LETTERS TO THE EDITOR

Refrigeration preserves synovial fluid cytology

Sir: We recently published an algorithm based on combinations of synovial fluid (SF) cell number thresholds and types which should considerably extend the diagnostic use of SF cytology in non-septic/crystal arthritis.[1] Key branch points depend on accurate values for total and differential nucleated leucocyte counts (white cell count (WCC)), ragocytes, and Reiter's cells (cytophagocytic mononuclear cells (CPMs))[2]; and the recognition of specific cell types—for example, LE cells, tart cells. The receipt and analysis of clinical SF specimens is often delayed (60% of SFs in our laboratory are processed the same day as taken, 22% after overnight fridge storage, others arriving one or more days late). Schumacher's group reported large falls in WCC in SF kept at room temperature for a few hours, though other cytological changes were not described.[3]

We investigated the effects of fridge (4°C) storage over one to three days on these key cytological indices, and the accuracy of algorithm derived diagnoses in 51 knee aspirates chosen randomly from routine diagnostic specimens, satisfying the following: (*a*) receipt within four hours of arthrocentesis ('fresh'); (*b*) possessing sufficient cells (>0·2×10⁹/l) and volume (>1·5 ml), lacking bloodstaining or clots. There were 48 'inflammatory' (25 rheumatoid, seven spondyloarthritic, eight Reiter's/reactive, and eight miscellaneous, including crystal and septic) and three osteoarthritic SFs.

Fluids were examined 'fresh' and then refrigerated without dilution in the original 2 ml Li-heparin bottle. Aliquots (0·25 ml) were taken daily and processed for (*a*) wet preparation (ragocyte count and crystals); (*b*) total WCC by haemocytometer; (*c*) cytocentrifugation and Jenner-Giemsa staining for cytology. A differential WCC (percentage polymorphonuclear leucocytes, small lymphocytes, monocytes), CPM count (as percentage monocytes), and the presence or absence of eosinophils, mast, plasma, and inclusion body cells were noted as described.[1] Fluids were examined blind to clinical details, and serial assessments were carried out by the same investigators.

In the 48 inflammatory SFs (table 1) the total WCC fell by 45% over three days, owing to falling polymorphonuclear leucocyte numbers; this only became significant after 48 hours. Within 17 CPM forming fluids, numbers of these gradually fell, though CPM status (present or absent) did not change with time. Ragocyte numbers, though quite variable case by case, remained remarkably static in individual fluids. Similar results were obtained if fluids were stratified by initial WCC (fresh fluid WCC <10×10⁹/l<WCC). No fluid with an initial 'inflammatory' WCC (≥1·5×10⁹/l) fell into the non-inflammatory range during storage (or vice versa).

Inclusion body (LE, tart, and Dohle) and lymphoid variant (Mott, plasma, and Rieder) cells typical of rheumatoid arthritis[1] were only reliably present in fresh and one day stored SFs, and did not appear as storage artefacts. Eosinophils persisted for several days, as did CPMs typical of reactive and spondyloarthropathies.[2] A specific correct, or matching short differential diagnosis was derived in 52% of fresh fluids (58% if three crystal cases were included), falling only to 46% (52%) after two days (table 2). A further 42–46% were labelled as non-diagnostic inflammatory fluids, and only three incorrect diagnoses were made. Derived diagnoses did not change in 90% of fluids over 48 hours; four became non-diagnostic and only one incorrect. Cytological deterioration of the specimens and artefactual increases in pyknotic and 'ghost' cell numbers interfering with leucocyte counting only became a significant problem after two to three days.

These results suggest that SF can be stored (and transported) under refrigerated conditions for 24 hours without significant changes in cytological indices, and for 48 hours with only minimal loss of diagnostic accuracy. This should allow wider access to regional SF cytoanalytical services, and implies that SF specimens should be promptly refrigerated if any transport or analytical delays are expected.

S T M JONES
J DENTON
P J L HOLT
A J FREEMONT
Departments of Rheumatology and Pathology
Manchester Royal Infirmary and
Manchester Medical School
Oxford Road
Manchester M13 9WL
United Kingdom

1 Freemont A J, Denton J, Chuck A, Holt P J L, Davies M. Diagnostic value of synovial fluid microscopy: a reassessment and rationalisation. *Ann Rheum Dis* 1991; **50:** 101–7.
2 Freemont A J, Denton J. Disease distribution of synovial fluid mast cells and cytophagocytic mononuclear cells ('CPM') in inflammatory arrthritis. *Ann Rheum Dis* 1985; **44:** 312–5.
3 Kerolus G, Clayburne G, Schumacher H R. Is it mandatory to examine synovial fluid promptly after arthrocentesis? *Arthritis Rheum* 1989; **32:** 271–8.

Table 1 Changes in inflammatory synovial fluid leucocyte indices during fridge storage at 4°C. Results are given as mean (SEM)

Synovial fluid age (days)	*n*	*White cell count (×10⁹/l)*	*Polymorphonuclear leucocytes (×10⁹/l)*	*Lymphocytes (×10⁹/l)*	*Monocytes (×10⁹/l)*	*CPMs‡ (% monocytes)§*	*Ragocytes (%)*
Fresh	48	16·2 (2·1)	13·0 (1·9)	1·6 (0·2)	1·6 (0·4)	8·7 (2·8)	29·8 (7·9)
1	48	14·4 (1·9)	10·9 (1·7)	1·5 (0·2)	1·9 (0·4)	7·2 (2·4)	34·4 (7·2)
2	35	10·6 (1·7)*	7·2 (1·5)*	1·2 (0·2)	2·2 (0·7)	4·4 (1·4)	28·7 (7·4)
3	30	9·0 (1·7)†	5·5 (1·5)†	1·0 (0·2)*	2·5 (0·9)	4·5 (1·6)	29·3 (8·8)

*p<0·05; †p<0·01, both compared with fresh synovial fluid. Other results were not significant.
‡CPMs=cytophagocytic mononuclear cells.
§Seventeen synovial fluid samples containing CPMs were studied.

Table 2 Effect of synovial fluid storage on algorithm derived diagnoses. Results are given as number (percentage)

Diagnosis	*Fresh (n=48)*	*1 Day old (n=48)*	*2 Days old (n=39)*
Correct	25 (52)	23 (48)	18 (46)
'Inflammatory'	20 (42)	22 (46)	18 (46)
Wrong	3 (6)*	3 (6)	3 (8)

*Two Reiter's, one reactive arthritis diagnosed as rheumatoid.

Antineutrophil cytoplasmic antibodies in polychondritis

Sir: Two major types of antineutrophil cytoplasmic antibodies (ANCA) have been recognised by indirect immunofluorescence. C-ANCA are defined by a diffuse granular neutrophilic staining pattern and recognise a 29 kilodalton serine protease (proteinase 3); these antibodies seem to be markers for Wegener's granulomatosis.[1] P-ANCA are defined by a perinuclear neutrophilic pattern and mainly recognise myeloperoxidase. Antinuclear antibodies must be recognised because they can be confused with P-ANCA. P-ANCA are seen in serum of patients with various vasculitides or in rapidly progressive glomerulonephritis.[2]

Relapsing polychondritis seemed an interesting disease to investigate for the presence of ANCA as it is sometimes associated with vasculitis, glomerulonephritis, and several connective tissue diseases. Moreover, besides crescentic glomerulonephritis, many clinical features can be shared by both relapsing polychondritis and Wegener's granulomatosis—for example, acquired saddle nose deformity, laryngotracheal disease, episcleritis, and even auricular chondritis. Specks *et al* reported P-ANCA positivity in eight of 22 patients with relapsing polychondritis, but there was no mention of testing for the presence of antinuclear antibody.[3] Furthermore, we found no study reporting the use of a solid phase assay to define antigen specificity of ANCA in association with relapsing polychondritis.

We investigated 33 patients with relapsing polychondritis—22 women, 11 men, aged 27–77 years. Relapsing polychondritis was defined by proved inflammatory episodes affecting at least two of three sites (auricular, nasal, or laryngotracheal cartilage) or one of those sites and two other manifestations, including ocular inflammation, hearing loss, vestibular dysfunction, or seronegative inflammatory arthritis.[4] Glomerulonephritis was present in three. Antineutrophil cytoplasmic antibodies were determined by immunofluorescence.[5] We then tried to define the antigenic target of ANCA by an enzyme linked immunosorbent assay (ELISA) (Bio-Carb, Lund, Sweden) specific for the 29 kilodalton myeloperoxidase antigens.

Relapsing polychondritis was associated with Sjögren's syndrome in three cases, lupus in two, and dysmyelopoiesis in two. Two patients had overlap between relapsing polychondritis and Wegener's granulomatosis. Antineutrophil cytoplasmic antibody immunofluorescence was positive in 8/33 serum samples from patients with relapsing polychondritis (three C-ANCA, five P-ANCA). Titres were low (C-ANCA range 1/10 to 1/50; P-ANCA range 1/10 to 1/100). All immunofluorescence positive serum

samples were tested by both solid phase assays. None of the 25 serum samples tested was positive for the anti-29 kilodalton ELISA. Four out of 27 serum samples were positive for the anti-myeloperoxidase ELISA and negative for antinuclear antibody testing. Anti-myeloperoxidase positive serum samples were P-ANCA positive (n=3) or immunofluorescence negative (n=1). No marked hypergammaglobulinaemia could explain 'false' positive results by non-specific Fc receptor binding. No association was found between the presence of ANCA and either vasculitis or glomerulonephritis. The serum of one patient with relapsing polychondritis-Wegener's granulomatosis overlap contained P-ANCA at a titre of 1/20. Among the eight patients positive for ANCA, relapsing polychondritis was active in seven, whereas it was active in only 12 out of 25 ANCA negative patients (p=0·1, two tailed Fisher's test).

These results suggest that low titres of C-ANCA are not specific for Wegener's granulomatosis and that ANCA (either diffuse or perinuclear) may be present in 24% of serum samples from patients with relapsing polychondritis, especially during the active phase of the disease.

T PAPO
J C PIETTE
LE THI HUONG DU
P GODEAU
Service de Médecine Interne
Groupe Hospitalier Pitié-Salpêtrière
75651 Paris Cedex 13, France

O MEYER
M F KAHN
Service de Rhumatologie
Hôpital Bichat
75018 Paris, France

P BOURGEOIS
Service de Rhumatologie
Groupe Hospitalier Pitié-Salpêtrière
75651 Paris Cedex 13, France

Correspondence to: Dr Thomas Papo, Service de Médecine Interne, Groupe Hospitalier Pitié-Salpêtrière, 83 bd de l'Hôpital, 75651 Paris Cedex 13, France.

1 Ludeman J, Utecht B, Gross W. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. *J Exp Med* 1990; **171:** 357–62.
2 Hagen E C, Ballieux B E, Daha M R, van Hes L A, van der Woude F J. Fundamental and clinical aspects of antineutrophil cytoplasmic antibodies. *Autoimmunity* 1992; **11:** 199–207.
3 Specks U, Wheatley C L, McDonald T J, Rohrbach M S, Deremee R A. Anti-cytoplasmic autoantibodies in the diagnosis and follow-up of Wegener's granulomatosis. *Mayo Clin Proc* 1989; **64:** 28–36.
4 Michet C J, McKenna C H, Luthra H S, O'Fallon W M. Relapsing polychondritis. *Ann Intern Med* 1986; **104:** 74–8.
5 Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. *Acta Pathol Microbiol Immunol Scand* 1989; **97:** 12–13.

Two sisters with ANCA positive vasculitis

Antineutrophil cytoplasmic antibodies (ANCAs) have been shown to be associated strongly with microscopic polyarteritis nodosa and Wegener's granulomatosis.[1 2] These antibodies have also been found in patients with other vasculitic disorders, including Kawasaki disease, Churg-Strauss syndrome, and relapsing polychondritis.[3 4] Factors governing production of ANCA remain unknown, though infection has been implicated as a possible trigger for onset of relapse of Wegener's granulomatosis.[5 6] We describe two sisters with ANCA positive vasculitic diseases.

The first patient, aged 55 years, presented with arthralgia and subsequently developed uveitis and scleritis, swelling of the nasal cartilage, chondritis of her ear, and costochondritis. She had an episode of transient diplopia and a Bell's palsy. A diagnosis of relapsing polychondritis was made. She was initially treated with steroids and a non-steroidal anti-inflammatory drug but subsequently required azathioprine to control her symptoms. She remains well six years later, having had two minor relapses in the interim. There has been no evidence of renal disease.

The second sister presented at the age of 54 years with nasal stuffiness, impaired hearing, episcleritis, myalgia, abnormal liver function, and proteinuria. A diagnosis of Wegener's granulomatosis was made. She has responded well to cyclophosphamide and remains in remission 18 months later.

Investigations have shown that both sisters have been repeatedly positive for ANCA. Patient 1 had a perinuclear pattern of staining (pANCA) with a maximum titre of 1/64 (on treatment), whereas patient 2 had a cytoplasmic pattern of staining (cANCA) with a maximum titre of 1/512. The sisters are HLA identical—A2, A10, B27, Bw4, Cw1, DR8, DR9.

As far as we know, this is the first report of ANCA positive vasculitis in siblings. There is a striking similarity between the age of onset and the clinical features of the two cases despite the different diagnoses. They are HLA identical. These cases suggest a role for genetic factors in the development of ANCA positive vasculitis, which merits further study. They also highlight the fact that within the spectrum of vasculitis there is considerable overlap in the clinical features of patients with different vasculitic disorders.

It is now recognised that the immunofluorescent staining patterns obtained when testing for ANCA reflect different antibody specificities—cANCA being associated with antibodies to proteinase-3 and pANCA being associated with antibodies to myeloperoxidase in some cases. It is therefore of interest that our two cases had different staining patterns.

There have been two recent reports of an HLA association in patients with ANCA positive vasculitis. Spencer *et al* found an association between HLA-DQw7 and susceptibility to ANCA related disease, while possession of HLA-DR5 or DR6 seemed to prolong the duration of ANCA synthesis.[7] Papiha *et al* reported an association between Wegener's granulomatosis and HLA-DR1.[8] It is therefore of interest that these two sisters are HLA identical, though they do not possess any of the HLA antigens previously associated with ANCA positive vasculitis.

E A MURPHY
R D STURROCK
Centre for Rheumatic Diseases
University Department of Medicine
Glasgow Royal Infirmary
Glasgow G31 2ER
United Kingdom

J G FOX
J M BOULTON-JONES
Renal Unit
University Department of Medicine
Glasgow Royal Infirmary
Glasgow G31 2ER
United Kingdom

1 Van der Woude F J, Rasmussen N, Lobatto S, *et al.* Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985; **i:** 425–9.
2 Lockwood C M, Bakes D, Jones S, Whitaker K B, Moss D W, Savage C O S. Association of alkaline phosphatase with an autoantigen recognised by circulating anti-neutrophil antibodies in systemic vasculitis. *Lancet* 1987; **i:** 716–9.
3 Venning M C, Arfeen S, Bird A G. Antibodies to neutrophil cytoplasmic antigen in systemic vasculitis. *Lancet* 1987; **ii:** 850.
4 Savage C O S, Lockwood C M. Autoantibodies in primary systemic vasculitis. *Ann Intern Med* 1990; **35:** 73–92.
5 Pinching A J, Rees A J, Pussell B A, *et al.* Relapses in Wegener's granulomatosis: the role of infection. *BMJ* 1980; **281:** 836.
6 Csernok E, Schmitt W H, Ernst M, Bainton D, Gross W L. Translocation of proteinase-3 on the cell membrane of polymorphonuclear leucocytes in Wegener's granulomatosis: correlation with disease activity. *Clin Rheumatol* 1992; **11:** 124.
7 Spencer S J, Burns A, Gaskin G, Pusey C, Rees A J. Influence of HLA class II genes on susceptibility to vasculitis. *Nephrol Dial Transplantation* 1991; **6:** 900.
8 Papiha S S, Murty G E, Ad'Hia A, Mains B T, Venning M. Association of Wegener's granulomatosis with HLA antigens and other genetic markers. *Ann Rheum Dis* 1992; **51:** 246–8.

Acquired Brown's syndrome in a patient with SLE

Sir: We read with interest the recent article by Alonso-Valdivielso *et al*[1] describing a patient with systemic lupus erythematosus (SLE) who later developed Brown's syndrome. In view of the authors' statement that this association has not previously been described we wish to draw your attention to two previously published reports.

In 1990 we published a case report[2] in which the patient, a 30 year old man, initially sought medical advice as a result of his ocular symptoms of variable double vision on upgaze. Only the left eye was affected and on examination restricted elevation of the adducted left eye produced diplopia associated with pain and a palpable click over the trochlea. A diagnosis of left Brown's syndrome was made following Hess chart assessment. The condition settled during treatment with ibuprofen 1200 mg daily. A five month history of arthralgia affecting the hands, wrists, elbows, shoulders, hips, and knees and acute alopecia one year before was noted. Later examination during febrile flare disclosed rash, lymphadenopathy, and synovitis of the hands, left elbow, shoulders, and knees. Investigations, including antinuclear antibody, dsDNA antibody, complement and immune complex levels, confirmed SLE.

In our discussion we made reference to an earlier review of Brown's syndrome which included a description of the condition occurring in a patient with established SLE.[3] We agree with Alonso-Valdivielso *et al* that Brown's syndrome should be considered in patients with diplopia and SLE. We consider that the true incidence of clinical disease may well be higher than these three reports suggest, perhaps because of mild or transient symptomatology and difficulty in diagnosis without orthoptic assistance.

JAMES McGALLIARD
Royal Liverpool University Hospital
Liverpool 7
United Kingdom