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FECAL PYRUVATE KINASE IS NOT SUITABLE FOR DISCRIMINATION BETWEEN INFLAMMATORY BOWEL DISEASE EXACERBATION AND ACUTE GASTROENTERITIS*

POMIAR STĘŻENIA KINAZY PIROGRONIANOWEJ W KALE NIE POZWALA NA ROZRÓŻNIENIE MIĘDZY ZAOSTRZENIEM NIESWOISTEGO ZAPALENIA JELIT A OSTRYM NIEŻYTEM ŻOŁĄDKOWO-JELITOWYM

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Abstract

Introduction: *In inflammatory bowel diseases (IBD) diarrhea can be caused by exacerbation and/or infectious agents. Fecal calprotectin (FC) is a well-established biomarker of intestinal inflammation in IBD. However, its usefulness in depiction of IBD exacerbation from infectious diarrhea is limited. The value of fecal pyruvate kinase isoenzyme type 2 (M2-PK) in this application remains unknown.*

Aim: *To compare the performance of M2-PK and FC in discriminating between diarrhea caused by IBD and infectious agents.*

Materials and methods: *One hundred three patients were enrolled for the study, including 32 with ulcerative colitis (UC), 21 with Crohn's disease (CD), 29 with acute diarrhea caused by rotavirus (AD-RV), and 21 with acute diarrhea caused by Salmonella enteritidis (AD-SE). M2-PK and FC were measured using ELISA. Areas under receiver operating characteristic curves (AUCs), sensitivities and specificities for both tests in distinguishing between patient subgroups with moderate to severe UC and CD from AD-RV and AD-SE were calculated.*

Results: *Differences in AUCs between M2-PK and FC for distinguishing UC [CD] from AD-RV were -0.06 ($p < 0.028$) [-0.10 ($p < 0.0018$)] and for differentiating UC [CD] from AD-SE were 0.03 (NS) [-0.19 ($p < 0.0011$)].M2-PK sensitivities and specificities in distinguishing UC [CD] from AD-RV were 75.0% [71.4%] and 89.7% [89.7%] and for differentiation of UC [CD] from AD-SE were 56.3% [71.4%] and 95.2 [57.1%].*

Conclusions: *The performance of M2-PK in distinguishing between children with moderate-to-severe IBD and patients with infectious gastroenteritis was inferior to FC. Neither test had sensitivity and specificity sufficient for everyday clinical application.*

Key words: ulcerative colitis, Crohn's disease, calprotectin, fecal biomarker, rotavirus, *Salmonella*

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Streszczenie

Wstęp: *Ostra biegunka jest objawem występującym zarówno w nieswoistych zapaleniach jelit, jak i w ostrym nieżycie żołądkowo-jelitowym. Oznaczenie stężenia kalprotektyny w kale służy jako biomarker procesów zapalnych toczących się w obrębie jelita. Wykazano, że podobne zastosowanie może mieć izoenzym 2 kinazy pirogronianowej.*

Cel: *Porównanie wartości oznaczenia stężeń izoenzymu 2 kinazy pirogronianowej i kalprotektyny w kale w różnicowaniu autoimmunologicznej i zakaźnej etiologii ostrej biegunki.*

Materiał i metody: *W badaniu wzięły udział 103 osoby w wieku do 19 lat, w tym: 32 z wrzodziejącym zapaleniem jelita grubego, 21 z chorobą Leśniowskiego-Crohna (aktywność umiarkowana lub znaczna), 29 z ostrym nieżytem żołądkowo-jelitowym o etiologii rotawirusowej i 21 z ostrym nieżytem żołądkowo-jelitowym wywołanym przez Salmonella enteritidis. Stężenia izoenzymu 2 kinazy pirogronianowej i kalprotektyny w stolcu oznaczano metodą ELISA.*

Wyniki: *Porównanie pól pod krzywymi ROC (Receiver Operating Characteristic) wykazało mniejszą wartość izoenzymu 2 kinazy pirogronianowej w porównaniu z kalprotektyną w rozróżnianiu między ostrą biegunką wywołaną nieswoistymi zapaleniami jelit a nieżytem żołądkowo-jelitowym o etiologii rotawirusowej (wrzodziejące zapalenie jelita grubego: -0,06 [p<0,028; w chorobie Leśniowskiego-Crohna -0,10 (p<0,0018)], a także między ostrą biegunką w przebiegu choroby Leśniowskiego-Crohna a wynikającą z zakażenia Salmonella enteritidis [-0,19 (p<0,0011)]. Czułości (swoistości – podane w nawiasach) w rozróżnianiu między osobami z wrzodziejącym zapaleniem jelita grubego a biegunką o etiologii rotawirusowej i Salmonella enteritidis były równe odpowiednio 75,0% (89,7%) i 56,3% (95,2%). Czułości (swoistości) dla rozróżniania między uczestnikami badania z chorobą Leśniowskiego-Crohna a biegunką o etiologii rotawirusowej i Salmonella enteritidis były równe odpowiednio 71,4% (89,7%) i 71,4% (57,1%).*

Wnioski: *W rozróżnianiu między ostrą biegunką, u podłoża której było nieswoiste zapalenie jelit lub infekcja rotawirusowa albo Salmonella enteritidis badane testy nie cechowały się czułością i swoistością wystarczającymi dla codziennego zastosowania w praktyce lekarskiej.*

Słowa kluczowe: wrzodziejące zapalenie jelita grubego, choroba Leśniowskiego-Crohna, kalprotektyna, biomarkery kałowe, rotawirus, *Salmonella*

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INTRODUCTION

Diarrhea is one of the main symptoms of inflammatory bowel disease (IBD) exacerbation [1, 2]. However, it can also be caused by viral or bacterial gastroenteritis. Differentiating diagnosis between these two main causes of diarrhea in IBD patients often presents difficulty and may require extensive laboratory work-up [3]. So far, it was shown that distinguishing between IBD and noninflammatory diarrhea could be achieved using blood gene expression profiling [4]. However, as oligonucleotide arrays technology is still too expensive to be widely used in clinical setting, a fast, reliable and inexpensive test that would enhance differential diagnostics in IBD patients with diarrhea is needed.

Pyruvate kinase isoenzyme type M2 (M2-PK) is a protein expressed in proliferating cells of many tissues, including leukocytes and tumour cells [5]. It takes part in glycolysis by facilitating a phosphate group transfer [6]. Destruction of cells containing M2-PK that are present in the gastrointestinal tract leads to release of the enzyme to the fecal stream, where it remains stable [7]. M2-PK was investigated as a marker in colorectal cancer [8]. We proposed that M2-PK could serve as a biomarker of IBD activity and severity [7].

Calprotectin is a low molecular weight calcium-binding protein that is also one of at least twenty-one S100 family proteins [9]. It is present in neutrophils and exerts bacteriostatic action [10]. Fecal calprotectin (FC) concentration measurement can be used to assess the severity of IBD [11, 12] and to predict its course [13, 14]. It was shown that FC has sensitivity of 87% and specificity of 83% in identifying patients with bacterial acute diarrhea among patients with acute diarrhea [15]. The study was carried out in a group of 2383 adults with acute diarrhea. In a similar study that included 66 children with acute gastroenteritis FC's sensitivity and specificity were 93% and 88% [16].

So far, comparison of clinical utility of M2-PK and FC in patients with IBD was the subject of two studies. In 2008 Shastri et al. demonstrated lower specificity of M2-PK in a population of 276 patients with Crohn's disease (CD) and ulcerative colitis (UC) [17]. In 2010 Turner et al. described the use of M2-PK, FC, lactoferrin and S100A12 for outcome prediction and response monitoring in 101 children with UC [18]. Although the performance of M2-PK was similar to that of other fecal markers, it differed from them in that it had construct and predictive validity.

FC is a well-established biomarker of intestinal inflammation, However, its usefulness in depiction of

IBD exacerbation from infectious diarrhea is limited. The value of pyruvate kinase isoenzyme type 2 (M2-PK) in this application remains unknown. The aim of the study was to determine whether M2-PK could be superior to FC in differentiating between underlying disease exacerbation and infective causes of diarrhea in children with IBD.

MATERIALS AND METHODS

One hundred three subjects were enrolled for the study, including 32 with UC (age range 2-19 years), 21 with CD (age range 9-19 years), 29 with acute diarrhea caused by rotavirus (AD-RV; age range 1-7 years) and 21 with acute diarrhea caused by *Salmonella enteritidis* (AD-SE; age range 1-12 years).

UC and CD were diagnosed according to Porto criteria using a combination of information coming from the physical examination, endoscopy, radiologic investigations, histologic analysis and supplementary laboratory tests [19-21]. The severity of UC was assessed using the pediatric ulcerative colitis activity index (PUCAI) [20, 22]. The severity of CD was described using The Pediatric Crohn's Disease Activity Index (PCDAI) [1]. PUCAI value greater than 34 and PCDAI value greater than 29 were considered as indicators of moderate-to-severe disease [23].

AD was diagnosed on basis of clinical criteria [24]. Rotaviral infection was diagnosed when typical symptoms were present and a latex agglutination test gave positive result. *S. enteritidis* presence in stools was determined by stool cultures.

The median patients' age (interquartile range [IQR]) was: 14.0 (11.7-15.0) in UC, 14.0 (12.0-15.0) in CD, 2.0 (1.3-3.0) in AD-RV, and 3.5 (2.6-8.0) in AD-SE. Median PUCAI score was 42.5 (IQR: 35-55), and median PCDAI score was 60 (42.5-62.5).

The study took place in four tertiary hospitals and one secondary care centre. Fresh stool specimens were obtained from hospitalized patients and stored in 4 degrees Celsius initially and in -70 degrees Celsius in the laboratory. The concentration of dimeric M2-PK was determined using a commercially available sandwich ELISA (ScheBo Biotech, Giesen, Germany). The cut-off value was 4 U/g. The concentration of FC was measured employing PhiCal ELISA Test (Calpro, Lysaker, Norway). The cut-off concentration was 15 μg/mL. Analyses were carried out by the same laboratory technician unaware of diagnosis, in the same stool samples for M2-PK and FC.

Statistical analyses were carried out in 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). Sensitivities, specificities and areas under (AUC) receiver operating characteristic curves were calculated. The AUCs for M2-PK and FC were then compared after equaling M2-PK specificity to FC specificity at 15 μg/mL. The level of significance was set at $p<0.05$.

Parents of all patients and patients at least 16 years old have expressed their written, informed consent to participation in the study after receiving full information on its scope and purpose. The study plan was approved by the Bioethical Committee at Poznan University of Medical Sciences (decision 1740/04). The study adhered to the tenets of the Declaration of Helsinki.

RESULTS

The median concentrations of M2-PK and FC in all four groups are shown in Table I. The sensitivities, specificities, and AUCs for M2-PK and FC in distinguishing children with IBD from children with AD-RV are shown in Tables II and III. The performance of FC was superior in UC and CD when AUCs were compared. Although the performance of both tests was good, it was still insufficient when analyzed in the context of the needs of clinical practice.

The sensitivities, specificities, and AUCs for M2-PK and FC in discriminating between children with IBD and children with AD-SE are shown in Tables IV and V. The AUCs plotted for FC in this application were greater than for M2-PK in CD, but not in UC. However, none of the two tests was sensitive and specific enough to have practical value in differentiating between patients with IBD exacerbation and *S. enteritidis* infection.

DISCUSSION

This is the first study to investigate the potential value of M2-PK and FC in identifying causes of diarrhea in children with IBD. AD-RV served as a model of viral gastroenteritis,

Table I. Median concentrations (interquartile range) of fecal pyruvate kinase isoenzyme M2 (M2-PK) and fecal calprotectin (FC) in children with moderate-to-severe ulcerative colitis (UC), moderate-to-severe Crohn's disease (CD), acute diarrhea caused by *Salmonella enteritidis* (AD-SE), and rotaviral acute diarrhea (AD-RV).

Tabela I. Mediany stężeń (w nawiasach podano rozstępy międzykwartylowe) izoenzymu M2 kinazy pirogronianowej (M2-PK) i kalprotektyny (FC) w kale dzieci z wrzodziejącym zapaleniem jelita grubego o umiarkowanej lub znacznej aktywności (UC), chorobie Leśniowskiego-Crohna o umiarkowanej lub znacznej aktywności (CD), ostrej biegunce wywołanej przez Salmonella enteritidis (AD-SE) lub rotawirus (AD-RV).

	UC	CD	AD-SE	AD-RV
M2-PK, U/g	152.9 (26.6-408.3)	96.3 (20.8-130.7)	22.3 (16.3-113.0)	8.7 (2.8-18.8)
FC, μg/mL	187.5 (52.7-334.2)	167.0 (78.0-256.0)	55.0 (22.0-62.5)	20.0 (4.0-40.0)

Table II. The sensitivities*, specificities*, and the areas under receiver operating curves (AUC) for fecal pyruvate kinase isoenzyme M2 (M2-PK) and fecal calprotectin (FC) in distinguishing children with moderate-to-severe inflammatory bowel diseases from children with rotaviral acute diarrhea; 95% confidence intervals for all data are shown.

Tabela II. Czułości i swoistości oraz pola pod krzywymi ROC (Receiver Operating Characteristic) (AUC) oznaczenia stężenia izoenzymu M2 kinazy pirogronianowej (M2-PK) i kalprotektyny (FC) w kale w diagnostyce różnicowej dzieci z nieswoistymi zapaleniami jelit o umiarkowanej lub znacznej aktywności i ostrą biegunką o etiologii rotawirusowej; w nawiasach kwadratowych podano przedziały 95% ufności.*

		M2-PK	FC
UC	Sensitivity *Czułość*	93.8% [79.2-99.2%]	95.3% [81.5-99.6%]
	Specificity *Swoistość*	25.6% [11.6-45.6%]	44.8% [26.4-64.3%]
	AUC, p	0.82 [0.71-0.94], p < 0.0001	0.89 [0.81-0.97], p < 0.0001
	AUC difference, p *Różnica AUC, p*	-0.06 [-0.12-(-0.01)], p < 0.028	
CD	Sensitivity *Czułość*	100% [83.9-100%]	97.6% [76.2-99.9%]
	Specificity *Swoistość*	26.7% [11.9-46.1%]	44.8% [26.4-64.3%]
	AUC, p	0.82 [0.70-0.94], p < 0.0001	0.92 [0.84-0.99], p < 0.0001
	AUC difference, p *Różnica AUC, p*	-0.10 [-0.16-(-0.04)], p < 0.0018	

CD – Crohn's disease/*choroba Leśniowskiego-Crohna*, UC – ulcerative colitis/*wrzodziejące zapalenie jelita grubego.*
*According to manufacturers' cut-off levels./*Stosując próg odcięcia zalecony przez producentów.*

Table III. The sensitivities and specificities of fecal pyruvate kinase isoenzyme M2 (M2-PK) and fecal calprotectin (FC) in distinguishing children with moderate-to-severe inflammatory bowel diseases from children with rotaviral acute diarrhea at optimal cut-off values. Ninety-five percent confidence intervals are shown for all data.

Tabela III. Czułości i swoistości oznaczenia stężenia izoenzymu M2 kinazy pirogronianowej (M2-PK) i kalprotektyny (FC) w kale w diagnostyce różnicowej dzieci z nieswoistymi zapaleniami jelit o umiarkowanej lub znacznej aktywności i ostrą biegunką o etiologii rotawirusowej przy zastosowaniu optymalnych progów odcięcia; w nawiasach kwadratowych podano przedziały 95% ufności.

		M2-PK	FC
UC	Cut-off *Próg odcięcia*	28.2 U/g	42.0 µg/mL
	Sensitivity *Czułość*	75.0% [56.6-88.5%]	81.3% [63.6-92.8%]
	Specificity *Swoistość*	89.7% [72.6-97.8%]	79.3% [60.3-92.0%]
CD	Cut-off *Próg odcięcia*	38.0 U/g	78.0 µg/mL
	Sensitivity *Czułość*	71.4% [47.8-88.7%]	81.0% [58.1-94.6%]
	Specificity *Swoistość*	89.7% [72.6-97.8%]	93.1% [77.2-99.2%]

CD – Crohn's disease/*choroba Leśniowskiego-Crohna*, UC – ulcerative colitis/*wrzodziejące zapalenie jelita grubego.*

Table IV. The sensitivities*, specificities*, and the areas under receiver operating curves (AUC) for fecal pyruvate kinase isoenzyme M2 (M2-PK) and fecal calprotectin (FC) in distinguishing children with moderate-to--severe inflammatory bowel diseases from children with acute diarrhea caused by *Salmonella enteritidis*; 95% confidence intervals for all data are shown.

Tabela IV. Czułości i swoistości oraz pola pod krzywymi ROC (Receiver Operating Characteristic) (AUC) oznaczenia stężenia izoenzymu M2 kinazy pirogronianowej (M2-PK) i kalprotektyny (FC) w diagnostyce różnicowej dzieci z nieswoistymi zapaleniami jelit o umiarkowanej lub znacznej aktywności i ostrą biegunką wywołaną przez Salmonella enteritidis; w nawiasach kwadratowych podano przedziały 95% ufności.*

		M2-PK	**FC**
UC	Sensitivity *Czułość*	94.7% [80.6-99.4%]	93.8% [79.2-99.2%]
	Specificity *Swoistość*	0% [0-16.1%]	0% [0-16.1%]
	AUC, p	0.69 [0.55-0.84], p < 0.0046	0.72 [0.58-0.87], p < 0.0013
	AUC difference, p *Różnica AUC, p*	-0.03 [-0.10-0.04], p = 0.4192	
CD	Sensitivity *Czułość*	100% [83.9-100%]	95.2% [83.9-100%]
	Specificity *Swoistość*	0% [0-16.1%]	0% [0-16.1%]
	AUC, p	0.59 [0.41-0.77], p = 0.1536	0.78 [0.62-0.94], p = 0.0003
	AUC difference, p *Różnica AUC, p*	-0.19 [-0.30-(-0.08)], p < 0.0011	

CD – Crohn's disease/*choroba Leśniowskiego-Crohna,* UC – ulcerative colitis/*wrzodziejące zapalenie jelita grubego*.
*According to manufacturers' cut-off levels./*Stosując próg odcięcia zalecony przez producentów.*

Table V. The sensitivities and specificities of fecal pyruvate kinase isoenzyme M2 (M2-PK) and fecal calprotectin (FC) in distinguishing children with moderate-to-severe inflammatory bowel diseases from children with acute diarrhea caused by *Salmonella enteritidis* at optimal cut-off values. Ninety-five percent confidence intervals are shown for all data.

Tabela V. Czułości i swoistości oznaczenia stężenia izoenzymu M2 kinazy pirogronianowej (M2-PK) i kalprotektyny (FC) w kale w diagnostyce różnicowej dzieci z nieswoistymi zapaleniami jelit o umiarkowanej lub znacznej aktywności i ostrą biegunką wywołaną przez Salmonella enteritidis przy zastosowaniu optymalnych progów odcięcia; w nawiasach kwadratowych podano przedziały 95% ufności.

		M2-PK	**FC**
UC	Cut-off Próg odcięcia	146.8 U/g	112.0 µg/mL
	Sensitivity Czułość	56.3% [37.7-73.6%]	65.6% [46.8-81.4%]
	Specificity Swoistość	95.2% [76.2-99.9%]	90.5% [69.6-98.8%]
CD	Cut-off Próg odcięcia	38.0 U/g	78.0 µg/mL
	Sensitivity Czułość	71.4% [47.8-88.7%]	81.0% [58.1-94.6%]
	Specificity Swoistość	57.1% [34.0-78.2%]	90.5% [69.6-98.8%]

CD – Crohn's disease/*choroba Leśniowskiego-Crohna*, UC – ulcerative colitis/*wrzodziejące zapalenie jelita grubego.*

and AD-SE represented bacterial gastrointestinal inflammation. Here, as in previous research, M2-PK and FC levels in moderate-to-severe IBD were markedly elevated [25, 26]. M2-PK and FC concentrations in AD-RV were lower than in AD-SE, as in a study by Shastri et al. [15]. Therefore, the potential value of both markers in distinguishing between exacerbation and infectious diarrhea in IBD patients would be greater in case of viral than bacterial origin. We conclude that M2-PK and FC served as markers of intestinal inflammation, and this was comparable in IBD exacerbation and gastroenteritis caused by *S. enteritidis* [27].

Because of the relatively low number of patients in whom IBD and acute gastroenteritis co-occured we decided not to enroll them in the study. However, it must be underscored that although the numbers of such patients are small, bacterial and viral infections need to be excluded in almost all IBD patients with diarrhea because of the differences in treatment. It is known that infectious gastroenteritis may cause exacerbations of IBD [28]. However, the causes of IBD relapses are varied, including chronic and acute stress, infections in sites other than the gastrointestinal tract and exposure to allergens [29].

CONCLUSION

The study showed that the performance of M2-PK in distinguishing between children with moderate-to-severe IBD and patients with infectious gastroenteritis was inferior to FC. Neither test had sensitivity and specificity sufficient for everyday clinical application.

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