Serological markers to differentiate between ulcerative colitis and Crohn's disease


Abstract

**Aim**—To assess prospectively the value of three serological tests for differentiating between ulcerative colitis and Crohn's disease, used either alone or combined.

**Methods**—Coded serum samples from 63 patients with ulcerative colitis and 67 patients with Crohn's disease were analysed. Detection assays for the presence of perinuclear antineutrophil cytoplasmic antibodies (pANCA), serum agglutinating antibodies to anaerobic coccoid rods, and specific IgG antibodies against a Kd-45/48 immunological crossreactive mycobacterial antigen complex (ImCrAC) were studied. Sensitivity, specificity, pre- and post-test probabilities, likelihood ratios, and predictive values of each of these serological tests were determined.

**Results**—The sensitivity and specificity of the pANCA test for the diagnosis of ulcerative colitis were 61 and 79%, respectively. The serum agglutination test for anaerobic coccoid rods had a sensitivity of 42% and a specificity of 89% for a diagnosis of Crohn's disease. The sensitivity of specific IgG antibodies against Kd-45/48 ImCrAC in diagnosing Crohn's disease was 70% and specificity 60%. Although 100% specificity was achieved by combining all three tests in a small group of patients with Crohn's disease (n = 20), combining two or more tests had no additive clinical value. No correlation was found between the presence of any one of these antibodies and disease activity, duration, or localisation of disease. Surgery or medical treatment did not influence the presence of antibodies or the antibody titre.

**Conclusions**—The value of these tests in the differential diagnosis between ulcerative colitis and Crohn's disease is limited, but the high predictive values and specificities of different tests for both diseases suggest that these tests may be of help in studying disease heterogeneity and in defining different subgroups of patients with different pathogenesis.

(Keywords: Crohn's disease, ulcerative colitis, serological markers.)

Chronic inflammatory bowel diseases (IBD) can be subdivided into ulcerative colitis and Crohn's disease. Several lines of evidence suggest that these are different diseases. A search for serological tests to differentiate between ulcerative colitis and Crohn's disease has been underway since the 1950s. An ideal serological marker should meet the following criteria: the test should be positive in most patients suffering from the disease (high sensitivity) and only be present in that particular disease (high specificity). A marker with these features offers a high positive likelihood ratio for disease and optimal possibilities as a screening assay. The predictive value for disease using such a test would be high and a negative test result would suggest that a patient is not suffering from the disease.

Since 1990, perinuclear antineutrophil cytoplasmic antibodies (pANCA) have been consistently found in patients with ulcerative colitis. Another possible useful test for the diagnosis of ulcerative colitis is based on the detection of autoantibodies against a Kd-40 protein, which appears to be specific for ulcerative colitis and primary sclerosing cholangitis. This test, however, is not widely available.

In Crohn's disease, patients with antibodies to intestinal bacteria and elevated antibody titres against different food antigens have been described. In the early 1980s a Dutch group found that *Eubacterium, Peptostreptococcus* and *Coprococcus cuniculatus* were present in the faeces of patients with Crohn's disease. This observation resulted in the development of a serum agglutination test against these anaerobic coccoid rods. More recently, based on the hypothesis that Crohn's disease might be a mycobacterial disease, a new serological test was developed recognising specific IgG antibodies against a Kd-45/48 immunological crossreactive antigen complex (ImCrAC), isolated from the cell matrix of several strains of mycobacteria, which appeared to be present in a high percentage of serum samples of patients with Crohn's disease.

In this study the value of detecting pANCA in diagnosing ulcerative colitis, and of antibodies to anaerobic rods and ImCrAC in diagnosing Crohn's disease has been evaluated by single or combined use of these tests. The relation between serological tests and clinical and biochemical parameters, such as disease activity, extent of disease, and medical and/or surgical treatment, was studied.
Serological markers in IBD

outpatient clinic of the Gastroenterology Department over a two year period. Classification of disease was based on conventional clinicopathological criteria, according to Leonard-Jones et al. Disease activity in Crohn’s disease was assessed using the Crohn’s disease activity index (CDAI), according to Best et al, and the van Hees activity index. The Sutherland score was used to grade ulcerative colitis. All serum samples were stored at -20°C until analysed. Antibody screening was performed using coded serum samples for ulcerative colitis in laboratories with ample experience in performing these tests.

PANCA INDIRECT IMMUNOFLUORESCENCE ASSAY
The standard pANCA indirect immunofluorescence assay was performed according to the protocol described by First International Workshop on ANCA. Briefly, human peripheral blood neutrophils were either cytospun or smeared on eight-well Nutacon slides and fixed in 96% ethanol (15 minutes at 4°C). Slides were incubated with patient serum (diluted 1 in 16) and stained with rabbit anti-human IgG-fluorescein isothiocyanate (FITC) conjugate (Dakopatts, Glostrup, Denmark). The slides were evaluated by fluorescence microscopy. All serum samples were tested in duplicate and scored by two observers who were unaware of the patient’s diagnosis. Depending on the brightness of the immunofluorescence staining pattern, the reactions were graded as negative (-), weakly positive (+), positive (+ +), and strongly positive (+ + +). Positive versus negative results are reported here as the immunofluorescence staining pattern has not proved to be of value in the clinical setting. Antinuclear antibody positive serum samples were excluded from the study (n = 6).

SERUM AGGLUTINATION TEST TO ANAEROBIC COCCOID RODS
Three strains of Gram positive anaerobic coccoid rods were isolated from the faeces of patients with Crohn’s disease: two Eubacterium conferturn strains and Coprococcus catus; the Peptostreptococcus productus strain was obtained from the Virginia Polytechnic Institute and State University, Anaerobic laboratory, Blacksburg, Virginia, USA. The agglutinating antibodies to coccoid rods are predominantly of the IgG isotype and less frequently IgM. Briefly, two drops of serum and one drop of bacterial suspension were mixed and the results scored after five minutes. Using a nephelometer, titres were determined with doubling dilutions of serum in 0.85% saline. On a scale from 0-00 to 1-00, ≤ 0.80 was considered negative and > 0.80 positive.

ANTIBODIES TO I1MCRAC
This solid phase enzyme linked immunosorbent assay (ELISA) detects human IgG antibodies to a Kd-45/48 mycobacterial antigen doublet. These common antigens were derived from three mycobacterial strains: Mycobacterium avium, M tuberculosis and M paratuberculosis. Serum samples were added to precoated wells containing the Kd-45/48 antigen and then incubated with peroxidase labelled antihuman IgG antibodies. Subsequently, the peroxidase reaction was carried out with hydrogen peroxide and 2,2,4,4 tetramethyl benzidine. Optimal density was measured using a spectrophotometer at 450 nm. On a scale from 0-00 to 2-00, ≤ 0.5 was considered negative and >0.5 positive.

STATISTICS
Sensitivity was defined as the probability of a positive test result in a patient with the disease under investigation. Specificity was defined as the probability of having a negative result in a patient without the disease under investigation. Pretest probability is the probability of disease in the patient to be tested. Pretest odds is the odds of having the disease and can be calculated by dividing the pretest probability by 1 minus the pretest probability.

The likelihood ratio is a measure of the validity of a test, given its sensitivity and specificity. It was calculated as the ratio of the probability of getting a result in patients with the condition of interest to the probability of getting that same result in patients without that condition.

Predictive values were calculated for each of the test results. The predictive value of a test result is the probability of a true test result in the patients with a given test result. The predictive value depends on the pretest probability and can be determined from the formula based on the Bayes theorem of conditional probability.

Post-test probability is the probability of disease in a patient with a given test result. For positive test results, it is the predictive value of a positive test result. For negative test results, it is 1 minus the predictive value of a negative test.

The correlation between the pre- and post-test probabilities and the likelihood ratio was calculated as follows:

\[
\text{Pretest odds} = \frac{\text{pretest probability}}{1 - \text{pretest probability}}
\]

\[
\text{Post-test odds of a test result} = \text{pretest odds} \times \text{likelihood ratio}
\]

\[
\text{post-test probability} = \frac{\text{post-test odds}}{\text{post-test odds} + 1}
\]

Sensitivity, specificity and likelihood ratios are given with 95% confidence intervals (CI). SPSS/PC 4.01 was used for statistical analysis.

RESULTS
The results of testing for pANCA in 130 consecutive, unrelated patients with IBD are shown in table 1. The pretest probability for a
Table 1  Test results for determining ulcerative colitis in IBD in 130 unrelated consecutive patients, 63 (48%) with ulcerative colitis and 67 (52%) with Crohn's disease

<table>
<thead>
<tr>
<th>Test</th>
<th>Ulcerative colitis positive</th>
<th>Crohn's disease positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
<th>Likelihood ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pANCA+</td>
<td>38</td>
<td>14</td>
<td>61</td>
<td>79</td>
<td>73</td>
<td>69</td>
</tr>
<tr>
<td>pANCA+ or ImCrAC−</td>
<td>51</td>
<td>28</td>
<td>84</td>
<td>58</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>pANCA+ and ImCrAC−</td>
<td>22</td>
<td>6</td>
<td>36</td>
<td>91</td>
<td>79</td>
<td>66</td>
</tr>
<tr>
<td>pANCA+ or Aggl−</td>
<td>58</td>
<td>42</td>
<td>95</td>
<td>37</td>
<td>58</td>
<td>89</td>
</tr>
<tr>
<td>pANCA+ and Aggl−</td>
<td>11</td>
<td>44</td>
<td>54</td>
<td>82</td>
<td>74</td>
<td>56</td>
</tr>
<tr>
<td>pANCA+ and ImCrAC−/Aggl−</td>
<td>33</td>
<td>12</td>
<td>54</td>
<td>82</td>
<td>74</td>
<td>56</td>
</tr>
<tr>
<td>pANCA+ and ImCrAC−/Aggl−</td>
<td>16</td>
<td>5</td>
<td>26</td>
<td>93</td>
<td>76</td>
<td>55</td>
</tr>
</tbody>
</table>

Aggl− = absence of serum agglutinating antibodies to anaerobic coccoid rods; * corrected for convergence where necessary.

Table 2  Test results for determining Crohn's disease in IBD in 130 unrelated consecutive patients, 63 (48%) with ulcerative colitis and 67 (52%) with Crohn's disease

<table>
<thead>
<tr>
<th>Test</th>
<th>Ulcerative colitis positive</th>
<th>Crohn's disease positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
<th>Likelihood ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggl−</td>
<td>7</td>
<td>28</td>
<td>42</td>
<td>89</td>
<td>80</td>
<td>58</td>
</tr>
<tr>
<td>ImCrAC+</td>
<td>25</td>
<td>47</td>
<td>70</td>
<td>60</td>
<td>65</td>
<td>64</td>
</tr>
<tr>
<td>ANCA− and Aggl−</td>
<td>3</td>
<td>25</td>
<td>37</td>
<td>95</td>
<td>89</td>
<td>58</td>
</tr>
<tr>
<td>ANCA− and ImCrAC+</td>
<td>10</td>
<td>39</td>
<td>58</td>
<td>84</td>
<td>80</td>
<td>65</td>
</tr>
<tr>
<td>ANCA− or ImCrAC+/Aggl−</td>
<td>7</td>
<td>45</td>
<td>67</td>
<td>89</td>
<td>87</td>
<td>71</td>
</tr>
<tr>
<td>ANCA− and ImCrAC−/Aggl+</td>
<td>0</td>
<td>20</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>57</td>
</tr>
</tbody>
</table>

Aggl+ = presence of serum agglutinating antibodies to anaerobic coccoid rods, *corrected for convergence where necessary.

diagnosis of ulcerative colitis in our study group was 48%. Thirty eight of 63 patients with ulcerative colitis and 14 of 67 with Crohn's disease were positive for pANCA. The sensitivity and specificity of the pANCA test were 61% (95% CI 48.2-72.4) and 79% (95% CI 69.4-88.8), respectively, with a likelihood ratio of 2.9 (95% CI 1.75-4.8) for a positive test result. The post-test probability for a diagnosis of ulcerative colitis after the testing for pANCA was 73%. The figure shows the post-test probability as a function of the pretest probability.

The results of the agglutination test in 130 patients with IBD are shown in table 2. The pretest probability for a diagnosis of Crohn's disease in our study group was 52%. Twenty eight patients with Crohn's disease and seven with ulcerative colitis had a positive agglutination test, with a sensitivity of 42% (95% CI 30-53.6) and a specificity of 89% (95% CI 81.1-96.6), which corresponds to a positive likelihood ratio of 3.6 (95% CI 1.77-7.7). The post-test probability curve for a diagnosis of Crohn's disease after the agglutination test was 80% (figure).

The results of testing for ImCrAC in 130 patients with IBD are shown in table 2. Forty seven patients with Crohn's disease and 25 with ulcerative colitis had a positive result, giving a sensitivity of 70% (95% CI 59.2-81.1), a specificity of 60% (95% CI 48.2-72.4), and a positive likelihood ratio of 1.7 (95% CI 1.2-2.4). The post-test probability for a diagnosis of Crohn's disease after testing for ImCrAC was 65% (figure).

The agglutination test for Crohn's disease and the pANCA test for ulcerative colitis showed the highest predictive values and likelihood ratios. In ulcerative colitis, starting with a positive pANCA result, the combination of all tests had a lower likelihood ratio than when just the pANCA and ImCrAC sets were combined (table 1). A small subgroup of patients with Crohn's disease (n = 20) could be defined with 100% specificity when both the serum agglutination and ImCrAC tests were positive and the pANCA test negative (table 2).

Discussion

In the present study, we assessed the value of three serological tests for differentiating between ulcerative colitis and Crohn's disease. At present, it appears that only the pANCA test may be of diagnostic value with reproducible results.5-24,25

Only 61% of serum samples from patients with ulcerative colitis were positive for the presence of pANCA (table 1); this was in contrast to the 79% pANCA positivity demonstrated previously in a much larger study by our group.4 However, the results of the present study are still comparable to the incidence figures obtained in western Europe, as analysed recently.26 To date, no major progress in the localisation of the pANCA antigen has been made;27,28 it is therefore almost impossible to characterise pANCA positive or negative patients more accurately. The observed differences in pANCA prevalence in this and in previous studies in our region may be attributed to the inclusion of different patient subpopulations (see later).
Previous studies using agglutination tests demonstrated positivity in 58% of serum samples from patients with Crohn's disease and significantly less in control serum samples (healthy subjects and patients with diarrhoea). Sensitivity for Crohn's disease in the present study was 42%; therefore, the test seems to have little value in the routine clinical assessment of patients with Crohn's disease, but should be reserved for studies, in which a more accurate definition of subgroups is needed.

In a previous study ImCrAC antibodies were found in 70% of patients with Crohn's disease, whereas in our sera population, 48% of patients with ulcerative colitis also had a positive response. The low specificity makes this test of limited value as a screening assay (table 2).

After combining the three serological tests, sensitivities dropped and, therefore, combinations appear to have no clinical value (tables 1 and 2).

No correlation was found between the presence of any antibody and disease activity, duration and localisation of disease, previous surgery, or use of corticosteroids (results not shown).

Is the presence of serum antibodies in 40–50% of patients with IBD merely due to the process of inflammation, or do they play a causative role in pathogenesis? Different findings may be due to various aetiological causes or may be the result of genetic predisposition as suggested by familial and epidemiological studies. The finding of pANCA in healthy relatives of patients with ulcerative colitis, the prevalence of pANCA after total colectomy and the lack of correlation with disease activity showed that at least these antibodies are not simply an epiphenomenon of inflammation.

Different autoantibodies in subgroups of patients may be explained by the heterogeneity of both Crohn's disease and ulcerative colitis, in which a positive pANCA test in ulcerative colitis may indicate a different disease compared with the pANCA negative subgroup. Indirect evidence in favour for this heterogeneity came from a genetic study. Susceptibility to ulcerative colitis seems to be influenced by the major histocompatibility complex, contained in over 100 genes on chromosome 6. Associations between a positive pANCA result and HLA-DR2, and between a negative pANCA test result and HLA-DR4 have been found.

In conclusion, the present study has clearly shown that use of the available serological tests, either singly or combined, does not aid diagnosis. However, a positive pANCA response is of value in establishing a diagnosis of ulcerative colitis. The hypothesis that the above antibodies are markers of subgroups of IBD is highly attractive and should be pursued vigorously. The elucidation of the influence of genetic factors on the immune system, and the definition of patient subgroups by serological and genetic markers will be a major diagnostic research project, eventually resulting in a better insight into the behaviour of IBD in the respective disease subgroups.

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