EXTENDED REPORT

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

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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 intercritical 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 of the specificity and sensitivity of SF microbiological assays, crystal identification, and cytology.

A 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”. 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. It was subsequently shown that detection of crystals in SF may change clinical diagnosis and treatment. 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.

The last major critical appraisal of SF analysis, published in 1994 by Shmerling, concluded that there are two important reasons for SF analysis. 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) 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 markers and a resurgence of interest in SF cytology. 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

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.44

We used the same classification when appraising the literature for this report. We found that recent publications fell into five main categories: total and differential white cell counts, Gram stain, SF culture, polarised light microscopy, and biochemical assays. Thus 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 story is similar for the identification of MSUM or CPPD crystals by polarised light microscopy. Thus there is a mass of

<table>
<thead>
<tr>
<th>Groups of infective agents</th>
<th>Prior antibiotics</th>
<th>SF culture</th>
<th>SF Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>Non-gonococcal infections (mostly)</td>
<td>No</td>
<td>75–95</td>
<td>90 plus</td>
</tr>
<tr>
<td>Staphylococcus aureus and streptococci</td>
<td>Yes</td>
<td>Much lower</td>
<td>Much lower</td>
</tr>
<tr>
<td>Gonococcal infections</td>
<td>No</td>
<td>10–50</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Table 1 Septic arthritis. Specificity and sensitivity of culture and Gram stain. Figures quoted are from the Shmerling review,14 which relies on data from his previous study.15
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 crystals, whereas the odds for pseudogout were increased by a factor of 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 observers</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>
<tr>
<td>25</td>
<td>30 Heparinised SF</td>
<td>Reference laboratory only. Intra-laboratory study Two observers</td>
<td>Coefficients of variation (CV) for a given sample with total WBC counts &gt; 1500/mm$^3$ varied from 1 to 18%. For a given sample with a very low total WBC of &lt; 300/mm$^3$ the CV varied from 20 to 62%</td>
<td>Good</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>26 Laboratories</td>
<td>Mean total WBC was 6683/mm$^3$ (SD 1992/mm$^3$ (range 2467–12000/mm$^3$)</td>
<td>Poor</td>
</tr>
</tbody>
</table>

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>Comment</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 (2SD) 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>
<tr>
<td>25</td>
<td>24 Heparinised SF</td>
<td>Reference laboratory only. Intra-laboratory study Two observers</td>
<td>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</td>
<td>Good</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>26 Hospital laboratories</td>
<td>The percentage of PMN cells identified varied from 0 to 30%, mean 13.8 (SD 8.6) (median 11%)</td>
<td>Considerable variation</td>
</tr>
</tbody>
</table>

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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

Table 4 Quality control studies for MSUM

<table>
<thead>
<tr>
<th>Study</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></td>
<td>3/7 MSUM crystal SF were correctly identified by 1 laboratory</td>
<td>Sensitivity was calculated as 62.5%</td>
<td></td>
<td></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></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) But the results for the other 2 laboratories were poor (50% correct and scored 2 false negatives and 3 false positives)</td>
<td></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></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></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 3 False positives One false negative</td>
<td></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 4 Laboratories good results (14/16 correct) 2 Laboratories poor, only 50% or less correct, and scored 3 false positives for CPPD</td>
<td></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% High false positive result</td>
<td></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></td>
</tr>
</tbody>
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Biochemical measures
SF biochemical and immunological assays have been used widely as research tools but have not generally been...
recommend 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.39 SF serology, assaying factors such as complement or rheumatoid factor, have also proved to be of little clinical value.40 Drugs levels have also been measured, and SF antibiotic levels can be of some practical use.41 However, it is diffi-
cult 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 42 and the raised levels of cartilage oligomeric matrix protein seen in osteoarthritis.43–45 But interpretation of these SF biomarkers, as with other biochemical measures, is difficult.46

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.46–51 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”,46 and mistakes continue to be made.52

Intercritical gout
It is over 20 years since crystals (both MSUM and CPPD) were first identified in SF from asymptomatic joints.53 More recent studies have confirmed this finding and emphasised its importance in identifying intercritical gout.54–57 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.57

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.).58 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.59 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.60

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,60–62 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 under researched 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;63 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,64 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.

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