ACTA ABP BIOCHIMICA POLONICA

Vol. 61, No 1/2014
99–102
on-line at: www.actabp.pl

Regular paper

Comparison of fecal pyruvate kinase isoform M2 and calprotectin in assessment of pediatric inflammatory bowel disease severity and activity

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Aims: Accurate assessment of inflammatory bowel disease (IBD) activity is the cornerstone of effective therapy. Fecal M2 isoform of pyruvate kinase (M2-PK) and fecal calprotectin (FC) are noninvasive markers of mucosal inflammation in IBD. The aim of this study was to compare performance of M2-PK and FC in assessment of pediatric ulcerative colitis (UC) and Crohn's disease (CD) severity and activity. Materials and methods: 121 patients with IBD, including 75 with UC and 46 with CD were recruited. Control group consisted of 35 healthy children (HS). Patients were assigned to groups depending on disease severity and activity. M2-PK and calprotectin concentration were determined in stool samples using ELISA. Areas under receiver operating characteristic curves (AUC) for FC and M2-PK with cut-off level at which M2-PK specificity was matching FC specificity were calculated and compared. Results: Performance of M2-PK at identifying patients with IBD, UC and CD among HS was inferior to FC. The differences in AUC were respectively: -0.10 (95% confidence interval [CI] [-0.13-(-0.06)], p<0.0001), -0.14 (95% CI [-0.19-(-0.09)], p<0.0001) and -0.03 (95% CI [-0.05-(-0.001)], p<0.02). M2-PK was inferior to FC in discriminating patients with mild UC from those with HS (AUC difference –0.23, 95% CI [-0.31-(-0.15)], p<0.0001). Conclusions: FC reflects pediatric IBD severity and activity better than M2-PK. This difference is particularly pronounced when identifying patients with mild UC and UC in remission.

Key words: pyruvate kinase, calprotectin, inflammatory bowel diseases, ulcerative colitis, Crohn's disease, pediatrics

Received: 11 September, 2013; revised: 12 March, 2014; accepted: 16 March, 2014; available on-line: 20 March, 2014

INTRODUCTION

Embryonic M2 isoform of pyruvate kinase (M2-PK) is a cytosolic enzyme catalyzing a phosphate group transfer in glycolysis (Walkowiak *et al*., 2005). Its tetrameric and dimeric forms are present in proliferating cells of many tissues, including leukocytes (Gupta & Bamezai, 2010). Upon leukocyte destruction in the gastrointestinal tract, the protein is released to the fecal stream (Walkowiak *et al*., 2005). M2-PK is stable in stools, which increases the potential value of fecal M2-PK concentration assessment both in intestinal inflammation and cancer. We postulated that fecal M2-PK could serve as a biomarker of inflammation in pediatric inflammatory bowel diseases (IBD) (Czub *et al*., 2007). Other groups concentrated on M2-PK utility in cancer diagnostics (Gupta & Bamezai, 2010).

Calprotectin is a calcium and zinc binding protein belonging to S100 protein family. It constitutes more than 40% of neutrophil cytosol proteins and exhibits bacteriostatic activity (Yui *et al*., 2003). It has been shown that fecal calprotectin (FC) concentration correlates with severity of inflammatory bowel disease endoscopic presentation (Aomatsu *et al*., 2011; D'Haens *et al*., 2012; Önal *et al*., 2012). It is also known that fecal calprotectin may be used to predict IBD relapses (Lasson *et al*., 2013; Mao *et al*., 2012). Although calprotectin's sensitivity and specificity in adult IBD is 93% and 96% respectively, it was demonstrated that in children the specificity is significantly lower (van Rheenen *et al*., 2010).

To date two studies comparing M2-PK and calprotectin were published. In 2008 Shastri *et al*. described fecal M2-PK and FC concentrations in a population of 276 adult patients with IBD, concluding that M2-PK had inferior specificity (Shastri *et al*., 2008). In 2010 Turner *et al*. investigated fecal M2-PK, FC, lactoferrin and S100A12 protein in children with severe ulcerative colitis (Turner *et al*., 2010). They showed that only M2-PK had constructive and predictive validity, while other markers failed to meet this criterion. Therefore the aim of this study was to compare the value of M2-PK and calprotectin in assessment of pediatric IBD activity.

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Abbreviations: AUC, area under receiver operating characteristic curves; CD, Crohn's disease; ELISA, enzyme-linked immunosorbent assay; FC, fecal calprotectin; HS, healthy subjects; IBD, inflammatory bowel diseases; IQR, interquartile range; M2-PK, M2 isoform of pyruvate kinase; PCDAI, the Pediatric Crohn's Disease Activity Index; ROC, receiver operating characteristic; TW, Truelove-Witts; UC, ulcerative colitis

Table 1. Group characteristics.
Data for age are presented as median (interquartile range). UC — ulcerative colitis, CD — Crohn's disease, HS — healthy subjects.

	Number of participants	Sex		Age, years	Disease severity		Disease activity	
		Male	Female		Mild	Moderate and severe	Remission	Active
UC	75	44 (58.7%)	31 (41.3%)	13.8 (11.25-16.08)	43 (57.3%)	32 (42.7%)	40 (53.3%)	35 (46.7%)
CD	46	28 (60.9%)	18 (39.1%)	15.4 (12.92-16.33)	25 (54.3%)	21 (45.7%)	22 (47.8%)	24 (52.2%)
HS	35	18 (51.4%)	17 (48.6%)	15.0 (11.3-16.0)				

MATERIALS AND METHODS

One hundred fifty-six children were enrolled for the study, including 75 with ulcerative colitis (UC), 46 with Crohn's disease (CD) and 35 healthy controls (HC). The diagnosis of IBD was based on the physical examination, endoscopic, histologic and radiologic evaluations, as well as supplementary laboratory tests (Kornbluth *et al*., 2004; Turner *et al*., 2007; Van Assche *et al*., 2010). Disease severity was described using Truelove-Witts (TW) scores in children with ulcerative colitis (Mahadevan *et al*., 2002) (Kobelska-Dubiel *et al*., 2007) and The Pediatric Crohn's Disease Activity Index (PCDAI) in Crohn's disease patients (Hyams *et al*., 1991). Whether disease was active or in remission was determined on basis of an extended set of criteria including endoscopy, magnetic resonance enterography, ultrasonography and biomarkers of inflammation in the blood, depending on the case studied and the clinical context. Group characteristics are presented in Table 1. Median TW score was 1.0 (interquartile range [IQR]: 0–5), median PCDAI score was 42.5 (7–60).

The study was conducted in four tertiary care centres and one secondary care centre. Fresh stool samples were initially stored at 4 degrees Celsius and at –70 degrees Celsius after transfer to the laboratory. Dimeric M2-PK concentration was assessed using commercially available sandwich ELISA with monoclonal antibodies (ScheBo Biotech, Giesen, Germany) and expressed in U/g. Although manufacturer-suggested cut-off value was 4 U/g, results for cut-off set at 5 U/g were analysed as well to increase the test specificity. FC levels were determined employing PhiCal ELISA Test (Calpro, Lysaker, Norway). Cut-off concentration for FC was 15 μg/mL. Both analyses were performed in the same stool specimens. All samples were assessed by the same observer who was unaware of group allocation.

Statistical analysis was carried out using STATISTICA data analysis software system v. 10 (StatSoft, Inc., Tulsa, United States of America) and Analyse-it v. 2.30 (Analyse-it Software, Leeds, United Kingdom). Sensitivity, specificity and area under receiver operating characteristic (ROC) curve were calculated. The areas under the receiver operating characteristic curves (AUC) for M2-PK and FC in different settings were compared. These comparisons were made after adjusting M2-PK cut-off level so that M2-PK specificity equaled that of FC at standard cut-off value of 15. M2-PK and FC performance was compared with the use of Whitney-Mann U-test. The level of significance was set at $p<0.05$. The data are presented as median (IQR), unless stated otherwise.

Parents of all patients, and patients at least 16 years old have expressed their written, informed consent to participation in the study. The study project received a positive opinion of the Bioethical Committee at Poznan University of Medical Sciences (decision 1740/04).

RESULTS

Fecal concentrations of M2-PK and FC in groups are shown in Table 2. The highest M2-PK concentration was 1849.0 U/g, while the highest FC concentration was 556.0 μg/mL, both in active, severe UC. Highest concentrations of M2-PK and FC in patients with CD was 770.4 U/g (mild CD in remission) and 456.0 μg/mL (active, severe CD), respectively.

Fecal concentrations of M2-PK and FC in subgroups of children with IBD depending on disease activity status and disease severity are shown in Table 3. and Table 4.

The performance of M2-PK in distinguishing UC patients from those with HS was inferior to FC (AUC difference -0.14, 95% confidence interval [CI] [-0.19-(-0.09)], $p<0.0001$). M2-PK was also inferior to FC in distinguishing CD patients from HS (AUC difference -0.03, 95% CI [-0.05-(-0.001)], $p<0.02$). In general, the performance of M2-PK in identifying patients with IBD among HS was poorer in comparison with FC (AUC difference -0.10, 95% CI [-0.13-(-0.06)], $p<0.0001$). M2-PK

Table 2. Fecal concentrations of pyruvate kinase isoform M2 (M2-PK) and calprotectin (FC) in groups.
Data are presented as median (interquartile range). CD — Crohn's disease, HS — healthy subjects, IBD — inflammatory bowel disease, UC — ulcerative colitis.

	M2-PK, U/g		FC, μg/mL	
HS	0	(0.0–1.3)	2.5	(2.0–2.5)
IBD	22.0	(2.4–194.2)	56.0	(12.5–218.0)
UC	10.0	(0.6–189.5)	45.0	(11.8–211.0)
CD	63.5	(12.9–182.8)	78.0	(25.2–212.0)

Table 3. Fecal concentrations of pyruvate kinase isoform M2 (M2-PK) and calprotectin (FC) in groups of children with inflammatory bowel diseases depending on activity status.
Data are presented as median (interquartile range). A — active, CD — Crohn's disease, R — remission, UC — ulcerative colitis.

	M2-PK, U/g		FC, μg/mL	
UC-R	1.5	(0.0–7.1)	12.5	(5.0–32.9)
UC-A	148.8	(12.3–375.1)	176.0	(88.5–329.5)
CD-R	32.6	(5.0–76.8)	29.0	(12.5–71.2)
CD-A	96.3	(23.9–200.2)	189.5	(74.8–275.0)

Table 4. Fecal concentrations of pyruvate kinase isoform M2 (M2-PK) and calprotectin (FC) in children with inflammatory bowel diseases depending on disease severity.
Data are presented as median (interquartile range). CD — Crohn's disease, M — mild, UC — ulcerative colitis, S — moderate to severe.

	M2-PK, U/g		FC, µg/mL	
UC-M	1.95	(0.0–10.0)	12.5	(5.0–88.2)
UC-S	152.9	(26.6–408.3)	187.5	(52.7–334.2)
CD-M	40.2	(7.8–200.2)	32.5	(12.5–80.0)
CD-S	96.3	(20.8–130.7)	167.0	(78.0–256.0)

was less efficacious than FC in discriminating patients with mild CU from HS (AUC difference -0.23, 95% CI [-0.31-(-0.15), $p < 0.0001$] and patients with mild CD from HS (-0.04, 95% CI [-0.08-(-0.01)], $p=0.025$). M2-PK was inferior to FC in identifying patients with UC in remission among HS (AUC difference -0.22, 95% CI [-0.30-(-0.14)], $p<0.0001$) and patients with CD in remission among HS (AUC difference -0.05, 95% CI (-0.09-(-0.01), $p=0.02$).

DISCUSSION

This is the first study to directly compare M2-PK and FC in assessment of severity and activity of pediatric IBD. It presents a response to questions that arose after we found that M2-PK is a marker of pediatric IBD activity (Czub *et al*., 2007). The main concern was to determine whether M2-PK could improve IBD diagnostics in children.

The data obtained lend support to the notion that M2-PK is inferior to FC in IBD diagnostics. This was clearly visible in two areas: identification of UC patients among HS and recognition of UC patients in remission among HS. Both were expressed in marked AUC differences. With regard to CD, the AUC differences were smaller, yet significant. Given the high sensitivity and good specificity of FC, M2-PK would present no added value in CD (Henderson *et al*., 2013).

In general, observations regarding FC that were made in this study are in line with what was previously described (Bunn *et al*., 2001; Komraus *et al*., 2012; Kostakis *et al*., 2013). They seem to contradict the conclusions drawn by Shaoul *et al*. who found no correlation between clinical IBD activity and FC concentrations (Shaoul *et al*., 2012) and to agree with a report by D'Haens *et al*. in which a correlation between clinical score in UC and FC was described (D'Haens *et al*., 2012).

One of the limitations of this study is that it investigated M2-PK and FC in the context of disease severity and activity without detailed analysis of response to treatment in a longer period of observation. This was rendered impossible by the settings.

The future directions of research should include questions, such as: how fecal markers could be used to decrease the number of endoscopic investigations without compromising diagnosis (Aomatsu *et al*., 2011; van Rheenen *et al*., 2010), to predict relapses in patients that do not exhibit symptoms (Kallel *et al*., 2011; Mao *et al*., 2012), and to predict the course of disease in general (Lasson *et al*., 2013; Önal *et al*., 2012).

In conclusion, these data show that in children, calprotectin reflects IBD severity and activity better than M2-PK.

Acknowledgements

This research was supported by a grant from Poznan University of Medical Sciences.

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