
OBJECTIVE: To evaluate the diagnostic accuracy of perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA), as single agents and in combination, for the diagnosis of Crohn’s disease (CD) and ulcerative colitis (UC), including in cases of indeterminate colitis (IC).

METHODS: The sera from a total of 98 patients were studied: 77 with CD, 16 with UC and five with IC. The medical records of these patients were reviewed for disease diagnosis, demographic data, and patient symptoms and medications. ELISAs were utilized to detect the presence of ASCAs and deoxyribonuclease-sensitive pANCA, and these results were then compared with the patients’ clinical data.

RESULTS: For UC, a positive pANCA test alone provided a sensitivity of 50% and a specificity of 82%. For CD, a positive ASCA test alone provided a sensitivity of 40% and a specificity of 100%. A combination of pANCA-positive and ASCA-negative results showed a sensitivity of 50% and specificity of 90% for the diagnosis of UC. Similarly, the combination of ASCA-positive and pANCA-negative results provided a sensitivity and specificity of 32% and 100% for the diagnosis of CD, respectively. Interestingly, 80% of IC patients showed serology results consistent with UC.

CONCLUSIONS: Although this combination of serological markers provides a diagnostic tool with generally high specificities, the low sensitivities of these serological markers, most notably in terms of CD, preclude the possibility that they can replace the tools currently used for inflammatory bowel disease diagnosis and management. It is possible, however, that these serological markers may prove beneficial in the management of IC.

Key Words: ANCA; Antineutrophil cytoplasmic antibody; Anti-Saccharomyces cerevisiae antibody; ASCA; Crohn’s disease; ELISA; Ulcerative colitis
examination, and a multitude of physiological investigations, including endoscopy, histology of any endoscopically obtained tissue specimens and diagnostic radiography. In the past several years, however, much attention has been paid to the possible clinical significance of serum markers, which may be useful in the diagnosis and management of IBD, most notably perinuclear antineutrophil cytoplasmic autoantibodies (pANCAs) and anti-Saccharomyces cerevisiae antibodies (ASCA).

Since 1990, we have seen the emergence of studies on pANCAs and their association with UC. Most literature reports that between 60% and 80% of adults and children with UC test positive for serum pANCAs (1,2). The pANCA is an autoantibody made by the lamina propria and mesenteric node lymphocytes, and although its exact epitope remains unknown, its antigen is suspected to be located on the inner side of the nuclear periphery and to be deoxyribonuclease-sensitive (2).

Since 1988, ASCAs have also been gaining recognition in terms of their association with CD. An antibody that targets the phosphopeptidomannan part of the cell wall of S cerevisiae (ie, Baker’s yeast), ASCAs have consistently been found in 50% to 80% of CD patients (3).

Because the number of studies evaluating these serological markers is limited, especially those studies that consider these markers’ combined applicability, we evaluated their precision as diagnostic tools for subsets of our IBD patient population. Specifically, we researched the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pANCAs and ASCAs, used alone and in combination, for the diagnosis of both CD and UC.

**PATIENTS AND METHODS**

**Patient population**
A total of 98 patients enrolled in the present study between December 2004 and May 2005. Serum samples were collected from all participants. No specific inclusion or exclusion criteria were used in the identification of prospective participants. After each patient’s serum was tested, all medical records of that patient were reviewed for data regarding the final disease diagnosis, basic demographic data, and patient symptoms and medications. In all cases, disease diagnosis was confirmed using endoscopic and histological criteria.

**ASCA**
The ASCA kits were obtained from Euroimmun AG, Germany. The traditional indirect immunofluorescent assay method, in which slides with biochips of S cerevisiae are incubated with either a 1:100 or 1:1000 dilution of patient serum for 30 min at room temperature, was followed. Different serum dilutions were used to correspond with either immunoglobulin

| TABLE 1 | Test results for determining ulcerative colitis in 93 inflammatory bowel disease patients |
|---------------|---------------------------------|----------------|----------------|---------------|----------------|
| Test result   | Crohn’s disease (n=77), n | Ulcerative colitis (n=16), n | Sensitivity | Specificity | PPV | NPV |
| pANCA+        | 14                             | 8                             | 0.50         | 0.82         | 0.36           | 0.89           |
| pANCA+ and ASCA+ | 8                             | 8                             | 0.50         | 0.90         | 0.50           | 0.90           |

ASCA – Anti-Saccharomyces cerevisiae antibody-negative; PPV – Positive predictive value; pANCA+ – Perinuclear antineutrophil cytoplasmic autoantibody-positive; NPV – Negative predictive value

| TABLE 2 | Test results for determining Crohn’s disease in 93 inflammatory bowel disease patients |
|---------------|---------------------------------|----------------|----------------|---------------|----------------|
| Test result   | Crohn’s disease (n=77), n | Ulcerative colitis (n=16), n | Sensitivity | Specificity | PPV | NPV |
| ASCA+        | 31                             | 0                             | 0.40         | 1.00         | 1.00           | 0.26           |
| pANCA– and ASCA+ | 25                             | 0                             | 0.32         | 1.00         | 1.00           | 0.24           |

ASCA+ – Anti-Saccharomyces cerevisiae antibody-positive; PPV – Positive predictive value

**RESULTS**

Of the 98 patients included in the present study, 77 had CD, 16 had UC and five had indeterminate colitis (IC). The results of the study as they relate to these specific disease groups are summarized in Tables 1 and 2, as well as in Figure 1.

For the UC group, the pANCA test was found to be positive in eight of the 16 known UC patients. Thus, the pANCA test had a sensitivity and specificity of 50% and 82%, respectively. In other words, the pANCA test, when used on its own, had a PPV of 36% and a NPV of 89% for UC.

Of the patients with CD (n=77), 31 were ASCA-positive (+) and 46 were ASCA-negative (--). Of the 31 CD patients who were ASCA+, 13 expressed both IgA and IgG, 16 expressed only IgA and two expressed only IgG. Therefore, when examined on its own, the ASCA test for CD had a sensitivity and specificity of 40% and 100%, respectively, providing a PPV of 100% and a NPV of 26%. Remarkably, none of the UC patients tested positive for ASCA IgA or ASCA IgG.
DISCUSSION

The fact that pANCA and ASCA antibodies are associated with UC and CD, respectively, has been well proven in the literature to date. The question that remains is whether this association is strong enough for these serological markers to be used as primary diagnostic tools for the different subtypes of IBD.

Our results indicate that, for the diagnosis of UC, the pANCA+ and ASCA− combination is more accurate than the pANCA test results alone, because the combination test has a 50% sensitivity and 90% specificity, with a PPV of 50%. These results are similar to those seen in the literature to date, in which ANCA+ titres have been seen in approximately 80% of UC patients (4). In fact, our results are virtually identical to those of Linskens et al (2), who found a sensitivity and specificity for the pANCA+ and ASCA− combination of 51% and 94%, respectively.

In contrast to the more accurate results seen with the combination test for the diagnosis of UC, the CD identification results did not improve significantly with the combination of tests. In fact, for the pANCA+ and ASCA− combination, the sensitivity decreased in comparison to the ASCA+ test, and the specificity and PPV remained unchanged at 100%. Our results contrast with those found in the literature to date, which show serum ASCA expressed in up to 70% of CD patients (1). In fact, the majority of the literature to date gives a sensitivity of 44% to 57% and a PPV between 75% and 92% for the combination test for CD (5).

In general, our results follow the same trends of low sensitivity and high specificity describing these serological markers that have predominated in the literature. In fact, our results are very similar to a recently published meta-analysis that examined the sensitivity and specificity of pANCA and ASCA serology in IBD (6). Where our results deviated from the available literature, however, is in the exceptionally high PPV seen with both the ASCA+ test, as well as with the combination pANCA− and ASCA+ test for CD. However, the PPV of these tests is often increased when a study’s participants are taken exclusively from an IBD program. In these cases, the referring physician’s clinical expertise acts as an effective screening mechanism, and therefore, the subject pool has a reliably high prevalence of IBD (4).

In comparing our results with those in the literature to date, we also need to note that result variability often stems from the use of different cut-off values for a positive titre, differences in the types of assays being used and differences in the types of patients were selected for the study. For example, Zholudev et al (4) found that CD patients with ileal or ileocecal involvement were more likely to have an ASCA+ test. Therefore, a study with a higher number of patients with this CD phenotype is likely to show inflated ASCA+ test results (4).

CONCLUSIONS

The low sensitivity and high specificity pattern seen in our study suggest that there may be limitations to the applicability of pANCA and ASCA for the diagnosis and management of IBD patients. Although our results indicate a statistically more significant PPV for CD than for UC, we suggest that, in both cases, these serological markers may be better used as adjuncts to the clinical workup than as primary tools.
diagnostic tools. Whether these serological markers may be more useful in family or population screening needs to be addressed with prospective studies. Indeed as the study of these serological markers continues, it is possible that they will prove useful in predicting disease patterns rather than definitively identifying disease itself.

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REFERENCES
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