Table 1 MPO-ANCA and PR3-ANCA ELISA, ANCA Combi-kit® ELISA, and ANCA IIF results

<table>
<thead>
<tr>
<th>MPO-ANCA and PR3-ANCA IgG ELISA (U/mL)</th>
<th>ANCA Combi-kit IgG ELISA (OD ratio)</th>
<th>MPO</th>
<th>PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO positive (58)</td>
<td>MPO (6.4)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td>MPO (2.9)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>MPO positive (8)</td>
<td>MPO (1.6)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td>MPO (1.3)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>MPO positive (6)</td>
<td>MPO (9.1)</td>
<td>P (1/640)</td>
<td>P (1/640)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td>Lysozyme (1.7)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>MPO positive (36)</td>
<td>MPO (3.2)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td>Lactoferrin (1.1)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO positive (53)</td>
<td>PR3 (6.44)</td>
<td>C (1/40)</td>
<td>C (1/40)</td>
</tr>
</tbody>
</table>

IIF staining pattern (tire) on ethanol fixed neutrophils using polyclonal FITC conjugated antihuman IgG

1 MPO positive, 2 PR3 negative, 3 MPO positive, 4 PR3 negative, 5 MPO positive (>100), 6 MPO positive, 7 PR3 negative.

Correspondence

MPO-ANCA may produce a combination of P-ANCA and atypical cytoplasmic ANCA indirect immunofluorescent patterns on certain ethanol fixed neutrophil substrates

The P-ANCA pattern is defined as perinuclear indirect immunofluorescent (IF) staining on ethanol fixed normal human neutrophils. This pattern is an artefact of ethanol fixation, dependent on the redistribution of certain cationic neutrophil granule proteins (such as myeloperoxidase (MPO), lactoferrin, and lysozyme) around the negatively charged nuclear membrane. 

However, certain MPO-ANCA can produce cytoplasmic rather than perinuclear IIF staining, possibly related to a subpopulation of epitopes on MPO that do not redistribute with ethanol fixation.

We now report that MPO-ANCA positive sera may produce a combination of P-ANCA and atypical cytoplasmic ANCA IIF patterns on certain ethanol fixed neutrophil substrates, potentially leading to interpretive and diagnostic difficulties.

Sera from six patients with biopsy confirmed microscopic polyangiitis (at different stages of disease activity) were present, all sera were tested on the ORGenTec ANCA Combi-kit ELISA containing proteinase-3, MPO, lactoferrin, elastase, cathepsin G, lysozyme, and bactericidal/permeability increasing protein (BPI). IIF was then repeated on all sera on two separate occasions using in house (kindly supplied by the Division of Immunology, Royal Brisbane Hospital) and two commercial (Inova Diagnostics (different batch) and Medical and Biological Laboratories (MBL, Nagoya, Japan)) ethanol fixed neutrophil slides.

The IIF staining patterns and end point titres were determined by consensus. Table 1 summarised the results. In four of the six sera, no reactivity other than MPO-ANCA was detected using the ANCA Combi-kit ELISA. Of the other two sera, one also contained lactoferrin-ANCA and the other lysozyme-ANCA. Nevertheless, in addition to P-ANCA staining, atypical cytoplasmic staining was consistently produced by all six MPO-ANCA sera on the Inova slides, but not on the MBL or in house slides. These findings were reproducible on two different batches of neutrophil slides from the former manufacturer.

Our small study demonstrates that sera containing MPO-ANCA may produce a combination of P-ANCA and atypical cytoplasmic IIF patterns on certain ethanol fixed neutrophil substrates. The recent International Consensus Statement recommends that such combined patterns be reported as “atypical ANCA”. Because atypical ANCA are not strongly associated with microscopic polyangiitis or Wegener’s granulomatosis, an atypical ANCA IIF report on these sera could potentially erroneously lead the requesting clinician away from the correct diagnosis.

We have subsequently found that these combined IIF patterns do not occur with all MPO-ANCA positive sera on the Inova slides, and thus speculate that the phenomenon might be caused by factors in the ethanol fixation conditions of these slides resulting in the differential redistribution of different MPO epitopes. Therefore, we recommend that laboratories using this brand (and possibly other commercial brands) of ethanol fixed neutrophil slides be aware of this phenomenon, and consider repeating any sera producing such combined “atypical ANCA” IIF patterns on alternative ethanol fixed neutrophil substrates to clarify their “true” IIF pattern.

Furthermore, antigen specific ELISA testing for MPO-ANCA and PR3-ANCA should also be performed on all such sera because combining IIF and ELISA in ANCA testing improves overall diagnostic specificity/predictive value compared with using either test alone.


High prevalence of serum markers of coeliac disease in patients with chronic fatigue syndrome

There has been recent interest in the possibility that undiagnosed coeliac disease (CD) might be the cause of diverse clinical symptoms, most particularly “tired all the time”. A recent study reported a prevalence of three in 100 cases in a primary care environment in which samples were taken from patients with a range of symptoms and signs. The second most frequent symptom reported by the endomyosal antibody (EMA) positive patients was “being tired all the time”. We decided to examine the prevalence of EMA in patients attending our tertiary referral centre with the diagnosis of chronic fatigue syndrome (CFS).

We tested serum from 100 consecutive patients (47 men, 53 women; median age, 40 years; range, 18–57) referred to our specialist clinic and satisfying the standard CDC criteria for a diagnosis of CFS, and from 100 healthy control subjects (45 men, 55 women; median age, 40 years; range, 18–68) who were blood donors at the South East Thames Blood Transfusion Service. The CFS samples had been stored as part of other studies, and were analysed retrospectively. EMA of the IgA class were detected by indirect immunofluorescence (IF) using cryostat sections of distal primate oesophagus as substrate (Binding Site, Birmingham, UK). Positive samples were confirmed using an enzyme linked immunosorbent assay (ELISA) for the detection of antitissue transglutaminase antibodies (Menarini Diagnostics, Wokingham, England).
of CD being misdiagnosed as CFS.

Only been two reports concerning three cases illnesses is very unusual. Until now there have investigations, and history are unremarkable, particular, if basic physical examination, review that a higher index of suspicion is needed with non-specific symptoms and a suggestion primary care of a surprisingly high frequency diagnosed as CFS (see Wessely jejunal biopsy after the retrospective identifi- cases, CD was subsequently confirmed on patient was symptom free at the end of treat- ard treatment for CFS. In both cases, CBT cognitive behaviour therapy (CBT), a stand- currently euthyroid. Before the diagnosis of CD taking long term thyroxine, and were cur- Both had histories of hypothyroidism, were 2.1 g/litre (0.98). Neither of the positive cases, both women aged 27 and 54, had reported symptoms typical of CD, although one had a history of constipation. Routine blood tests including serum proteins and full blood count were normal, and both had been seen by consultant physicians before referral. Both had histories of hypothyroidism, were taking long term thyroxine, and were cur- rently euthyroid. Before the diagnosis of CD was made retrospectively, both had received cognitive behaviour therapy (CBT), a stan- dard treatment for CFS. In both cases, CBT led to a substantial improvement in the quality of life and physical activity, and neither patient was symptom free at the end of treat- ment or at six months follow up. In both cases, CD was subsequently confirmed on jejunal biopsy after the retrospective identifi- cation. In general, it remains true that although a wide range of physical illnesses can be misdi- agnosed as CFS (see Wessely et al for review), in practice this is uncommon. In particular, if basic physical examination, investigations in suspected cases of CFS are unremarkable, misdiagnosis of CFS and other physical illnesses is very unusual. Until now there have only been two reports concerning three cases of CD being misdiagnosed as CFS.

However, there is now evidence from primary care of a surprisingly high frequency of unsuspected positive EMA tests in people with non-specific symptoms and a suggestion that a higher index of suspicion is needed when assessing such patients. We now extend that observation to our CFS clinic. Indeed, given our prevalence of 2%, and the fact that there is a treatment for CD, we now suggest that screening for CD should be added to the relatively short list of mandatory investigations in suspected cases of CFS.

Correction


C Visser (Department of Cardiology, Free University Hospital, 1007 MB Amsterdam, The Netherlands) was mistakenly omitted from the list of authors of this paper. The journal apologises for any inconvenience that this may have caused.

Diagnostic Histopathology of breast Disease

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BSCC London Spring Tutorial: Lung and Pleural Cavity Fluid Cytology

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Professional Standards of Pathologists in a Modern NHS Pathology Service

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Infectious Hazards of Donated Organs

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Recent Advances in Genetics

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