



Comparison of a rapid test and an automated method for faecal calprotectin measurement



Sandra Martins^{a,b,*}, David Garcia^a, Rui Farinha^{a,b}, João Tiago Guimarães^{a,b,c}

^a Clinical Pathology Department, São João University Hospital Centre, Porto, Portugal

^b EPIUnit, Institute of Public Health, University of Porto, Porto, Portugal

^c Biochemistry Unit, Biomedicine Department, Faculty of Medicine, University of Porto, Porto, Portugal

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1. Introduction

Faecal calprotectin (FC) is a sensitive and non-invasive marker for gastrointestinal mucosal inflammation [1]. Since Calprotectin is a small calcium-binding protein released by neutrophils (S100 family) as a result of an inflammatory process [2], it is an excellent inflammatory marker to distinguish organic inflammatory bowel disease (IBD) - Crohn's disease (CD) or Ulcerative Colitis (UC) from functional disease- Irritable Bowel Syndrome (IBS) [1,2]. In pediatric patients, FC is useful for an early diagnosis of IBD significantly reducing the number of invasive procedures in children with gastrointestinal symptoms. Endoscopic procedures are unpleasant for the patient, time-consuming and bear the risk of intestinal perforation. In addition, FC is more sensitive than C-reactive protein (CRP) in reflecting IBD activity [3], being for this reason also used to monitor IBD therapy and to predict relapse in these patients [1,3].

Because of its enormous potential as biomarker, many methods have been developed for its measurement. Although the quantitative enzyme-linked immune sorbent assay (ELISA) is considered the "gold" standard method to quantify FC, there are also immunochromatographic tests that are regularly used. More recently, automated immunoassays have been developed as an alternative, leading to an improved solution in the routine laboratory in view of an exponential increase in workload [4,5].

In our hospital, we have been using a rapid test to measure FC in pediatric samples. As the number of tests for these samples has been increasing in the last years, the possibility to automate this parameter was considered as worth to evaluate.

The main purpose of this study was to determine FC levels with an immunoassay test adapted to an automated equipment and compare with a rapid routine test.

2. Material and methods

2.1. Sample collection

Between October 2016 and February 2017, we evaluated 132 consecutive stool samples of children and adolescents (71 male and 61

* Corresponding author. Clinical Pathology Department, São João Hospital Centre, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal.
E-mail address: smvmartins@gmail.com (S. Martins).

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female, aged 10–18 years) followed-up in our Pediatric Inflammatory Bowel Disease's Clinic, and sent to the laboratory for routine calprotectin testing. For all these samples, the caring physician decided on ordering the test according to clinical symptoms for inpatients or according to the evaluation of remission for outpatients.

Faecal samples were refrigerated at 2–8 °C immediately upon receipt and the extraction was performed up to 24 h after the reception, according to the respective manufacturer's directions. Specific extraction devices, Bühlmann CALEX®Cap, were used for sample preparation for both methods. The stool extract was diluted and homogenized using a vortex. No additional pipetting and manual dilution steps were required. The extracts are stable at 2–8 °C for up to 6 days and can be frozen (at –20 °C) for at least 6 months. The extracted samples were stored in double at –20 °C for 7 days before testing. The tests were performed simultaneously at the same day.

2.2. Biochemical analysis

The samples extracted were measured with two kits from Bühlmann Laboratories (Basel, Switzerland): Quantum Blue®fCAL Extended and fCAL™ Turbo that was adapted to Abbott® Architect C4000 (Abbott Diagnostics, Lake Forest, IL, USA), according to the manufacturer's instructions.

The Quantum Blue®fCAL Extended is a rapid and quantitative immunochromatographic test based on lateral flow immunochromatography designed for the selective measurement of calprotectin antigen by sandwich immunoassay. The signal intensities of the "Test" line and the "Control" line are measured quantitatively by the Bühlmann Quantum Blue® Reader.

The new fCAL™ Turbo is a turbidimetric immunoassay (PETIA), applicable on all major clinical chemistry platforms. The extracts were incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies (immunoparticles). Calprotectin available in the sample mediates immunoparticle agglutination. Sample turbidity, measured by light absorbance, increases with calprotectin immunoparticle complex formation and is proportional to calprotectin concentration.

2.3. Statistical analysis

Precision, using quality control material was evaluated in 5 days study following CLSI guideline EP5-A2. The comparison between the two different calprotectin test kits was conducted using Spearman correlation and differences were evaluated by paired samples t-test. Test results were divided according to their FC concentration into five categories: Normal (<50 µg/g), Borderline (50–100 µg/g), Moderately High (100–250 µg/g), High (250–1000 µg/g) and Very High (>1000 µg/g). Concordance between the two tests into these five categories was assessed using Kendall's τ coefficient.

All statistical analyses were performed with the SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). The level of significance was set as p -value <0.05 for a confidence interval of 95%.

3. Results

The precision of fCAL™ Turbo test was determined by using two Quality Control levels (Low and High) assayed in 3 replicates per run during 5 days, following CLSI guideline EP5-A2. The precision was 3.8% for Control level Low and 1.4% for Control level High (Mean \pm SD, 76.3 \pm 3.3 µg/g and 245.8 \pm 3.4 µg/g, respectively). The Inter-lot precision determined by the manufacturer was 2.4% and 3.2% for Control Level Low and High, respectively. The assay was performed in a 20-day study using four extracted faecal samples and 2 controls.

The immunochromatography Quantum Blue®fCAL Extended test has two levels of Quality Control: Low, < 30 µg/g and High, 225–675 µg/g (Mean: 450 µg/g). The Inter-lot precision, according to the manufacturers, is 16.5–20.6% and the results showed linearity within the 30–1000 µg/g range. The assay was determined with four extracted stool samples, including extracts with calprotectin levels close to clinical decision points, using three different reagent lots. The measurements were performed over five days, in a single run each day, with two replicates per run. The manufacturer didn't refer specifically the calprotectin concentration and didn't repeated the test with QC material.

According to manufacturers, calibration range for fCAL™ Turbo is 0–2600 µg/g and the measuring range is 20–8000 µg/g.

For comparison of methods, 37 samples were excluded-samples with FC concentration \leq 30 µg/g ($n = 32$) and \geq 1000 µg/g ($n = 5$), for Quantum Blue®fCAL Extended and samples with FC concentration \leq 20 µg/g ($n = 23$) and \geq 2600 µg/g ($n = 28$), for fCAL™ Turbo. The remaining ninety five samples, not normally distributed ($p < 0.005$, 95%Cl) when evaluated for normality by the Kolmogorov-Smirnov test, had medians (IQR) of 243.5 (598.8) µg/g for Quantum Blue®fCAL Extended and 284.2 (1252.4) µg/g for fCAL™ Turbo, respectively. The Spearman rank correlation coefficient was 0.920 ($p < 0.005$), with a mean difference of 1174 µg/g [723.4 µg/g; 1626.1 µg/g] between the two tests.

The cut-off, generally accepted, between negative and positive, and suggested by the manufacturers, is 50 µg/g for both tests. All test results ($n = 132$) were divided according to their FC concentration into five categories, listed below in Table 1. Concordance between the two tests in these five categories was assessed using Kendall's τ coefficient. The contingency coefficient was 0.395 ($p = 0.000$). Our results show that for the 17 patients with FC concentration between 100 and 250 µg/g with Quantum Blue®fCAL, 4 patients had a FC concentration, with the fCAL™ Turbo, between 250 and 1000 µg/g. For the 67 patients with FC concentration between 250 and 1000 µg/g with Quantum Blue®fCAL, 39 had a concentration with fCAL™ Turbo superior to 1000 µg/g (and in 24 of these samples, the concentration was higher than 2600 µg/g).

We also compared both tests according to the clinical condition of the patients as defined in the laboratory information system by the attending pediatrician. Using this data, we classified them into three groups: (1) IBD-patients with diagnosed CD or UC; (2) Other GI

Table 1

Comparison of the two different methods categorizing children according to their faecal calprotectin concentrations in five categories (n = 132): Normal: 0–50 µg/g; Borderline: 50–100 µg/g; Moderately high: 100–250 µg/g; High: 250–1000 µg/g and Very high: >1000 µg/g.

		fCAL™Turbo				
		< 50	50-100	100-250	250-1000	>1000
Quantum Blue®fCAL	< 50	30 (22,7%)	4	0	0	0
	50-100	3	6 (4,5%)	1	0	0
	100-250	0	0	13 (9,8%)	4	0
	250-1000	0	0	2	26 (19,7%)	39 (29,5%) *
	>1000	0	0	0	0	4 (3,0%) **

* 24 samples have FC concentration > 2600 µg/g in fCAL™ Turbo.

** 3 samples have FC concentration > 2600 µg/g in fCAL™ Turbo.

disease-patients with another intestinal pathology (Irritable bowel syndrome and Celiac disease); (3) Control-children with no finding of any organic gastrointestinal disorder at the time of the measurements. The results of mean FC concentration in IBD, Others and Control groups are shown in Fig. 1. The Spearman rank correlation coefficient in IBD group was 0.59 (n = 77, p < 0.005) and 0.79 (n = 16, p < 0.005) in the Others GI disease group. The demographics of these patients are presented in Table 2.

4. Discussion

Faecal calprotectin levels are already being used in the monitoring of IBD and other bowel disease in adults [6]. Since FC has been validated as a non-invasive biomarker of local gastrointestinal inflammation in patients with IBD, its use has been extended to the pediatric population [7]. Even more, Kostakis et al. in a systematic study proposed that, in children, FC is able to diagnose or confirm a relapse of IBD [8].

In this comparison study, we can say that both methods have an acceptable agreement. They have the ability to distinguish IBD from other inflammatory diseases and can determinate the active phase in IBD. However, the bench top Quantum Blue device may present a disadvantage for the follow up of IBD patients in the active phase of the disease, as its results are limited to values up to 1000 µg/g. This may be explained by the fact that immunochromatographic methods, as Quantum Blue, only use a monoclonal antibody. Conversely, the fCAL® Turbo is a particle-enhanced turbidimetric immunoassay based on the standardization of the ELISA method, which is the gold standard calprotectin measurement. According to the manufacturer, fCAL® Turbo shows a good correlation with ELISA method with a linear regression coefficient of 0.91 (manufacturer information) and lacks a hook effect for calprotectin concentrations up to 8000 µg/g.

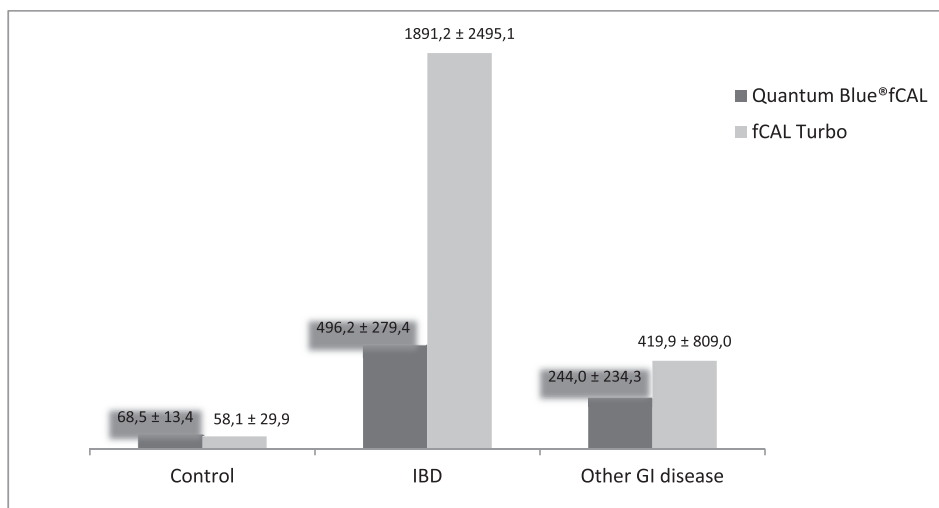


Fig. 1. Distribution of FC concentrations (µg/g) for the two different tests (Quantum Blue®fCAL and fCAL™ Turbo) in the different diagnostic groups (IBD, Other GI disease and Control group).

Table 2

Patient's characteristics by diagnosis group. Descriptive data are reported as mean±SD.

Group	Diagnosis	N	Male (n)	Female (n)	Age (years) Median (min-máx)
1	IBD	95	52	43	14 (11–17)
2	Other GI disease	21	11	10	12 (7–17)
3	Control	16	8	8	14 (10–18)

1- IBD, patients with diagnosed CD or UC; 2- Other GI disease, patients with another intestinal pathology; 3- Control, without organic gastrointestinal disorder.

For the purpose of this comparison, we considered the widely used professional guidelines, which refer four categories in interpreting the results: Normal: 0–50 µg/g, Borderline: 50–100 µg/g, Moderately high: 100–250 µg/g and Very high >250 µg/g [1]. The 50 µg/g cut-off used by both tests, represents the generally accepted delimitation between a negative and a positive test result. However, although there is still some controversy surrounding the cut-off values, a calprotectin value > 250 µg/g is already considered as a signal of an active organic disease, with inflammation of the gastrointestinal tract [1,9]. For this reason, and because of the need to monitor the activity of the disease, we classified values between 250 µg/g and 1000 µg/g as high levels and added another category for values over 1000 µg/g, labeled as very high [10].

When evaluated with fCAL® Turbo, 39.5% of patients changed calprotectin categories. This reclassification occurs in IBD patients, moving from the fourth to the fifth level (High to Very High), and has no clinical relevance when considering diagnostic purposes. However, when we are considering, as stated above, the possibility to monitor active disease and the effect of treatment, then this capacity to better separate patients with high and very high concentrations is clinically useful. In addition, these differences may turn to be even more striking when we consider the possibility of having changes in the methods used for FC measurement, that are not communicated to clinicians and patients.

5. Conclusion

The fCAL® Turbo method has the advantage of automation, has a broader range of results, proved to be simple to use and easily incorporated into existing workflows.

This comparison points that the usefulness of FC determination is not only dependent of the clinical condition but also of the specific test used.

Conflict of interest

None of the authors have any conflict of interest to report.

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