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SEED Body fluids

Synovial fluid – part 2: laboratory evaluation

In part 1 of this SEED article on synovial fluid the anatomy and composition of synovial fluid was described together with the indications for analysis, plus some useful tips on specimen collection and handling. This second part describes the common laboratory analyses.

Laboratory evaluation

Similar to the processing of other body fluid samples, common laboratory evaluation of synovial fluid involves the following steps:

1. Physical examination
2. Cellular analysis – automated or microscopically
3. Chemical analysis
4. Microbiological tests
5. Serologic tests

1. Physical examination

Colour and clarity

A report of the overall appearance is an essential part of synovial fluid analysis. Normal synovial fluid appears clear and colourless to pale yellow (Fig. 1 left image). The colour becomes a deeper yellow in the presence of non-inflammatory effusions (Fig. 1 centre image) and may have a greenish tinge with bacterial infection (Fig. 1 right image).

If blood is present, the colour ranges from red to brown or xanthochromatic. One must distinguish between blood stemming from haemorrhagic arthritis and traumatic aspiration. This is accomplished primarily by observing the uneven distribution of blood typical of specimens obtained from traumatic aspiration.

Turbidity is a strong indicator for inflammatory conditions. Cloudy yellow samples point to inflammation mainly due to the presence of white blood cells (WBC); however, synovial cell debris and fibrin also produce turbidity. The fluid may appear whitish and milky when crystals are present.

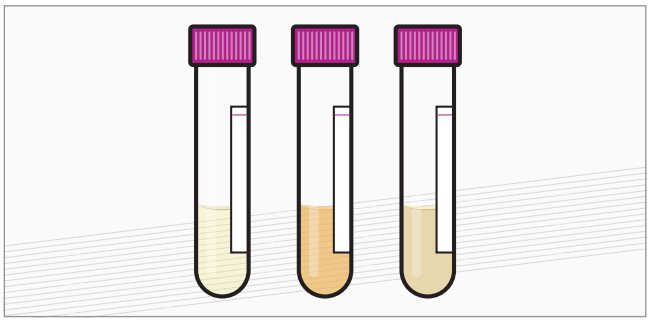

Fig. 1 *The visual appearance of synovial (or: joint) fluid*

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Viscosity

The viscosity of synovial fluid derives from the polymerisation of hyaluronic acid and is essential for the proper lubrication of the joints. Arthritis affects both the production of hyaluronic acid and its ability to polymerise, thus decreasing the viscosity of the fluid. Several methods are available to measure the viscosity of the fluid. The simplest is to observe the ability of the fluid to form a string from the tip of a syringe, a test that can be done at the bedside. A string that measures 4 – 6 cm is considered normal.

A semi-quantitative measurement of the precipitation can be performed using the mucin clot test, also known as 'Ropes test'. When added to a solution of 2–5 % acetic acid, normal synovial fluid forms a solid clot surrounded by clear fluid. As the ability of the hyaluronic acid to polymerise decreases, the clot becomes less firm, and the surrounding fluid increases in turbidity. The mucin clot test is reported in terms of 'good' (solid clot), 'fair' (soft clot), 'low' (friable clot), and 'poor' (no clot). The mucin clot test is not routinely performed anymore, because all forms of arthritis decrease viscosity and little diagnostic information is obtained. The formation of a mucin clot following the addition of acetic acid can be used to identify a questionable fluid as synovial fluid.

Very viscous fluids may need to be pre-treated for further analysis by adding 400 units of the enzyme hyaluronidase to 1 mL of fluid and incubating the mixture at 37° C for 10 minutes [1].

2. Cellular analysis

Cell counts

For cell counting, the specimen should be anticoagulated, either using heparin or EDTA. The total WBC count is the most frequently performed cell count from synovial fluid. Red blood cell (RBC) counts are not often requested. The available literature shows that usually the total WBC count decreases over time, which may produce misleading results preventing a correct patient diagnosis [2]. Therefore, it is of utmost importance to run these analyses without delay once samples have been obtained. Generally speaking, once obtained, it is important to analyse aspirated synovial fluid samples as quickly as possible to avoid spurious results. In particular the WBC count and differential should ideally be performed on fresh specimens.

Manual counts from thoroughly mixed specimens of synovial fluid are performed using the Neubauer counting chamber in the same manner as for cerebrospinal fluid (CSF) counts. Usually clear fluids can be counted without diluting them, but dilutions are necessary when fluids are turbid or bloody. If it is necessary to lyse the RBC prior to counting, hypotonic saline (0.3 %) or saline containing saponin are suitable diluents. Methylene blue added to isotonic saline will stain the WBC nuclei, permitting to distinguish between RBC and WBC when counting specimens that contain RBC.

Despite optical microscopy still being considered the reference technique for WBC enumeration, many drawbacks have been emphasised. These include high cost, low throughput, long turn-around time, lack of inter-laboratory harmonisation, high imprecision (especially in samples with low cell concentrations) and need of specialised staff performing the analysis [3, 4].

Latest performance evaluations confirm that automated counting of WBC in synovial fluid displays excellent performance, which makes it a reliable and practical alternative to optical microscopy [3–5]. The majority of the manufacturers of the analysers available in the market also provide quality control material to verify a high precision of their cell counts.

Differential

Differential WBC counts are generally performed from cyto-centrifuge preparations or on thinly smeared slides followed by May-Grünwald-Giemsa staining.

Approximately 50 % of the nucleated cells are monocytes, 25 % lymphocytes and the rest is made up of neutrophils, macrophages and synovial lining cells. The evidence regarding the use of the WBC and differential counts in synovial fluid analysis varies. The findings of the total WBC count and its differential differ noticeably, but in general, the majority of textbooks and publications emphasises that the combination of WBC and polymorphonuclear cell (PMN) counts are important diagnostic markers for a fast discrimination of non-inflammatory from inflammatory and septic disorders. Yet, both WBC and PMN alone are limited in distinguishing between these specific disease categories because of their wide and partially overlapping distributions. Today, many textbooks and publications quote the following traditional classification system composed by the American Rheumatism Association [4]:

- Normal: WBC < 200 x 10^6/L, PMN < 25 %
- Non-inflammatory: WBC < 2,000 x 10^6/L, PMN < 25 %
- Inflammatory: WBC 2,000 – 50,000 x 10^6/L, PMN > 50 %
- Septic: WBC > 50,000 x 10^6/L, PMN > 75 %

The most frequently encountered cells and inclusions in synovial fluid are summarised in Table 1 [6]. When reviewing both normal and abnormal specimens under the microscope, one should bear in mind that cells may appear more vacuolated than they do on a blood smear.

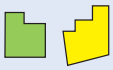
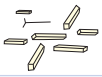
Crystal identification

The microscopic examination of synovial fluid using polarised light to detect the presence of crystals is an important diagnostic test in the evaluation of arthritis. Crystal formation in a joint frequently results in an acute, painful inflammation. It can also become a chronic condition. Causes of crystal formation include metabolic disorders and decreased renal excretion rates that produce elevated blood levels of crystallising chemicals, the degeneration of cartilage and bone, and the injection of medications, such as corticosteroids, into a joint. Table 2 [6] presents the crystals most commonly seen in synovial fluid.

Table 1 *Cells and inclusions seen in synovial fluid*

Cell/inclusion	Description	Increased levels with:
Cartilage cells	Large, multinuclear cells	Osteoarthritis
Fat droplets	Refractile intracellular and extracellular globules that can be stained with Sudan dyes	Traumatic injury and chronic inflammation
Haemosiderin	Inclusions within clusters of synovial cells	Pigmented villonodular synovitis
LE cell	Neutrophil containing a characteristic ingested 'round body'	Lupus erythematosus
Lymphocyte	Mononuclear white blood cell	Non-septic inflammation
Macrophage (monocyte)	Large mononuclear white blood cell that may be vacuolated	Viral infections
Neutrophil	Polymorphonuclear white blood cell	Bacterial sepsis, crystal-induced inflammation
Reiter cell	Vacuolated macrophage with ingested neutrophils	Reiter syndrome, non-specific inflammation
Rheumatoid arthritis cell (ragocyte)	Polymorphonuclear phagocyte with dark cytoplasmic granules containing aggregated immunoglobulins, fibrin, complement and rheumatoid factor	Rheumatoid arthritis, immunologic inflammation
Rice bodies	Macroscopically resembling polished rice, they show collagen and fibrin	Tuberculosis, septic and rheumatoid arthritis
Synovial lining cell	Similar to macrophage,but may be multinuclear	Always physiological

Table 2 *Characteristics of crystals found in synovial fluid*

Crystal	Shape		Cause
Monosodium urate (MSU)		Needles	Gout
Calcium pyrophosphate		Rhombic squares and rods	Pseudogou
Cholesterol		Notched, rhombic plates	High levels of blood cholesterol
Corticosteroid		Flat, variably shaped plates	Injections
Calcium oxalate		Envelopes	Renal dialysis
Apatite (calcium phosphate)		Small particles	Osteoarthritis

3. Chemical analysis: glucose, protein, uric acid

The most frequently requested test is glucose determination, as markedly decreased values are indicative of inflammatory or septic disorders. Since under normal conditions synovial fluid glucose values are related to the blood glucose level, blood and synovial fluid samples should be simultaneously obtained, preferably after the patient has fasted for eight hours to allow equilibration between the two fluids. Under these conditions, a normal synovial fluid glucose level should not be more than 10 mg/dL below the plasma value. To prevent falsely decreased values caused by glycolysis, specimens should be analysed within one hour of collection or preserved with sodium fluoride [6].

Synovial fluid contains all proteins found in plasma, except for various high-molecular weight proteins, such as fibrinogen, beta-2-macroglobulin and alpha-2-macroglobulin. Most commonly used serum protein procedures can be used to measure synovial fluid protein. The range for synovial fluid protein is 1–3 g/dL [6]. Increased protein levels are found in inflammatory and haemorrhagic disorders; however, measuring synovial fluid protein does not contribute greatly to the classification of these disorders.

An elevated level of serum uric acid in cases of gout is well known; therefore, the demonstration of an elevated uric acid level in synovial fluid may be used to confirm the diagnosis when the presence of crystals cannot be demonstrated in the fluid. The measurement of serum uric acid is often performed as a first evaluation with suspected cases of gout.

4. Microbiological tests [6]

An infection may occur as a secondary complication of inflammation caused by trauma or through dissemination of a systemic infection; therefore, Gram stains and cultures are two of the most important tests performed on synovial fluid. Both tests must be performed on all specimens because some organisms will be missed if only Gram staining is carried out. Bacterial infections are most frequently seen; however, fungal and viral infections may occur too. When they are suspected, special culturing procedures should be used. Patient history and other symptoms can aid in requesting additional testing.

Routine bacterial cultures should include an enrichment medium, such as chocolate agar, because besides *Staphylococcus* and *Streptococcus*, the common organisms that infect synovial fluid are the fastidious *Haemophilus* species and *Neisseria gonorrhoeae.*

5. Serologic tests [6]

Because joint disorders often have an immunologic component, serologic testing plays an important role in their diagnosis. However, the majority of the tests are performed on serum, with the actual analysis of synovial fluid merely serving as a confirmatory measure in cases that are difficult to diagnose. The autoimmune diseases rheumatoid arthritis and lupus erythematosus cause very serious inflammation of the joints and are diagnosed by demonstrating the presence of specific autoantibodies in the patient's serum. The same antibodies can also be determined in the synovial fluid, if necessary. Arthritis is a frequent complication of Lyme disease. Therefore, demonstrating the presence of antibodies to the causative agent Borrelia burgdorferi in the patient's serum can confirm this cause of arthritis. The extent of inflammation can be determined by measuring the concentration of acute phase reactants, such as fibrinogen and C-reactive protein (CRP).

Conclusions

- Laboratory testing of synovial fluids is complex and requires skilled staff. Poor standards of synovial fluid analysis may be partly due to not including it in routine pathology services, as well as the relatively low throughput of such samples in most units.
- Automating laboratory procedures can contribute to improving the standardisation of testing performance and reduce not only the turnaround time but also transcription errors [3, 4, 7].

References

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