

## 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.<sup>1</sup> 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))<sup>2</sup>; 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.<sup>3</sup>

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 ( $>0.2 \times 10^9/l$ ) 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) cyto-centrifugation 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.<sup>1</sup> 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

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.

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  $<10 \times 10^9/l < WCC$ ). No fluid with an initial 'inflammatory' WCC ( $\geq 1.5 \times 10^9/l$ ) 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<sup>1</sup> 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.<sup>2</sup> 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 cytological services, and implies that SF specimens should be promptly refrigerated if any transport or analytical delays are expected.

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### Antineutrophil cytoplasmic antibodies in polycondritis

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.<sup>1</sup> 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.<sup>2</sup>

Relapsing polycondritis 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 polycondritis 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 polycondritis, but there was no mention of testing for the presence of antinuclear antibody.<sup>3</sup> Furthermore, we found no study reporting the use of a solid phase assay to define antigen specificity of ANCA in association with relapsing polycondritis.

We investigated 33 patients with relapsing polycondritis—22 women, 11 men, aged 27-77 years. Relapsing polycondritis 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.<sup>4</sup> Glomerulonephritis was present in three. Antineutrophil cytoplasmic antibodies were determined by immunofluorescence.<sup>5</sup> 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 polycondritis was associated with Sjögren's syndrome in three cases, lupus in two, and dysmyelopoiesis in two. Two patients had overlap between relapsing polycondritis and Wegener's granulomatosis. Antineutrophil cytoplasmic antibody immunofluorescence was positive in 8/33 serum samples from patients with relapsing polycondritis (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

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 ( $\times 10^9/l$ )	Polymorphonuclear leucocytes ( $\times 10^9/l$ )	Lymphocytes ( $\times 10^9/l$ )	Monocytes ( $\times 10^9/l$ )	CPMs <sup>‡</sup> (% monocytes) <sup>§</sup>	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.