Higher Titers of Anti–Saccharomyces Cerevisiae Antibodies IgA and IgG are Associated with More Aggressive Phenotypes in Romanian Patients with Crohn’s Disease

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Abstract

Background & Aims. Serological markers have been widely used for diagnostic purposes and disease stratification in inflammatory bowel diseases (IBD). The aim of this study was to investigate the seroprevalence and the correlations of anti–Saccharomyces cerevisiae antibodies (ASCA) titers with different clinical phenotypes in Romanian patients with Crohn’s disease (CD).

Methods. The study included 107 CD and 86 ulcerative colitis (UC) patients from the Gastroenterology Departments of three University Hospitals, and 60 healthy subjects. ASCA IgA and IgG titers were determined using ELISA test. For CD patients the phenotype was established according to the Montreal classification. The differences in ASCA titers for different CD phenotypes were assessed using the Mann-Whitney U test.

Results. ASCA prevalence was 33.6% in CD group, 12.8% in UC group and 6.6% in the control group. Significantly higher IgA (p=0.05) and IgG (p=0.03) titers were found in patients from the Montreal A1+A2 groups (age at onset below 40) compared with the older patients (A3). Higher titers were found in patients with extensive ileo-colonic lesions (L3) and upper gastrointestinal tract involvement (L4) than in patients having only colonic disease (L2). Significantly higher IgA (p=0.03) and IgG (p=0.03) titers were observed in patients with stenosing (B2) and penetrating (B3) disease compared with the nonstricturing, nonpenetrating (B1) phenotype. No correlation between ASCA titers and disease duration was found.

Conclusion. ASCA seropositivity in Romanian CD patients is lower than in Western Europe. Higher ASCA IgA and IgG titers are associated with a younger age at diagnosis and more aggressive phenotypes.

Key words

Inflammatory bowel disease – Crohn’s disease – phenotype – anti-Saccharomyces cerevisiae antibodies (ASCA).

Introduction

Crohn’s disease (CD) and ulcerative colitis (UC) are heterogeneous inflammatory disorders of unknown cause affecting the gastrointestinal tract. The current etiopathogenetic theory suggests that inflammatory bowel diseases (IBD) are the result of an altered immune response to bacterial microflora in genetically susceptible individuals [1]. These interactions may also be important determinants of the disease phenotype and the progression to complications [2]. It is now widely accepted that both CD and UC are characterized by a loss of the mucosal tolerance to microbial components caused by defects in innate immunity [3]. In the latter decades, various antibodies against microbial and self antigens have been extensively studied in CD and UC. However, their clinical value in diagnosing, stratifying and predicting specific forms of IBD remains questionable [4]. Recent studies have shown that, in particular for CD, there are subsets of patients with widely different immune responses to microbial antigens, this pattern being related to the genetic and phenotypic heterogeneity [5, 6].

Anti-Saccharomyces cerevisiae antibodies (ASCA) are antibodies directed against oligomannosidic epitopes of S. cerevisiae, a strain of brewer’s yeast [7]. In Western countries, increased levels of ASCA were found in 50-70% of patients with CD, in 20–25% of unaffected first degree CD relatives, and only in a minority of UC patients (5-15%) and healthy subjects (0-5%) [8-10]. Approximately two-thirds of CD patients with ASCA IgG are also positive for ASCA IgA and 0% to 19% of the patients have only ASCA IgA antibodies. This suggests that both ASCA IgG and IgA antibodies should be measured in order to increase specificity.

The aim of this study was to investigate the prevalence of ASCA positivity and the correlations of IgA and IgG ASCA...
titers with the different phenotypes in an extended cohort of Romanian CD patients.

Methods

Study population

Between August 2007 and September 2009, 107 unrelated adult patients with a diagnosis of CD, 86 UC patients and 60 healthy controls were included in the study. Patients were recruited from the Gastroenterology Departments of three University Hospitals: Fundeni Clinical Institute (Bucharest), Elias University Emergency Hospital (Bucharest) and 3rd Medical Clinic, University of Medicine and Pharmacy (Cluj-Napoca). The diagnosis of CD was established on clinical, endoscopic and histopathological criteria, at least 6 months prior to inclusion. Patients with CD were stratified to different subgroups according to the Montreal classification of IBD [11]. Informed consent was obtained from all participants before enrolment and the study protocol was approved by the local ethics committees.

ASCA determination

Patient serum samples were analyzed by a standardized enzyme-linked immunosorbent assay (ELISA) using antibodies against mannan protein within S. cerevisiae wall, according to the manufacturer’s specifications (QUANTA Lite, INOV A Diagnostics, San Diego, CA). ASCA IgA and IgG titers were assessed quantitatively with a cutoff level for positivity of 25 U/mL [12].

Statistical analysis

Quantitative variables were expressed as means +/- standard deviations. The statistical analysis was performed by means of the package SPSS 12.0 for Windows. Significant differences in ASCA titers for different CD phenotypes were assessed using the Mann-Whitney U test. A p-value less than or equal to 0.05 was considered statistically significant.

Results

From the 107 patients with CD included in the study, 62 (58%) were females, the mean age at diagnosis was 39.5 years (range 15-64) and the mean duration from the time of diagnosis was 4.4 years. Only 2 patients had a family history of CD. According to the Montreal classification of CD, localization was ileal (L1) in 8 (7.4%), colonic (L2) in 48 (44.9%), ileo-colonic (L3) in 44 (41.2%) and proximal (L4) in 7 (6.5%) patients. Sixty patients (56%) had a non-stricturing, non-penetrating (B1) pattern of the disease, 24 (22.5%) had a stricturing (B2) and 23 (21.5%) had a penetrating (B3) CD behaviour. Only 13 (12.1%) patients had perianal disease and 29 (27.1%) had a history of surgery.

ASCA (IgA and/or IgG) were found positive in 36 (33.6%) patients with CD, vs 11 (12.8%) patients with UC (p=0.001, OR=3.457, 95% CI: 1.634-7.313) and 4 (6.6%) healthy controls (p<0.001, OR=7.099, 95% CI: 2.385-21.130). The specificity, positive predictive value and negative predictive value for ASCA in our CD patients were 93.3%, 90% and 44.1%, respectively.

Concerning the values of ASCA titer in different age subgroups of CD, we found significantly higher levels of ASCA IgA (p=0.05) and ASCA IgG (p=0.03) in the Montreal A1+A2 groups in comparison with A3 group (Fig. 1). Higher ASCA titers were found in patients with extensive ileo-colonic lesions (L3) and upper gastrointestinal tract involvement (L4) in comparison with patients having only ileal (L1) or colonic disease (L2) (Fig. 2). Patients with colonic CD lesions (L2) had significantly lower ASCA IgA (p=0.0006) and IgG (p=0.00008) titers in comparison with patients with other phenotypes of disease. Significantly higher ASCA IgA (p=0.03) and ASCA IgG (p=0.03) titers were observed in patients with stricture (B2) and penetrating (B3) disease, compared with patients with nonstricturing, nonpenetrating (B1) phenotype (Fig. 3). Also, CD patients with perianal disease had higher ASCA IgA (p=0.003) and IgG (p=0.05) titers in comparison with patients with no perianal lesions (Fig. 4). On the other hand, we found that the ASCA titers were not significantly different in patients who underwent surgery and those with no history of surgery for CD. Moreover, the spreading of IgA and IgG ASCA titers over the time from the diagnosis, shown in Fig. 5, did not exhibit any particular pattern and appeared to be rather uniform. Therefore, no correlation between the disease duration and ASCA titers was observed in our patients.

Discussion

During the latter decades, various antibodies (ASCA, p-ANCA, anti-I2, anti-OmpC, anti-CBir1, anti-glycans, etc) and serotype-phenotype associations have been identified in IBD, but the diagnostic role of these serological markers
ASCA IgA and IgG in Crohn’s disease patients

Fig 1. ASCA IgA (A) and IgG (B) titers in the age groups A1 (age<17) + A2 (age 17-40) vs A3 (age > 40) CD; C-controls.

Fig 2. ASCA IgA (A) and IgG (B) titers according to location of CD. L1 ileal, L2 colonic, L3 ileocolonic, L4 upper GI.

Fig 3. ASCA IgA (A) and IgG (B) titers according to the pattern of the disease. B1 nonstricturing, nonpenetrating, B2 stricturing, B3 penetrating CD.

Fig 4. ASCA IgA (A) and IgG (B) titers in patients with perianal (P+) vs no perianal (P-) CD.
appears to be limited due to their low sensitivity [13]. However, the combination of atypical p-ANCA and ASCA is useful in clinical practice, supporting the differentiation of CD from UC when conventional diagnostic tools are equivocal [10].

ASCA positivity has been more frequently associated with ulcerative involvement, earlier age at onset, complicated CD, and the need for surgery [13-15]. It was also suggested that there might be an association of ASCA status and NOD2/CARD15 mutations to CD phenotypes [16-18], but it is not clear whether there is an interaction between genetic factors and the serologic response to intestinal bacteria, or they represent independent factors for disease progression.

Concordant with the genetic and clinical heterogeneity of CD, studies have shown that immune response heterogeneity exists among different IBD populations. For example, the sensitivity of ASCA IgA is lower in Japanese and Chinese CD patients when compared with Caucasian CD patients, suggesting that the ASCA response may be influenced by several distinct genetic determinants and/or environmental risk factors [19, 20].

Vasiliauskas et al [21] introduced the notion of immune response stratification when first reporting that high ASCA levels are associated with an earlier age of disease onset, fibrostenosing and penetrating disease forms, as well as the need for small bowel surgery. These observations indicated that subgroups of patients can be defined on the basis of the immune response patterns. The same associations have been observed also in the pediatric CD population [22]. Interestingly, the number of antibodies produced against microbial antigens in CD shows a positive correlation with the severity of the disease course. The study of Mow et al [15] indicated that the cumulative reactivity to ASCA, anti-I2 and anti-OmpC was associated with small bowel complications.

Serological data from Eastern Europe and particularly from Romanian IBD patients are limited. In a study published in 2005, Preda et al [23] found a much lower prevalence of ASCA in Romanian CD patients (12.5%) than reported in Western cohorts. The authors also found a correlation of ASCA positivity with a younger age at diagnosis (< 40 years), colonic (L2) and ileo-colonic (L3) location, but not with the behaviour of the disease or the clinical severity. On the other hand, a multicentre study indicated a low incidence and prevalence of IBD and particularly of CD in Romania [24]. Furthermore, clinical observations have shown that the Romanian CD population is characterized by a lower proportion of severe and complicated disease (penetrating and fistulizing), and a low prevalence of familial CD [24]. This may explain the relatively low prevalence of ASCA positivity in our CD patients.

In this study we found a prevalence of ASCA positivity in 33.6% of patients with CD, which is almost three times higher than that reported in the 2005 study [23]. The Romanian epidemiological findings [25] are consistent with recent data from other countries in Eastern Europe [26], which show a constant increase in incidence of CD during the recent years. The trend may be explained by the “westernization” of the lifestyle in these countries. These changes may explain also the changes of the serological patterns in CD patients.

We found that ASCA IgA and IgG titers are associated with complicated (stricturing and penetrating) disease, in agreement with previous reports [12, 14, 15, 21, 22]. The absence of a significant association of ASCA titers with ileal disease may be explained by the low prevalence of isolated small bowel (L1) location in our CD patient population. However, we noticed higher ASCA titers in patients with ileo-colonic (L3) compared with colonic (L2) location, suggesting a correlation of ASCA positivity with the disease extension. On the other hand, we found no correlation between the ASCA titers and the time from diagnosis of CD, or the presence of a surgical event. As observed already in other clinical [27, 28] and experimental [29] studies, the ASCA titers are stable throughout the disease regardless of medical or surgical treatment. Therefore, they are not suitable for monitoring the disease activity, but may have a prognostic value as a surrogate marker for severe disease. The prospective monitoring of clinical events and of ASCA levels in our cohort of patients will allow us to have a better assessment of the predictive value of these markers.
Conclusion

Although an increasing trend in recent years is noticed, ASCA seropositivity in Romanian CD patients is still lower than that reported in Western Europe. Higher ASCA IgA and IgG titers are associated with a younger age at diagnosis, ileo-colonic location and complicated (stricturing and penetrating) behaviour. Further studies on the stability of the titers over time in correlation with the disease severity are necessary in order to establish the involvement of ASCA in CD pathogenesis and in the assessment of the prognosis.

Conflicts of interests

None to declare.

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