Synovial fluid analysis in the diagnosis of joint disease

Paul Hermansen
Tony Freemont

Abstract

Normal synovial fluid consists of a transudate of plasma from synovial blood vessels supplemented with high molecular weight lipid and saccharide-rich molecules. This produces a paucicellular, viscous fluid, which behaves like a tissue. In primary (e.g. rheumatoid disease) and secondary (e.g. septic and crystal-induced) inflammatory arthropathies, changes occur to the cell numbers and cell type in the fluid forming the basis of a diagnostic test. The diagnostic value is enhanced by the appearance of endogenous and exogenous particles, particularly those associated with degenerative, crystal-induced, and prosthesis-associated arthropathies (e.g. fibrin, cartilage, crystals, metal and plastic). Cells and particles can be characterised under the microscope leading to a simple, inexpensive, "cytological" test for every type of joint disease.

Keywords arthritis; crystals; microscopy; polarisation; synovial fluid

Why perform synovial fluid microscopy?

Histopathologists are frequently called upon to diagnose inflammatory or non-inflammatory arthropathies based on the examination of a synovial biopsy. Even experienced histopathologists can have difficulty in distinguishing inflammatory from non-inflammatory arthropathies since the latter frequently have a lymphocyte infiltrate in the synovium. Even if they can be distinguished it is usually impossible to make specific diagnoses as there are few differences that can be detected histologically between disorders in the same broad group.

By contrast, synovial fluid (SF) microscopy is of greatest value in these disorders, supporting clinicians in making early and accurate diagnoses of a spectrum of inflammatory and non-inflammatory arthropathies often before the full blown syndrome develops.

SF microscopy, on as little as a 0.5 ml sample, permits the rapid diagnosis of joint disease (the full test takes between 10 minutes for crystal arthropathies and 2 hours for full cytological analysis), particularly disorders such as sepsis and crystal related arthropathy where the prognosis is inversely related to delay in diagnosis.[1]

The properties of synovial fluid

SF consists of a transudate of plasma from synovial blood vessels, supplemented with high molecular weight lipid and saccharide-rich molecules,[2] produced by one of the two main types of synovial cells (type B synoviocytes derived from synovial fibroblasts). Type A synoviocytes are tissue macrophage-derived phagocytes that remove debris from the synovial fluid.

SF differs from all other body fluids in that the synovium and cartilage, which are the tissues it contacts, do not have an intact surface cellular layer seated on a basement membrane. This means that the matrix of both tissues is in direct contact with and through SF allowing an homogenous biological environment to develop within the joint. Because of this it is probably better to regard SF as a semi-liquid, avascular, hypocellular, connective tissue rather than a true body fluid such as is seen in other situations (e.g. pericardial effusion).

Cytological analysis of SF differs in three important ways from other body fluids. Firstly synovial joints are only very rarely affected by primary or secondary malignancies. Secondly the term "cytological analysis" of SF would be better described as "microscopy" of SF, as many of the diagnostic features found are not cellular but are particles such as cartilage, crystals and prosthetic wear particles. Thirdly the greatest diagnostic information comes from the recognition of individual cell types and their quantification.

Normal SF:

- Is a supplemented ultra-filtrate of plasma
- Contains no clotting factors
- Is viscous
- Is paucicellular (<500 cells/mm^3)
- Contains no particles

Any or all of these may change with joint disease.

High molecular weight clotting factors are introduced into the joint as a result of vascular leakage following trauma or in inflammation. This results in the need to anticoagulate specimens. The choice of anticoagulant can have profound influence on the final diagnosis. Anticoagulants such as EDTA will, because of their sequestering properties, dissolve complex calcium phosphates such as seen in bone-derived hydroxyapatite and calcium pyrophosphate making the diagnosis of osteoarthritis and pseudogout difficult, if not impossible. Other anticoagulants, such as sodium heparin, can crystalise out leading to the false diagnosis of crystal arthritis. Lithium heparin, by contrast, has none of these faults and is the anticoagulant of choice.

As, at the time of aspiration it is impossible to predict which SF will clot or contain crystals, all SF samples should be anticoagulated with lithium heparin.

The viscosity of the SF is dependent on the concentration and size of the proteoglycans it contains. Normal SF and that from non-inflammatory arthropathies is very viscous. By contrast, in inflammatory arthropathies, inflammatory mediators and/or products cause abnormal synthesis or breakdown of existing proteoglycans, reducing viscosity.

Paul Hermansen *MSc FIBMS is Consultant BMS in the Cytology Department, Central Manchester University NHS Foundation Trust, Manchester, UK. Conflicts of interest: none declared.*

Tony Freemont *BSc MD FRCP FRCPath is Professor of Osteoarticular Pathology, University of Manchester, Director of the Manchester MRC/EPSRC Molecular Pathology Node, Manchester NIHR Biomedical Research Centre, Manchester, UK. Conflicts of interest: none declared.*

Viscosity of SF can be assessed by mixing SF and a 2% solution of acetic acid leading to the formation of a white precipitate, produced by aggregation of proteins and hyaluronans (the mucin clot test). The nature and amount of precipitate varies from good to poor and reflects the quality and quantity of the protein/hyaluronan complex, such that in inflammatory joint diseases and haemarthrosis there is poor clot formation. Non-inflammatory arthropathies exhibit a good mucin clot.

The mucin clot test is not normally performed on routine specimens, but it is very useful in identifying surgical joint washouts or dilution with local anaesthetic, where, in the presence of a low cell count no clot will form. This is an important observation as dilution of SF with water will affect cell counts and remove monosodium urate crystals, adversely affecting diagnostics.

Why examine synovial fluid?

Basic examination of SF will result in identifying combinations of cellular and non-cellular biomarkers, which can have diagnostic, prognostic and theranostic (following responses to treatment) value, in primary, secondary and tertiary care, where SF analysis can be used to influence the choice of care pathway, and assess treatment options.

This valuable role for SF analysis is made possible because the absence of a cell or basement membrane barrier between synovium, cartilage and SF means that pathological changes in the tissues surrounding the joint are reflected in the volume, cellular composition and particulate load within the SF.

SF is examined "fresh" requiring careful handling in the laboratory and a need to minimise cell and crystal loss, which can dramatically adversely affect diagnosis, during transport to the laboratory. Storage at 4 °C is well tolerated by the SF and prolongs transport times, but even so, adequate diagnosis that even cooled samples must reach the laboratory within 48 hours of aspiration.[3]

Basic approach to synovial fluid analysis

In order that no part of the analysis is omitted there is a sequential examination of SF specimens arriving in the laboratory. It follows four steps:

- Gross analysis
- Nucleated cell count
- The "wet prep"
- Preparation and examination of a stained cell monolayer

Gross analysis relies on the visual inspection of the SF to determine colour, clarity and viscosity.

Colour will change, particularly following haemorrhage such as that seen in trauma, anticoagulant use and in primary disorders of joints such as pigmented villonodular synovitis.

Clarity of SF is variable and a cloudy or opaque appearance generally indicates an increase in cellular concentration, crystal content or the presence of lipid, microscopic clarification is then necessary.

Total nucleated cell count

A total nucleated cell count is performed in order that the degree of inflammation can be assessed and so that an optimally diluted cell suspension can be achieved for the subsequent cytocentrifuge preparation.

The traditional method of counting cells is to use a graduated pipette to dilute the SF by a known amount with normal saline, to which crystal violet has been added as a supravital stain. This cell suspension is then introduced into a haemocytometer counting chamber where the cells are manually counted on the microscope.

With the advent of newer technologies for cell counting, assessing cell number has now changed. The nature of the proteoglycans makes microbore based cell counting techniques tricky but computerised chamber counting methodologies are highly practical and accurate. These automated methods, although in themselves more costly in terms of initial investment and consumables, can make SF analysis quicker and more accurate than the manual method, and, by reducing the amount of biomedical scientist time required, reduce the overall cost of the test.

The number of nucleated cells contained in the SF is the primary indicator of inflammation. Where the cell count is less than $500/mm^3$ the patient can be assumed to have a non-inflammatory arthropathy, whereas a cell count greater than $1000/mm^3$ indicates an inflammatory arthropathy. If between these figures, the percentage of neutrophils in a differential count distinguishes inflammatory from noninflammatory arthropathies, where more than 50% of the nucleated cells in the sample being polymorphs indicates inflammation.

Wet preparation

The "wet prep" is an important part of the examination of SF and can often be relied upon to give a definitive diagnosis. Two preparations are made each serving a different purpose. Firstly using a glass Pasteur pipette for clarity, an aliquot of SF is removed from the container and by slowly returning the SF to the container visible particles of fibrin, cartilage or crystal aggregates can be seen in the thin part of the pipette and placed onto a microscope slide, this we call the "thick preparation". It can be of great help if the viewing background provides contrast, for example fragments of cartilage are most easily seen against a dark background whereas prosthetic debris is most easily seen against a pale background, white laboratory coats and dark laboratory bench surfaces usually provide this contrast. It is of great importance that any pieces of fibrin clot are found, as frequently identification of crystal arthropathies with a low crystal burden relies on finding crystals trapped in the fibrin clot.

A second, much thinner preparation, is made avoiding particulate matter and using only a few microlitres of SF. Coverslipping this preparation flattens the cells and allow intracytoplasmic inclusions to be identified. Large inclusions with specific refractile properties characterise a functional group of cells called ragocytes. Ragocytes have diagnostic significance (see below).

"Wet preps" can be preserved for 24 hours by painting around the edge of the coverslip with nail varnish to minimise evaporation. This also helps provide additional safety features, as the SF is a potential infection hazard.

Thick preparation examination

Crystals

When screening for crystals it is of the greatest importance that aggregates of fibrin and other particles are included in the slide preparation, as it is these micro clots that will often contain the

