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 enable us to 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 changes 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 algohith made 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 (>2×10⁶/ml) 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) cytospin centrifugation and Jenner-Giemsa staining for cytology. A differential WCC (percentage polymorphonuclear leucocytes, small lymphocytes, monocytcs), CPM count (as percentage monocyes), and the presence or absence of eosinophils, mast, plasma, and inclusion body cells were noted as described.4 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 stable in individual fluids. Similar results were obtained if fluids were stratified by initial WCC (fresh fluid WCC <10×10⁶/l<WCC). No fluid with an initial ‘inflammatory’ WCC (>1×10⁶/l) fell into the non-inflammatory range during storage (or vice versa).

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

<table>
<thead>
<tr>
<th>Synovial fluid age (days)</th>
<th>White cell count (×10⁶/ml)</th>
<th>Polymorphonuclear leucocytes (×10⁶/ml)</th>
<th>Monocytes (×10⁶/ml)</th>
<th>Total WCC (×10⁶/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>48</td>
<td>16.2 (2.1)</td>
<td>13.0 (1.9)</td>
<td>1.6 (0.2)</td>
</tr>
<tr>
<td>Absent</td>
<td>14.8 (1.8)</td>
<td>10.9 (1.7)</td>
<td>0.9 (0.2)</td>
<td>2.3 (0.3)</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>10.6 (1.7)*</td>
<td>7.2 (1.5)*</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>9.0 (1.7)*</td>
<td>5.5 (1.5)</td>
<td>1.0 (0.2)*</td>
</tr>
</tbody>
</table>

*p<0.05; *p<0.01, both compared with fresh synovial fluid. Other results were not significant. CPMs: cytophagocytic mononuclear cells.

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

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fresh (n=48)</th>
<th>1 Day old (n=48)</th>
<th>2 Days old (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct</td>
<td>25 (52)</td>
<td>23 (48)</td>
<td>18 (46)</td>
</tr>
<tr>
<td>‘Inflammatory’</td>
<td>20 (42)</td>
<td>22 (46)</td>
<td>18 (46)</td>
</tr>
<tr>
<td>Wrong</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
</tr>
</tbody>
</table>

Two Reiter’s, one reactive arthritis diagnosed as rheumatoid.

Antineutrophil cytoplasmic antibodies in polyarthritis

Sir: Two major types of antineutrophil cytoplasmic antibodies (ANCA) have been recognised by indirect immunofluorescence. C-ANCA are defined by a diffuse granular neutrophil 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 neutrophil pattern and mainly recognise myeloperoxidase. Anti-neutrophil 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 polyarthritis 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 polyarthritis and Wegener’s granulomatosis—for example, acquired saddle nose deformity, laryngotraheal disease, episcleritis, and even auricular chondritis. Specks et al reported P-ANCA positivity in eight of 22 patients with relapsing polyarthritis, but there was no mention of testing for the presence of antineutrophil antibody.3 Furthermore, we found no study reporting the use of a solid phase assay to detect antigen specific ANCA in association with relapsing polyarthritis.

We investigated 33 patients with relapsing polyarthritis—22 women, 11 men, aged 27–77 years. Relapsing polyarthritis was defined by proved inflammatory episodes affecting at least two of three sites (auricular, nasal, or laryngotraheal 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 specificity of ANCA by enzyme linked immunosorbant assay (ELISA) (Bio-Carb, Lund, Sweden) specific for the 29 kilodalton myeloperoxidase antigens.

Relapsing polyarthritis was associated with Sjögren’s syndrome in three cases, lupus in two, and dysmyelopoesis in two. Two patients had overlap between relapsing polyarthritis and Wegener’s granulomatosis. Antineutrophil cytoplasmic antibody immunofluorescence was positive in 8/33 serum samples from patients with relapsing polyarthritis (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 microscopy: a reassessment and rationalisation. Ann Rheum Dis 1991; 50:101–7.
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.

Two sisters with ANCA positive vasculitis

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

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 chostochondritis. She had had a 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 anti-thrombin 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 of the disease six years 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, B4w, 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 and DR6 seemed to prolong the duration of ANCA synthesis. Papia et al reported an association between Wegener's granulomatosis and HLA-DR1. 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.

Acquired Brown’s syndrome in a patient with SLE

SIR: We read with interest the recent article by Alonso-Valdivieso et al 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 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 head movement. 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 a 30 lb weight loss over one year 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 acute active lupus.

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. We agree with Alonso-Valdivieso 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 reported. Our reports suggest, perhaps because of mild or transient symptomatology and difficulty in diagnosis without orthoptic assistance.
Antineutrophil cytoplasmic antibodies in polychondritis.
T Papo, J C Piette, D u Le Thi Huong, P Godeau, O Meyer, M F Kahn and P Bourgeois

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