Research article/Raziskovalni prispevek

CLINICAL UTILITY OF SERODIAGNOSTIC TESTING IN PEDIATRIC INFLAMMATORY BOWEL DISEASE

KLINIČNA UPORABNOST SEROLOŠKIH OZNAČEVALCEV V DIAGNOSTIKI KRONIČNE VNETNE ČREVESNE BOLEZNI V PEDIATRIJI

Darja Urlep-Žužej[1], Jernej Dolinšek [1], Dušanka Mičetić-Turk[1], Evgenija Homšak[2], Boris Zagradišnik[3]

[1] Odsek za gastroenterologijo in prehrano, Klinični oddelek za pediatrijo, Splošna bolnišnica Maribor, Ljubljanska 5, 2000 Maribor

[2] Oddelek za laboratorijsko diagnostiko, Splošna bolnišnica Maribor, Ljubljanska 5, 2000 Maribor

[3] Laboratorij za genetiko, Splošna bolnišnica Maribor, Ljubljanska 5, 2000 Maribor

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Key words *serological markers; inflammatory bowel disease; children; adolescents; Crohn's disease; ulcerative colitis; NOD2/CARD15 mutations*

Abstract

Background *Among children and adolescents, the diagnosis of inflammatory bowel disease (IBD) is often missed or delayed because of the nonspecific nature of the clinical symptoms. In such instances noninvasive and accurate diagnostic tests that would accurately distinguish IBD from functional disorders would be most valuable to clinicians. Several serological markers have been used as non-invasive diagnostic tools in IBD pediatric patients.*
The aim of our study was to determine the prevalence and diagnostic accuracy of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), anti-Saccharomyces cerevisiae antibodies (ASCA), anti-exocrine pancreatic antibodies (PAB) and anti-goblet cells antibodies (GAB) alone and in combination in children and adolescents with IBD.

Patients and methods *Serum specimens were analyzed for p-ANCA, ASCA IgG, ASCA IgA, PAB and GAB antibodies in 49 children and adolescents with confirmed IBD and 53 non-IBD controls. P-ANCA, PAB and GAB antibodies were determined by indirect immunofluorescent test and ASCA by enzyme-linked immunosorbent assay. All patients with Crohn's disease (CD) had genotyping performed using a sequence specific PCR directed against the wild type and the three principal mutations of NOD2/CARD15 gene. Disease location, body mass index (BMI) and disease activity by pediatric Crohn's disease activity index (PCDAI) at the time of diagnosis were determined in CD patients.*

Results *The prevalence of p-ANCA in patients with UC and ASCA in CD patients was high (82.3 % and 67.9 %, respectively). Positivity for PAB antibodies in CD and GAB in UC was lower (35.7 % and 23.5 %, respectively). Accuracy data (sensitivity, specificity, PPV, NPV, respectively) for differentiating IBD from non-IBD controls were as follows: p-ANCA: 82 %, 100 %, 100 %, 94 %; ASCA IgG: 68 %, 94 %, 86 %, 84 %; ASCA IgA: 54 %, 100 %, 100 %, 80 %; PAB: 36 %, 98 %, 91 %, 74 %; GAB: 23 %, 100 %, 100 %, 80 %. In distinguishing CD from UC we found out the following accuracy data (sensitivity, specificity, PPV, NPV, respectively): p-ANCA: 82 %, 82 %, 74 %, 88 %; ASCA IgG: 68 %, 100 %, 100 %, 65 %; ASCA IgA: 54 %, 100 %, 100 %, 57 %; PAB: 36 %, 100 %, 100 %, 49 %; GAB: 23 %, 100 %, 100 %, 68 %. There*

Corresponding author / Avtor za dopisovanje:
Asist. mag. Darja Urlep-Žužej, dr. med., Klinični oddelek za pediatrijo, Splošna bolnišnica Maribor, Ljubljanska 5, 2000 Maribor

were no significant association between ASCA positivity and the three major mutations of NOD2/CARD15 gene, disesase location and family history in CD patients, however an association between BMI and disease activity at the time of diagnosis was found out.

Conclusions *Specificity and positive predictive value of serological markers p-ANCA, ASCA IgG, ASCA IgA, PAB and GAB for IBD alone and in combination are high and which make them useful in diagnosis of inflammatory bowel disease in day-to-day clinical practice, particulary in making decision about performing invasive diagnostic procedures. Because of low sensitivity they are less useful as screening tests for inflammatory bowel disease in pediatric population.*

Ključne besede *serološki označevalci; kronična vnetna črevesna bolezen; otroci; mladostniki; Crohnova bolezen; ulcerozni kolitis; NOD2/CARD15 mutacije*

Izvleček

Izhodišča *Kronična vnetna črevesna bolezen (KVČB) pri otrocih in mladostnikih pogosto poteka z nespecifičnimi kliničnimi znaki, ki so lahko podobni funkcionalnim motnjam, zato je diagnoza KVČB pogosto postavljena pozno ali pa celo spregledana. V pediatriji so v klinični praksi še posebno zaželjeni zanesljivi neinvazivni diagnostični testi, ki ločijo KVČB od funkcionalnih motenj. Številni serološki označevalci se že uporabljajo kot neinvazivni diagnostični testi v diagnostiki KVČB v pediatriji.*
Namen naše raziskave je bil določiti prevalenco ter diagnostično uporabnost seroloških označevalcev – protiteles proti citoplazemskim antigenom nevtrofilcev (Antineutrophil Cytoplasmic Antibodies – ANCA), protiteles proti kvasovki Saccharomyces cerevisiae (Anti-Saccharomyces Cerevisiae Antibodies – ASCA), protiteles proti eksokrinemu delu pankreasa (Pancreas Antibodies – PAB) in protiteles proti čašastim celicam (Goblet cells Antibodies – GAB) posamezno in v kombinaciji pri otrocih in mladostnikih s KVČB.

Bolniki in metode *V vzorcih serumov 49 otrok in mladostnikov s KVČB in 53 otrok in mladostnikov kontrolne skupine, ki niso imeli KVČB, smo ugotavljali prisotnost protiteles p-ANCA, ASCA IgG, ASCA IgA, PAB and GAB. Protitelesa p-ANCA, PAB in GAB smo določali z metodo indirektne imunofluorescence, protitelesa ASCA IgG in IgA pa z encimsko-imunsko metodo ELISA. Dodatno smo vsem bolnikom s Crohnovo boleznijo določali morebitno prisotnost treh najpomembnejših mutacij v genu NOD2/CARD15 z metodo polimerazne verižne reakcije (PCR), opredelili umestitev vnetja, določili indeks telesne mase in stopnjo aktivnosti vnetja s pediatričnim indeksom aktivnosti bolezni (PCDAI) ob postavitvi diagnoze.*

Rezultati *Ugotovili smo visoko prevalenco p-ANCA protiteles pri bolnikih z ulceroznim kolitisom (UK) (82,3 %), medtem ko je bila prevalenca ASCA protiteles pri bolnikih s Crohnovo boleznijo (CB) nekoliko nižja (67,9 %). Prevalenca PAB protiteles pri CB in GAB pri bolnikih z UK je bila še nižja (35,7 % za PAB pri CB in 23,5 % za GAB protitelesa pri UK). Občutljivost, specifičnost, pozitivna napovedna vrednost (PPV) in negativna napovedna vrednost (NPV) omenjenih seroloških označevalcev v diagnostičnem razlikovanju KVČB od zdravih preiskovancev so bile sledeče: za p-ANCA: 82 %, 100 %, 100 %, 94 %; za ASCA IgG: 68 %, 94 %, 86 %, 84 %; za ASCA IgA: 54 %, 100 %, 100 %, 80 %; za PAB: 36 %, 98 %, 91 %, 74 % in za GAB: 23 %, 100 %, 100 %, 80 %. V razlikovanju med CB in UK pa so občutljivost, specifičnost, pozitivna napovedna vrednost (PPV) in negativna napovedna vrednost (NPV) znašale: za p-ANCA: 82 %, 82 %, 74 %, 88 %; za ASCA IgG: 68 %, 100 %, 100 %, 65 %; za ASCA IgA: 54 %, 100 %, 100 %, 57 %; za PAB: 36 %, 100 %, 100 %, 49 % in za GAB: 23 %, 100 %, 100 %, 68 %. V naši študiji nismo potrdili statistično pomembne povezave med prisotnostjo ASCA protiteles in treh najpogostejših mutacij v genu NOD2/CARD15 pri bolnikih s CB. Prav tako nismo potrdili povezave med ASCA pozitivnim serološkim statusom in ilealno umestitvijo vnetja ter pozitivno družinsko anamnezo. Ugotovili pa smo statistično pomembno povezavo med prisotnostjo ASCA protiteles z večjo aktivnostjo CB in nižjim indeksom telesne mase pri bolnikih s CB.*

Zaključki *Zaradi visoke specifičnosti ter pozitivne napovedne vrednosti so serološki označevalci p-ANCA, ASCA IgG, ASCA IgA, PAB in GAB tako samostojno kot v kombinaciji pomembni neinvazivni diagnostični testi. V vsakodnevni klinični praksi nam koristijo predvsem v diagnostični razmejitvi med kronično vnetno črevesno boleznijo in funkcionalnimi motnjami ter pri odločitvi za invazivne diagnostične metode. Zaradi prenizke občutljivosti pa niso primerni za uporabo kot presejalni testi za kronično vnetno črevesno bolezen v pediatrični populaciji.*

Introduction

Inflammatory bowel disease (IBD) has become an increasingly important diagnostic consideration in pediatric patients. Among children, the diagnosis of IBD – particular CD is often missed or delayed because of the unspecific nature of both intestinal and extraintestinal symptoms at presentation. These overlap with functional bowel disorders such as recurrent abdominal pain in childhood. In other cases joint pains, pubertal delay or growth deceleration with short stature may be the clinical presentation of IBD, in the absence of any digestive signs or symptoms (1, 2). In such instances noninvasive and accurate diagnostic tests that would accurately distinguish IBD from functional disorders would be most valuable to clinicians.

Several serological markers have been used as noninvasive diagnostic tools in IBD patients. Since 1990, perinuclear anti-neutrophil cytoplasmatic antibodies (p-ANCA) which show a specific staining on indirect immunoflourescence, have been consistently found in 50–80 % of patients suffering from ulcerative colitis (3–10). These are spontaneusly produced by lamina propria and mesenteric node lymphocytes (11). The exact epitopes recognized by pANCA antibodies remain as yet unknown. Some studies showed p-ANCA react the antigens localized in the inner side of the nuclear periphery of neutrophils and specially with a 50-kilodalton nuclear envelope protein (12, 13). It is hypothesized that epitopes for p-ANCA are expressed during the process of apoptosis of neutrophils (14). P-ANCA expression is not associated with disease activity (15).

Antibodies to baker's yeast and brewer's yeast anti-Saccharomyces cerevisiae antibodies (ASCA) have been reported to be highly specific for Crohn's disease and described in up to 65 % of patients with Crohn's disease (16–25). It has been demonstrated that specific antigen is a mannan localized in the yeast cell wall (26). The reason for generation of ASCA in Crohn's disease remains unknown. Some recent studies suggest that expression of ASCA is a familial trait in CD and show that a significant fraction of unaffected first-degree relatives of CD patients also display ASCA (27–29). The expression of ASCA in these families may represent a genetic immunologic trait (29).

Autoantibodies against exocrine pancreas (PAB) have been described in patients with CD since 1984, however exact role and the reason for the generation is completely unknown. They have been reported to be highly specific for CD, albeit at a low prevalence (30–34). Specific antigen reacting with PAB has not yet been identified. Some studies suggest the antigen to be a large protein complex consisting of several units (32, 35).

Autoantibodies against intestinal goblet cells (GAB) were found in a subgroup of patients with ulcerative colitis and exhibit reactivity against a > 200 kD antigen of goblet cells (36–38).

Recently, the IBD1 gene on chromosome 16 has been mapped. The mutations in the gene NOD2/CARD15 have been identified to be associated with Crohn's disease. The three major mutations within the coding region of NOD2/CARD15-R702W, G908R and 3020insC have been found out to be associated with susceptibility for Crohn's disease (39–40).

In our study we determined the prevalence of positive serological tests, p-ANCA, ASCA IgG, ASCA IgA, ASCA IgG and/or IgA, PAB and GAB in children and adolescents with IBD. We compared the serological status with non-IBD controls. The accuracy of these serological markers alone and in combination in identifying IBD and in differentiating CD from UC was evaluated. In addition, the possible associations of ASCA positivity with family history, disease phenotype and the three principal mutations of NOD2/CARD15 were investigated.

Patients and methods

There were 49 children and adolescents with confirmed IBD (6–19 years) and 53 non-IBD controls matched by age and sex included in our study. From patients with IBD there were 28 children and adolescents with Crohn's disease, 17 with ulcerative colitis and 4 with indeterminate colitis (Table 1).

Table 1. *The number of children and adolescents with inflammatory bowel disease according to sex and the type of inflammatory bowel disease.*

Razpr.1. *Število otrok in mladostnikov s kronično vnetno črevesno boleznijo glede na spol in vrsto vnetne črevesne bolezni.*

	Crohn's disease Crohnova bolezen	Ulcerative colitis Ulcerozni kolitis	Indeterminate colitis Nedeterminirani kolitis	Total Skupaj	
				N	%
Boys Dečki	19	13	2	34	69.4
Girls Deklice	9	4	2	15	30.6
Total Skupaj	28	17	4	49	100

In all patients included in our study ASCA IgA, ASCA IgG, p-ANCA, PAB and GAB antibodies were detected by indirect immunofluorescence and ELISA immunosorbent test in the period from January 1, 2001 till November 1, 2003. At the same time the clinical history, laboratory tests and also the three major mutations of NOD2/CARD15 gene were estimated.

The positive familial diagnosis in patients was defined as having at least one relative with inflammatory bowel disease in first and second-degree relatives. In patients with Crohn's disease the activity of the disease was determined according to Pediatric Crohn's Disease Activity Index (PCDAI) at the time of diagnosis before starting the therapy. All patients had also body mass index (BMI) calculated at the time of diagnosis. The localization of the inflammation was determined by endoscopic (upper endoscopy, ilecolonoscopy), ultrasound, scintiscanning and a small-bowel follow-through.

From patients with Crohn's disease 49.9 % had ileocolonic distribution of the disease, 39.3 % had only ileum and 10.7 % had only colon affected (Figure 1). From 52.9 % patients with UC, pancolitis was found in 52.9 %.

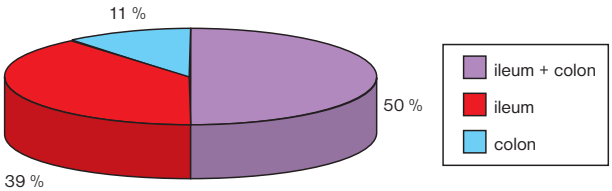

Figure 1. *The localization of the mucosal inflammation in CD patients.*

Sl. 1. *Umeščenost sluzničnega vnetja pri bolnikih s Crohnovo boleznijo.*

Determination of ANCA antibodies was performed by an indirect immunofluorescence using ethanol-fixed neutrophil slides (Euroimmune). Sera were incubated at a 1:40 dilution for 30 minutes at room temperature, washed and incubated with fluorescein-labeled antihuman immunoglobulin. Sera that exhibited fluorescence on indirect immunofluorescence were titrated to endpoint and classified as perinuclear (p-ANCA) or cytoplasmic (c-ANCA). Interference by antinuclear antibodies, which may mimic the p-ANCA pattern, was ruled out by using formalin fixed cells. Formalin fixation renders the neutrophil cell membrane permeable to antibodies, but does not solubilize proteins such as MPO, so staining of MPO appears as cytoplasmic fluorescence.

The ASCA were measured by ELISA (Euroimmun) using the crude mannan from Saccharomyces cerevisiae as the antigen. In the first reaction step, diluted patient samples were incubated with the phosphopeptidomannan antigen. In the case of positive samples, specific IgA and IgG antibodies were bound to the antigen. To detect the bound antibodies, a second incubation was carried out using an enzyme-labelled antihuman antibodies (enzyme conjugate; peroxidase-labelled) which was capable of promoting a colour reaction. Colour intensity was determined by photometric measurement.

The detection of PAB and GAB antibodies were carried out at the same time by IIF method (Euroimmun) using pancreating tissue of primates as antigen for detection of PAB and bowel mucosa tissue of primates as antigen for GAB antibodies. The assay were performed in the same way as detection of ANCA antibodies.

All patients had genotyping performed using sequence specific PCR directed against the wild tipe and the R702W, G908R and 3020insC variants of NOD2/CARD15 gene.

Statistical analyses. Comparison of the frequences of discrete variables was analyzed by the χ^2 test, or, when appropriate by Fisher exact test. A p value of < 0.05 was considered statistically significant. Sensitivity was defined as the probability of a positive test result in a patient with the disease under investigation. Specificity was defined as the probability of having a negative result in a patient, without the disease under investigation. The positive predictive value (PPV) was defined as the probability of being affected with the disease in a patient with a positive test result. The negative predictive value (NPV) was defined as the probability of not being affected with the disease in a patient with a negative test result.

Results

The prevalence of serological markers ASCA, PAB, p-ANCA and GAB in our study population is presented in Figure 2.

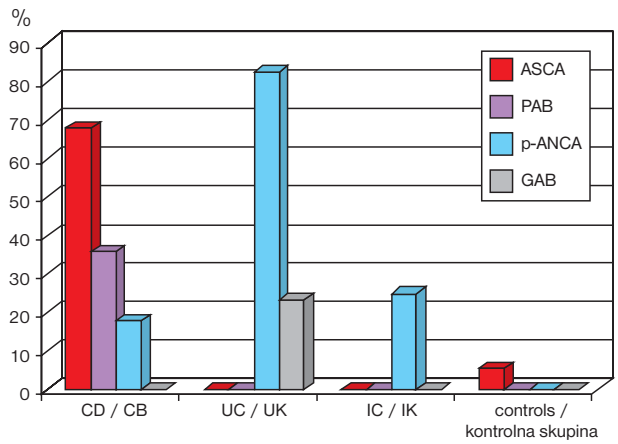

Figure 2. *The prevalence of serological markers (ASCA, PAB, p-ANCA, GAB) in patients with Crohn's disease (CD), ulcerative colitis (UC), indeterminate colitis (IC) and controls.*

Sl. 2. *Prevalenca seroloških označevalcev (ASCA, PAB, p-ANCA, GAB) pri bolnikih s Crohnovo boleznijo (CB), ulceroznim kolitisom (UK), nedeterminiranim kolitisom (IK) ter kontrolnih preiskovancih.*

A significant higher proportion of patients with Crohn's disease (67.9 %) were ASCA positive as compared to ulcerative colitis patients ($p < 0.001$) and non-IBD controls ($p < 0.001$). ASCA were also found in three control patients. On the contrary, p-ANCA positivity was strongly associated with ulcerative colitis (82.3 %) compared to patients with Crohn's disease ($p < 0.001$) and non-IBD controls ($p < 0.001$). Five of 28 patients with Crohn's disease were p-ANCA positive, however no patients with ulcerative colitis had ASCA positivity. PAB antibodies were present in 35.7 % patients with Crohn's disease and in one non-IBD control. GAB antibodies were strongly associated with ulcerative colitis, albeit at a low prevalence (23.5 %).

Table 2 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ASCA, p-ANCA, PAB and GAB antibodies in distinguishing patients with Crohn's disease and ulcerative colitis from non-IBD controls.

Table 2. *Sensitivity, specificity, positive predictive value and negative predictive value of serological markers (ASCA IgG, ASCA IgA, p-ANCA, PAB and GAB) for distinguishing pediatric patients with Crohn's disease and ulcerative colitis from non-IBD controls.*

Razpr. 2. *Občutljivost, specifičnost, pozitivna napovedna vrednost in negativna napovedna vrednost pri razlikovanju bolnikov s Crohnovo boleznijo in ulceroznim kolitisom od kontrolnih preiskovancev.*

Diagnosis	Serological markers	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Diagnoza	Serološki označevalci	Občutljivost	Specifičnost	Pozitivna napovedna vrednost	Negativna napovedna vrednost
		%	%	%	%
CD/CB	ASCA IgA ali IgG	67.9	94.3	86.4	84.7
CD/CB	ASCA IgG	67.9	94.3	86.4	84.7
CD/CB	ASCA IgA	53.6	100	100	80.3
CD/CB	PAB	35.7	98.1	90.9	74.2
UC/UK	p-ANCA	82.3	100	100	94.6
UC/UK	GAB	23.5	100	100	80.3

CD = Crohn's disease, UC = ulcerative colitis
CB = Crohnova bolezen, UK = ulcerozni kolitis

Table 3. *Sensitivity, specificity, positive predictive value and negative predictive value of serological markers (ASCA IgG, ASCA IgA, p-ANCA, PAB and GAB) for distinguishing between Crohn's disease and ulcerative colitis.*

Razpr. 3. *Občutljivost, specifičnost, pozitivna napovedna vrednost in negativna napovedna vrednost seroloških označevalcev (ASCA IgG, ASCA IgA, p-ANCA, PAB and GAB) pri razlikovanju med Crohnovo boleznijo in ulceroznim kolitisom.*

Diagnosis	Serological markers	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Diagnoza	Serološki označevalci	Občutljivost	Specifičnost	Pozitivna napovedna vrednost	Negativna napovedna vrednost
		%	%	%	%
CD/CB	ASCA IgA ali IgG	67.9	100	100	65.4
CD/CB	ASCA IgG	67.9	100	100	65.4
CD/CB	ASCA IgA	53.6	100	100	56.6
CD/CB	PAB	35.7	100	100	48.6
UC/UK	p-ANCA	82.3	82.1	73.7	88.5
UC/UK	GAB	23.5	100	100	68.3

CD = Crohn's disease, UC = ulcerative colitis
CB = Crohnova bolezen, UK = ulcerozni kolitis

The specificity for all investigated serological markers was > 90 %, the highest for p-ANCA and GAB in distinguishing patients with ulcerative colitis from non-IBD controls and IgA ASCA for Crohn's disease patients versus non-IBD controls. The positive predictive value (PPV) was also high for all investigated serological markers, the highest for ASCA IgA for Crohn's disease and p-ANCA and GAB for ulcerative colitis. On the other hand, sensitivity figures for all serological markers with the exception of p-ANCA for ulcerative colitis were rather disappointing. The highest sensitivity was obtained for p-ANCA for distinguishing ulcerative colitis patients from controls (82.3 %), however sensitivity of other serological markers was lower (67.9 % for IgG and/or IgA ASCA, 35.7 % for PAB and 25.5 % for GAB).

Table 3 shows the accuracy of each test in distinguishing Crohn's disease patients from ulcerative colitis patients. The sensitivity of all serological markers were unchanged, however the specificity and PPV reached 100 % for all serological markers except for p-ANCA. The reason for lower specificity and PPV of p-ANCA for ulcerative colitis in distinguishing between ulcerative colitis from Crohn's disease patients compared to specificity for distinguishing UC from non IBD-controls are five p-ANCA positive Crohn's disease patients. The negative predictive value was lower for all serological markers.

In our study we wanted to improve a diagnostic accuracy by using a combination of serological markers. Table 4 shows the accuracy of the combinations of serological markers in distinguishing IBD patients from non-IBD controls.

By using combinations of serological markers we did not improve the sensitivity for determing Crohn's disease, just the opposite, the sensitivity was lower. In determing ulcerative colitis the sensitivity of combinations p-ANCA+ASCA- and p-ANCA+GAB+ was the same as in using only p-ANCA. The specificity of the combinations of serological markers was in almost all combinations 100 % and PPV and NPV also improved.

We also wanted to investigate the relationship between ASCA, positive familial history, disease phenotype (location of the inflammation, disease activity and body mass index at the time of diagnosis) and

Table 4. *Sensitivity, specificity, positive predictive value and negative predictive value of the combinations of serological markers (ASCA IgG, ASCA IgA, p-ANCA, PAB and GAB) for distinguishing pediatric patients with Crohn's disease and ulcerative colitis from non-IBD controls.*

Razpr. 4. *Občutljivost, specifičnost, pozitivna napovedna vrednost in negativna napovedna vrednost kombinacije seroloških označevalcev pri razlikovanju bolnikov s Crohnovo boleznijo in ulceroznim kolitisom od kontrolnih preiskovancev.*

Diagnosis	Serological markers	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Diagnoza	Serološki označevalci	Občutljivost	Specifičnost	Pozitivna napov. vrednost	Negativna napov. vrednost
		%	%	%	%
CD/CB	ASCA+pANCA-	60.7	94.4	85.0	81.9
CD/CB	ASCA+PAB+	35.7	100	100	74.6
CD/CB	ASCA+PAB+pANCA-	32.1	100	100	73.6
CD/CB	ASCA+PAB+GAB-	35.7	100	100	74.6
CD/CB	ASCA+PAB+pANCA-GAB-	32.1	100	100	73.6
UC/UK	p-ANCA+ASCA-	82.3	100	100	94.6
UC/UK	p-ANCA+GAB+	82.3	100	100	94.6
UC/UK	p-ANCA+ASCA-PAB-	23.5	100	100	80.3
UC/UK	p-ANCA+GAB+ASCA-	23.5	100	100	80.3
UC/UK	p-ANCA+GAB+ASCA-PAB-	23.5	100	100	80.3

CD = Crohn's disease UC = ulcerative colitis
CB = Crohnova bolezen UK = ulcerozni kolitis

NOD2/CARD15 genotype in our study cohort of Crohn's disease patients.

The positive familial history was found out in 20.4 % patients with IBD (21.4 % in Crohn's disease patients and 17.6 % in ulcerative colitis patients). We did not confirm the positive relationship between ASCA positivity and positive familial history (p = 0.468), however.

ASCA positive patients had significantly lower BMI (p = 0.012) and higher pediatric Crohn's disease activity index – PCDAI (p = 0.022) at the time of diagnosis compared to ASCA negative patients. We found a negative association between the presence of ASCA and ileal location of the disease (p = 0,001).

Table 5. *The prevalence of positive familial history, ileal location of the disease, low body mass index (lower than 3rd percentile for sex and age) and moderate to severe activity of the disease at diagnosis (PCDA higher than 30) in ASCA positive and ASCA negative Crohn's disease patients.*

Razpr. 5. *Prevalenca pozitivne družinske anamneze, umeščenosti vnetja v ileumu, nizkega indeksa telesne mase (pod tretjo percentilo za starost in spol) ter aktivnosti vnetja zmerne in hude stopnje ob postavitvi diagnoze (PCDA večji od 30) pri ASCA pozitivnih in ASCA negativnih bolnikih s Crohnovo boleznijo.*

CD patients (n = 28) Bolniki s CB (n = 28)	Positive familial history Pozitivna družinska anamneza	Ileal location of the disease Umeščenost vnetja v ileumu	BMI < 3P ITM < 3P	PCDAI > 30
	%	%	%	%
ASCA positive CD patients ASCA pozitivni bolniki s CB	15.8	21.1	47.3	68.5
ASCA negative CD patients ASCA negativni bolniki s CB	33.3	77.7	0	22.2
p value p vrednost	0.468	0.001	0.012	0.022

PCDAI = pediatric Crohn's disease activity index BMI (body mass index)
PCDAI = pediatrični indeks aktivnosti Crohnove bolezni ITM (indeks telesne mase)

In our study more ASCA positive CD patients had mucosal inflammation of colon compared to ASCA negative patients. We found a negative association between the presence of ASCA and ileal location of the disease (p = 0,001) (Figure 3).

We were also interested if ASCA positivity was associated with the presence of three principal mutations of NOD2/CARD15 gene. In our study cohort 9 of 28 (32.2 %) pediatric CD patients had at least one of the three principal mutations of NOD2/CARD15 gene. All the patients with positive mutation of NOD2/CARD15 gene were heterozygotes for one of the three major mutations except one who was combined heterozygote (with R702W and G908R mutations). We did not find the association between ASCA positivity and mutations of NOD2/CARD15 gene (p = 0.926).

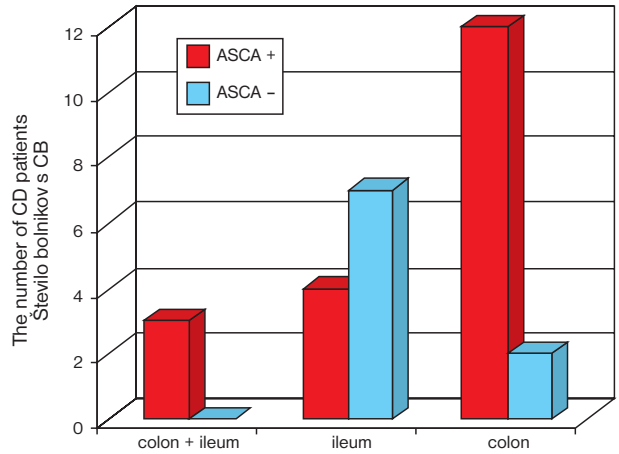

Figure 3. *The disease localization in ASCA positive and ASCA negative patients.*

Sl. 3. *Umeščenost vnetja pri ASCA pozitivnih in ASCA negativnih bolnikih.*

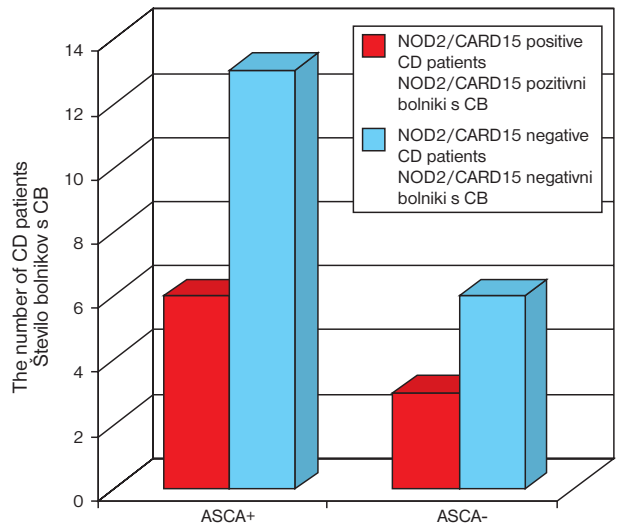

Figure 4. *The presence of NOD2/CARD15 mutations in ASCA positive and ASCA negative CD patients.*

Sl. 4. *Prisotnost NOD2/CARD15 mutacij pri ASCA pozitivnih in ASCA negativnih bolnikih s CB.*

Discussion

Although important progress has been made in understanding the disturbances of the immune system and, more recently, genetic susceptibility, inflammatory bowel disease still remains an important chalenge to the clinician. In children, the onset of the disease is frequently insidious, and presenting symptoms are nonspecific. The use of noninvasive diagnostic tools in this population is preferable. In our study we wanted to find out if determination of serological markers p-ANCA, IgG and IgA ASCA, PAB and GAB and their combinations could help us in establishing which children with nonspecific symptoms suggestive of IBD should undergo further invasive diagnostic procedures.

As expected, p-ANCA and GAB were strongly associated with ulcerative colitis and ASCA and PAB with Crohn's disease. The prevalence of p-ANCA in patients with ulcerative colitis (82.3 %) and also ASCA in Crohn's disease patients (67.9 %) were high. These prevalences are comparable with those previously reported (18–19, 21–25, 41–43). PAB antibodies in Crohn's disease and GAB in ulcerative colitis (35.7 % and 23.5 %, respectively) were found in much lower percentages as was expected. These findings are in agreement with available data from other groups (30–34, 37–38, 44). It should be emphasized that interpretation of our results in comparison with other studies is hazardous, since different assays are being used with different cut-off values and, moreover, standards are not available (45). Because of this reason it is very important that we have our own data about the accuracy of serological markers which are determined in our laboratory.

More important than prevalence data is the diagnostic accuracy of these serological markers. Our study showed very high specificity for all investigated serological markers. The highest specificity was obtained for p-ANCA (100 %) and GAB for distinguishing UC from non-IBD controls, IgA ASCA (100 %) and PAB (98.1 %) for distinguishing CD from non-IBD controls. IgG ASCA showed lower specificity than IgA ASCA for CD (94.3 %), but still enough high to be an important serological marker for distinguishing CD from healthy controls. We observed also high positive predictive value of IgA ASCA for CD (100 %), p-ANCA (100 %) for UC, GAB (100 %) for UC and PAB (90.9 %) for CD. The specificity for IgG ASCA and PAB even improved in differentiating UC from CD. On the other hand specificity of p-ANCA in distinguishing between UC and CD was lower because of five p-ANCA positive CD patients. Vasiliauskas et al. described a subgroup of CD patients who were p-ANCA positive. These patients were characterised by an UC-like phenotype (46). Three of five CD patients from our study cohort also present with predominating inflammation of colon and UC-like symptoms.

In our study we confirmed rather low sensitivity for all investigated serological markers (67.9 % for ASCA IgG, 53.6 % for ASCA IgA, 35.7 % for PAB and 23.5 % for GAB) except for p-ANCA (82.3 %). These values are consistent with reports in literature (19, 22–24, 30–34, 41–42), however our study showed a bit higher sensitivity for p-ANCA in distinguishing UC from non-IBD controls and also from CD in comparison with previous reports but still not high enough to be suitable for screening test.

In literature there are only few data in which more than two serological tests are combined (47, 48). Combining all investigated serological markers did not improve sensitivity in distinguishing IBD from non-IBD controls, however sensitivity and PPV reached 100 % in all combinations except for ASCA+ANCA- (sensitivity 94.4 % and PPV 85.0 %). These findings have shown that serological markers have high accuracy in differentiating IBD from non-IBD patients and CD patients from UC patients. This is very important in day-to-day clinical practice in making decision about performing invasive diagnostic procedures like ileocolonoscopy.

It is unclear what role the investigated serological markers have in ulcerative colitis and Crohn's disease. The antigen(s) of p-ANCA associated with ulcerative colitis seem to be localised in most cases in the neutrophil nucleus. Intracellular DNA redistribution during neutrophil apoptosis may play a role in antigen exposure to the immune system and p-ANCA production in ulcerative colitis (14). It is yet not known if ASCA antibodies are linked to increased intestinal permeability in patients with Crohn's disease, which in turn allows increased exposure of the intestinal immune system to ingested yeats (and other antigens). Konrad et al. demonstrated that immune reactivity toward mannan of ASCA-positive patients is disturbed (49). However, some recent studies have shown increased prevalence of ASCA in healthy first-degree relatives of patients with Crohn's disease (27–29). The expression of ASCA in these families probably represents a genetic immunologic trait. In our study cohort we did not find the association between ASCA positivity and three principal mutations of NOD2/CARD15 gene. This finding suggests that some other genes and not NOD2/CARD15 may be involved in generation of ASCA. On the other hand, we observed positive association of ASCA positivity with lower BMI and higher disease activity determined by PCDAI at the time of diagnosis in Crohn's disease patients. This finding is in agreement with recent study which has found out the association of ASCA positivity with the duration of Crohn's disease (50). Some studies have reported the connection of ASCA positivity with ileal localization of the inflammation in Crohn' s disease (48, 50), however our study did not confirm these findings. We also did not find out any significant association of ASCA positivity with the positive family history. Because of rather small group of our Crohn's disease pediatric patients, these findings should be confirmed with larger studies before any conclusions about these associations will be drawn.

Conclusions

Our study has shown that serological markers are useful tests to distinguish between IBD and functional non-IBD disorders in children and adolescents. They are noninvasive and simple tests what is preferable in this population. Serological markers (p-ANCA, IgG and IgA ASCA, PAB and GAB) and their combinations help us in establishing which children with nonspecific symptoms suggestive of IBD should undergo further invasive diagnostic procedures. They are also useful in distinguishing between ulcerative colitis and Crohn's disease. However, low sensitivity of all investigated serological markers makes them less suitable for IBD screening in the general population.

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