EXTENDED REPORT

The value of synovial fluid assays in the diagnosis of joint disease: a literature survey

A Swan, H Amer, P Dieppe

Objective: To carry out a critical appraisal of the literature in an attempt to assess the current value of synovial fluid (SF) analysis in the diagnosis of joint disease.

Methods: A literature search was undertaken using the Medline, Biomed, Bids, Pubmed, and Embase electronic databases using the keywords: synovial fluid (SF) analysis, SF crystals, joint sepsis, acute arthritis, and SF cell counts, cytology, biomarkers, and microbiology.

Results: Publications fell into three main categories. Firstly, reports assessing the value of the three traditional assays (microbiology, white blood cell counts, and microscopy for pathogenic crystals). For these quality control evidence was found to be sparse, and tests for sensitivity, specificity, and reliability showed worrying variations. These poor standards in SF analysis may be due to lack of inclusion of some tests within routine pathology services. Secondly, claims for the usefulness of “new” assays (cytology and biochemical markers). For cytology, the supporting evidence was mainly anecdotal and there were no reports on specificity, sensitivity, and reliability. Interpretation difficulties are a major hindrance to the clinical use of biochemical assays, which remain primarily research tools. Finally, work on the diagnostic value of SF analysis in general. The appraisal confirmed that SF analysis remains of major diagnostic value in acute arthritis, where septic arthritis or crystal arthropathy is suspected, and in intercultural gout.

Conclusions: Given the importance of SF tests, rationalisation of their use, together with improved quality control, should be immediate priorities. Further investigation is recommended into the contribution of SF inspection and white cell counts to diagnosis, as well as the specificity and sensitivity of SF microbiological assays, crystal identification, and cytology.

Analysis of synovial fluid (SF) has long been recommended as a routine procedure to assist in the diagnosis of arthritis. Ropes and Bauer were among the first to point out that differences in the appearance and cell content of abnormal SF could be related to different disease categories, in particular distinguishing inflammatory and non-inflammatory forms of arthritis. Hollander et al promulgated the routine use of SF analysis as an aid to diagnosis, documenting in detail the main findings of SF in different forms of arthritis and introducing the term “synovianalysis”.3 The recommended procedure included evaluation of the gross appearance of SF, cell counts, microbiology, and biochemical tests such as glucose levels. Hollander and McCarty introduced polarised light microscopy of SF to allow the identification of urate (monosodium urate monohydrate (MSUM)) and pyrophosphate (calcium pyrophosphate dihydrate (CPPD)) crystals as the definitive way of diagnosing gout or pseudogout.4 It was subsequently shown that detection of crystals in SF may change clinical diagnosis and treatment.5 Moreover, analysis of aspirated joint fluid is regarded as the key to the diagnosis of infectious arthritis. The analysis of SF is recommended by textbooks and publications as an important aid to diagnosis.6–13

The last major critical appraisal of SF analysis, published in 1994 by Shmerling, concluded that there are two important reasons for SF analysis.14 The first is to identify joint infection by SF Gram stain and culture; the second is to diagnose crystal-induced arthritis by polarised light microscopy. However, Shmerling concurred that useful additional information about disease “category” can be gained from total and differential white cell counts. He concluded (mainly from his own data,15) that chemical tests promoted previously, including SF glucose, protein, and lactic dehydrogenase, were insensitive and non-specific.

There have been many developments since 1994, including the introduction of new SF biochemical markers16 and a resurgence of interest in SF cytology.17 In addition, the availability of other new diagnostic techniques (such as magnetic resonance imaging), associated with an increasing awareness of potential problems with routine joint aspiration and the handling of body fluids, has led to a general decrease in the numbers of patients who have their SF analysed (including in our own laboratory). We have therefore reviewed the literature critically to try to assess the current value and position of SF analysis in the diagnosis of joint diseases.

METHODS
A literature search was undertaken using the Medline, Biomed, Bids, Pubmed, and Embase electronic databases. Keywords used included SF analysis, SF crystals, joint sepsis, acute arthritis, and SF cell counts, cytology, biomarkers, and microbiology. The abstracts of identified articles were scanned, and copies of those that appeared to be relevant obtained. The references quoted by all review articles and other references obtained were also scanned to provide evidence of other reports. The many general references on culture and Gram stain methodology for other body fluids were also scanned for potential relevance. In this report we have concentrated on reports that relate directly to the value of SF analysis in clinical diagnosis, and on the more recent publications, wishing to build on the authoritative review of Shmerling, and update it.

Abbreviations: CPM, cytophagocytic mononuclear; CPPD, calcium pyrophosphate dihydrate; MSUM, monosodium urate monohydrate; PMN, polymorphonuclear; SF, synovial fluid; WBC, white blood cell


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Accepted
7 December 2002
RESULTS

The volume of literature on SF tests

Using the key words “synovial fluid” we identified a total of 6556 papers for the period 1980–2001 inclusive. When the search for SF was combined with other keywords and related to our diagnostic interest numbers were far smaller: we identified a total of 300 relating to SF crystals (a small minority of which were concerned with their diagnostic value), 67 on cell counts, 33 on cytology, 10 on the diagnostic value of biomarkers, and only five on the value of microbiology. Many of the remaining SF publications were concerned with biomarkers, most of them concentrating on features such as assay characteristics rather than potential clinical value. There is a vast literature on the general application of cultures and Gram stain to body fluids, but a scan of these papers indicated that very few gave any evidence based recommendations that could be applied to SFs.

The number of articles related directly to the value of SF analysis in clinical diagnosis was about 200. We have not quoted those that are outdated or appeared to add little to other papers in this report.

What tests are available and in use?

In a previous report of the use of SF tests by UK practitioners we divided the types of assay into five categories: total and differential white cell counts, Gram stain and culture, polarised light microscopy for crystals, cytology, and biochemical assays. We found that only the first three of these were in general use, and that acute arthritis was the main indication for SF analysis.

We used the same classification when appraising the literature for this report. We found that recent publications fell into three main categories: reports assessing the value of longstanding SF assays (microbiology, white cell counts, and polarised light microscopy), anecdotal reports of the value of new SF assays (cytology and biochemical markers), and papers assessing the overall value of SF analysis in the diagnosis of arthritis.

Critical appraisal of traditional SF analysis tests

SF Gram stain and culture for infective arthritis

Many authors recommend the use of SF Gram stain and culture in cases of suspected septic arthritis. There are few data available to enable assessment of the sensitivity or specificity of these tests as applied to joint fluids, little quality control information, and little evidence based recommendation on their use. Gram stain and culture techniques are widely used to detect and identify infective agents in blood and other body fluids. In the UK the Public Health Laboratory Services issue detailed methodological guidelines for these tests, and cross checking procedures are in place.

However, the mortality rate for septic arthritis remains high (about 10%) despite the use of antibiotics, the outcome being particularly poor in older patients and those with hip joint infections. There is an additional morbidity rate of 20–30%, and this literature highlights delay in diagnosis as a key factor influencing bad outcomes. In a review of a large series of cases of septic arthritis in the UK (n = 242), Weston et al reported that simple SF Gram staining was positive in 50% of cases, SF culture was positive in 67%, and blood culture was positive in an additional 9% when SF culture was negative. Shmerling's paper is the only published attempt to assess the sensitivity and specificity of SF microbiological tests. These were estimated from a study comparing SF results from 19 cases of septic arthritis with those of 100 samples from patients with other disorders. The sensitivity was estimated to be 75–95% for culture, and 50–75% for Gram stain, and the specificity was estimated to be over 90% for culture and “quite high” for Gram stain (table 1). These data concerned non-gonococcal infections, without prior antibiotic treatment. However, as both these and other publications note, problems arise with atypical organisms and with patients who have received antibiotics before joint aspiration. Freemont et al estimated that 30–80% of SF cultures may give false negative results owing to prior use of antibiotics. The use of blood culture bottles for SF, using a high volume of inoculum and a high volume of medium, is likely to increase the chances of a positive SF culture. There are also recent reports that commercial blood culture systems (for example, “BACTEC plus Aerobic/F” medium) increase the chances of culturing aerobic pathogens from joint fluids, particularly in patients receiving antibiotic treatment. Freemont and others have emphasised the value of other SF tests, such as the proportion of polymorphonuclear (PMN) cells, as important adjuncts to the diagnosis of septic arthritis.

Total and differential white cell counts

It is generally taught that SF total and differential white cell counts provide a simple way of distinguishing non-inflammatory from inflammatory arthritides, and that the percentage of PMN cells is a good guide to the degree of joint inflammation, and thus a pointer to severe acute conditions such as gout or septic arthritis. However, there are few published data to support this contention, although the work of both Schumacher and Shmerling suggests that a reasonable degree of differentiation is achieved if the same laboratory assesses a large number of synovial fluids in the same way.

There are two published assessments of the reliability of such data, both of which report a high degree of variation in reports from different laboratories looking at the same fluids (tables 2 and 3). For example, total white blood cell (WBC) counts on the same fluid varied from 2467/mm³ to 12 000/mm³ in one study (table 2). In both these studies, synovial fluid sample(s) were analysed “blind” by different hospital laboratories and the results compared. In the Hasselbacher study one SF sample was analysed by 26 different hospital laboratories. In the Schumacher study results from three different hospital laboratories were compared with those recorded by an expert “reference” laboratory. Reproducibility of results within the reference laboratory was also checked (“blind” assessments by two experienced operatives). This constitutes an intralaboratory study and tables 2 and 3 show the results.

Urate and pyrophosphate crystal identification

The story is similar for the identification of MSUM or CPPD crystals by polarised light microscopy. Thus there is a mass of
literature recommending the procedure for the diagnosis of gout or pseudogout, but only a handful of tests of sensitivity, specificity or reliability, most of which show worrying problems and variations.

There are four quality control studies in the literature for both MSUM and CPPD crystals, and one for MSUM alone. Four studies involved a number of hospital laboratories assessing the same SF samples for crystal content. In the fifth, six observers of variable experience within the same hospital laboratory, examined “blind”, a range of synthetic crystal preparations. A sixth study gave figures for sensitivity and specificity, and compared results for Gram stained versus “wet” slide preparations of SF containing MSUM. Segal and Albert carried out a statistical analysis based mainly on the data of Gordon et al.

For MSUM crystals the results were modestly encouraging (table 4). Of the 25 laboratories in the Von Essen study, 19 identified all MSUM crystals correctly, and in the Hasselbacher study crystals were correctly detected in 39 out of a possible 50 samples. Equally good results for MSUM identification were obtained from both Gram stained and wet preparations of SF. However, some laboratories did very badly, particularly in the McGill and Schumacher studies. In general, results for CPPD crystals were a lot worse than for MSUM (table 5); for example, in the Hasselbacher study CPPD crystals were correctly identified in only six of a possible 50 slides. Gordon’s study shows that the crystal concentration is important—the higher the crystal load in the SF the more likely it is that observers will get it right. Segal et al carried out statistical analysis of data from the Schumacher and Gordon publications. When the Gordon data were used, likelihood ratios were calculated and curves constructed using the solutions to Bayesian equations that have been applied to a wide variety of diagnostic tests and procedures in evidence based medicine.

It was found that the odds of a diagnosis of gout could be increased by ×14 through the identification of SF MSUM crystals, whereas the odds for pseudogout were increased by a mere ×2.9 after identification of SF CPPD. However, the high false positive rate for identification of the synthetic CPPD used in the Gordon study might have led to the falsely low odds ratio for pseudogout.

Table 2 Summary of quality control studies for total WBC count

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Number of SF samples examined</th>
<th>Number of participating laboratories or observer</th>
<th>Statistical result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>30 Heparinised SF</td>
<td>3 Laboratories compared with an expert “reference laboratory”</td>
<td>Correlation of total counts results of 3 laboratories with those of the reference laboratory. Correlation coefficients from 0.76 to 0.80</td>
<td>Only fair</td>
</tr>
</tbody>
</table>

In SF from 4 patients, the difference in WBC between laboratories caused the fluids to be erroneously classified as either “non-inflammatory” (WBC <2000/mm³) or “inflammatory”.

25 | 30 Heparinised SF | Reference laboratory only. | Intraobserver study Two observers | Coefficients of variation (CV) for a given sample with total WBC counts >1500/mm³ varied from 1 to 18%. For a given sample with a very low total WBC of < 300/mm³ the CV varied from 20 to 62% | Good |

None of the variations would have clinical significance.

24 | 1 | 26 Laboratories | Mean total WBC was 6683/mm³ (SD 1992/mm³ (range 2467–12000/mm³) | Poor |

Fairly wide range of cell counts reported

Table 3 Summary of quality control studies for differential WBC count

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Number of SF samples examined</th>
<th>Number of participating laboratories or observers</th>
<th>Statistical result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>24 Heparinised SF</td>
<td>3 Laboratories compared with an expert “reference laboratory”</td>
<td>Comparing the 3 “test” laboratories with the reference laboratory. In one or more of the test laboratories, the %PMN cells in 12/24 SF samples fell outside (25D) of the 95% confidence limits of the value determined by the reference laboratory (Correlation coefficients for %PMN cell findings in 3 laboratories compared with the reference laboratory were 0.73, 0.84, 0.94, respectively)</td>
<td>Variable</td>
</tr>
</tbody>
</table>

Occasional wide variation in results and misidentification of cells

25 | 24 Heparinised SF | Reference laboratory only | Intraobserver study Two Observers | The mean %PMN cells counted by 2 observers were similar—maximum difference of 25%. When a 2 sided significance test for difference from previous value was applied, 23/24 percentages were within 95% confidence interval | Good |

24 | 1 | 26 Hospital laboratories | The percentage of PMN cells identified varied from 0 to 30%, mean 13.8 (SD 8.6) (median 11%) | Considerable variation |

Other particles
There have been many reports of other crystals and particles in SF, such as cartilage fragments (which in osteoarthritis may show the crimping of early fibrillation and clustered chondrocytes), “rice bodies”, lipid crystals, and basic calcium phosphates, but the literature is in the main anecdotal and we have not attempted a full review of it. However, detailed microscopic examination of a wet preparation of SF and identification of these particles form part of the “SF cytopathology” promulgated by Freemont et al and described in the next section.

Claims for usefulness of “new” SF tests
SF cytopathology
There has been interest in SF cell cytology in specialised laboratories for many years. The suggestion that detailed
cytopathology might be useful in routine SF analysis has been revitalised by the work of Freemont et al.\(^7\). This group recommended, firstly, microscopic examination of a wet preparation of SF for total WBC count, and detection/identification of particles. The particles include the less commonly occurring SF crystals (oxalate, cystine, etc) as well as tissue fragments which can give clues to systemic disease.\(^35\) Secondly, microscopy of a stained cytocentrifuged preparation of the SF to identify cell types and numbers. Although the cost effectiveness of this method has been questioned,\(^36\) cytocentrifuge preparations of SF (rather than the traditional smear) plus special staining techniques are said to improve the ability to identify a range of cells having diagnostic implications.\(^37\) For example, the presence of apoptotic PMN cells, cytophagocytic mononuclear (CPM) cells, and mast cells in SF distinguish seronegative arthropathies from rheumatoid arthritis, the latter having apoptotic cells but no CPM cells.\(^38\) SF eosinophilia suggest parasitic infection, allergy, Lyme disease, or tumour. Using such criteria, Freemont et al reported that retrospective analysis of SF from 1100 “proven cases” of arthritis, disclosed microscopic patterns that in 41% were specific for a range of joint diseases. The authors claimed an 87% success rate in the diagnosis of septic arthritis in “under one hour” using cytopathology. Unfortunately, the specificity, sensitivity, and reliability of these tests have not been widely discussed, and Freemont’s group is the only one to have reported them extensively in the past 10 years.

**Biochemical measures**

SF biochemical and immunological assays have been used widely as research tools but have not generally been

### Table 4: Quality control studies for MSUM

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Number of SF samples examined</th>
<th>Numbers of participating laboratories or observers</th>
<th>Results</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>11 SF of which 7 contained MSUM crystals</td>
<td>2 Laboratories compared with reference laboratory</td>
<td>5/7 MSUM crystal SF were correctly identified by 1 laboratory</td>
<td>Fair</td>
</tr>
<tr>
<td>24</td>
<td>4 SF of which 1 contained MSUM alone, and 1 contained both MSUM and CPPD</td>
<td>25 Laboratories</td>
<td>39/50 MSUM crystal SF correctly identified, 78% Accuracy</td>
<td>Good</td>
</tr>
<tr>
<td>27</td>
<td>12 SF of which 4 contained MSUM</td>
<td>6 Laboratories</td>
<td>19/24 MSUM crystal SF correctly identified, 15/16 by 4 laboratories (good)</td>
<td>4 Laboratories good</td>
</tr>
<tr>
<td>26</td>
<td>4 SF of which 1 contained MSUM</td>
<td>25 Laboratories</td>
<td>19/25 Laboratories identified MSUM crystal SF correctly. 24% False positives</td>
<td>Good</td>
</tr>
<tr>
<td>28</td>
<td>96 slide preparations of SF, 41 with synthetic MSUM added. Concentrations of MSUM 0.1–100 µg/ml</td>
<td>1 Laboratory 6 Observers</td>
<td>Mean sensitivity 69% Mean specificity 93%</td>
<td>MSUM identification improved at higher crystal concentrations</td>
</tr>
<tr>
<td>29</td>
<td>11 samples, 3 with MSUM Gram stained and wet preparation set up for each sample</td>
<td>1 Laboratory 3 Observers</td>
<td>78% Sensitivity 100% Specificity</td>
<td>Results equally good for Gram stained and wet preparations of SF</td>
</tr>
</tbody>
</table>

### Table 5: Quality control studies for SF CPPD

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of SF samples examined</th>
<th>Numbers of participating laboratories or observers</th>
<th>CPPD</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>11 SF of which 2 contained CPPD</td>
<td>2 Laboratories compared with reference laboratory</td>
<td>Only 1/4 CPPD crystal SF correctly identified</td>
<td>Poor. One laboratory failed to identify CPPD at all BUT too few test samples</td>
</tr>
<tr>
<td>24</td>
<td>4 SF of which 1 contained CPPD and 1 contained both CPPD and MSUM</td>
<td>25 Laboratories</td>
<td>Only 6/50 CPPD crystal SF correctly identified</td>
<td>Very poor CPPD missed or misidentified</td>
</tr>
<tr>
<td>27</td>
<td>12 SF of which 4 contained CPPD</td>
<td>6 Laboratories</td>
<td>17/24 CPPD crystal SF correctly identified</td>
<td>Variable</td>
</tr>
<tr>
<td>28</td>
<td>96 Slide preparations of SF, 42 with synthetic CPPD added Concentration ranged from 1 to 1000 µg/ml</td>
<td>1 Laboratory 6 Observers</td>
<td>Mean sensitivity 83% Mean specificity 78%</td>
<td>Improved with higher concentrations</td>
</tr>
<tr>
<td>29</td>
<td>11 SF, 3 containing CPPD Gram stained and wet preparations of each SF</td>
<td>1 Laboratory 3 Observers</td>
<td>Sensitivity 56% Specificity 96%</td>
<td>Good for both Gram stained and wet preparations</td>
</tr>
</tbody>
</table>
recommended as of clinical value in the diagnosis or treatment of arthritis. There was considerable early interest in small molecules, such as glucose, and their possible value in the diagnosis of septic arthritis, but the assays do not discriminate between different types of inflammatory arthritis. SF serology, assaying factors such as complement or rheumatoid factor, have also proved to be of little clinical value. Drugs levels have also been measured, and SF antibiotic levels can be of some practical use. However, it is difficult to interpret the meaning of a single measurement of the concentration of any of these molecules in SF, because of our lack of understanding of the dynamics of their movement through the joint space. Currently, there is interest in new types of SF biomarker. A biomarker can be defined as a biochemical measure that is indicative of a biological process or the response to an intervention. In a joint with arthritis, abnormal matrix turnover results in the release into the joint fluid of many molecules and fragments of matrix components derived from tissues such as the articular cartilage. Some of these fragments can be assayed. They are of great research interest as a way of detecting the nature of abnormalities in tissue turnover in different types of arthritis, but have yet to be proved to be of clinical value in the diagnosis or management of patients with musculoskeletal diseases. However, several different biomarkers are currently being investigated further for potential clinical use; examples include measures of collagen breakdown that are known to be associated with the accelerated breakdown of cartilage in inflammatory arthritis and the raised levels of cartilage oligomeric matrix protein seen in osteoarthritis. But interpretation of these SF biomarkers, as with other biochemical measures, is difficult.

**Value of SF tests in diagnosing acute monarthritis and intercritical gout**

The only conditions in which the value of SF analysis has been critically evaluated are acute monarthritis and intercritical gout.

**Diagnosis of acute monarthritis**

The difficulty in diagnosing acute monarthritis, and the inherent dangers in misdiagnosis, are reflected in the extensive publications on this topic. SF analysis by compensated light microscopy for crystals, together with Gram staining and culture, are universally and strongly recommended. Ho and De Nuccio, in a 1993 study of 67 patients admitted to hospital with gout or pseudogout, found that 25% encountered errors in diagnosis, resulting in “misdirected investigation, inappropriate treatment, pain, and increased costs”, and mistakes continue to be made.

**Intercritical gout**

It is over 20 years since crystals (both MSUM and CPPD) were first identified in SF from asymptomatic joints. More recent studies have confirmed this finding and emphasised its importance in identifying intercritical gout. The study of Pascual and Jovani of 101 SFs from the asymptomatic joints of patients with gout, disclosed MSUM crystals in 43/43 SF samples from patients not receiving hypouricaemic drugs and in 34/48 patients receiving hyperuricaemic drugs. Slightly raised WBC numbers were sometimes seen and might indicate low grade inflammation, on top of which “bursts” of acute inflammation take place.

**Routine SF analysis**

Pal and colleagues carried out an audit on 408 SF samples (consecutive, all diagnoses from all sources—that is, hospital wards, and outpatients, orthopaedic and accident and emergency departments, etc.). Samples were analysed for cell counts and crystals: crystals were identified in 25 SFs only (6.1%). The authors concluded that routine SF analysis does not contribute to diagnosis or management of osteoarthritis or inflammatory arthritis and should be performed only when the underlying cause is uncertain or in new patients. Similarly, they argued that routine SF culture and Gram stain, when infection is not clinically suspected, should be abandoned. Other authors concur that the laboratory analysis of SF is not a good “screening" procedure, but is most valuable when used selectively in patients with clinical likelihood of crystal arthropathy or sepsis.

**DISCUSSION**

This appraisal confirms that SF analysis can be of major diagnostic value in acute arthritis, when a crystal arthropathy or septic arthritis is suspected, and in intercritical gout. The widespread recommendation of the use of microbiological assays and polarised light microscopy of SFs in these situations seems supported by the literature. There are plenty of data to suggest that misdiagnosis of septic arthritis or crystal arthropathies is costly, and SF analysis makes correct diagnosis more likely, although far from certain. In contrast, there seems little justification for the wider use of SF assays as an adjunct to diagnosis or assessment.

Despite some individual claims, the value of assays other than microbiology or crystal identification is not supported by the literature. It is also apparent that there are few data on the sensitivity or specificity of any of the assays (including microbiological assays and crystal analysis), and that there is considerable interobserver and intraobserver error in the assessment of cells and identification of crystals in SF.

This is a sorry state of affairs. Clearly, SF analysis is relatively underresearched and generally excluded from routine diagnostic services. In the UK, for example, we found that only SF microbiology is standardised within the health service, other assays being left to the whim of local people and services; discussions with colleagues suggest that a similar problem exists in other countries. Poor standards in SF analysis may be partly due to this lack of inclusion within routine pathology services, as well as the relatively low throughput of samples in most units. Hasselbacher, for example, found that the mean number of SF samples seen each month was only 3.9 in 42 laboratories participating in a quality control study, and in 15 hospitals less than one sample a month was examined.

There is a need for further investigation of the value of SF inspection and cell counts (do they help clinicians with diagnosis or assessment of the degree of inflammation?), as well as of the specificity and sensitivity of microbiological assays, crystal identification, cytology, and biochemical markers. Without such data we will remain ignorant as to the value of SF analysis. In addition, given the importance of SF assays in acute arthritis, we need to rationalise their use and improve quality control.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge the financial support given to the Bristol SF Crystal Project by the Medical Research Council and the Arthritis and Rheumatism Research Council.

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The value of synovial fluid assays in the diagnosis of joint disease: a literature survey

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doi: 10.1136/ard.61.6.493

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