UTILITY AND SIGNIFICANCE OF SEROLOGIC DETECTION OF IMMUNE MARKERS OF INFLAMMATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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INTRODUCTION: Classically, three major entities of inflammatory bowel disease (IBD) have been defined based on symptoms of disease and standard clinical laboratory, endoscopic, radiologic and histologic parameters: Crohn’s disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC). The diagnosis of indeterminate colitis is usually a temporary diagnosis, and many patients with IC will be diagnosed with UC or CD over time [1]. The recent advance in the area of diagnostic testing is focusing on serologic immune markers. Numerous studies have investigated the utility of 2 serologic markers in differentiating between UC and CD: atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and anti-Saccharomyces cerevisiae antibody (ASCA). These determinations represent ideal non-invasive diagnostic test for IBD patients, especially in severe exacerbation, in cases where immediate endoscopy may be contraindicated or areas of the bowel are otherwise inaccessible [1]. Given the CD and UC-specific characteristics of ASCA and pANCA, these markers were initially introduced as markers that have increased and also improved test sensitivity, considered has been given to these tests are adjunctive diagnostic tools and as possible prognostic indicators given their association with disease phenotype [2]. The aim of this study was to investigate the diagnostic value of pANCA and ASCA in IBD diagnosis and for the differential diagnosis of UC from CD.

MATERIAL AND METHOD: A prospective study was performed at the Gastroenterology Clinic, Târgu Mureș, in 2010. The reason for admission were workup of abdominal pain, altered bowel habit, and/or anorectal bleeding. In the first phase (phase I) a diagnosis of colonic disease was prospectively established based on clinical history and examination, laboratory findings (hemoglobin level, leukocyte count, platelet counts, electrolytes, serum albumin, ESR and CRP), abdominal ultrasonography and stool samples for exclusion of infectious diseases (fecal microbiology and fecal test for Clostridium difficile toxin). Out of the total of 164 IBD patients, in study were included 31 patients with new or established diagnoses of UC (n=15), CD (n=9) or IC (n=7) and also controls (n=7). Antibodies status has been measured with ELISA. A definitive diagnosis was reached using conventional techniques (colonoscopy or ileoscopy).

RESULTS: Sensitivity and specificity of pANCA for UC diagnosis was 66.67% and 77.78%, respectively; and ASCA for CD: 20% and 22.22%, respectively. The combined use of these two markers gave changes in diagnosis accuracy: pANCA+/ASCA- in UC and pANCA-/ASCA+ in CD: 75% and 72.73%, respectively. In phase II, for 23 of 38 patients a definitive diagnosis was reached using conventional techniques (colonoscopy and ileoscopy). In IC group, after 1-year follow-up, a definitive diagnosis was reached in 5 of the 7 patients.

CONCLUSION: The combined use of atypical pANCA and ASCA test results substantially affects pretest-posttest probability in distinguishing UC from CD in patients with IBD. This may be of help in patients in whom distinction between CD or UC is not obvious with the classic diagnostic tools.

Keywords: Ulcerative colitis, Crohn’s disease, Indeterminate colitis, perinuclear anti-neutrophil cytoplasmic antibody, anti-Saccharomyces cerevisiae antibody.

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ileocolonic and isolated upper disease. The severity of disease was determined on clinical basis according to the Crohn’s Disease Activity Index (CDAI) and categorized into mild-moderate, moderate-severe and severe-fulminant disease. CDAI higher than 150 was predicted as active disease in CD. The endoscopic Activity Score for CD was determined by SES-CD (Simplified endoscopic score for Crohn’s disease).

Statistical analysis: Data were processed by statistical tools in Excel (Microsoft Excel 2003), and with statistical program GraphPad Prism 5. A p-value <0.05 was considered to be significant.

**RESULTS:** A total of 38 patients were participated in the study. All patients were including in three subgroups: 31 in IBD groups respectively: UC group (n=15), CD group (n=9) and IC group (n=7), and 7 healthy subjects – in the control group (CG). Demographic characteristics of the included patients are shown in Table 1.

There were 21 women and 17 men, with a mean age of 38.54 years. In the UC group, proctitis was present in 20%, left-sided colitis in 46.66% and extensive colitis in 33.33% (Figure 1).

Clinical activity were analyzed by UCDAI score: in the UC, no patient was in clinical remission; 2 patients had mild disease (4-5 points); 10 moderate disease (6-10 points); and 2 severe disease (11-12 points) (Fig. 2).

In the CD group small bowel location was present in 11.11%, ileocolonic location in 33.33% and colonic location in the 55.55% (Fig. 3).

Clinical disease activity index in CD group were calculated by SES-CD and were in the range at 150 to 460, respectively mild-moderate disease in 22%, moderate-severe in 67% and severe in 11%.

pANCA was detected by ELISA in 10 of 15 (66.6%) samples from UC patients, in 2 of 9 (22.2%) samples from CD patients and in 3 of 7 (42.85%) samples of IC patients. pANCA was not detected in the CG (Table II). The difference between the prevalence of positive value pANCA in IBD and control groups was statistically significant (P value <0.0007). 5 (33%) patients with pancolitis and 7 (47%) patients with left-sided colitis were positive for pANCA. ASCA was detected by ELISA in 3 of 15 (20%) samples of UC patients, in 7 of 9 (77.7%) samples of CD group and in 2 of 7 (28.57%) samples of IC patients. Not found positive value for ASCA in CG. (Table 2).

The difference between the prevalence of positive value ASCA in IBD and control groups was statistically significant. The pANCA negative value in UC group were associated with mild disease cases of proctitis and left-sided colitis. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pANCA for UC diagnosis was 0.666, 0.7778, 0.8333 and 0.583; and ASCA for CD: 0.2000, 0.2222, 0.3000, 0.1429, respectively. The highest sensitivity for detecting IBD was achieved by using both pANCA and ASCA antibodies together. The combined use of these two markers gave changes in diagnosis accuracy: pANCA+/ASCA- in UC: 0.7500, 0.7273, 0.7500 and 0.7273, and for pANCA-/ASCA+ in CD: 0.7273, 0.7500, 0.7500 and 0.7273 respectively. pANCA-/ASCA- value were detected in 2 patients with IC.
In phase II, for 23 of 38 patients a definitive diagnosis was reached using conventional techniques (colonoscopy and ileoscopy). In IC group (n=7) endoscopic and histological diseasewere UC (n=3), CD (n=2) and IC (n=2). After 1-year follow-up, a definite diagnosis was reached in 5 of the 7 patients.

**DISCUSSIONS:** Serologic testing using pANCA and ASCA has also been proposed as a method to reach a definitive diagnosis of Crohn’s disease or ulcerative colitis in patients with indeterminate colitis [3-7]. pANCA has been shown repeatedly to be prevalent in the sera of approximately 60% and 20% of UC and CD patients, respectively [8-10]. In our study pANCA were detected in 10 of 15 patients (66.66%) with UC, in 2 of 9 cases (22.22%) with CD, and in 3 of 7 patients (42.85%) with IC. A distinct subset of patients manifesting left-sided Crohn’s colitis may be positive for pANCA [11]. Koutrobakis et al. reported pANCA positivity as 30% in colonic involvement of CD patients [12]. We found 22.22% (2/9) in colonic involvement and these findings are in accordance with data from literature. The combination of atypical pANCA and ASCA may be useful in the differential diagnosis of UC and CD in patients with IBD. The UC associated pattern was pANCA+/ASCA−, whereas the CD associated pattern was ASCA+/pANCA−. The combined evaluation of pANCA and ASCA had a higher specificity (>90% in most studies and > 80% in all studies) to differentiate CD from UC than the separate use of either pANCA or ASCA. In patients with IBD, the PPV of the combination of a positive ASCA test with a negative pANCA test for UC has been reported to be 92.5% by Quinton et al. [9], 95% by Peeters et al. [13] and 77% by Koutrobakis et al. [12]. The PPV places test specificity in the context of disease prevalence and indicates what percentage of patients with positive test results actually have the disease. The our data for specificity and sensitivity is in accordance with literature.

Thus, the combined use of pANCA and ASCA results could be an addition to conventional technique (the patient’s history, radiologic examination, endoscopy and biopsy) in the differential diagnosis between UC and CD.

**CONCLUSIONS:** In summary, the testing for pANCA, ASCA and other antibodies appears to be a promising approach to diagnose inflammatory bowel disease, and to distinguish UC from CD. Serologic evaluation of pANCA and ASCA could be of help in patients with indeterminate colitis. In this patients, early knowledge of the exact diagnosis could be of clinical importance with regard to therapeutic decisions and prognosis. The combination pANCA+/ASCA− predicted UC in 57.1% of IC patients, whereas pANCA−/ASCA+ predicted CD in 42.8% of the patients. The 64.27% of patients did not have antibodies to either ASCA or pANCA and these seronegative patients remained indeterminate.

The combined use of atypical pANCA and ASCA test results substantially affects pretest-posttest probability in distinguishing UC from CD in patients with IBD. This may be of help in patients in whom distinction between CD or UC is not obvious with the classic diagnostic tools.

**References:**

Table 2 - Prevalence of pANCA and ASCA in patients with UC, CD, IC and CG

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