Clinical Pathology of Synovial Fluid

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ABSTRACT

Examination of synovial fluid should be performed in a systematic manner so as to derive the maximum of information. Synovial fluids should be divided into (1) non-inflammatory, (2) inflammatory, (3) purulent and (4) hemorrhagic types. In addition to general description, analysis should include mucin clot test, fibrin clot formation, microscopic examination for cell count and differential cell count, microscopic examination for crystals of gout and pseudogout and microbiological examination. Chemical examination should include estimation of glucose and uric acid. Immunochemical examination may include determination of immunoglobulins, antinuclear factor and LE factor.

Introduction

Although specific laboratory tests useful in the diagnosis of various forms of arthritis are generally lacking, intelligent appraisal of available laboratory procedures will permit an accurate diagnosis in a high percentage of cases. Synovial fluid analysis is unfortunately approached too frequently in a "hit or miss" fashion and, consequently, yields disappointing results of limited clinical value. However, when done in a systematic manner and when used in conjunction with other laboratory tests, a maximum of information can be obtained of diagnostic importance.1,9,10,11,13,23,24,25,28

Classification of Joint Diseases

On the basis of gross and microscopic examination of the synovial fluid, categories of joint fluid shown in table I can be distinguished.14,23,24,25 Thus synovioanalysis will permit categorization of a particular patient with joint disease into four broad groups suggesting further laboratory analysis or clinical investigation.

Collection of Specimen

No attempt will be made to describe the actual technique of aspiration of synovial fluid. Abnormal synovial fluid may clot spontaneously and therefore should be anticoagulated either with ethylenediaminetetraacetic acid (EDTA) or heparin.14,23,24,25 Oxalate anticoagulants should preferably not be used since calcium oxalate crystals are formed which may interfere with the identification of crystals. When the synovial fluid is unusually viscid, the high viscosity may cause difficulties in the performance of tests for rheumatoid factor, electrophoresis and chemical tests. The fluid may be digested with testicular hyaluronidase for a period of four hours prior to such analytical procedures in order to depolymerize the hyaluronides responsible for the viscosity.
TABLE I
Classification of Joint Diseases

<table>
<thead>
<tr>
<th>Group</th>
<th>Diseases</th>
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<tbody>
<tr>
<td>Normal</td>
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<td>I. Non-inflammatory (Group I)</td>
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<tr>
<td>Osteoarthritis</td>
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<td>Traumatic arthritis</td>
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<td>Avascular necrosis</td>
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<td>Osteochondralacia</td>
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<td>Hypertrophic osteoarthropathy</td>
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<td>Osteochondritis dissecans</td>
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<td>Systemic lupus erythematosus</td>
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<td>Early rheumatoid arthritis</td>
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<td>Chronic crystal synovitis</td>
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<td>II. Inflammatory (Group II)</td>
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<tr>
<td>Rheumatoid arthritis</td>
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<tr>
<td>Acute pseudogout</td>
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<td>Ankylosing spondylitis</td>
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<td>Psoriatic arthritis</td>
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<td>Acute rheumatic fever</td>
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<td>Serum sickness</td>
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<td>III. Purulent (Group III)</td>
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<tr>
<td>Bacterial infections</td>
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<td>IV. Hemorrhagic (Group IV)</td>
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<tr>
<td>Traumatic arthritis</td>
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<tr>
<td>Neuroarthropathy (Charcot's joint)</td>
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<tr>
<td>Hemophilic arthropathy</td>
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<tr>
<td>Pigmented villonodular synovitis</td>
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</table>

Dorner et al. have suggested the use of ultrasound in reducing viscosity.

As a routine procedure, the following schema is suggested:

1. Microbiological examination. Two ml of joint fluid are placed in soy broth or other appropriate broth. An aliquot may be used for fungus cultures or acid fast cultures using the appropriate concentration techniques. Smears are made for routine Gram stains, acid fast stains or fungus stains as indicated. If gonorrheal arthritis is suspected, the synovial fluid is placed immediately on a chocolate agar plate or Thayer-Martin medium.

2. White cell count. Use white blood cell pipet and appropriate diluting fluid.

3. Microscopic wet smear examination. One or two drops are placed on a slide and covered with a cover slip.

4. Remainder of the fluid may be used for various chemical and immunological analyses.

Gross Appearance

An evaluation of the gross appearance of the synovial fluid immediately on withdrawal from the joint space is easily and quickly done and will frequently dictate the future direction of the analysis. Volume and color should be recorded. Normal synovial fluid is clear, colorless or pale yellow and viscid. It does not normally clot spontaneously after removal. The hemorrhagic fluid of a traumatic arthritis or hemophilic arthropathy must be distinguished from the streaks of bloody fluid within an otherwise nonbloody fluid signifying a traumatic tap. Clarity is best evaluated by transillumination or by attempting to read print through a tube of fluid. Turbidity is usually proportional to the leukocyte or red blood cell count. Viscosity can easily be judged with a little experience by the length of the string it forms when the fluid is expressed through the aspirating needle. It can also be easily evaluated by placing a drop between the thumb and index finger and noting the string produced when they are separated. An inflammatory fluid of low viscosity forms a very short string whereas normal or noninflammatory fluid produces a long string of 3 cm or more. A more accurate determination of viscosity may be obtained by use of a viscosimeter. Viscosity is, in general equivalent to the mucin hyaluronate content.

Mucin Clot Formation

This test is performed by mixing two parts of synovial fluid with one part of 3 percent acetic acid. The character of the clot is interpreted in two hours as (1) good (a firm ropy clot with a clear supernatant fluid); (2) fair (a soft clot with a slightly turbid supernatant fluid); (3) poor (a friable small clot with a turbid supernatant fluid); (4) very poor (a few flecks of precipitate in a cloudy supernatant fluid). Normally, the mucin clot
test is positive, i.e., a firm mucin clot is formed. 9, 10, 11, 15, 23, 24, 25, 28

Fibrin Clot Formation

Normal synovial fluid will not clot spontaneously because it does not contain fibrinogen. The development of a clot spontaneously in an unanticoagulated specimen indicates the presence of fibrinogen owing to inflammatory changes in the synovial fluid. 9, 23, 24, 25 The clotting phenomenon is graded from 1+ to 4+. A large firm fibrin clot which forms soon after aspiration is indicative of a 4+ reaction. The formation of a small soft clot at the end of an hour is designated as a 1+ reaction. The tendency of synovial fluid to clot spontaneously can be related directly to the severity of the inflammatory reaction of the synovial tissue.

Microbiological Examination

An aliquot of synovial fluid is submitted to the microbiology laboratory when bacterial or mycotic etiological agents are suspected. Any synovial fluid specimen having a white blood cell count which is high, which clots spontaneously owing to fibrin formation, or which has a negative mucin clot test should be examined bacteriologically. 10, 11 Preliminary smears are made for Gram stains, acid fast stains and fungus stains. A wet mount with lactophenol cotton blue or with 20 percent potassium hydroxide may be made if a fungal etiology is suspected. Two ml of fluid are inoculated into soy broth for routine examination and after 24 to 48 hours is subcultured into appropriate solid media. If a gonorrheal arthritis is suspected, the material is plated on warm chocolate agar or on Thayer-Martin medium and incubated under 6 percent carbon dioxide. Preferably, plating should be done promptly at the bedside because of the fastidious nature of the Gonococcus. Acid fast stains and acid fast cultures after centrifugation and sedimentation of the fluid should be obtained if tuberculous arthritis is suspected. Inoculation of mycologic media is indicated if there is a suspicion of a mycotic infection. Although rarely anaerobic organisms may be involved, the failure to recover a bacterial organism by conventional techniques may require the inoculation of anaerobic culture media such as thioglycollate media or chopped meat glucose broth under anaerobic conditions. Special cultures, such as those for viral or mycoplasmal organisms, may occasionally be indicated.

Microscopic Examination

The acetic acid solution used for ordinary peripheral white blood cell counting is not satisfactory as a diluting fluid for cell counts since a mucin clot will be formed with the acetic acid. Isotonic saline solution or the regular red blood cell diluting fluid (Hayem's solution) may be used. Red cells and white cells may be counted separately. The counting technique is identical to that used for the leukocyte count in peripheral blood.

Differential cell counts may be done on a smear stained with Wright's stain or Giemsa stain. 10, 29 Unless the specimen is distinctly turbid with a high total cell count, it is advisable to centrifuge the fluid and concentrate the cells, resuspending them in an appropriate amount of isotonic saline solution or of synovial fluid.

Leukocytes of synovial fluid from patients with rheumatoid arthritis have been shown to have cytoplasmic granules or inclusions. 1, 11, 17, 20, 28 Studies by immunofluorescence techniques have identified components of these inclusions as IgG and IgM immunoglobulins. Rheumatoid factor has also been obtained from disrupted synovial leukocytes. 1 The synovial fluid in pseudogout, traumatic arthritis and osteoarthritis may also rarely contain intraleukocytic cytoplasmic inclusions.

The examination of a drop of fresh uncentrifuged synovial fluid may reveal valuable information on the differential diagnosis of
CLINICAL PATHOLOGY OF SYNOVIAL FLUID

arthritis. A drop of fluid is placed on a slide, is covered with a cover slip and the edges sealed with petroleum jelly, clear nail polish or wax to prevent evaporation and drying. Under low power examination an estimate of the number of cells can be made as well as the general distribution of cellular elements. The preparation may be viewed through a regular light microscope (with the light appropriately reduced by lowering the condenser and closing the iris diaphragm) or preferably through a phase contrast microscope.

As indicated, oxalate must not be used as an anticoagulant because of the possibility of recrystallization of the oxalate producing confusion with other clinically important crystals. The value of examination of a wet mount of synovial fluid may be of crucial diagnostic importance in the differentiation of the crystal-induced synovitides, gout and pseudogout.7'10'11'13'14'17 Under polarized light, monosodium urate crystals diagnostic of gout are seen as needle-like or rod-like structures which show strong negative birefringence. Calcium pyrophosphate crystals diagnostic of pseudogout or articular chondrocalcinosis are rod-like or plate-like and show weak negative birefringence.

Occasionally cholesterol crystals may appear in joint or bursa fluid, indicating chronicity of the effusion or cell necrosis.14 Steroid crystals may occasionally be seen in synovial fluid after intra-articular injection of corticosteroids.

Chemical Analysis

Glucose concentration of synovial fluid may be of considerable aid in cases of difficulty in differential diagnosis. Generally decreased levels of synovial fluid glucose will be associated with Group II and III fluids, i.e., in the inflammatory types of synovial disease. The difference in glucose concentration between blood and synovial fluid in inflammatory conditions may be the result of decreased glucose transport into the joint or may be the result of increased glucose utilization by tissue cells, leukocytes and, possibly, microorganisms. simultaneous samples of blood and synovial fluid must be obtained with the patient fasting preferably for a period of 12 hours. This precaution is necessary owing to formal time lag of glucose equilibration between the two compartments. If samples are taken too soon after ingestion of carbohydrate, the lag in glucose transfer to the joint space or exit from the joint space may cause an exaggeration in differential concentration between the two compartments.

The determination of synovial fluid protein,2,21,28 protein electrophoresis,2,12 A1-A2 globulin ration,2 haptoglobin218 and various immunoglobulins,12,19,21 while demonstrating differences in concentration between non-inflammatory vs. inflammatory and non-rheumatoid vs. rheumatoid fluids, usually do not reveal data of sufficient diagnostic value for the individual patient. Generally, comparison of the serum/synovial fluid ratios of various proteins indicates that in rheumatoid synovial fluids all proteins are present in higher concentrations than in the synovial fluid of degenerative joint disease.21

The synovial fluid uric acid level may be of considerable diagnostic value. The joint fluid uric acid is considered by some22 as a better single diagnostic factor than the serum uric acid level. A joint fluid uric acid level significantly above the serum uric acid level is considered as diagnostic of gout.

Studies on joint fluid zinc,3 acid phosphatase,16 alkaline phosphatase,16 haptoglobin,2,18 lactic dehydrogenase,5 glutamic oxalacetic transaminase5 and other components have been carried out. The concentration of iron in the synovial membrane in patients with rheumatoid arthritis has been found to be consistently high.26

In a study of zinc concentration in biological fluids from normal persons and patients with rheumatoid arthritis, the latter were found to have a significantly increased concentration of zinc in synovial fluid,
normal zinc concentration in serum and significantly increased urinary zinc concentration. Other studies demonstrated elevated acid phosphatase activity in the synovial fluid of many patients with rheumatoid arthritis. The suggestion has been made that the presence of acid phosphatase in increased concentration may be a reflection of chondroclastic activity and, therefore, be a measure of active articular destruction.

In the same study, alkaline phosphatase activity was not noted to be increased in inflammatory arthritides. Lactic dehydrogenase activity of the synovial fluid but not of serum has been described as elevated in patients with rheumatoid arthritis, infectious arthritis and gout but normal in fluids of patients with degenerative joint disease. Glutamic oxalacetic transaminase activity was described as normal in both serum and synovial fluids in both non-inflammatory and inflammatory arthritis.

With the exception of glucose and uric acid estimation, chemical analysis of synovial fluid usually does not yield significant information not obtainable by simpler tests used in synovianalysis.

Immunochemical Analysis

Changes occurring in the concentration of immunoglobulins have been alluded to. Serum IgG and IgA and synovial fluid IgG antigammaglobulin levels have been described as significantly higher in patients with rheumatoid arthritis than in other individuals with highest levels occurring in patients with a positive latex fixation test. IgM antigammaglobulins were elevated only in patients with latex positive rheumatoid arthritis. Increased serum and synovial fluid levels of IgG and IgM antigammaglobulins were associated with diminished serum and synovial fluid complement levels.

In another study an increase of immunoglobulin IgG was found in 15 percent of patients with rheumatoid arthritis, IgA in 21 percent and IgM in 6 percent. In rheumatoid synovial fluids, all proteins tested for were present in higher concentration than in the synovial fluids of degenerative joint disease. In one patient with rheumatoid arthritis, a non-myeelomatous monoclonal IgG—lambda gam-mopathy in the synovial fluid is described.

In patients with systemic lupus erythematosus, Wright's stained smears of the cells of synovial fluid nearly always show numerous lupus erythematous cells of typical morphology. No incubation techniques are necessary since incubation has already taken place in the joint space.

Antinuclear factor (ANF) of one or more immunoglobulin classes may be identified in the synovial fluid of patients with rheumatoid arthritis. ANF may also be demonstrated in the serum of similar patients.

Exfoliative Cytology

Cytologic examination for tumor cells has not been extensively studied perhaps because of the rarity of invasion of the joint space by tumor. Smears may be prepared by the usual technique and stained by the method of Papanicolaou. Millipore preparations may be utilized although the extreme viscosity of the specimen may preclude use of this technique. In general, exfoliative cytology offers little additional information except in the rare instance where tumor invasion of the synovial cavity is suspected.

Conclusions and Summary

Synovianalysis done in a systematic way is capable of yielding information of value in the differential diagnosis of arthritis. The basic panel of synovianalysis includes general appearance, volume, viscosity, mucin clot test, fibrin clot test, cell count and examination of a wet mount for cell types and crystal identification. Optional tests which may yield diagnostic information
include microbiological investigation for bacteria, acid fast organisms or fungi. Chemical examination may include determination of synovial fluid glucose and uric acid. The identification of antinuclear antibody, rheumatoid factor and LE cell phenomenon may add information of crucial diagnostic importance.

References