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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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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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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