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Fecal calprotectin is a good biomarker of mucosal healing in monitoring of children with IBD

Stężenie kalprotektyny w stolcu jako dobry biomarker gojenia śluzówkowego w monitorowaniu przebiegu choroby u dzieci z nieswoistymi zapaleniami jelit

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Słowa kluczowe
kalprotektyna, gojenie śluzówkowe, nieswoiste zapalenie jelit

Summary

Introduction. Fecal calprotectin (FC) concentrations of patients with inflammatory bowel diseases (IBD) are much higher than those of healthy controls or patients with functional disorders or other gastrointestinal diseases. Thus FC is a good biomarker of gut inflammation in differential diagnosis of IBD as well as mucosal healing in monitoring of IBD in adults. There is shortage of data concerning predictive value of FC in mucosa status assessment in children with IBD.

Aim. The aim of the study was to assess the usefulness of FC as a biomarker of endoscopy proven mucosal healing in monitoring of children with IBD.

Material and methods. 46 patients (25M, 21F; aged 13.7 ± 3.8) with IBD (24 ulcerative colitis – UC, and 22 Crohn's disease – CD) were involved to the study and had elective colonoscopy performed and FC within a week before endoscopy measured. Mucosa status during endoscopy were assessed with SES-CD in case of CD and with Baron score in case of UC. Full mucosal healing was defined as SES-CD = 0 or Baron score = 0. The ROC curves was used as a statistical method to establish cut off points and AUC (area under curve) was regarded as assessment of discrimination between subgroup with full mucosal healing vs. subgroup with mucosal inflammation present.

Results. The AUC was 0.95. The optimal cut-off level of discrimination between subgroup with full mucosal healing vs. subgroup with mucosal inflammation present was 233 μg/g with sensitivity 1 and specificity 0.79. When specificity was outweighed over sensitivity the cut-off point was 54 μg/g with sensitivity 0.77 and specificity 0.97.

Conclusions. FC is a good biomarker of mucosal healing in monitoring of children with IBD. Values below 54 μg/g enable to select 77% patients with full mucosal healing.

Streszczenie

Wstęp. Pacjenci z nieswoistymi zapaleniami jelit (IBD) mają dużo wyższe stężenie kalprotektyny w stolcu (FC) niż osoby zdrowe i chorzy z czynnościowymi chorobami jelit. Wykazano, że FC może być wykorzystana w diagnostyce różnicowej IBD, a także w monitorowaniu gojenia śluzówkowego u dorosłych pacjentów z IBD. Jednak nadal brak jest wystarczających danych oceniających ten marker u dzieci.

Cel pracy. Celem pracy jest ocena przydatności FC jako biomarkera gojenia śluzówkowego u dzieci chorych na IBD.

Materiał i metody. Do badania włączono 46 pacjentów (25 chłopców i 21 dziewczynek) w wieku 13,7 ± 3,8 lat chorujących na nieswoiste zapalenia jelit (24 na wrzodziejące zapalenie jelita grubego i 22 na chorobę Crohna). Wszyscy oni mieli wykonaną kolonoskopię i oznaczone w ciągu tygodnia przed badaniem stężenie kalprotektyny w stolcu. Endoskopowa ocena stanu zapalnego śluzówki opierała się na skali SES-CD w przypadkach choroby Crohna i skali Barona w przypadkach wrzodziejącego zapalenia jelita grubego. Całkowite wygojenie śluzówki było definiowane jako 0 punktów w skali SES-CD i 0 punktów w skali Barona. Dla oceny zdolności dyskryminacyjnej FC i optymalnych punktów odcięcia stężenia kalprotektyny w monitoringu wygojenia śluzówki wykorzystano analizę krzywych ROC.

Wyniki. Pole pod krzywą ROC (AUC) wyniosło 0,95. Optymalny punkt odcięcia różnicujący grupę chorych z całkowitym wygojeniem śluzówki od chorych z aktywnym

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zapaleniem wyniósł FC = 233 μg/g z czułością 1,0 i swoistością 0,79. Przy maksymalizacji swoistości natomiast punkt odcięcia wyniósł FC = 54 μg/g z czułością 0,77 i swoistością 0,97.

Wnioski. FC jest dobrym biomarkerem gojenia śluzówkowego w monitorowaniu przebiegu choroby u dzieci z nieswoistymi zapaleniami jelit. Stężenie kalprotektyny w stolcu poniżej 54 μg/g pozwala zidentyfikować 77% pacjentów z całkowitym wygojeniem śluzówki.

INTRODUCTION

Calprotectin is a calcium-binding protein with *in vitro* bacteriostatic and fungistatic properties. It is found in abundance in neutrophils, where it accounts for 60% of the protein in the cytosol. Lower calprotectin concentrations are found in monocytes and reactive macrophages (1, 2). Calprotectin is involved in the inflammatory process regulation. Study evidence has shown that its level is significantly increased in inflammatory bowel disease (IBD) and neoplasms, whereas normal values are found in patients with irritable bowel syndrome (IBS) and in healthy subjects (3, 4). Although, calprotectin is found in cerebrospinal fluid, colonic biopsies, saliva, plasma, synovial fluids, urine and faeces (5), only its fecal concentration (fecal calprotectin FC) seems to be an useful biomarker of intestinal inflammation, because, it is not influenced by extraintestinal inflammatory processes. Costa et al. (3, 4), proved that patients with clinically active IBD presented higher FC levels than those in remission or with quiescent disease. This highlighted FC as a promising non-invasive, cheep, and simple tool for predicting relapse and monitoring therapy. Study of Tibble et al. (6) showed strong correlation between FC levels and histopathologic findings in biopsies of IBD patients, and weak correlation with clinical condition expressed by CDAI (Crohn's Disease Activity Index), respectively. Other studies (7-9) proved that FC tends to correlate stronger with endoscopic activity than with aforementioned clinical indices. These results suggested that FC is useful not only in predicting relapse but also in monitoring mucosal healing, which is considered an endpoint for evaluation of efficacy in IBD treatment nowadays. However, there is shortage of data concerning predictive value of FC in mucosa status assessment, specially in children. The aim of the study was to assess the usefulness of FC as a biomarker of endoscopy proven mucosal healing in monitoring of children with IBD.

AIM

The aim of the study was to assess the usefulness of fecal calprotectin as a biomarker of endoscopy proven mucosal healing in monitoring of children with IBD.

MATERIAL AND METHODS

Patients

46 patients (25M, 21F; aged 13.7 ± 3.8) with IBD (24 ulcerative colitis – UC, and 22 Crohn's disease – CD) were involved to the study and had elective colonoscopy performed and FC within a week before endoscopy measured. Mucosa status during endoscopy were assessed with SES-CD in case of CD and with Baron score in case of UC. Full mucosal healing was defined as SES-CD = 0 or Baron score = 0. The ROC curves was used as a statistical method to establish cut off points and AUC (area under curve) was regarded as assessment of discrimination between subgroup with full mucosal healing vs. subgroup with mucosal inflammation present. Table 1 presents patients characteristics.

Table 1. Characteristics of study participants (n = 46).

Parameter	Characteristic
Gender: – males – females	 25 (54.3%) 21 (46.7%)
Age (years)	13.7 ± 3.8
Type of IBD: – UC – CD	 24 (52.2%) 22 (47.8%)

Methods

The level of fecal calprotectin was assessed with Bühlmann Quantum Blue Calprotectin test. It is an immunologic test which serves quantitive assessment of fecal calprotectin level. The test is a disposable cartridge which allows FC assessment within only about 45 minutes. After assessment, the outcome is registered with reader.

Quantum Blue Calprotectin is an immunologic test of double-binding which uses 2 types of mouse monoclonal antibodies (mAb) highly specific to human calprotectin. First of the antibodies (marked with colloid gold) is unchained and deposited within membrane in the distal part of the cartridge. After covering the membrane by properly prepared samples containing calprotectin's molecules, the specific antibodies immediately bind the molecules and form complexes. These complexes quickly move along the cartrigde toward the test window, where constantly membrane bound antibodies bind them. The increased number of such gold marked complexes within small space become visible as a single line (testing Line). The intensity of this Line is proportional to the level of calprotectin within analyzed sample. Since the quantity of gold conjugeted antibody is in the number adequate to saturate calprotectin molecules within the sample, its excess (not calprotectin bound) moves forward along the cartridge reaching the point where goat anti-mouse antibody is deposited. This antibody binds all anti calprotectin antibodies forming the second single line (control line). The intensity of both lines is measured with Quantum Blue® Reader.

Statistics

Descriptive statistics of analyzed variables (calprotectin, endoscopic scales, clinical scales, histological assessment, demographic variables) was performed with Statistica for Windows software. ROC curve analysis was performed to assess the discriminant ability of fecal calprotectin concentration between groups with mucosal remission vs. no mucosal remission respectively as well as to establish cut off levels: optimal and with specifity outweighted.

RESULTS

13 out of 46 patients presented with full mucosal remission assessed during full colonoscopy with ileum terminale intubation. The clinical characteristics of both subgroups are listed in tables 2 and 3 below.

Table 2. Patients with full mucosal remission.

	Mean	Median	Q1	Q3	SD
Age	13.8	13.8	11.8	17.0	3.5
Height	157.6	165.0	130.0	174.0	22.2
Weight	53.1	53.9	43.0	63.0	18.2
BMI	20.9	19.7	18.6	24.5	4.1
CRP	0.4	0.0	0.0	0.6	0.6
ESR	12.9	10.0	2.0	16.0	13.5
Hematocrit	37.6	36.8	35.3	39.3	3.7
Platelets	250.4	238.0	187.0	343.0	107.8

Table 3. Patients with no mucosal remission.

	Mean	Median	Q1	Q3	SD
Age	13.6	15.0	12.0	16.6	3.9
Height	157.7	165.5	153.5	174.5	23.5
Weight	47.3	49.0	37.5	58.8	16.3
BMI	18.2	18.4	15.4	19.8	3.2
CRP	0.7	0.3	0.1	1.1	1.0
ESR	16.8	15.0	7.0	26.5	11.5
Hematocrit	35.6	37.4	34.3	38.8	6.5
Platelets	379.6	344.0	282.0	420.0	168.1

ROC curve analysis plotted to distinguish groups with mucosal remission vs. no mucosal remission is listed in figure 1 below.

The area under curve (AUC) was 0.95. The optimal cut-off level of discrimination between subgroup with full mucosal healing vs. subgroup with mucosal inflammation present was 233 μg/g with sensitivity 1 and specificity 0.79. When specificity was outweighed over sensitivity the cut-off point was 54 μg/g with sensitivity 0.77 and specificity 0.97.

DISCUSSION

FC has been proposed as an ideal marker of disease activity in IBD. The test is cheap, and simple to perform, with a marker that is stable at room temperature for up to seven days (thereby permitting postage of samples) (6). FC correlates well with endoscopic disease activity and histologic inflammation, thus it was proposed as marker of mucosal healing which is a major therapeutic goal in IBD treatment nowadays. However, to introduce FC as a routine test, thresholds for the prediction of mucosal healing are required. There is shortage of data on this subjects, especially in children. Lobaton Ortega et al. (10) observed 88 CU patients to evaluated the capacity of FC in discriminating between the different levels of endoscopic disease activity. They also established a cut-off level of FC in order to predict mucosal healing. In this study FC correlated closest with Mayo endoscopic subscore with a cut-off level of 250 μg/g, the sensivity was 94% and specificity 80%, respectively. Bojic et al. (11) studied 124 patients (62 with CD and 62 with UC) to evaluated the significance of FC in IBD activity assessment by comparing it with endoscopic activity index (CDEIS/Baron score) and histologic disease activity. Sensitivity and specificity, for CD FC concentration at cut-off value of 250 μg/g was 73, 91% in predicting endoscopicaly active disease, and 70, 83% in predicting histologicaly active disease. Sensitivity, specificity, for UC FC at cut-off value of 250 μg/g was 88, 100%, in predicting endoscopicaly active disease, and 78, 90%, in predicting histologicaly active disease. These results suggested that FC is a good marker of endoscopic/histologic activity in both subtypes of IBD with slightly higher sensitivity and specificity in UC. In our study, we observed both UC and CD patients, mucosal status was assessed with SES-CD in case of CD and with Baron score in case

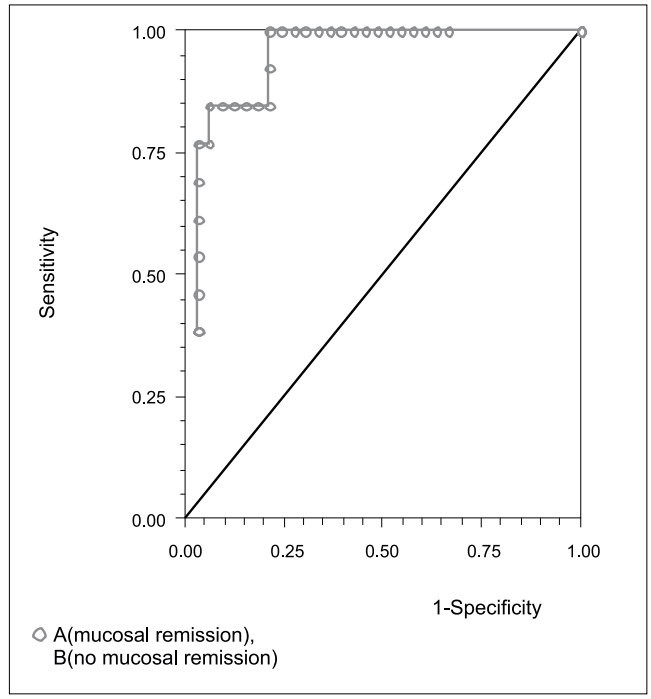

Fig. 1. ROC curve analysis plotted to distinguish groups with mucosal remission vs. no mucosal remission.

of UC. According to literature reports accuracy of FC is comparable in both entities thus, we did not evaluate results separately for UC and CD. We obtained sensitivity 100% and specificity 79% at the optimal cut-off level 233 μg/g with sensitivity 100%. When specificity was outweighed over sensitivity the cut-off point was 54 μg/g with sensitivity 77% and specificity 97%. This means that values below 54 μg/g enable to select 77% patients with full mucosal healing. We focused on assessing the usefulness of FC as a biomarker of endoscopy proven mucosal healing, therefore we compared subgroup with full mucosal healing versus subgroup with mucosal inflammation of any severity. The results proved that FC is a good marker to identify patients in full remission, including histologic healing. Because the test is non-invasive and cheap, it can be a promising monitoring tool in IBD patient. It is especially important in children, when omitting unnecessary colonoscopic examination is of great concern. The limitation of our study is relatively small number of the patient group, we analyzed only 46 children. Nonetheless, the size of each subgroup was comparable. These results are the baseline for the further analysis.

CONCLUSIONS

FC is a good biomarker of mucosal healing in monitoring of children with IBD. Values below 54 μg/g enable to select 77% patients with full mucosal healing.

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