

Original Research Article

Diagnostic Value of Fecal Calprotectin Point of Care Testing in the Pediatric Practice

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Abstract

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We evaluated 54 children – 24 children with inflammatory bowel disease, 15 with other non-infectious intestinal diseases and 15 healthy controls. All of the children provided fresh fecal samples for measurement of Fecal Calprotectin (FC). FC concentration was assayed by two methods – quantitative point of care test and ELISA test. The mean FC levels were significantly increased in all of the patients with intestinal inflammation. There was a strong correlation between the results obtained from the point of care test and the ELISA assay. FC point of care testing is a useful non-invasive screening tool in the pediatric practice.

Key words: Fecal calprotectin, Intestinal inflammation, Point of care testing

INTRODUCTION

Fecal Calprotectin (FC) is a small 36.5 KDa calcium and zinc binding heterocomplex protein that belongs to the S-100 protein family (Johns et al., 1997; Varela et al., 2009). FC is mainly found in neutrophils granulocyte accounting for up to 60% of the cytosolic proteins but also in monocytes, macrophages and epithelial cells. In the presence of bowel inflammation the amount of calprotectin in the feces is proportional to the amount of neutrophil migration from the inflamed bowel wall (Schoepfer et al., 2008). FC is resistant to colonic bacterial degradation and stable in stool for up to 1 week at room temperature (Roseth et al., 1992). In children it has been shown that the levels of FC may guide the need for an endoscopy (Bunn et al., 2001a,b, Fagerberg et al., 2007).

FC can be measured by a commercially available enzyme-linked immunosorbent assay (ELISA) and recently by a rapid quantitative immunochromatographic point of care test (Quantum Blue®).

The aims of this study were to confirm the validity of

FC as a marker of intestinal inflammation in children and to compare the FC point of care testing (Quantum Blue® Calprotectin/ Quantum Blue® Calprotectin High Range, Bühlmann Laboratories AG, Switzerland) with a standard ELISA method (Ridascreen® Calprotectin, R-Biopharm AG, Germany).

MATERIALS AND METHODS

We evaluated 54 children—24 boys (44,4%) and 30 girls (55,6%) with a median age of 11,35 years. Of the enrolled patient group 24 were diagnosed with inflammatory bowel disease (IBD)- 15 with ulcerative colitis (UC) and 9 with Crohn's disease (CD), 15 with other non-infectious intestinal diseases and 15 served as a control group (with no known gastrointestinal problems). We divided the patients into three groups: Group I- IBD, Group II- Non-IBD and Group III- Healthy controls.

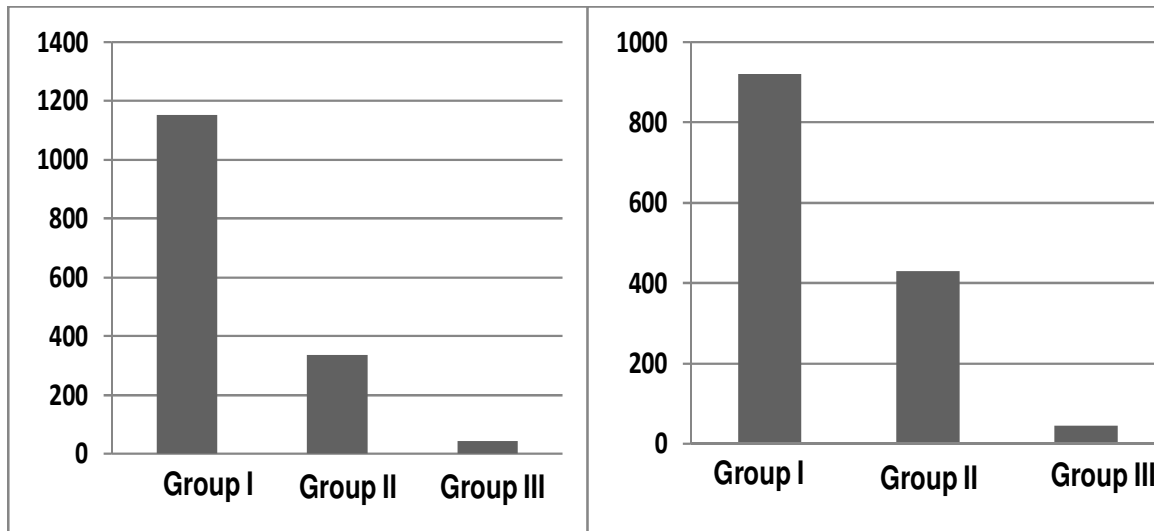


Figure 1a). Mean FC levels measured by Quantum Blue[®] 1b) Mean FC levels measured by standard ELISA

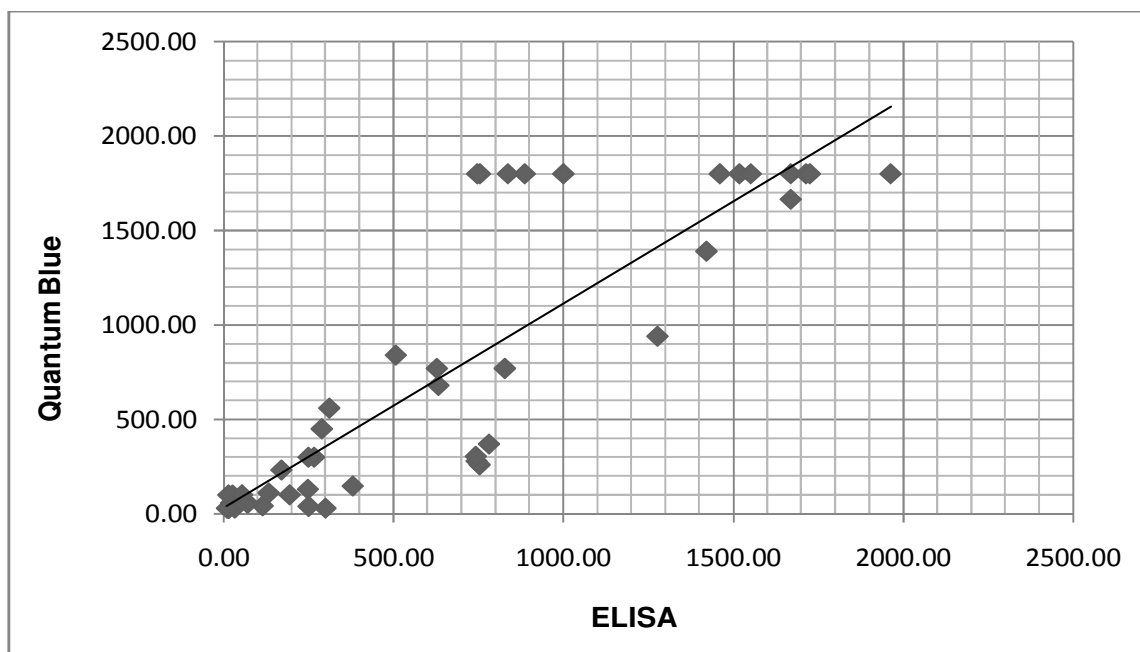


Figure 2. Correlation between the FC levels measured by Quantum Blue[®] Calprotectin and standard ELISA

All participants provided fresh fecal samples for FC measurement. Each sample was distributed into two containers – one container for ELISA assay and one container for point of care testing. The containers for ELISA assay were stored at -20°C. After thawing the samples were prepared and analyzed according to the manufacturer’s instructions (Ridascreen[®] Calprotectin, R-Biopharm AG, Germany).

The samples for the point of care testing were processed immediately or within 24 hours. They were prepared and analyzed according to the manufacturer’s

instructions (Quantum Blue[®] Calprotectin, Bühlmann Laboratories AG, Switzerland). We used both the normal range cartilage (Quantum Blue[®] Calprotectin 30-300 µg/g) as well as the high range cartilage (Quantum Blue[®] Calprotectin High Range 100-1800 µg/g). FC values above the upper limit of the measurement ranges were registered as 1800 µg/g and FC values below the lower limit were accordingly registered as 30 µg/g.

The association between the results obtained from both tests was assessed by the determination of

Spearman's rank correlation coefficient (*r*). We used an ANOVA analysis to assess the differences between the mean FC levels in the separated groups.

All analyses were performed using SPSS V22.0. A *p*-value <0.05 was considered statistically significant.

RESULTS

Both methods the standard ELISA and the rapid quantitative point of care test Quantum Blue[®] produced an identical outcome with regards to FC levels. The mean FC levels were significantly increased in patients with IBD when compared to the patients with other non-infectious intestinal diseases (*p*<0.01) and the healthy controls (*p*<0.01): 1151.25 µg/g vs. 336.13 µg/g vs. 42.00 µg/g when measured with Quantum Blue[®] Calprotectin and 920.34 µg/g vs. 429.64 µg/g vs. 43.45 µg/g when measured with standard ELISA (Figure 1a and b).

There was a strong correlation between the FC levels measured by Quantum Blue[®] Calprotectin and those measured by the standard ELISA (*r*=0.91, *p*<0.01, *n*=54) (Figure 2). Considering the absolute FC values there was a poor correspondence between the methods and this is not hardly surprising as different methodologies were involved.

DISCUSSION

A number of studies have demonstrated that FC is a valuable surrogate marker of intestinal inflammation in adults and children (Bunn et al., 2001, Konikoff and Denson 2006, Nakov 2012). Our findings are consistent with this data. The mean FC levels in the group with severe intestinal inflammation (the IBD group) are considerably higher than the group with less severe intestinal inflammation (the Non-IBD group) and the healthy controls.

The ELISA method is a time-consuming procedure requiring special laboratory equipment and specially trained personnel (Coorevits et al., 2013; Elkjaer et al., 2010). The point of care testing, also called a near-patient testing or a bedside testing, is an analytical procedure performed for patients by healthcare professionals outside the conventional laboratory (Kost 2002). The advantages of the point of care testing are simpler sample collection, simpler pre-analytical process and faster test results available. The Quantum Blue[®] Calprotectin was able to produce results in less than 30 minutes and allowed the assay of individual samples. The ELISA analysis took more than 6 hours and required multiple samples (optimum 40 samples). Although the point of care testing may not be as accurate as the "gold standard" – the ELISA assay, its value in offering faster results in the conventional setting cannot be ignored.

Similar to other authors we found out a very good correlation between the results obtained from the rapid quantitative test Quantum Blue[®] Calprotectin and the standard ELISA test (Coorevits et al., 2013; Elkjaer et al., 2010; Sydora et al., 2012; Nakov et al., 2013). An advantage of our study is that we used both the normal range cartilage as well as the high range cartilage and were able to assess the relationship between the higher FC concentrations. We believe that FC point of care testing is an excellent alternative to the time consuming ELISA in the everyday pediatric practice.

CONCLUSION

Fecal calprotectin point of care testing is a useful screening tool to detect children with intestinal inflammation and to identify those requiring further endoscopic assessment. It is simple and has a good diagnostic performance comparable to the time-consuming ELISA assay.

REFERENCES

- Bunn SK, Bisset WM, Main MJ, Golden BE (2001). Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr. Gastroenterol. Nutr.*; 32: 171–7.
- Bunn SK, Bisset WM, Main MJ, Gray ES, Olson S, Golden BE (2001). Fecal calprotectin: validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. *J Pediatr. Gastroenterol. Nutr.*; 33: 14–22.
- Coorevits L, Baert FJ, Vanpoucke HJ (2013). Faecal calprotectin: comparative study of the Quantum Blue rapid test and an established ELISA method. *Clin. Chem. Lab. Med.*; 51 (4): 825-31.
- Elkjaer M, Burisch J, Voxen Hansen V, DeibjergKristensen B, Slott Jensen JK, Munkholm P (2010). A new rapid home test for faecal calprotectin in ulcerative colitis. *Aliment Pharmacol. Ther.*; 15; 31 (2): 323-30.
- Fagerberg UL, Lööf L, Lindholm J, Hansson LO, Finkel Y. Fecal calprotectin: a quantitative marker of colonic inflammation in children with inflammatory bowel disease. *J Pediatr. Gastroenterol. Nutr* 2007;45:414–20
- Johne B, Fagerhol MK, Lyberg T, Prydz H, Brandtzaeg P, Naess-Andresen CF, Dale I (1997). Functional and clinical aspects of the myelomonocyte protein calprotectin. *MolPathol*; 50: 113–123.
- Konikoff M, Denson L (2006). Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. *Inflamm Bowel Dis*; 12 (6): 524-34.
- Kost GJ (2002). Goals, guidelines and principles for point of care testing. In: Kost GJ, ed. *Principles and practice of point of care testing*. Philadelphia, PA: Lippincott Williams and Wilkins; 3-12.
- Nakov V (2012). Surrogate markers of intestinal inflammation – fecal calprotectin. *Bulgarian Medical Journal*; 6 (3): 26-30.
- Nakov V, Shishenkov M, Nakov R, Kundurzhiev T (2013). Bulgarian Hepatogastroenterology; 1: 34-38
- Quantum Blue[®] Calprotectin High Range Quantitative Lateral Flow Assay LF-CHR20 package insert (2011-04-11).
- Quantum Blue[®] Calprotectin Normal Range Quantitative Lateral Flow Assay LF-CAL20 package insert (2010-11-10).
- Roseth AG, Fagerhol MK, Aadland E, Schjønsby H (1992). Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scand J Gastroenterol*; 27:793–798.
- Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F (2008). Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD

antibodies. *Inflamm Bowel Dis*;14:32–39.
Sydora M., Sydora B, Fedorak R (2012). Validation of a point-of-care desk top device to quantitate fecal calprotectin and distinguish inflammatory bowel disease from irritable bowel syndrome. *J Crohns Colitis*; 6(2): 207-14.

Varela E, Antolín M, Guarner F, Verges R, Giralt J, Malagelada JR (2009). Faecal DNA and calprotectin as biomarkers of acute intestinal toxicity in patients undergoing pelvic radiotherapy. *Aliment Pharmacol. Ther.*; 30:175-185.