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 IF staining, possibly related to a subpopulation of epitopes on MPO that do not redistribute with ethanol fixation. We report that MPO-ANCA positive sera may produce a combination of P-ANCA and atypical cytoplasmic ANCA IF 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 IF 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 commercially available ORGenTec MPO-ANCA and PR3-ANCA IgG ELISA. MPO-ANCA positive sera may produce a combination of P-ANCA and cytoplasmic ANCA IF patterns on certain ethanol fixed neutrophil substrates. The recent International Consensus Statement recommends that such combined patterns be reported as ‘atypical ANCA’.

We have subsequently found that these combined IF 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’ IF patterns on alternative ethanol fixed neutrophil substrates to clarify their ‘true’ IF pattern. Furthermore, antigen specific ELISA testing for MPO-ANCA and PR3-ANCA should also be performed on all such sera because combining IF 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, 18–78) 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 immunosorbant assay (ELISA) for the detection of antitrace transfugalmische antibodies (Menarini Diagnostics, Wokingham, England).

Table 1 MPO-ANCA and PR3-ANCA ELISA, ANCA Combi-kit® ELISA, and ANCA IF 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 cytopsin</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>
<td>P (1/160)</td>
<td>P (1/160)</td>
</tr>
<tr>
<td>MPO negative (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>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>MPO negative (6)</td>
<td>MPO (1.3)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO positive (40)</td>
<td>MPO (0.9)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO negative (36)</td>
<td>MPO (5.2)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO negative (31)</td>
<td>Lysozyme (1.7)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO negative (36)</td>
<td>Lactoferrin (1.1)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
<td>P (1/40)</td>
</tr>
<tr>
<td>MPO negative (53)</td>
<td>PR3 (6.44)</td>
<td>C (1/40)</td>
<td>C (1/40)</td>
<td>C (1/40)</td>
<td>C (1/40)</td>
</tr>
</tbody>
</table>

ORGenTec MPO-ANCA and PR3-ANCA IgG ELISA: positive, >5 U/ml; negative, <5 U/ml.

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only been two reports concerning three cases of misdiagnosis of CFS and other physical investigations, and history are unremarkable, particularly if basic physical examination, review of systems, and review of investigations in suspected cases of CFS. 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 II, Dade Behring, 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 selective 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 referral. Both had histories of hypothyroidism, were taking long term thyroxine, and were currently euthyroid. Before the diagnosis of CD was made retrospectively, both had received cognitive behaviour therapy (CBT), a standard treatment for CFS. In both cases, CBT led to a substantial improvement in quality of life and physical activity, but neither patient was symptom free at the end of treatment or at six months follow up. In both cases, CD was subsequently confirmed on jejunal biopsy after the retrospective identification of undiagnosed celiac disease with atypical features using antiretulcin and antigliadin antibodies.

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.

Calendar of events

Diagnostic Histopathology of breast disease
23–27 April 2001, Hammersmith Hospital (Imperial School of Medicine), London, UK
Further details: Wolfson Conference Centre, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK. (Tel +44 020 8383 3117/3227/3245; fax +44 020 8383 2428; email wcc@ic.ac.uk)

Gynecologic and Obstetric Pathology
26–29 April 2001, Fairmont Copley Plaza, Boston, Massachusetts, USA
Further details: Department of Continuing Education, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA. (Tel +1 617 432 1525; fax +1 617 432 1562; email hms-cme@hms.harvard.edu)

BSCC London Spring Tutorial: Lung and Pleural Cavity Fluid Cytology
27 April 2001, Guy’s Hospital, London, UK
Further details: BSCC Office, PO Box 352, Uxbridge UB10 9TX, UK. (Tel +44 01895 274 020; fax +44 01895 274 080; email lesley.couch@psilink.co.uk)

International Consultation on the Diagnosis of Noninvasive Urothelial Neoplasms
11–12 May 2001, University of Ancona School of Medicine, Torrette, Ancona, Italy
Further details: R Montironi, Ancona Italy (email r.montironi@popsi.unian.it), DG Bostwick, Richmond, VA, USA (email bostwick@bostwicklaboratories.com), P-F Bassi, Padua, Italy (email bassip@ux1.unipd.it), M Droller, New York, USA (email michael_droller@smtpink.msm.edu), or D Waters, Seattle, WA, USA (email waters@vet.vet.purdue.edu)

Professional Standards of Pathologists in a Modern NHS Pathology Service
7 June 2001, Royal College of Pathologists, London, UK
Further details: Michelle Casey, Academic Activities Coordinator, 2 Carlton House Terrace, London SW1Y 5AF, UK. (Tel +44 020 7451 6700; fax +44 020 7451 6701; www.rcpath.org)

Infectious Hazards of Donated Organs
28 June 2001, Royal College of Pathologists, London, UK
Further details: Michelle Casey, Academic Activities Coordinator, 2 Carlton House Terrace, London SW1Y 5AF, UK. (Tel +44 020 7451 6700; fax +44 020 7451 6701; www.rcpath.org)

Recent Advances in Genetics
5 July 2001, Royal College of Pathologists, London, UK
Further details: Michelle Casey, Academic Activities Coordinator, 2 Carlton House Terrace, London SW1Y 5AF, UK. (Tel +44 020 7451 6700; fax +44 020 7451 6701; www.rcpath.org)

BSCC Annual Scientific Meeting
9–11 September 2001, Majestic Hotel, Harrogate, UK
Further details: BSCC Office, PO Box 352, Uxbridge UB10 9TX, UK. (Tel +44 01895 274 020; fax +44 01895 274 080; email lesley.couch@psilink.co.uk)