



Surrogate Markers of Intestinal Inflammation in Paediatric Patients with Inflammatory Bowel Disease

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Abstract

Background: Endoscopic evaluation is the gold standard for monitoring the disease activity in inflammatory bowel disease (IBD) but the procedure is invasive and not appropriate for frequent use, especially in the paediatric population. The aim of the present study was to assess the correlation between the levels of several inflammatory biomarkers and the degree of intestinal inflammation in paediatric patients with IBD.

Materials and methods: A single center study including 31 children with ulcerative colitis (UC) and 22 children with Crohn's disease (CD) with different disease duration and activity. All participants provided blood samples to measure the levels of white blood cell count, platelets, C-reactive protein, erythrocyte sedimentation rate, albumin and fibrinogen, and faecal samples for measurement of faecal calprotectin and faecal alpha-1 antitrypsin. All participants underwent endoscopic evaluation. Endoscopic disease activity was assessed according to the Mayo Endoscopic Subscore and Simple Endoscopic Score for Crohn's Disease in UC and CD patients, respectively.

Results: 135 visits were included: 73 for UC patients and 62 for CD patients. In UC patients the strongest correlation was between the Mayo Endoscopic Subscore and the faecal calprotectin ($r=0.867$, $p<0.001$) followed by the albumin ($r=0.523$, $p<0.001$) and the C-reactive protein ($r=0.487$, $p<0.001$). In CD the strongest correlation was between the Simple Endoscopic Score for Crohn's disease and the faecal calprotectin ($r=0.872$, $p<0.001$) followed by the C-reactive protein ($r=0.708$, $p<0.001$) and the erythrocyte sedimentation rate ($r=0.605$, $p<0.001$).

Conclusions: The faecal calprotectin is a valuable surrogate marker of intestinal inflammation that is useful for monitoring of a disease activity in paediatric patients with IBD.

Keywords

faecal calprotectin, surrogate markers, paediatric inflammatory bowel disease

BACKGROUND

The treatment and follow-up strategies of patients with IBD have changed in the last years. Traditionally, the primary goal of IBD treatment was to achieve "a clinical remission", defined by the absence of signs and symptoms of

active disease.¹ More recently, the focus has shifted to the induction of "mucosal healing", a state defined not only by the absence of clinical symptoms, but also by the absence of ulcers and inflammatory lesions during colonoscopy.²⁻⁴ The endoscopic evaluation remains the gold standard for assessment of intestinal mucosa but the procedure is in-

vasive and not appropriate for frequent use, especially in children.⁵ The presence of an active gut inflammation in patients with IBD is associated with both a local and a systemic inflammatory response, causing migration of leucocytes to the gut and production of specific proteins, which may be detected in serum or stools.⁶ These biomarkers are often used in clinical practice in addition to the subjective clinical indices as a non-invasive or microinvasive tool for objective measurement of a disease activity.

AIM

The aim of our study was to evaluate the role of six blood tests: white blood cell count, platelets count, C-reactive protein, erythrocyte sedimentation rate, albumin, and fibrinogen, and two faecal tests: faecal calprotectin and faecal alpha-1 antitrypsin, as surrogate markers of intestinal inflammation in paediatric patients with IBD.

MATERIALS AND METHODS

Study Design

A single center study including 53 children with IBD with different disease duration and activity treated in our department between June 2012 and June 2016. The median age of participants at enrolment was 15 years (range 2-17 years), 60.37% were girls and 39.63% were boys. All of them had been diagnosed in accordance with the Porto criteria for a diagnosis of IBD in children and adolescents.⁸ The disease extent and severity had been assessed according to the Paris Classification – paediatric modification of the Montreal Classification for IBD.⁹ During each hospitalization, the patients provided blood samples for checking the levels of white blood cell (WBC) count, platelets (PLT) count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin and fibrinogen, and faecal samples for measurement of faecal calprotectin (FC) and faecal alpha-1 antitrypsin (A1-AT). In addition, they had a clinical assessment of the disease activity using an appropriate activity index – the Pediatric Ulcerative Colitis Activity Index and Pediatric Crohn's Disease Activity Index^{10,11}, and underwent an endoscopic evaluation for checking the mucosal status. The assessment of the endoscopic disease activity was based on the Mayo Endoscopic Subscore (MES) and Simple Endoscopic Score for Crohn's Disease (SES-CD) in UC and CD patients, respectively.^{12,13} All procedures were in accordance with the ethical standards of the local Ethics Committee and with the 1964 Helsinki declaration. Written informed consent was provided by each patient or his/her relatives.

Statistical analysis

Data were included and analyzed using statistical software

(SPSS version 19.0; SPSS Inc, Chicago, IL). The categorical variables are presented as percentages and the continuous variables are presented as medians with ranges. The association between the endoscopic disease activity and the biomarkers was assessed by determination of Spearman's rank correlation coefficient (r). A two-tailed p -value of less than 0.05 was considered significant.

RESULTS

Participants

Thirty-one children with UC (20 girls and 11 boys; median age at diagnosis 13 years, range: 2-17 years) and 22 children with CD (12 girls and 10 boys; median age at diagnosis 15 years, range: 6-17 years) took part in the study. Bloody diarrhoea was the debuting symptom in most of our UC patients 45.2% (14/31). Other presenting features were non-bloody diarrhoea 32.3% (10/31) and abdominal pain 12.9% (4/31). In 3 cases the disease presented with an extraintestinal manifestation. At the time of diagnosis 42% (13/31) of our patients presented with pancolitis (E4), 19% (6/31) with extensive colitis (E3), 32% (10/31) with left-sided UC (E2) and 7% (2/31) with ulcerative proctitis (E1). Severe disease (PUCAI>65) was observed in 45.2% (14/31) of our UC patients.

The most common presenting symptom at the time of diagnosis in our CD patients was abdominal pain 40.9% (9/22), followed by diarrhoea 22.5% (5/22). In 22.5% (5/22) of the cases the disease debuted with extraintestinal manifestation. Ileocolonic disease (L3) at presentation was seen in 54.5% (12/22), isolated terminal ileal disease (L1) in 27.3% (6/22) and isolated colonic disease (L2) in 18.2% (4/22) of our patients. Forty-five percent had an upper gastrointestinal tract involvement. Most of the patients 63.6% (14/22) had non-stricturing and non-penetrating disease (B1). Stricturing disease (B2) was observed in 13.6% (3/22) of the patients, penetrating disease (B3) in 9.0% (2/22) and stricturing and penetrating disease in 13.6% (3/22) of the patients. Perianal disease was identified in 13.6% (3/22) of the patients and 22.7% (5/22) of our CD patients had growth failure. A total of 135 hospitalizations were included in the final analysis: 73 for the UC patients and 62 for the CD patients. The demographic features and the clinical characteristics of the study population are summarized in **Table 1**.

Correlations

Despite of the achievement of a clinical remission, in 21.5% (29/135) of the cases we observed an endoscopically active disease. Analyzing the relationship between the endoscopic activity and the biomarkers in the UC patients we found that the strongest correlation was between the MES and the FC ($r=0.867$, $p<0.001$) followed by the albumin ($r=0.523$, $p<0.001$) and the CRP ($r=0.487$, $p<0.001$) (**Table 2**).

Table 1. Demographic features and clinical characteristics of study population

| | UC | CD |
|---------------------------------|--|--|
| Female/Male | 20/11 | 12/10 |
| Median age at diagnosis (range) | 13 years (2-17 years) | 15 years (6-17 years) |
| Disease localization | E1 = 2 E2 = 10 E3 = 6 E4 = 13 | L1 = 6 L2 = 4 L3 = 12 L4 = 14 |
| Disease severity and behavior | S0 = 17 S1 = 14 | B1 = 14 B2 = 3 B3 = 2 B2B3 = 3 G0 = 17 G1 = 5 |
| Clinical disease activity | n=73 | n=62 |
| Severe | 13 | 8 |
| Moderate | 17 | 9 |
| Mild | 10 | 14 |
| Remission | 33 | 31 |
| Endoscopic disease activity | n=73 | n=62 |
| Severe | 22 | 26 |
| Moderate | 30 | 17 |
| Mild | 8 | 5 |
| Remission | 13 | 14 |

In CD, the strongest correlation was between the SES-CD and the FC ($r=0.872$, $p<0.001$) followed by the CRP (0.708 , $p<0.001$) and the ESR (0.605 , $p<0.001$) (**Table 3**).

DISCUSSION

The surrogate markers are parameters measured to detect

a pathologic condition when a more specific test is missing or it is impractical or not cost-effective.⁶ The surrogate markers are biomarkers but not all biomarkers are surrogate markers. An ideal surrogate marker should be simple, easy to perform, non-invasive or microinvasive, cheap, rapid, and reproducible.⁷ In the past, CRP was the test most commonly used in clinical practice as a predictor of a disease activity in paediatric patients with IBD.⁷ Unfortunately, elevated CRP is not specific to IBD. The CRP levels are also increased in various viral and bacterial infections, other autoimmune disorders, malignancy, and other disorders resulting in tissue necrosis.¹⁴ In addition there is a big difference between the CRP response in CD and UC patients. CD patients have usually a strong CRP response while UC patients have a mild to absent CRP response.^{7,14} Our findings are consistent with these data. We found a strong correlation between CRP and SES-CD ($r=0.708$, $p<0.001$) and a moderate correlation between CRP and MES ($r=0.487$, $p<0.001$).

Other blood tests such as WBC and PLT count, ESR, acute phase proteins (albumin, fibrinogen) are also used as inflammatory biomarkers in IBD patients, but none of them is specific for intestinal inflammation and they may be increased by various other conditions.^{7,14}

In recent years, faecal markers have appeared as new diagnostic tools to detect inflammation within the gastrointestinal tract. They are a heterogeneous group of substances that either leak from or are actively released by the inflamed mucosa.¹⁵ The most studied faecal marker is the FC. It was recognized as a diagnostic marker and a marker of disease activity in adults and paediatric patients with IBD.¹⁵⁻²¹ In our study, FC demonstrated to be an excellent marker of intestinal inflammation, superior to other laboratory tests. We established a very strong positive correlation between the FC levels and the degree of endoscopic activity in both UC and CD patients. Our findings are in agreement with the results of Lobaton et al. who found that FC levels correlated more closely with the endoscopic activity than leucocytes, platelets or CRP in adult patients with UC and CD.^{22,23} Similarly, Schoepfer et al. reported that FC cor-

Table 2. Correlation between the inflammatory biomarkers and MES

| | FC | Alb* | CRP | Alpha-1 AT | Fibrinogen | WBC | Plt | ESR |
|-----|----|--------|--------|------------|------------|--------|--------|-------|
| MES | r | 0.867 | 0.523 | 0.487 | 0.468 | 0.434 | 0.421 | 0.388 |
| | p | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.001 |

* absolute value

Table 3. Correlation between the inflammatory biomarkers and SES-CD

| | FC | CRP | ESR | Alb* | Plt | Fibrinogen | Leu | Alpha-1 AT |
|--------|----|--------|--------|--------|--------|------------|-------|------------|
| SES-CD | r | 0.872 | 0.708 | 0.605 | 0.519 | 0.514 | 0.377 | 0.351 |
| | p | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.003 | 0.011 |

* absolute value

related more closely with the SES-CD than CRP and leucocytes in adult CD patients and more accurately reflects endoscopic activity than CRP, platelets, hemoglobin, and blood leucocytes in adult UC patients.^{24,25} An advantage of our study, including paediatric patients with IBD, is that we analyze a greater number of laboratory markers.

Based on our results, we believe that FC is a valuable surrogate marker of intestinal inflammation that plays an important role during the follow-up of the children with IBD. FC may predict endoscopic activity in clinically quiescent disease, may guide the medical therapy and may reduce the number of the invasive endoscopic evaluations. Further studies will determine the specific cut-off values requiring attention or action.

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Суррогатные маркеры воспаления кишечника у детей с воспалительными заболеваниями кишечника

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Абстракт

Введение: Эндоскопическая оценка является золотым стандартом для мониторинга активности заболевания при воспалительных заболеваниях кишечника (ВЗК), но процедура инвазивна и не подходит для частого использования, особенно среди детей. Целью настоящего исследования было оценить взаимосвязь между уровнями нескольких воспалительных биомаркеров и стадией воспаления кишечника у детей с ВЗК.

Материалы и методы: Одноцентровое исследование с участием 31 ребёнка с язвенным колитом (ЯК) и 22 детей с болезнью Крона (БК) различной продолжительности и активности. Все участники дали образцы крови для измерения уровня лейкоцитов, тромбоцитов, С-реактивного белка, скорости оседания эритроцитов, альбумина и фибриногена, а также образцы фекалий для измерения фекального кальпротектина и фекального альфа-1-антитрипсина. Все участники прошли эндоскопическую оценку. Эндоскопическую активность заболевания оценивали с помощью эндоскопической шкалы активности Мейо и обычной эндоскопической шкалы для болезни Крона, соответственно, у пациентов с ЯК и БК.

Результаты: Было включено 135 обследований: 73 пациента с ЯК и 62 пациента с БК. У пациентов с ЯК наиболее сильная корреляция существовала между эндоскопической шкалой активности Мейо и фекальным кальпротектином ($r = 0,867$, $p < 0,001$), за которым следовали альбумин ($r = 0,523$, $p < 0,001$) и С-реактивный белок ($r = 0,487$, $p < 0,001$). У пациентов с БК наиболее сильная корреляция была установлена между нормальной эндоскопической шкалой для болезни Крона и фекальным кальпротектином ($r = 0,872$, $p < 0,001$), за которым следовал С-реактивный белок ($r = 0,708$, $p < 0,001$) и скорость оседания эритроцитов ($r = 0,605$, $p < 0,001$).

Заключение: Фекальный кальпротектин является ценным суррогатным маркером воспаления кишечника, который полезен для мониторинга активности заболевания у детей с ВЗК.

Ключевые слова

фекальный кальпротектин, суррогатные маркеры, воспалительные заболевания кишечника у детей
