Advances in Medical Sciences 60 (2015) 246–252

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Determination of faecal inflammatory marker concentration as a noninvasive method of evaluation of pathological activity in children with inflammatory bowel diseases

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ARTICLE INFO

Article history:
Received 27 June 2014
Received in revised form 19 January 2015
Accepted 14 April 2015
Available online 29 April 2015

Keywords:
Inflammatory markers
M2-PK
Lactoferrin
Calprotectin
IBD

ABSTRACT

Purpose: The optimization of procedure evaluating the severity of inflammatory bowel diseases (IBD) using non-invasive methods.
Patients/methods: One hundred and nine children with IBD hospitalized in gastroenterology ward between 2009 and 2011 participated in the study. Activity of the disease was evaluated in each patient. Concentration of three inflammatory markers: dimeric form of tumor pyruvate kinase (M2-PK), calprotectin and lactoferrin was evaluated using immunoenzymatic tests.
Results: Existence of a significant correlation between the faecal level of all tested markers and the stage of clinical activity of the disease was demonstrated in children with IBD, both in Crohn's disease (M2-PK $p < 0.01$; calprotectin $p = 0.005$; lactoferrin $p < 0.01$) and in ulcerative colitis group (M2-PK $p < 0.01$; calprotectin $p = 0.004$; lactoferrin $p < 0.01$). A significant difference in the level of markers was found between children with unclassified colitis and the group of patients with ulcerative colitis and Crohn's disease, but there was no difference between Crohn's disease and ulcerative colitis. The increase in the level of one marker correlated with increasing level of other markers ($p < 0.01$).

Faecal markers seem to correlate well with majority of indicators of inflammatory condition in blood.
Conclusions: Measuring M2-PK, lactoferrin and calprotectin levels in faeces seem to be a useful indicator of the level of disease activity in children with IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic and incurable inflammation of the gastrointestinal tract. The most common forms are Crohn's disease and ulcerative colitis. Etiology of those diseases has not been determined yet. Possible causes include patient's genetic susceptibility and a defect in functioning of the immunological system of the gastrointestinal tract (gastrointestinal-associated lymphoid tissue – GALT). Majority of researchers agree that some microbiological agents (autochthonic flora or pathogenic microorganism) cause and/or maintain the inflammatory condition. Approximately 10–15% of patients with IBD do not fulfil a full range of criteria of ulcerative colitis and Crohn's disease. The symptoms of both pathologies may overlap. Those cases are commonly qualified as unclassified colitis (IBDU – Inflammatory bowel disease unclassified) [1]. The group of IBD includes also other – sporadically occurring pathologies – such as Behcet's disease, collagenous colitis, microscopic colitis and eosinophilic gastroenteritis. Diagnostic and therapeutic scheme of IBD is based mostly on invasive and painful procedures (gastroscopy, colonoscopy, rectoscopy, etc.). IBD diagnostics has been improved by histological examination of specimens obtained by endoscopic biopsy. Diagnostics is aimed at evaluating the extension and intensity of inflammatory lesions, and at detecting and treating the complications. Unfortunately, preliminary diagnosis is often blurred by similar clinical presentation of ulcerative colitis and Crohn's disease. Final diagnosis cannot be reached based on examination in as many as 10% of patients, and some other percent of diagnoses are erroneous [2,3]. Diagnostic scheme of IBD patients includes also imaging techniques and laboratory tests. Endoscopic procedures constitute a basis for diagnosis and selection of therapy. Considering the incurable character of the disease, those examinations and tests have to be performed repeatedly throughout the whole life of a

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http://dx.doi.org/10.1016/j.advms.2015.04.003
1896-1126/

patient. However, their high invasiveness limits their availability and is not accepted by patients, especially by children [4].

Such additional diagnostic methods are sought that – with maintained diagnostic parameters – would be non-invasive and non-burdening for a patient [5–7]. Markers of inflammatory condition determined in faeces could be the future of non-invasive IBD diagnostics. Those markers seem to be helpful in confirming IBD diagnosis and in evaluating the intensity of inflammatory condition. Their faecal level is positively correlated with activity of the pathological process. The test allows for identifying the inflammation onset in an asymptomatic form, and therefore gives a chance for effective therapy. The researchers are still searching for a marker of enteric inflammatory condition that would be specific for IBD and could provide a differential diagnosis from other conditions of the gastrointestinal tract. From the laboratory and practical point of view it is important that the marker would be highly stable. Lactoferrin and calprotectin are well studied markers [8–18]. A dimeric form of tumor pyruvate kinase (M2-PK) seems to be a new and promising marker [19–25]. That parameter proved effective in the screening of colonic carcinoma, polyps and adenomas. Rare available analyses indicate its possible use in IBD patients.

The main aim of this study was to analyze the simultaneous determination of several markers that seem to significantly increase the specificity of the test and to compare the results with clinical activity of IBD, which might mark the future of non-invasive IBD diagnostics.

2. Materials and methods

2.1. Patients

One hundred and nine children, between 3 and 16 years of age, with IBD hospitalized in the First Department of Pediatrics, Department of Pediatric Gastroenterology and Metabolic Diseases of Poznan University of Medical Sciences between 2009 and 2011 participated in the study. Diagnosis of the disease was made according to the generally accepted scheme of diagnostic proceedings, including medical history (anamnesis), physical (subjective) examination, laboratory tests, endoscopic examination, radiological imaging and histopathological analysis. We determined the following parameters in the studied patients: serum level of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), plasma fibrinogen level (FIBR), platelet count (PLT), blood iron level (Fe), blood hemoglobin level, plasma levels of total protein and albumins, and plasma concentration of immunoglobulins IgM, IgA, IgE and IgG. Additionally, Cole's nutrition index was determined for each patient.

2.2. Clinical disease activity

We evaluated the activity of the disease in each patient. In patients with Crohn's disease, we determined the Pediatric Crohn's Disease Activity Index (PCDAI – Table 1). In children with ulcerative colitis, we evaluated the activity of the disease using Truelove–Witts index. All patients – depending on time elapsed from diagnosis – were qualified into three groups: newly diagnosed disease, disease diagnosed within the last 12 months, and disease diagnosed earlier than within the last 12 months.

2.3. Laboratory studies

A sample of faeces was collected from each child to evaluate the inflammatory marker levels. All faeces samples were gathered, stored and prepared for immunoenzymatic testing strictly according to manufacturer's requirements and protocols. Concentration of analyzed markers was determined using immunoenzymatic tests (ELISA): M2-PK (cut-off point 4 U/ml; ScheBo – Biotech, Giessen, Germany), calprotectin (cut-off point < 15 mg/l; Immundiagnostik, Bensheim, Germany) and lactoferrin (cut-off point > 7.25 μg/g; Techlab, Blacksburg, VA, USA). The study involved determining a correlation between the level of faecal markers and: child's age and gender, type of pathology, activity of the disease, time of diagnosis and the level of classic blood inflammatory markers.

Faeces were collected after obtaining an informed consent for participation in the analysis (patient's or his/her guardian's for children under the age of 16 years). Other tests were completed within the framework of standard diagnostics realized in children hospitalized due to suspected or diagnosed IBD.

Table 1
Pediatric Crohn's disease activity index (PCDAI).

Symptoms	Points
Abdominal pains	
Absence	0
Weak	5
Strong	10
Stools	
<2 bloodless	0
2–5 diarrhea/blood	5
>5 or substantial bleeding	10
Fettle	
Good	0
Slightly worse	5
Bad	10
Hemoglobin concentration (g/dl)	
>12	0
10–12	2.5
<10	5
Erythrocyte sedimentation rate (ESR)	
<20	0
20–50	2.5
>50	5
Albumins	
>35	0
31–35	5
<31	10
Body mass index (BMI)	
>85	0
80–85	5
<85	10
Palpation of abdomen	
Without tenderness and resistance	0
Slight/tumor	5
Clear/and tumor	10
Perianal changes	
Absence	0
Minor, painless	5
Fistulas, pain or abscess	10
Parenteral symptoms	
Absence	0
1 of symptoms	5
2 or more symptoms	10

Activity evaluation:
No clinical activity of the disease: 0–10 points.
Mild form of the disease: 11–25 points
Moderate form of the disease: 26–50 points.
Severe form of the disease: >51 points.

2.4. Ethical considerations

The study gained approval from the Ethical Committee at the Poznan University of Medical Sciences. Determinations of inflammatory condition markers (calprotectin, lactoferrin and dimeric

Table 2
Number of patients at individual levels of clinical activity of disease.

Diagnosis	The level of clinical activity of disease				Total
	No activity	Mild	Moderate	Severe	
UC	0	19	9	8	37
CD	18	9	12	8	47
IBDU	–	–	–	–	25
Total	18	28	21	16	109

UC – ulcerative colitis.
CD – Crohn's disease.
IBDU – inflammatory bowel disease unclassified.

form of tumor pyruvate kinase) were completed at the Institute of Microecology in Poznan.

2.5. Statistical analysis

Analysis of faecal marker level was performed using measurements of descriptive statistics (arithmetic mean, standard deviation, median, lower and upper quartile). Verification of normality of distribution of variables was performed using Shapiro–Wilk test. Considering the lack of normality of distribution, differences in marker levels were evaluated using a nonparametrical Kruskal–Wallis test. Evaluation of differences in marker levels in relation to gender was performed using a non-parametrical Mann–Whitney *U* test. $p \leq 0.05$ has been considered statistically significant.

3. Results

The group of patients with IBD consisted of 64 boys and 45 girls. Table 2 summarizes the precise distribution of patients at individual levels of clinical activity of the disease.

3.1. Distribution of inflammatory condition marker levels in faeces

In children with IBD the levels of analyzed markers ranged from <1 to 3898 U/ml for M2-PK; from 1.6 to 53.8 mg/g for calprotectin, and from 0.27 to 1133 μg/g for lactoferrin. We estimated that in 49 patients the level of faecal M2-PK was within the normal range. In those patients no activity of the disease, its mild state or unclassified colitis was demonstrated. In the remaining 59 cases the marker's level was clearly increased. Fifty five physiological thresholds of calprotectin were obtained. In 52 children in that group there was no activity of the disease, mild condition or unclassified colitis. In remaining 50 patients the markers level was clearly increased. In 31 children, result for lactoferrin was within the normal range. In all of them there was no activity of the disease, its mild condition or unclassified colitis. In remaining 77 patients the level of the marker was clearly increased.

3.2. The level of inflammatory markers in faeces and gender and age

The level of determined markers does not depend on age. Existence of statistically significant differences in lactoferrin level depending on gender was found ($p = 0.02$). In girls a mean level of the marker was clearly lower compared to a group of boys (198 vs. 360).

3.3. Distribution of marker level depending on the type of diagnosis (ulcerative colitis, Crohn's disease, IBDU)

The existence of clear differences in the level of all three markers was demonstrated only between patients with unclassified colitis and ulcerative colitis and Crohn's disease ($p < 0.001$ for all the tested markers). There were no significant differences in the marker's concentration between ulcerative colitis and Crohn's disease (M2-PK $p = 1$; calprotectin $p = 1$; lactoferrin $p = 0.8$). The results of that analysis do not encourage for the use of markers as a tool useful for differentiation of IBD types (Table 3).

3.4. Distribution of marker levels depending on time elapsed from diagnosis of the disease

No statistically significant correlations were demonstrated between time elapsed from diagnosis and the level of M2-PK ($p = 0.387$) and lactoferrin in patients' faeces (0.0733). A positive correlation was demonstrated between time elapsed from diagnosis and the level of faecal calprotectin ($p = 0.0237$; $R = 0.221$). The longer time elapsed from diagnosis, the higher faecal level of the marker in children.

Table 3
Faecal marker levels for individual pathologies.

Diagnosis	Mean	*N*	SD	Minimum	Maximum	Q25	Median	Q75
Concentration of M2-PK (U/ml)								
UC	405.80	37	809.12	1.00	3433.80	1.92	16.65	461.46
CD	462.59	47	800.19	1.00	3897.80	1.89	17.23	846.25
IBDU	42.85	24	195.75	1.00	961.58	1.00	1.00	2.14
Total	349.86	108	729.09	1.00	3897.80	1.00	6.74	246.17
Concentration of calprotectin (mg/l)								
UC	19.75	37	12.90	2.50	53.75	8.75	17.50	27.50
CD	18.34	47	11.02	1.60	49.96	8.99	15.52	26.29
IBDU	8.25	24	6.82	2.86	32.50	3.50	6.00	10.00
Total	16.66	108	11.78	1.60	53.75	6.25	13.75	25.50
Concentration of lactoferrin (μg/ml)								
UC	299.99	36	317.37	0.34	957.74	20.28	170.63	551.63
CD	413.53	46	359.50	0.64	1132.84	38.18	395.65	781.09
IBDU	44.24	24	154.79	0.27	755.33	0.77	1.89	8.45
Total	291.35	106	338.72	0.27	1132.84	5.53	80.40	566.03

UC – ulcerative colitis.
CD – Crohn's disease.
IBDU – inflammatory bowel disease unclassified.
Q25 – Quartile 25.
Q75 – Quartile 75.
SD – standard deviation.
N – number of patients.

Table 4
Values of the Spearman' rank correlation coefficient *R* and levels of statistical significance *p* for correlation between the activity of Crohn's disease evaluated using the PCDAI scale and the faecal level of individual markers.

Crohn's disease	*N*	*R*	$t(N-2)$	p^*
Concentration of *M2-PK* (U/ml) and the level of clinical activity of the disease	47	0.820	9.621	0
Concentration of *calprotectin* (mg/l) and the level of clinical activity of the disease	47	0.403	2.951	0.005
Concentration of *lactoferrin* (μg/ml) and the level of clinical activity of the disease	46	0.534	4.185	0

* Correlation coefficients are significant with $p < 0.05$.
N – number of patients.
p – probability.
R – Spearman's rank correlation coefficient.

3.5. The distribution of marker levels depending on the level of clinical activity of the disease

In majority of children with IBD faecal marker levels significantly exceeded the physiological normal level. We strived to evaluate if the marker level is correlated with the level of activity of the disease in children. Existence of a strong correlation between the level of activity of the disease and concentration of all faecal markers was demonstrated. The strongest correlation for patients with Crohn's disease (evaluated using the PCDAI scale) was found for M2-PK ($R = 0.82$) (Table 4). A correlation between the level of markers and the level of clinical activity of ulcerative colitis (expressed in Truelove–Witts scale) was also statistically significant (Table 5). The next stage evaluated – in more detail – which stages of clinical activity of the disease were associated with significant differences in levels of tested markers. In patients with Crohn's disease there was a statistically significant difference in the level of M2-PK between all stages of clinical activity of the disease, except for:

(a) no activity of the disease and mild stage
(b) moderate and severe stages.

In case of calprotectin, a significant correlation was observed only between no activity and the severe stage of the disease ($p = 0.02$), and for lactoferrin – between the lack of activity and moderate and severe stages ($p = 0.002$; $p = 0.026$, respectively). Obtained results suggest that the most sensitive marker for Crohn's disease activity level evaluation is M2-PK. In children with ulcerative colitis, correlations between the level of markers and stages of activity of the disease were less commonly observed. A difference was significant for levels of M2-PK and calprotectin between mild and severe stages of the disease ($p = 0.001$ for M2PK; $p = 0.031$ for calprotectin), and the level of lactoferrin was significantly different in patients with mild and severe disease ($p = 0.001$; $p = 0.017$, respectively). At the same time, a strong, positive correlation was observed between the levels of the determined markers – the increase in one marker level was associated with the increase in the remaining ones (Table 6).

3.6. Concentration of faecal inflammatory markers and selected parameters of inflammatory condition

Analyzed faecal markers correlate well with the selected laboratory parameters. A strong positive correlation was observed between concentration of all markers and levels of CRP, ESR, fibrinogen in blood and platelet count. A strong negative correlation was observed between the concentration of markers and blood levels of hemoglobin and iron and plasma level of albumin. M2-PK and lactoferrin were additionally correlated with Cole's nutrition index. A negative correlation with the plasma level of total protein was observed for M2-PK. In children with Crohn's disease, markers were positively correlated with blood levels of CRP, ESR, fibrinogen and platelet count, and negatively correlated with hemoglobin and plasma albumin level. In children with ulcerative colitis there was a positive correlation with ESR and blood fibrinogen level, and a negative correlation with hemoglobin and plasma albumin level. In patients with unclassified colitis only rare correlations between faecal markers and parameters of inflammatory condition were observed.

Table 5
Values of the Spearman' rank correlation coefficient *R* and levels of statistical significance *p* for correlation between the activity of ulcerative colitis expressed in Truelove–Witts scale and the faecal level of individual markers.

Ulcerative colitis	*N*	*R*	$t(N-2)$	p^*
Concentration of *M2-PK* (U/ml) and the level of clinical activity of the disease	36	0.772	7.091	0
Concentration of *calprotectin* (mg/l) and the level of clinical activity of the disease	36	0.474	3.137	0.004
Concentration of *lactoferrin* (μg/ml) and the level of clinical activity of the disease	35	0.689	5.458	0

* Correlation coefficients are significant with $p < 0.05$.
N – number of patients.
p – probability.
R – Spearman's rank correlation coefficient.

Table 6
Correlations between concentrations of analysed faecal markers.

Correlations	*N*	*R*-Spearman	$t(N-2)$	*p*
Concentration of *M2-PK* (U/ml) and concentration of calprotectin (mg/l)	107	0.60	7.71	0
Concentration of *M2-PK* (U/ml) and concentration of lactoferrin (μg/ml)	106	0.78	12.79	0
Concentration of *calprotectin* (mg/l) and concentration of lactoferrin (μg/ml)	105	0.58	7.29	0

N – number of patients.
p – probability.
R – Spearman's rank correlation coefficient.

4. Discussion

Introduction of faecal markers into the routine scheme of diagnostics of patients with IBD requires numerous analyses confirming their real usefulness. Lactoferrin and calprotectin have already been well studied. Lactoferrin in a group of patients with IBD was first evaluated in 1993 [10]. Studies evaluating usefulness of that marker in pediatric group are infrequent, but demonstrate a good correlation between activity of disease and faecal lactoferrin level [11,12]. Results obtained in a group of adult patients have not always been equally promising. For example, the study by Schröder et al. [13] did not confirm the existence of assumed correlation between marker level and the activity of disease in any enteric pathology. Another tested marker – calprotectin is currently the only marker recommended by the European Crohn's and Colitis Organization (ECCO). A correlation between calprotectin and the level of exacerbation of the disease has been well confirmed, also in pediatric population [14–16]. There are reports, however, that the marker is less useful in children with IBD, compared to adult population [17]. On the other hand, Kobelska-Dubiel et al. [16] demonstrated a strong correlation between the level of calprotectin and the clinical activity in children with ulcerative colitis, and a total lack of any correlation in Crohn's disease. The identical correlation is presented by Bremner et al. [18]. The authors of this analysis decided to examine a real role of the marker in evaluating the activity of the disease in children. Usefulness of M2-PK, a changed form of the enzyme engaged in basic cellular energetic processes has also been analyzed. Its primary purpose was an early detection of gastrointestinal tumors [19–22]. It seems however, that the marker is also valuable for evaluating the intensity of inflammatory changes in the course of IBD. Only two analyses assessing the usefulness of M2-PK in patients with IBD have been performed so far – one in children and the other in adults [23,24]. The marker concentration proved to correlate well with the level of activity of the disease in adults, both for Crohn's disease and ulcerative colitis. In patients at remission the marker concentration was significantly lower compared to the active form of the disease. On the other hand, evaluation of the level of M2-PK in pediatric population demonstrated a better correlation of the marker with disease activity in children with ulcerative colitis [23]. In children with Crohn's disease the correlation was less significant. An increased level of kinase was observed in children at remission. The authors correctly plead that a seemingly good clinical condition of a patient might be misguiding. Increased marker concentration in faeces may also forecast future exacerbation of the disease that remains asymptomatic so far. Therefore, diagnostic potential of M2-PK in a population of children with IBD requires urgent evaluation. In this study, all analyzed markers proved their good correlation with the level of clinical activity of disease in patients with IBD. Those correlations were observed both in case of Crohn's disease and ulcerative colitis. Marker levels were in the majority of cases increased in patients with exacerbation of the disease. Their physiological levels were characteristic for patients at remission and with mild form of the disease. The markers seem to be a potentially useful tool to control the efficacy of introduced therapy. They also allow indirect evaluation of patient's condition. M2-PK seems to be the best candidate for evaluating the activity of inflammatory process in Crohn's disease. Only in case of that marker, statistically significant differences in faecal levels were observed between majority of stages of the disease activity. In children with ulcerative colitis, on the other hand, patient's condition seems to be best evaluated with lactoferrin level which is significantly different at mild stage and severe and moderate stages of the disease. It should be noted, that Czub et al. [26] obtained different results. Their study showed that faecal calprotectin reflects IBD severity and activity in pediatric populations in a better way than M2-PK, especially in a group with mild ulcerative colitis and ulcerative colitis in remission. In our present study, calprotectin concentration showed significantly worse correlation with the severity of IBD than M2-PK or lactoferrin. These discrepancies must be explained in order to determine the best faecal marker in pediatric IBD. It is necessary to conduct further studies in this area.

In the presented analysis, M2-PK and lactoferrin levels were independent of the time elapsed since diagnosis. A correlation between time of diagnosis and the level of faecal marker was observed for calprotectin ($p = 0.023$). We found no other reports on correlation between faecal markers and the time of diagnosis. Possible introduction of calprotectin into a routine scheme of IBD diagnostics requires further research. For clinical practice, observation that child's age has no significant effect on the level of any of the analyzed markers, seems to be irrelevant. Another stage involved evaluating a correlation level between faecal marker concentration and the type of pathology. No significant difference in marker concentration was demonstrated between ulcerative colitis and Crohn's disease. Sparse available reports analyzing the above mentioned aspect present conclusions analogous to ours [12,23]. Kane et al. [27] indicates, however, some potential differences in the level of lactoferrin in patients with ulcerative colitis and Crohn's disease. Therefore, further studies in that field are necessary. On the other hand, some significant differences were observed between unclassified colitis and both, ulcerative colitis and Crohn's disease. That observation may be helpful in the diagnostic process. Diagnosing unclassified colitis is recognized as the period of differentiation towards Crohn's disease or ulcerative colitis. Since reaching a unanimous diagnosis is difficult, the availability of diagnostic markers would be valuable due to necessary constant monitoring of possible progress of the disease in the discussed group of children. It is possible that an increased level of faecal markers may indicate differentiation of unclassified colitis into another pathology. For that reason, special attention should be paid to the usefulness of faecal markers in the group of patients with IBDU. Correlations between analyzed markers and traditional blood indicators of inflammatory condition have been studied bearing in mind increase of diagnostic standards in children with IBD. General blood tests are one of basic analyses performed during hospitalization. Events of exacerbation of inflammatory condition are evaluated using the following parameters: ESR, CRP, platelet count and others. Clinical condition of a patient may be indirectly evaluated by the measurement of iron and hemoglobin concentration or plasma albumin level. This study demonstrated a good correlation between faecal markers and the majority of traditional parameters of inflammatory condition. The correlation of markers with the higher number of parameters measured in blood was observed in children with Crohn's disease, and the weakest correlation was found in case of patients with unclassified colitis. Most likely, the lack of correlation was a result of specific group of patients, in which the final character of disease remained unspecified. Due to an innovative character of the analysis, studies regarding that type of correlation are rare.

The correlation between activity of disease evaluated in Truelove–Witts scale and biochemical markers of inflammatory condition was described in the study by Kobelska-Dubiel et al. [16], but the correlation was not detected for the disease evaluated using PCDAI scale. A direct correlation between the level of biochemical parameters and the faecal level of calprotectin was not evaluated at all. Borkowska demonstrated the lack of correlation between blood inflammatory parameters and the level of faecal lactoferrin [12]. Those results are inconsistent with the observations of our present study. The lack of correlation in the

study by Borkowska [12] could be caused by small population size of pediatric patients (60 vs. 108). On the other hand, the study by Walker et al. [11] demonstrated a significant correlation between the level of lactoferrin and traditional indicators of inflammatory condition in children and adolescents. Possibly, a relatively short period that elapsed since the first emergence of M2-PK is a reason for the lack of any studies on its correlation with the parameters of inflammatory condition in blood. The ambiguity of obtained results and low number of completed studies indicate the necessity for further research on the correlation between faecal markers and blood markers of inflammatory condition. Introducing faecal markers into the routine diagnostic scheme of IBD could significantly reduce medical expenses and the number of invasive endoscopic and anesthesia procedures. It should be noted however, that faecal markers are not specific for IBD only. Their levels increase in response to any inflammatory condition in the gastrointestinal tract, or development of a tumor in the large intestine. Rare occurrence of colonic carcinoma in pediatric population increases the usefulness of faecal markers in diagnosing IBD. Maybe extending IBD diagnostics by simultaneous measurement of several markers will improve the usefulness of the analysis. Combined analysis of M2-PK and calprotectin demonstrated the reduction of sensitivity of the test down to 64%, but with simultaneous increase in specificity up to 98% [25]. Considering the lack of a control group, this study could not provide the evaluation of sensitivity and specificity of individual tests. Therefore, it is impossible to provide an unambiguous estimation of how simultaneous performance of several measurements could influence the correctness of diagnosis. However, existence of a strong and positive correlation was demonstrated between all analyzed faecal markers. Therefore, it may be assumed that analysing several markers will increase the probability of detecting the disease and correctly evaluating patient's current condition. Another limitation of the test is the use of partially subjective scales for evaluating the activity of pathological process. The result of that evaluation depends largely on patient's own sensations (pain threshold, mental conditions). Those limitations have to be taken into account when evaluating the correlation between the marker level and disease exacerbation in an individual patient. Additionally, the necessity of multiple dilutions of markers to achieve a result falling within the measurement range (especially in patients on active stage of the disease) poses some problems, extends time of analysis and increases its cost. The comparison of advantages and disadvantages of applying faecal markers analysis brings the following conclusions:

Advantages of faecal markers' assessment

- High sensitivity in the diagnosis of the intestine changes (faecal markers reflect the dynamics of the inflammatory process ongoing mainly in the intestine, as nondigestible inside the intestine).
- The biological material (faeces) is easy to obtain.
- Non-invasiveness of the assay – particularly in case of patients with IBD who have invasive tests as part of routine diagnosis. Especially important in children.
- The ability to reduce the frequency of invasive testing, due to the implementation of routine assessment of faecal markers concentrations as part of diagnostic control of patients with IBD.
- Substantial correlation with the disease activity.
- The diagnostic evaluation is easy to implement in common clinical practice.
- The diagnostic evaluation is useful in the differentiation of functional states of organic changes.
- The possibility of early evaluation of therapy results and possible disease recurrence.
- Stability of marker proteins in faeces gives the possibility of shipping the material for further analysis to any distant laboratory.

Disadvantages of faecal markers' assessment

- Relatively low specificity – concentration of the markers is increased not only in IBD exacerbation, but also in other types of diseases, such as bacterial diarrhea, cancers, etc. (due to the fact that proteins and enzymes, which are markers of inflammation are released into the faeces during any inflammatory process, occurring in the intestinal mucous membranes, food allergy, celiac disease).
- Relatively high price – in some patients obtaining the exact concentration values requires several dilution of stool samples (additional costs).
- Extended waiting time for the result – the need to perform and analyze a certain number of samples due to the diagnostic procedure being based on enzymatic immunoassay (time of collection, required number of material from several patients).
- The spread of results caused by different water content in faeces.
- The inability to evaluate diarrheal faeces – often present in patients with IBD – due to dilution and potential influence on the result.

5. Conclusions

Although the measurement of M2-PK, lactoferrin and calprotectin as a diagnostic evaluation is relatively expensive and not without flaws, its numerous advantages such as high sensitivity, efficiency, relative precision and non-invasiveness, indicate that faecal markers of inflammation are worthy of including in the diagnostic process in children with IBD.

Conflict of interests

The authors have no conflict of interest to declare.

Financial disclosure

The study was supported by State Committee for Scientific Research funding – KBN (SCSR) 4248/B/PO1/2010/38.

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