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 (IIF) 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.\(^1\) However, certain MPO-ANCA can produce cytoplasmic rather than perinuclear IIF staining,\(^1\) possibly related to a subpopulation of epitopes on MPO that do not redistribute with ethanol fixation.\(^3\) We report that MPO-ANCA positive sera may produce a combination of P-ANCA and atypical cytoplasmic\(^1\) ANCA IIF patterns on certain ethanol fixed neutrophil substrates, potentially leading to interpretative and diagnostic difficulties.

Sera from six patients with biopsy confirmed microscopic polyangiitis (at different stages of disease activity) were selected because of initial difficulties in the interpretation of their IIF patterns on ethanol fixed neutrophil slides from Inova Diagnostics (San Diego, California, USA). All six sera were MPO-ANCA positive and proteinase 3-ANCA (PR3-ANCA) negative by the corresponding ORGenTec (Mainz, Germany) enzyme linked immunosorbent assay (ELISA). PR3-ANCA positive serum from a patient with biopsy confirmed Wegener’s granulomatosis was also tested. To establish whether other ANCA antigen specificities were present, all sera were tested on the ORGenTec ANCA Combi-kit\(^\text{®}\) 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 tites 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”.\(^4\) Because atypical ANCA are not strongly associated with microscopic polyangiitis or Wegener’s granulomatosis,\(^5\) an atypical ANCA IIF report on these sera could potentially erroneously lead the requesting clinician away from the correct diagnosis. However, in all six sera, the positive MPO-ANCA ELISA result would hopefully redirect attention towards a possible diagnosis of systemic necrotising vasculitis.

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 of assay take note 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.\(^6\)


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.\(^1\) 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, UK) (and possibly other commercial brands) of ethanol fixed neutrophil slides to 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.\(^1\)

**Table 1** MPO-ANCA and PR3-ANCA ELISA, ANCA Combi-kit\(^\text{®}\) ELISA, and ANCA IIF results

<table>
<thead>
<tr>
<th>Sera</th>
<th>ELISA (U/mL)</th>
<th>ANCA Combi-kit IgG ELISA (OD ratio)</th>
<th>Inova Diagnostics</th>
<th>MBL</th>
<th>In house cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO-ANCA and PR3-ANCA IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 MPO positive (58)</td>
<td>MPO (6.4)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/160)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 MPO positive (61)</td>
<td>MPO (2.9)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/160)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 MPO positive (8)</td>
<td>MPO (1.6)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 MPO positive (6)</td>
<td>MPO (1.3)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/160)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 MPO positive (&gt;100)</td>
<td>MPO (9.1)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/160)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 MPO positive (36)</td>
<td>MPO (3.2)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>PR3 negative</td>
<td></td>
<td>P (1/160)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 MPO negative</td>
<td>PR3 (6.44)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
</tbody>
</table>

ORGenTec MPO-ANCA and PR3-ANCA IgG ELISA: positive, >5 U/mL; negative, <5 U/mL. ORGenTec ANCA Combi-kit IgG ELISA OD ratio: positive, >1; negative, <1 (only positive results shown). IIF staining pattern: C, classic granular cytoplasmic IIF staining with central/interlobular accentuation. P, perinuclear.

Sera 1-6 were from patients with biopsy confirmed microscopic polyangiitis. Serum 7 was from a patient with biopsy confirmed Wegener’s granulomatosis.

ANCA, antineutrophil cytoplasmic antibody; ELISA, enzyme linked immunosorbent assay; FITC, fluorescein isothiocyanate; IF, indirect immunofluorescence; MPO, myeloperoxidase; OD, optical density; PR3, proteinase 3.
tissue transglutaminase being the auto-
antigen responsible for the IF pattern of
EMA. To exclude selective IgA deficiency,
serum IgA concentrations were measured by
laser nephelometry using specific antisera
according to the manufacturer’s instructions
(Behring Laser nephelometer III, Dade Be-
erring, Dortmund, Germany).

Two of the 100 CFS samples were positive for
EMA using IF, and this was confirmed by
ELISA, but none of the 100 control samples
was positive. None of the subjects had select-
ive IgA deficiency. Mean (SD) serum IgA
concentrations among patients with CFS
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
hospitalization. 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 stand-
tard treatment for CFS. In both cases, CBT
led to a substantial improvement in the qual-
ity of life and physical activity, but 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-
gnosed as CFS (see Wesley et al for
review), in practice this is uncommon. In
particular, if basic physical examination,
imaging, and history 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.

Diagnostic Histopathology of Breast Disease
23–27 April 2001, Hammersmith Hospital
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Gynecologic and Obstetric Pathology
26–29 April 2001, Fairmont Copley Plaza,
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BSCC London Spring Tutorial: Lung
and Pleuric Fluid Cytology
27 April 2001, Guy’s Hospital, London,
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International Consultation on the
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11–12 May 2001, University of Ancona
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Professional Standards of Pathologists
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Recent Advances in Genetics
5 July 2001, Royal College of Pathologists,
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