OPEN

Can 2 Different Fecal Calprotectin Assays be Used Interchangeably in IBD Treatment?

Elsa A. van Wassenaer, MD,† Kay Diederen, MD, PhD,*† Ester M.M. van Leeuwen, PhD,‡ Geert R. D'Haens, MD, PhD,§ Marc A. Benninga, MD, PhD,*† Bart G.P. Koot, MD, PhD,*† and Angelika Kindermann, MD, PhD*†*

Background: Fecal calprotectin (FC) is a biomarker for inflammation in inflammatory bowel disease (IBD). Interpretation of results can be complicated because of the use of different assays to determine FC.

Goals: To assess the agreement between 2 different assays for determining FC in patients with IBD.

Methods: Samples from adults and children with IBD were tested with 2 assays: (1) EliA 2 Calprotectin and (2) EK-Cal. Samples were uniformly tested on the same day. Interassay variability was displayed in a Bland-Altman plot. The difference in categorization of the FC result (1: 0 to 250 mg/kg, 2: 250 to 500 mg/kg, 3: > 500 mg/kg) was assessed with the linear weighted κ for adults and children separately.

Results: A total of 171 patients [mean age: 33 (range: 7 to 81); 92 (54%) female; 117 (68%) Crohn's disease; 53 (31%) ulcerative colitis] were included. Median (interquartile ranges) FC levels were 281 mg/kg (70 to 971) (EK-Cal) and 159 mg/kg (31 to 778) (EliA 2), and the mean delta FC was 89 mg/kg. In the adult population, there was substantial agreement between the 2 assays (κ: 0.72; SE: 0.06; 95% confidence interval, 0.60-0.83) and for pediatric patients, the agreement was almost perfect (κ: 0.83; SE: 0.06; 95% confidence interval: 0.70-0.95). Five of 171 patients (all aged ≥ 17 y and all with colonic disease) had a difference of 2 categories (1 vs. 3) between assays. Interassay variability was the highest in category 3.

Conclusions: The agreement between the EliA 2 and EK-Cal assay in this cohort of IBD patients is substantial to almost perfect. Interassay variability is higher in the highest FC category.

Key Words: inflammatory bowel disease, fecal calprotectin, disease monitoring, agreement

(*J Clin Gastroenterol* 2022;56:e27–e30)

Fecal Calprotectin (FC) levels can be used as a surrogate marker for disease activity in patients with inflammatory bowel disease (IBD).[1] Calprotectin is released into the intestinal lumen from the neutrophils in the mucosa during inflammation, and measuring the levels of FC thus reflects intestinal inflammation. Not only can FC levels be used to discriminate between IBD and functional gastrointestinal disorders in patients with chronic abdominal pain, these measurements also have a prominent place in today's clinical care of patients with IBD[2,3]; FC is used to discriminate between active and quiescent IBD, and has a higher accuracy than other biomarkers such as C-reactive protein (CRP).[4]

Despite its widespread use, there is currently no consensus on the optimal cutoff value for discriminating active disease from remission in patients with IBD.[5] This lack of consensus is partly caused by questions about the variability between different assays used to measure FC. Several studies have demonstrated varying accuracies when comparing different FC assays head-to-head,[6–10] but these studies mainly focused on discriminating between healthy controls and patients with IBD.

As in practice patients frequently switch between different laboratories where different assays are used, we need to know whether different assays classify disease activity in the same way in order to correctly monitor the disease activity of our patients with IBD. This study aimed to answer the following research question: "Can two different FC assays be used interchangeably in IBD monitoring?" Our primary outcome was the agreement between the 2 assays, as assessed with the weighted κ.

METHODS

Design and Patient Population

This was a single-center cross-sectional study. Consecutive patients of any age with known IBD, in whom FC was requested by their treating physician for general care between January 2017 and April 2017 were included. Feces were collected by patients, brought to the hospital, stored at −20°C upon arrival for 1 to 5 days, and not homogenized before testing, as is common practice in many laboratories. The samples were tested with the following assays: (1) EliA 2 Calprotectin (Thermo Fisher

Received for publication April 21, 2020; accepted September 24, 2020.
From the Departments of *Pediatric Gastroenterology, Emma Children's Hospital; ‡Experimental Immunology; §Gastroenterology and Hepatology, Amsterdam UMC, University of Amsterdam; and †Amsterdam Reproduction and Development and Amsterdam Gastroentrology and Metabolism, Amsterdam, The Netherlands.
E.A.v.W., K.D., E.M.M.v.L., B.G.P.K., and A.K.: substantial contributions to conception and design of the study. E.A.v.W., E.M.M.v.L., M.A.B., and G.R.D.: acquisition of data. E.A.v.W., K.D., E.M.M.v.L., G.R.D., M.A.B., B.G.P.K., A.K.: analysis and interpretation of data, drafting the article or making critical revisions related to the important intellectual content of the manuscript, and final approval.

The authors declare that they have nothing to disclose.
Address correspondence to: Elsa A. van Wassenaer, MD, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, Amsterdam 1105 AZ, The Netherlands (e-mail: e.a.vanwassenaer@amsterdamumc.nl).
Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.jcge.com.

DOI: 10.1097/MCG.0000000000001460

Scientific Phadia, Sweden), which is a commonly used automated fluorescent enzyme immunoassay test; and (2) EK-Cal (Bühlmann Laboratories, Switzerland), which is a manually performed enzyme-linked immunosorbent assay test. Samples were tested with both methods on the same day according to the manufacturer's instructions. Baseline characteristics of patients, including clinical disease phenotype and CRP were obtained from their medical charts. The clinical disease phenotype was recorded using the Montreal classification.[11] CRP results were analyzed within 14 days of the FC tests. Disease activity of patients according to their FC level was categorized as follows: (1) *low* (indicating biochemical remission; FC: 0 to 250 mg/kg), (2) *dubious* (indicating suspicion of biochemical flare; FC: 250 to 500 mg/kg), and (3) *high* (indicating a biochemical flare; FC > 500 mg/kg). These cutoff values were based on guidelines for monitoring IBD patients.[5]

Ethical Considerations

This study complied to the declaration of Helsinki. All included patients were informed about the study and got the possibility to withdraw consent for participation. As this study regarded reuse of care data, no official informed consent procedure was required by our ethical committee.

Statistical Analyses

Normally distributed variables were displayed as means with SDs, and non-normally distributed variables were displayed as medians with interquartile ranges. Interassay variability was first displayed in a Bland-Altman plot, and then the difference in the categorization of the FC result was assessed with the linear weighted κ. The agreement was assessed for the whole population together and also separately for the pediatric (ie: <18 y) and adult population. Sensitivity analyses were performed to assess the agreement for patients with Crohn's disease (CD) and ulcerative colitis (UC) separately, and patients with small bowel disease (CD L1) and large bowel disease (CD L2 and UC) separately. Values for the agreement were judged as follows: κ <0.0: poor; κ 0.0 to 0.20: slight; 0.21 to 0.40: fair; 0.41 to 0.60: moderate; 0.61 to 0.80: substantial; > 0.80, almost perfect. For the analysis, FC measurements > 1800 mg/kg were displayed as 1800 mg/kg, as the EK-Cal test could not measure higher values.

RESULTS

A total of 171 patients were included in this study: 125 adults and 46 children, all diagnosed with IBD. Baseline characteristics of the included patients are displayed in Table 1.

FC Measurements

Median (interquartile ranges) FC levels were 281 mg/kg (70 to 971) for the measurements by EK-Cal and 159 mg/kg (31 to 778) for the measurements by the EliA 2. The mean difference between the 2 assays was 89 mg/kg (range, −1140 to 1341). Figure 1 displays the Bland-Altman plot in which the mean FC level per patient is plotted against the difference (ΔFC) between the 2 measurements. It demonstrates a wide range between the limits of agreement (−487 to 666 mg/kg), resulting from an increase in ΔFC in patients with a higher mean FC level. This indicates a larger interassay variability in patients with a high FC level. The categorized FC results per assay are displayed in Table 2. In 5 of 171 patients (3%), there was a difference of 2 categories between the 2 assays and in 25 of 171 (15%), there was a difference of 1 category. For the whole population and the adult population, there was substantial agreement between the 2 assays [κ: 0.78; SE: 0.04; 95% confidence interval (CI), 0.71-0.86; and κ: 0.72; SE: 0.06; 95% CI: 0.60-0.83, respectively], as assessed with the linear weighted κ. For the pediatric population the agreement was almost perfect (κ: 0.83; SE: 0.06; 95% CI: 0.70-0.95).

TABLE 1. Patient Demographics at Baseline

Patient Characteristics	n = 171, n (%)
Age at baseline (SD) (y)	33 (17); range: 7-81
Females	92 (54)
CRP (SD), n = 170 (mg/L)	4 (5.5); range: 0-27
FC measured by EliA 2 (IQR) (mg/kg)	159 (31-778)
FC measured by EK-Cal (IQR) (mg/kg)	281 (70-971)
Crohn's disease patients	117 (68)
IBD-U	1 (1)
A1 age at onset < 17 y	51 (43)
A2 age at onset 17-40 y	38 (32)
A3 age at onset > 40 y	57 (33)
L1 distal 1/3 ileum ± limited cecal disease	33 (28)
L2 colonic disease	28 (24)
L3 ileocolonic disease	56 (47)
B1 nonstricturing nonpenetrating	72 (61)
B2 stricturing	27 (23)
B3 penetrating	19 (16)
P perianal disease	21 (18)
Ulcerative colitis patients	53 (31)
E1 proctitis	7 (13)
E2 left-sided colitis	18 (34)
E3 extensive colitis	28 (53)
Medication at baseline	
Aminosalicylates	45 (26)
Immunomodulators	77 (45)
Biologicals	84 (49)

CRP indicates C-reactive protein; FC, fecal calprotectin; IBD-U, inflammatory bowel disease-unclassified type; IQR, interquartile ranges.

In the 5 patients with a difference of 2 categories, the age ranged from 17 to 52 years. One of these patients was aged <18 years, 3 were female, 4 of 5 were diagnosed with UC [proctitis (E1): n = 1; left-sided colitis (E2): n = 1; extensive colitis (E3): n = 2], and 1 with CD [colonic disease (L2): n = 1].

CD Versus UC

Sensitivity analyses were performed, to assess the agreement between the 2 assays in patients with CD and UC, respectively, and to assess the agreement in patients with small bowel disease (CD L1) and large bowel disease (CD L2 and UC), respectively.

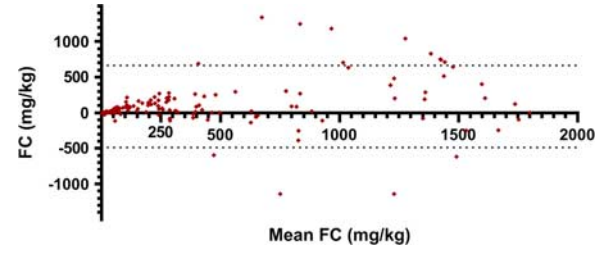

FIGURE 1. Bland-Altman plot displaying the variability between fecal calprotectin (FC) values measured by the EliA 2 and EK-Cal. *X*-axis: mean FC and the *y*-axis: difference between the 2 assays. The dotted lines represent the upper limit and lower limit of the 95% confidence interval (−487 to 666 mg/kg). The mean difference between the 2 assays is 89 mg/kg. full color online

TABLE 2. Agreement Between FC Measurement by EK-Cal and EliA 2 Assay

	FC Measured by EK-Cal (mg/kg)			
	0-250	**250-500**	**> 500**	**Total**
(A) For the whole population				
FC measured by EliA 2 (mg/kg)				
0-250	77	17	3	97
250-500	3	12	5	20
> 500	2	0	52	54
Total	82	29	60	171
(B) For the pediatric (< 18 y) population				
FC measured by EliA 2 (mg/kg)				
0-250	18	5	1	24
250-500	0	2	1	3
> 500	0	0	19	19
Total	18	7	21	46
(C) For the adult population				
FC measured by EliA 2 (mg/kg)				
0-250	59	12	2	73
250-500	3	10	4	17
> 500	2	0	33	35
Total	64	22	39	125

FC indicates fecal calprotectin.

For CD (32 children, 85 adults), the agreement was almost perfect (κ: 0.83; SE: 0.039; 95% CI: 0.75-0.91). For UC (15 children, 39 adults), the agreement was substantial (κ: 0.68; SE: 0.088; 95% CI: 0.51-0.85). For patients with small bowel disease (7 children, 26 adults), the agreement was almost perfect (κ: 0.90; SE: 0.053; 95% CI: 0.80-1), and for patients with large bowel disease (25 children, 57 adults) the agreement was substantial (κ: 0.73; SE: 0.064; 95% CI: 0.60-0.85). The categorized FC results per assay for these analyses can be found in the Supplementary File (Supplemental Digital Content 1, http://links.lww.com/JCG/A636).

DISCUSSION

In this cohort of patients with IBD, the agreement between FC levels measured by the EK-Cal assay and EliA 2 assay was substantial for adults and almost perfect for children, when categorizing the FC results in low (< 250 mg/kg), medium (250 to 500 mg/kg), and high (> 500 mg/kg). In only 3% of patients, there was a difference of 2 categories between the 2 assays. These results suggest that both assays could be used interchangeably in IBD treatment. Comparing the agreement between different assays is of importance for the management of patients with IBD, as in practice patients frequently switch between different laboratories where different assays are used, for practical considerations.

Our results are in line with previous studies on this topic, although to our knowledge, this study is the first to assess the agreement between different therapeutic categories of FC in solely patients with IBD and also the first to compare agreement between adults and children. Previous studies in which agreement between several assays was studied, either focused on the determination between healthy controls and IBD,[8–10,12] or assessed the overall agreement without categorizing results.[6] Since treating to a target FC level is common practice, assessing the agreement in categorizing FC results is essential. As was stated by the authors of the ECCO-ESGAR guideline for diagnostic assessment in IBD,[5] there are 3 ranges of FC: a target range, an uncertain or gray range, and an action range. To date, there are no generally accepted or applied cutoff values between the 3 ranges, although 250 mg/kg is often used in prospective studies and also suggested in guidelines.[5] For this reason we also chose 250 mg/kg as a cutoff value between the target and uncertain range.

Interestingly, the agreement seemed to be better in pediatric patients as compared with adults and all 5 patients with a 2 category difference in FC measurement where aged ≥ 17 and all had colonic disease. In addition, there was a trend toward a better agreement in patients with small bowel compared with large bowel disease. A possible hypothesis could be that calprotectin mixes through the feces less if it is excreted in the colon, as it has less time to mix, compared with the small bowel. However, this remains to be proven by future research. Another interesting finding of our study was the higher interassay variability in the highest FC category (> 500 mg/kg). This could imply that the variability of FC levels increases when FC levels increase. A possible explanation of this finding might be a difference in neutrophil concentrations in different parts of the inflamed bowel. This finding is not in line with the results of a study in 50 patients with IBD that showed that the within stool variability was highest in patients in remission.[13] However, poor agreement of FC in the high range between assays has been demonstrated before,[14] and our results are also in line with the advice of De Vos et al[15] to perform > 1 FC measurement before changing therapy when a relapse is suspected, as she demonstrated that 2 consecutive raised FC measurements are more accurate than 1 for predicting relapse in UC patients.

In the present study, we did not aim to determine the most accurate cutoff value for the different assays, nor did we aim to compare the diagnostic performance of the assays at a preset cutoff level. The substantial agreement does suggest that the same cutoff level could be used for the EK-Cal and the EliA 2 assay, which is in line with a previous study comparing the accuracy of the same 2 FC assays in diagnosing IBD using 50 mg/kg as a cutoff level.[12]

It should be taken into consideration that the EK-Cal is a manually performed assay, whereas the EliA 2 assay is automated. Therefore one could expect the automated one to be more standardized; however, we do not expect this to be the case in our laboratory, as the manual process is done by well-trained lab staff with calibrated pipets. Another point that should be taken into consideration is that the samples in the present study were not homogenized before analysis, which is a limitation of this study as FC levels have been demonstrated to show within-sample variation.[13] In contrast, we are convinced that this represents the routine diagnostic setting of daily diagnostics in laboratories worldwide. However, to truly reflect the variability between the assays in a future study, stool samples should be homogenized, although we believe that the finding of substantial agreement without homogenizing the samples even underlines our conclusion more.

In summary, our results suggest that the EK-Cal assay and EliA 2 assay could be used interchangeably when monitoring patients with IBD, both in children and in adults. In addition, the finding of higher interassay variability in the highest FC category supports the common practice to perform a second measurement when a high FC level is found before changing therapy.

REFERENCES

1. Colombel JF, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *Lancet*. 2017;390:2779–2789.

2. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ*. 2010;341:c3369.
3. Holtman GA, Lisman-van Leeuwen Y, Kollen BJ, et al. Diagnostic accuracy of fecal calprotectin for pediatric inflammatory bowel disease in primary care: a prospective cohort study. *Ann Fam Med*. 2016;14:437–445.
4. Mosli MH, Zou G, Garg SK, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol*. 2015; 110:802–819.
5. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. 2019;13:144–164.
6. Kittanakom S, Shajib MS, Garvie K, et al. Comparison of fecal calprotectin methods for predicting relapse of pediatric inflammatory bowel disease. *Can J Gastroenterol Hepatol*. 2017; 2017:1450970.
7. Labaere D, Smismans A, Van Olmen A, et al. Comparison of six different calprotectin assays for the assessment of inflammatory bowel disease. *United European Gastroenterol J*. 2014;2: 30–37.
8. Acevedo D, Salvador MP, Girbes J, et al. Fecal calprotectin: a comparison of two commercial enzymoimmunoassays and study of fecal extract stability at room temperature. *J Clin Med Res*. 2018;10:396–404.
9. Goll R, Heitmann R, Moe ØK, et al. Head to head comparison of two commercial fecal calprotectin kits as predictor of Mayo endoscopic subscore and mucosal TNF expression in ulcerative colitis. *PLoS One*. 2019;14:e0224895.
10. Mirsepasi-Lauridsen HC, Holmetoft UB, Halkjær SI, et al. Comparison of three commercial fecal calprotectin ELISA test kits used in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 2016;51:211–217.
11. Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut*. 2006;55:749–753.
12. Prell C, Nagel D, Freudenberg F, et al. Comparison of three tests for faecal calprotectin in children and young adults: a retrospective monocentric study. *BMJ Open*. 2014;4:1–6.
13. Du L, Foshaug R, Huang VW, et al. Within-stool and within-day sample variability of fecal calprotectin in patients with inflammatory bowel disease. *J Clin Gastroenterol*. 2018;52:235–240.
14. Haisma S-M, Galaurchi A, Almahwzi S, et al. Head-to-head comparison of three stool calprotectin tests for home use. *PLoS One*. 2019;14:e0214751.
15. De Vos M, Louis EJ, Jahnsen J, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis*. 2013;19:2111–2117.