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 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® 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 ANCA 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. 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 (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.

R C W WONG
K FIELD
Division of Immunology, Queensland Health Pathology Services, Level 3, Lions Pathology Building, Alexandra Hospital, Woolloongabba 4102, Queensland, Australia
richard.wong@health.qld.gov.au


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 antitransglutaminase antibody bodies (Menarini Diagnostics, Wokingham, England).
UK), tissue transglutaminase being the auto-

tigen 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 II, Dade Be-

hring, 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 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 stand-

ard 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-

agnosed as CFS (see Wessely et al for review), 

in practice this is uncommon. In particular, if 

basic physical examination, investigation, and 

history are unremarkable, misdiagnosis of CFS 

and other physical illnesses is very unusual. Until now there have been 

very few incidents of CD being misdiagnosed as CFS.

Our findings are in keeping with the 

recent studies of goes et al, and 100 CFS 

samples were positive using IF, and this was 

confirmed by ELISA. It is interesting to note 

that both women had a history of constipation. 

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and other physical illnesses is very unusual. Until now there have been 

very few incidents 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

Niessen HWM, Lagrand WK, Rensink 

HJAM, et al. Apolipoprotein H, a new 

mediator in the inflammatory changes ensuing 

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 lesley.couch@psilink.co.uk)

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@popcsi.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.mssm.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 Patholo-

gists, 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 Patholo-

gists, 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 274020; fax +44 01895 274080; 

email lesley.couch@psilink.co.uk)