# Evaluating IBD-specific antiglycan antibodies in serum of patients with spondyloarthritis and rheumatoid arthritis: are they really specific?

V. Aloush[1], I. Dotan[2,3], J.N. Ablin[1,3], O. Elkayam[1,3]

*[1]Rheumatology Department, [2]Gastroenterology Department, Tel Aviv Medical Center, Israel; [3]Sackler Faculty of Medicine, Tel Aviv University, Israel.*

## Abstract

### Objective

*The presence of serological markers associated with inflammatory bowel disease (IBD) has been studied in spondyloarthritis with conflicting results. The anti-glycan antibodies: anti-laminaribioside, anti-chitobioside, and anti-mannobioside carbohydrate antibodies (ALCA, ACCA, and AMCA) are serological markers previously associated with IBD. We aim to investigate the prevalence of these antibodies in spondyloarthritis in comparison with rheumatoid arthritis (RA) patients*

### Methods

*Serum samples were obtained from consecutive patients with spondyloarthritis and were compared to RA and healthy controls. Anti-glycan antibodies – ALCA, ACCA and AMCA – were assessed using ELISA (Glycominds Ltd, Israel). Demographic characteristics, family history, disease pattern, skin evaluation (for PsA), disease activity and a questionnaire for gastrointestinal symptoms were recorded.*

### Results

*Seventy patients were recruited: 36 ankylosing spondylitis (AS) and 28 psoriatic arthritis (PsA). No difference in ALCA or AMCA levels was observed between all the study groups. Significantly higher levels of ACCA were observed in RA patients, compared to healthy controls (p=0.002). One or more of the anti-glycan antibodies was found in 16.7%, and 3.6% of patients with AS and PsA, respectively, compared to 7.3% in healthy controls and 27% in RA (p=0.09). No correlation was found between the presence of anti-glycan antibodies and gastrointestinal symptoms.*

### Conclusion

*Our data fail to show an increased prevalence of anti-glycan antibodies in AS or PsA patients. ACCA were found to be significantly higher in RA patients than in controls, and may serve as an inflammatory biomarker. The present results do not support a role for antiglycan antibodies as biomarkers for spondyloarthritis.*

**Key words**

ankylosing spondylitis, psoriatic arthritis, antiglycan antibodies

*Valerie Aloush, MD*
*Iris Dotan, MD*
*Jacob N. Ablin, Prof.*
*Ori Elkayam, Prof.*

*Please address correspondence and reprint requests to:*
*Dr Valerie Aloush,*
*Rheumatology Department,*
*Tel Aviv Medical Center,*
*Weizman 6,*
*64239 Tel Aviv, Israel.*
*E-mail: valerie.aloush@gmail.com*

*Received on November 2, 2017; accepted in revised form on March 26, 2018.*



*Competing interests: none declared.*

## Introduction

The relationship between inflammatory bowel diseases (IBD) and spondyloarthritis is well recognised (1). Ankylosing spondylitis (AS) or sacroiliitis are estimated to be present in 5–25% of patients with IBD, and subclinical axial involvement is even more frequent (2). Conversely, the frequency of true IBD in AS is about 5%, but subclinical gut inflammation may be present in up to 60% of patients with spondyloarthritis (3). About 7% of these asymptomatic patients may subsequently develop Crohn's disease (CD) or ulcerative colitis (UC) (4).

*Anti Saccharomyces Cerevisiae* antibodies (ASCA), directed against the cell wall mannan of *Saccharomyces Cerevisiae*, are present in approximately two thirds of patients with CD and are considered to be a serological marker for that disorder. Although their pathogenetic role is unclear, these antibodies along with perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), may contribute to the discrimination between CD and UC (5).

Because of the pathophysiological relationship between CD and spondyloarthritis, ASCA serology has been previously studied in patients with spondyloarthritis including AS, psoriatic arthritis (PsA) and undifferentiated spondyloarthritis (uSpA), with conflicting results (6-9).

Like IBD, AS is a condition characterised by an increase in intestinal permeability and mucosal dysregulation (10). This increased permeability is associated with an increased exposure of the immune system to antigens related to luminal bacteria, leading to the formation of specific serological markers which represent the loss of tolerance to intestinal bacteria, a process which has been shown to be common to both IBD and AS (11).

The anti-glycan carbohydrate antibodies anti-laminaribioside (ALCA), anti-mannobioside (AMCA) and anti-chitobioside (ACCA) are serological markers of CD, specifically of a complicated phenotype. (12, 13). These antibodies have been proposed to have potential use as biomarkers in IBD, particularly in CD, and are of potential use both for diagnosis and for follow-up (14). However, their prevalence and diagnostic utility in AS are not known.

In view of this background, the aim of the current study was to investigate whether anti-glycan antibodies are present in the sera of patients with AS and PsA, in comparison with healthy controls and with patients suffering from a non-spondyloarthritis associated inflammation such as RA. Identifying such serological markers could serve as a clinical biomarker in the diagnosis and treamtent of AS.

## Methods

*Study group*

Consecutive outpatients attending the Rheumatology Department for routine follow up, diagnosed with spondyloarthritis were enrolled in the study (Table I): Inclusion crieria for AS included patients meeting the New York criteria for the diagnosis of AS (15); and patients with PsA according to the CASPAR criteria (16).

We also included patients with RA, meeting the EULAR/ACR classification criteria for RA (17) and healthy controls, recruited from the hospital staff.

Serum samples were obtained from all patients and immediately stored at -20°C until further testing.

Clinical assessment included duration of disease, presence of peripheral synovitis, presence of axial disease for PsA and AS, extra-articular involvement, and evaluation of BASDAI.

Patients were interviewed regarding gastrointestinal-symptoms using a standardised questionnaire that assessed the following parameters: stool frequency (<3/day or ≥3/day), mucus or blood in stools, abdominal pain and weight loss. We computed a GI score which was defined as a sum of these parameters; thus the GI score may range from 0 to 4. Additional information regarding positive family history of IBD or spondyloarthritis and drug regimen was obtained.

Laboratory assessment included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and immunoglobulin levels. ANCA serology was performed in all patients, and Rheumatoid factor (RF) serology in RA patients.

The study was approved by the institutional Helsinki ethics committee (ethics approval number 07-318) and all patients signed informed consent.

*Detection of anti-glycan antibodies*

ALCA ACCA AMCA and ASCA were detected using the IBDx® panel (Glycominds, Ltd, Israel) according to manufacturer's instructions. Briefly, patients' samples were diluted (1:101) in Sample Diluent. Following dilution, the samples were incubated with appropriate horseradish peroxidase HRP conjugate. Colour development was achieved by using 3,3',5,5'-Tetramethylbenzidine (TMB) Chromogen. The absorbance (optical density) of each well was read at 450 nm within one half hour of stopping the reaction.

The cut-off values for anti-glycan antibodies are shown in Table II.

*Statistical analysis*

Chi-square test or Fisher`s exact test were used to compare categorical variables such as previous treatment for arthritis (*i.e*. ANTI TNF, steroids or DMARDS), family history of IBD, presence or absence of one of the antibodies mentioned above (*i.e*. ASCA, ALCA, ACCA, AMCA). Continuous variables (*e.g*. age) were analysed using ANOVA and Bonferroni multiple comparison when the variables were normally distributed or by the Kruskal-Wallis test for variables without a normal distribution (*e.g*. titre of antibodies, duration of disease, BASDAI score). All tests were two-sided, and a *p*-value of ≤0.05 was considered statistically significant. Statistical analysis was conducted using the SPSS package 17 (SSPS Inc., Chicago, IL, USA).

Sample size – as no prior data was available regarding the prevalence of the study tests in this population of patients, the current study was considered an exploratory study.

**Table I.** Study group.

| | AS | PsA | RA | Healthy controls | *p*-value |
|---|---|---|---|---|---|
| n. | 36 | 28 | 26 | 41 | |
| Mean age (range) | 44 (21-69) | 45 (22-84) | 62 (33-80) | 36 (15-48) | 0.001 |
| Female/Male | 19/17 | 8/19 | 19/7 | 21/20 | |

**Table II.** Cut-off values for anti-glycan antibodies.

| Result | Cut-off values for | | |
|---|---|---|---|
| | ALCA | ACCA | AMCA |
| Negative | <55 | <80 | <90 |
| Equivocal | 55-60 | 80-90 | 90-100 |
| Positive | ≥60 | ≥90 | ≥100 |

**Table III.** Clinical and laboratory characteristics of patients with spondyloarthritis.

| Characteristic | AS | PsA | *p*-value |
|---|---|---|---|
| Duration of disease (years, mean) | 11.5.6 ± 10.32 | 12.9 ± 6.4 | 0.14 |
| Presence of peripheral synovitis; n (%) | 23 (64) | 23 (82.1) | 0.4 |
| Axial disease; n (%) | 36 (100) | 10 (35.7) | <0.001 |
| Extra-articular involvement; n (%) | 8 (22) | 0 | 0.017 |
| Anti-TNF treatment; n %) | 26 (72.2) | 14 (50 | 0.09 |
| BASDAI mm (mean) | 4.09 ± 2.4 | 4.7 ± 2.2 | 0.43 |
| ESR mm/1st h (mean) | 26.1 ± 18.4 | 31.2 ± 19.3 | 0.161 |
| CRP mg/l (mean) | 8.5 ± 15.8 | 17.3 ± 29.4 | 0.14 |
| IgG levels (mean) (7-16 g/L) | 12.6 ± 3.1 | 13. ± 4.8 | 0.57 |
| IgA levels (mean) (0.7- 4 g/L) | 2.96 ± 1.2 | 2.7 ± 1.3 | 0.31 |
| Gastro-intestinal complaints[1]; n (%) | 10 (27.7) | 2 (7.1) | 0.009 |

## Results

Seventy patients were enrolled in the study, six of them were subsequently excluded because full clinical and laboratory information was not available. Thirty-six patients with AS and 28 patients with PsA were finally included in the study group; 26 patients with RA and 41 healthy subjects in the control group.

Clinical and laboratory characteristics of patients with spondyloarthritis are shown in Table III. As expected, patients with spondyloarthritis were younger than patients with RA. Mean disease duration was 11.5 (1–40) years for AS and 12.9 (1–28) years for PsA. The majority of patients in all groups had peripheral synovitis. Twenty-two per cent of patients with AS and none of the PsA patients had uveitis as an extra-articular involvement.

Most patients in the study group had clinically active disease with mean BASDAI values of 4.09 (range 0.7–9.05) in AS and 4.7 (1.3–8.9) in PsA, and mean CRP levels of 26.1 in the AS group and 31.2 in PsA group.

Sixty five% of RA patients were RF positive. Disease activity was moderate with mean DAS 28-CRP (3) of 4.19.

Total mean levels of IgA were within normal range in all groups. Four patients in each study group had slightly elevated IgA levels. As expected, GI complaints were significantly more frequent among AS patietns compared to PsA patients.

*ASCA IgA*

No significant difference was observed in mean levels of ASCA IgA between patients with spondyloarthritis and RA patients. Using a cut-off of 50, no patient in the study group, and one patient with RA was positive for ASCA IgA.

*ANCA*

None of the patients in the 4 study groups were positive for c-ANCA or p-ANCA. Two patients with AS and 4 patients with RA had a non-specific positivity for anti-neutrophil cytoplasmic antibodies.

*Anti-glycans antibodies*

• *Levels*

No significant difference was observed when comparing levels of ALCA and AMCA between patients suffering from AS, PsA, RA and healthy controls. When comparing levels of ACCA between these groups, a significantly

higher mean level was observed among patients suffering from RA compared with healthy controls ($p<0.001$), while no significant difference was observed between AS and PsA patients, compared with controls (Table IV). In RA patients, a significant correlation was observed between ACCA IgA levels and CRP levels ($p<0.05$).

• *Anti-glycan positivity*

As shown in Table V, when dichotomously analysing anti-glycan positivity according to manufacturer cut-off (Table II), ALCA was found to be positive in 5/36 AS patients, 0/28 PsA, 3/26 RA and 2/41 healthy controls. Despite a higher rate of ALCA positive serology in AS and RA patients than in healthy controls, the difference was not statistically significant.

ACCA was positive in only one patient with AS, 1 with PsA, 3 with RA and 1 in the control group. None of the patients with spondyloarthritis was positive for AMCA. When combined together, positivity for one or more anti-glycan antibody was found in 6 patients with AS, 1 with PsA, 6 with RA and 3 healthy controls ($p=0.09$).

**Table IV.** Mean levels of antiglycan antibodies, ASCA and ATTG.

| | AS | PsA | RA | Healthy controls | *p*-value |
|---|---|---|---|---|---|
| ALCA (mean ± SD) | 30.9 ± 22 | 25.6 ± 13 | 36.4 ± 23.1 | 27.9 ±22 | 0.28 |
| AMCA (mean ± SD) | 32.7 ± 13.6 | 31.7 ± 14.3 | 38.3 ± 23.3 | 29.3 ±12 | 0.22 |
| ACCA (mean ± SD)* | 41.9 ± 23.5 | 38.2 ± 39.7 | 54.4 ± 33.5 | 27.4 ±19.9 | <0.001 |
| ASCA IgA (mean ± SD) | 1.6 ± 1.3 | 2.3 ± 2 | 2.7 ± 3.3 | - | 0.68 |
| ATTG (mean ± SD) | 1.7 ± 0.6 | 2.4 ± 3.1 | 2.8 ± 5.5 | - | |

*ACCA-RA group *vs*. control group Bonferoni multiple comparison P.V= 0.002.

**Table V.** Prevalence of antiglycan antibodies, ASCA and ANCA in disease groups and controls

| | AS | PsA | RA | Healthy controls | *p*-value |
|---|---|---|---|---|---|
| ALCA; n (%) | 5 (13.9%) | 0 | 3 (11.5%) | 2 (4.9%) | 0.42 |
| AMCA; n (%) | 0 | 0 | 1 (3.9%) | 0 | |
| ACCA; n (%) | 1 (3.7%) | 1 (3.6%) | 3 (11.5%) | 1 (2.4%) | 0.43 |
| One or more positive antiglycan antibody | 6 (16.7%) | 1 (3.6%) | 6 (24%) | 3 (7.3%) | 0.09 |
| ASCA IgA; n (%) | 0 | 0 | 1 (3.9%) | - | 0.35 |
| c-ANCA; n (%) | 0 | 0 | 0 | - | |
| p-ANCA; n (%) | 0 | 0 | 0 | - | |
| x-ANCA; n (%) | 2 (5.5%) | 0 | 4 (15.4%) | - | 0.18 |
| Rheumatoid factor | - | - | 17 (65.4%) | - | |

## Discussion

Identifying a serological marker for spondyloarthritis remains an important target, with far-reaching clinical and epidemiological implications. Specific markers of bone metabolism and inflammation, and selected angiogenic cytokines have been studied in spondyloarthritis (18). In view of the pathogenetic and clinical overlap between spondyloarthritis and IBD, this endeavour has previously focused on ASCA, with limited and controversial results.

Hoffman et al found that ASCA IgA levels were significantly higher in patients with undifferentiated SpA and AS (but not in PsA), than in healthy controls or in patients with rheumatoid arthritis (RA) (6). No correlation between the presence of subclinical bowel inflammation and ASCA IgA levels was noted. Torok *et al*. compared 87 HLA- B27 positive spondyloarthritis patients with healthy controls (7). They found significantly higher levels of ASCA IgA in patients with AS and uSpA compared with controls. As in the previous study, the existence of antibodies was not associated with gastrointestinal symptoms. However, in their study, Riente *et al*. failed to show an increased prevalence of antibodies associated with CD in patients with AS or PsA, when compared to healthy controls and (RA) patients (8). Most recently, Maillet *et al*. have demonstrated an increased prevalence of ASCA among SpA patients, with an association between ASCA positivity and a specific phenotype characterised by peripheral involvement and uveitis (9). Notably, ASCA have been studied as potential markers for a wide variety of autoimmune and auto-inflammatory diseases ranging from Behçet's disease (19) to autoimmune hepatitis (20) and systemic lupus erythematosus (21).

Further attempts have been carried out in the effort to identify IBD- associated markers among patients with spondyloathritis. Wallis *et al*. have demonstrated higher anti- CBir1 (anti-flagellin) antibody positivity rates, associated with elevated acute phase reactants, among AS patients compared with patients with mechanical back pain (22). Serologic testing combined with calprotectin measurement was attempted in order to achieve optimal biomarkers for AS (23). In this study, 40% of AS patients were found to have elevated fecal levels of calprotectin, while no significant differences in IBD-specific antibodies (ASCA, anti-flagellin, anti I2 and anti OmpC) were found, when compared with controls.

In the current study we focused on the anti-glycan antibodies previously associated with IBD, specifically CD with a complicated phenotype (24, 25). In IBD, the presence of anti-glycan antibodies was assumed to reflect the loss of tolerance to commensal flora, considered a hallmark of the immunopathogenetic process (26). Notably, in IBD, serologic responses are qualitatively and quantitatively associated with a worse prognosis (27, 28).

Using these serologies, we screened patients with spondyloarthritis (AS and PsA) and RA. We did not find significant elevated rates of any anti-glycan antibodies in the spondyloarthritis group, when compared to healthy controls. Interestingly, we found elevated levels of ACCA IgA in RA patients, but this quantitatively significant difference did not translate into a significant result on a dichotomous analysis of the data. Higher levels of ACCA in RA patients were correlated with higher disease activity.

Previous studies pointed to the possibility, that cartilage breakdown and degradation, which causes exposure of carbo-

hydrate components of proteoglycans, may lead to the development of systematic antibodies reactive to glycosaminoglycans (GAGs) in RA (29). These anti-GAG antibodies showed significant cross-reactivity among different types of GAGs, as well as with bacterial peptidoglycans and fungal polysaccharides. Thus, while in IBD bacteria may form the target for development of anti-glycan antibodies, inflammatory joint disease accompanied by cartilage degradation may bring about a similar result through a different, non-bacterial mechanism, *i.e.* through molecular mimicry. An interesting correlate of this hypothesis would be examination of the presence of such antibodies in the sera of patients suffering from osteoarthritis, a condition characterised by massive cartilage degradation, with relatively low levels of systemic inflammation.

Identifying accurate serological markers for the diagnosis of spondyloarthropathies remain an unmet goal which carries significant clinical benefits. Such markers could enable early diagnosis in specific patients populations.

A number of limitations must be mentioned regarding the present study. The number of patients in each subgroup was relatively small, and potentially significant differences may thus have been undetected. In addition, a high proportion of AS patients in the current study were treated with anti-TNF-α medications, which may have had an influence on the results.

## Conclusion

In this study we have analysed for the first time the presence of anti-glycan antibodies among patients suffering from spondyloarthritis and compared them with RA patients and healthy controls. Our data fail to show an increased prevalence of these antibodies in AS or PsA patients, while demonstrating increased levels of ACCA IgA among RA patients. Developing serological markers for spondyloarthritis remains an important and unmet goal. Further studies on larger populations are needed to evaluate the clinical, diagnostic and prognostic impact of IBD serological markers in patients with spondyloarthritis.

## References


1. WRIGHT V: A unifying concept for the spondyloarthropathies. *Clin Orthop Relat Res* 1979; 143: 8-14.
2. GRAVALLESE EM, KANTROWITZ FG: Arthritic manifestations of inflammatory bowel disease. *Am J Gastroenterol* 1988; 83: 703-9.
3. MIELANTS H, VEYS EM: The gut in the spondyloarthropathies. *J Rheumatol* 1990; 17: 7-10.
4. DE VOS M, MIELANTS H, CUVELIER C, ELEWAUT A, VEYS E: Long-term evolution of gut inflammation in patients with spondyloarthropathy. *Gastroenterology* 1996; c110: 1696-703.
5. QUINTON JF, SENDID B, REUMAUX D *et al*.: Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998; 42: 788-91.
6. HOFFMAN IEA, DEMETTER P, PEETERS M *et al*.: Anti-Saccharomyces cerevisiae IgA antibodies are raised in ankylosing spondylitis and undifferentiated spondyloarthropathy. *Ann Rheum Dis* 2003; 62: 455-59.
7. TÖRÖK HP, GLAS J, GRUBER R *et al*.: Inflammatory bowel disease-specific autoantibodies in HLA-B27-associated spondyloarthropathies: increased prevalence of ASCA and pANCA. *Digestion* 2004; 70: 49-54.
8. RIENTE L, CHIMENTI D, PRATESI F *et al*.: Antibodies to tissue transglutaminase and Saccharomyces cerevisiae in ankylosing spondylitis and psoriatic arthritis. *J Rheumatol* 2004; 31: 920-24.
9. MAILLET J, OTTAVIANI S, TUBACH F *et al*.: Anti-Saccharomyces cerevisiae antibodies (ASCA) in spondyloarthritis: Prevalence and associated phenotype. *Joint Bone Spine* 2016; 83: 665-68.
10. MARTÍNEZ-GONZÁLEZ O, CANTERO-HINOJOSA J, PAULE-SASTRE P, GÓMEZ-MAGÁN JC, SALVATIERRA-RÍOS D: Intestinal permeability in patients with ankylosing spondylitis and their healthy relatives. *Br J Rheumatol* 1994; 33: 644-7.
11. AYDIN SZ, ATAGUNDUZ P, TEMEL M, BICAKCIGIL M, TASAN D, DIRESKENELI H: Anti-Saccharomyces cerevisiae antibodies (ASCA) in spondyloarthropathies: a reassessment *Rheumatology* 2008; 47: 142-4.
12. DOTAN I, FISHMAN S, DGANI Y *et al*.: Antibodies against laminaribioside and chitobioside are novel serologic marker in Crohn's disease. *Gastroenterology* 2006; 131: 366-78.
13. BONNEAU J, DUMESTRE-PERARD C, RINAUDO-GAUJOUS M *et al*.: Systematic review: new serological markers (anti-glycan, anti-GP2, anti-GM-CSF Ab) in the prediction of IBD patient outcomes. *Autoimmun Rev* 2015; 14: 231-45.
14. DOTAN N, ALTSTOCK RT, SCHWARZ M, DUKLER A: Anti-glycan antibodies as biomarkers for diagnosis and prognosis. *Lupus* 2006; 15: 442-50.
15. RUDWALEIT M, VAN DER HEIJDE D, LANDEWÉ R *et al*.: The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009; 68: 777-83.
16. TAYLOR W, GLADMAN D, HELLIWELL P, MARCHESONI A, MEASE P, MIELANTS H: Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006; 54: 2665-73.
17. ALETAHA D NEOGI T SILMAN AJ *et al*.: 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010; 62: 2569-81.
18. TERENZI R, MONTI S, TESEI G, CARLI L: One year in review 2017: spondyloarthritis. *Clin Exp Rheumatol* 2018; 36:1-14.
19. KRAUSE I, MONSELISE Y, MILO G, WEINBERGER A: Anti-Saccharomyces cerevisiae antibodies - a novel serologic marker for Behcet's disease. *Clin Exp Rheumatol* 2002; 20 (Suppl. 26): S21-24.
20. MURATORI P, MURATORI L, GUIDI M *et al*.: Anti-Saccharomyces cerevisiae antibodies (ASCA) and autoimmune liver diseases. *Clin Exp Immunol* 2003; 132: 473-6.
21. DAI H, LI Z, ZHANG Y, LV P, GAO XM: Elevated levels of serum antibodies against Saccharomyces cerevisiae mannan in patients with systemic lupus erythematosus. *Lupus* 2009; 18: 1087-90.
22. WALLIS D, ASADUZZAMAN A, WEISMAN M *et al*.: Elevated serum anti-flagellin antibodies implicate subclinical bowel inflammation in ankylosing spondylitis: an observational study. *Arthritis Res Ther* 2013; 15: R166.
23. MATZKIES FG, TARGAN SR, BEREL D *et al*.: Markers of intestinal inflammation in patients with ankylosing spondylitis: a pilot study. *Arthritis Res Ther* 2012; 14: R261.
24. FERRANTE M, HENCKAERTS L, JOOSSENS M *et al*.: New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007; 56: 1394-403.
25. DOTAN I: New serologic markers for inflammatory bowel disease diagnosis. *Dig Dis* 2010; 28: 418-23.
26. LANDERS CJ, COHAVY O, MISRA R *et al*.: Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto-and microbial antigens. *Gastroenterology* 2002; 123: 689-99.
27. MOW WS, VASILIAUSKAS EA, LIN YC *et al*.: Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004; 126: 414-24.
28. PAPP M, ALTORJAY I, DOTAN N *et al*.: New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008; 103: 665-81.
29. GYÖRGY B, TÓTHFALUSI L, NAGY G *et al*.: Natural autoantibodies reactive with glycosaminoglycans in rheumatoid arthritis. *Arthritis Res Ther* 2008; 10: R110.