The combination is positive if 1 of the 2 tests is positive and negative if both tests are negative.
[b] Percentage within adenomas.
[c] "Non–bowel related causes" and "non–OBD diagnosed by GP" are not shown (number of patients too small).
[d] "Fixated sigmoid or adhesions" and "diverticulosis" from Table 2 were merged to keep categories >10 patients.
[e] "Other benign findings" and "constipation" from Table 2 were merged to keep categories >10 patients.

Our results for calprotectin in the primary care setting are less optimistic than previous secondary care studies. A recent metaanalysis of studies with patients suspected of IBD in secondary care reported a pooled sensitivity of calprotectin of 0.93 (95% CI 0.85–0.97) and a pooled specificity of 0.96 (0.79–0.99) *(30)*. The case-mix of our study population differs from higher-risk secondary care patients, influencing the diagnostic estimates, which may partly explain the different study results. Our use of OBD as outcome also contributes to a different performance than in studies evaluating only IBD or only CRC. Sensitivity estimates of calprotectin did improve when a single outcome was evaluated in our study [ELISA 0.84 (0.62–0.94) for IBD and 0.95 (0.75–0.99) for CRC; POC 0.79 (0.57–0.91) for only IBD and only CRC], with corresponding lower specificities, however.

Patients with diverticulosis frequently had increased calprotectin concentrations in our study, possibly due to occult mild diverticulitis, contributing to the lower specificity. High concentrations of calprotectin have also been reported in healthy individuals and may be related to various individual variables including age, diet, and medication use (e.g., nonsteroidal anti-inflammatory drugs) *(15, 31, 32)*.

The diagnostic accuracy of iFOBT has been evaluated more extensively than that of calprotectin. A recent systematic review on secondary care populations with a low prevalence of CRC to resemble a primary care situation showed that the sensitivity of iFOBT for CRC ranged from 0.70 for an iFOBT strip device to 1.00 for automatic devices and specificity from 0.71 for a hemoglobin–albumin complex to 0.93 for an iFOBT strip device *(17)*. The sensitivity of the iFOB POC test for CRC in our study was comparable at 0.84 (0.62–0.94), as well as its specificity [0.76 (0.71–0.80)]. The relatively low specificity can be explained by the high prevalence of non-OBD patients with rectal bleeding.

Discriminating OBD from non-OBD in primary care may improve even further when these tests are used in combination with symptoms and signs such as those incorporated in scoring systems developed to

identify patients suspected of CRC or OBD *(33)*. Also, adaptation of calprotectin concentration cutoff concentrations for certain subgroups of patients, such as those who are older, on a certain diet, or on particular medications, could be of value *(15, 31, 32)*.

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