Autoantibodies pANCA, GAB and PAB in inflammatory bowel disease: prevalence, characteristics and diagnostic value

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Summary. Background: The diagnosis of inflammatory bowel disease (IBD), particularly the differentiation between ulcerative colitis (UC) and Crohn’s disease (CD), is difficult and delayed in many cases, despite invasive diagnostic tools.

Aims: To determine the presence and evaluate the diagnostic accuracy of the potential serological diagnostic markers perinuclear antineutrophil cytoplasmic antibodies (pANCA), autoantibodies to intestinal goblet cells (GAB) and autoantibodies to exocrine pancreas (PAB), together with combinations of these, in the diagnosis of IBD and differential diagnosis of UC and CD.

Methods: The presence of pANCA, GAB and PAB was determined in indirect immunofluorescence assay of serum samples from 71 patients with IBD (CD 43, UC 28) and 41 healthy controls. The antigen specificity of ANCA was determined using ELISA.

Results: Compared with the control group, we confirmed a statistically significant presence of pANCA (71.4%) and GAB (46.4%) in patients with UC and the presence of PAB only in patients with CD (30.2%) (P < 0.001). In healthy controls, neither PAB nor GAB was detected and pANCA was present in only 4.8%. The sensitivity, specificity and positive and negative predictive values in differentiation of IBD from healthy controls were as follows: pANCA+: 71%, 95%, 91%, 83%; GAB+: 46%, 100%, 100%, 73%; PAB+: 30%, 100%, 100%, 58%; combination of (pANCA+ or GAB+)/PAB–: 82%, 95%, 93%, 74%; and to distinguish UC from CD: pANCA +: 71%, 98%, 95%, 84%; GAB+: 46%, 98%, 93%, 74%; PAB+: 30%, 100%, 100%, 48%; (pANCA+ or GAB+)/PAB–: 82%, 98%, 96%, 89%.

Conclusions: All three autoantibodies may be helpful tools in non-invasive diagnosis and differential diagnosis of UC and CD. Combination of the autoantibodies may be particularly helpful, as the diagnostic sensitivity is considerably improved.

Key words: Inflammatory bowel disease, autoimmunity, autoantibodies, diagnostic markers, diagnostic value.

Introduction

Inflammatory bowel disease (IBD) comprises a group of chronic gastrointestinal inflammatory diseases of unknown etiology, usually with a lifelong and unpredictable course [1, 2]. These diseases are divided into two main groups according to their phenotypic and genotypic characteristics: ulcerative colitis (UC) and Crohn’s disease (CD). A clear definition and distinction between the two diseases is important because of the significant differences in treatment and prognosis [3]. Defining IBD and distinguishing it from other gastrointestinal diseases, as well as defining its subgroups UC and CD, is usually very difficult because the symptoms and clinical signs are non-specific and commonly overlap [4, 5]. Despite the use of very invasive examinations (endoscopy with biopsy, radiological examination), the percentage of cases that cannot be clearly defined is relatively high at 8–20% [3, 5].

The etiology of IBD is complex and as yet unexplained. Genetic [6] and environmental factors [7] are involved; thus, recently IBD has been connected with disorders of the immune system and autoimmunity [8, 9]. Growing evidence that IBD should be regarded as an autoimmune disease is confirmed by the reactivity of lymphocytes to their own antigens [10] and by autoimmune extra-intestinal manifestations [11], successful immunosuppressive therapy and the presence of a variety of autoantibodies [12–15]. Perinuclear antineutrophil cytoplasmic antibodies (pANCA) have been reported in most patients with UC and in a small subset of patients with CD [16, 17]. Autoantibodies against different colonic antigens have also been found in patients with UC [12, 15, 18–21]; for example, antibodies to intestinal goblet cells (GAB). Stöcker et al. reported that antibodies against exocrine pancreas (PAB) could be found only in the serum of patients with CD [18, 22]. However, data on the specificity and sensitivity of PAB and GAB in IBD are scarce and contradictory.
In our research we focused on the autoantibodies pANCA, GAB and PAB as potential serological markers of IBD, which could also define subgroups of IBD with similar etiology (autoimmunity). We determined the prevalence, specificity according to immunoglobulin classes, and significant concentrations of all three autoantibodies in groups of patients with IBD. We also analyzed the specificity of ANCA against the main known cytoplasmic antigens. In addition, we determined the diagnostic value of individual autoantibodies in the diagnosis of IBD and the differential diagnosis of UC and CD. As a new approach we also defined the diagnostic value of various combinations of these antibodies.

Materials and methods

Serum samples

The presence of ANCA, GAB and PAB was determined in serum samples from 71 patients with IBD. Based on standard criteria, with typical clinical, radiological, endoscopic and histological features [1], the IBD patients were classified as having UC (n = 28) or CD (n = 43). All IBD samples referred by gastroenterologists of the Maribor Teaching Hospital Department of Gastroenterology for routine ANCA testing were collected prospectively between January 2000 and December 2001 (we did not take the results of ANCA testing into account when including samples in the study). Control serum samples were obtained from 41 healthy donors.

All serum samples were stored at –80°C without preservatives until analysis. Samples were also coded so that investigators had no knowledge of patients’ diagnoses or clinical features at the time of serological testing. The study protocol was approved by the National Ethics Committee.

Indirect immunofluorescence assay

Presence of ANCA, GAB and PAB was determined in an indirect immunofluorescence (IIF) assay. We used cryoconserved slides with well defined areas of different tissue sections and differently prepared cells as relevant antigenic substrates: ethanol-fixed granulocytes, formalin-fixed granulocytes, Hep-2 cells, and primate intestinal, pancreatic and liver tissue (Biochip slides; Euroimmun, Lübeck, Germany). This configuration of the IIF test allowed us to simultaneously evaluate the immunofluorescence pattern of all three antibodies for each patient.

Patients’ samples and positive and negative control sera (each 75 μl of 1:10 dilution in phosphate-buffered saline containing 2% bovine serum albumin) were added to antigens on the slides and incubated at room temperature in a humid chamber for 30 min. After washing with the buffer, slides were then incubated with monovalent (IgA or IgG) fluorescein isothiocyanate-conjugated F(ab’)_2, goat anti-human immunoglobulin (Sigma, Munich, Germany) for another 30 minutes. After further washing, bound antibodies were detected by incubating the plates with chromogen/substrate solution (tetramethylbenzidine/H₂O₂) for 15 min in the dark. The enzymatic reaction was stopped by the addition of 0.5 M sulfuric acid and plates were read at 450 nm and reference wavelength 620 nm on an ELISA reader (Sunrise, Tecan GmbH, Austria). Samples were considered positive or negative according to the absorbance value of the specific calibration sera (cut-off extinction) for each antigen: thus, ratio of extinction of serum samples to cut-off extinction <1.0 = negative, ratio ≥ 1.0 = positive.

Statistical analysis

Comparison of the frequency of discrete qualitative variables was analyzed using the z-test. Differences were considered statistically significant when the P value was <0.001.

The diagnostic sensitivity of a test was defined as the probability of a positive result in a patient with the disease under investigation; the diagnostic specificity of a test was defined as the probability of a negative result in a patient without the disease. The positive predictive value (PPV) of a test was defined as the probability that a patient with a positive test result had the given disease; the negative predictive value (NPV) of a test was defined as the probability that a patient with a negative test result did not have the disease [23].