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  - The web

Chapter 14
Synovial Fluid
Professor A. S. Alhomida

Physiology and Composition

- **Movable Joints (diarthroses)**
  - Composed of
    1. Bones lined with articular cartilage
    2. Separated by a cavity containing synovial fluid enclosed in a synovial membrane
Physiology and Composition, Cont’d

• **Synovial Membrane**
  1. Synoviocytes
     • Phagocytic: synthesizes degradative enzymes
     • Synthesizes hyaluronate
  2. Connective tissue
     • Blood vessels, lymphatics and nerves

Physiology and Composition, Cont’d

• **Fluid Formation**
  1. Ultrafiltrate of plasma across synovial membrane
     • Non selective
     • Excludes proteins of high molecular weight
  2. Synoviocytes
     • Secrete mucopolysaccharide which contains:
       • Hyaluronic acid
       • Protein
Physiology and Composition, Cont’d

- **Cartilage and Fluid Function**
  1. Reduce friction between bones
  2. Lubricates joints
  3. Fluid provides nutrients to cartilage
  4. Lessens shock of walking and jogging impact

### Synovial Fluid: Normal Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>&lt;3.5 mL</td>
</tr>
<tr>
<td>Color</td>
<td>pale yellow</td>
</tr>
<tr>
<td>Clarity</td>
<td>clear</td>
</tr>
<tr>
<td>Viscosity</td>
<td>forms string 4-6 cm long</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>&lt;2000 cells/uL</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&lt;200 cells/uL</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;20% of diff.</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>&lt;15% of diff.</td>
</tr>
<tr>
<td>Monocytes &amp; macrophages</td>
<td>65% of diff.</td>
</tr>
<tr>
<td>Crystals</td>
<td>NONE</td>
</tr>
<tr>
<td>Glucose</td>
<td>&lt;10 mg/dL (&lt; blood glucose)</td>
</tr>
<tr>
<td>Lactate</td>
<td>&lt;250 mg/dL</td>
</tr>
<tr>
<td>Total protein</td>
<td>&lt;3 g/dL</td>
</tr>
<tr>
<td>Uric acid</td>
<td>= blood value</td>
</tr>
</tbody>
</table>
• **Arthrocentesis**
  1. It is the method for obtaining synovial fluids by using a needle aspiration of synovial fluid
  2. Volume
     - Normal = 3.5 mL
     - Diseased and inflamed = up to 25 mL

**Specimen Collection, Cont’d**

3. Collect 2 tubes
   - Heparin tube: microbiology
   - Plain top: chemistry and immunology
   - EDTA (liquid): hematology
   - Avoid all powdered anticoagulants – interfere with crystal analysis
Specimen Collection, Cont’d

- **Fluid Verification**
  1. Mucin clot test
     - Add fluid to dilute acetic acid → turbidity (clot formation) due to hyaluronate
  2. Metachromatic staining
     - Place fluid on filter paper + few drops of toluidine blue → metachromatic staining

Physical Examination

- **Color**
  1. Normal: clear, pale yellow
  2. Red to brown: indicates trauma of procedure or disorder
  3. Turbidity: associated with presence of WBCs
  4. Milky: may indicate presence of crystals
Physical Examination, Cont’d

• **Viscosity**
  1. Measured at bedside by ability to form a string from tip of syringe
  2. Normal: 4-6 cm

• **Ropes Test (Mucin Clot Test)**
  • Measurement of hyaluronate polymerization
    1. Fluid forms a clot surrounded by clear fluid when added to acetic acid
    2. Clot quality is reported:
       • Good = solid clot
       • Fair = soft clot
       • Poor = friable clot
       • Very poor = no clot
  3. Test is of questionable precision and seldom used
Specimen Collection, Cont’d

Synovial Fluid

The tougher the “clot”, the less hydrolyzed the hyaluronic acid.

- clear fluid, solid clot
- cloudy fluid, solid clot
- friable clot
- no clot

- normal
- osteoarthritis
- trauma
- bleed

Inseros
F.I.A.
Peters

Synovial Joint

Diagram of a synovial joint.
Synovial Joint, Cont’d

<table>
<thead>
<tr>
<th>Group Classification</th>
<th>Pathologic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Noninflammatory</td>
<td>Degenerative joint disorders; including Arthritic arthritis and hyperlymphocytosis</td>
</tr>
<tr>
<td>II. Inflammatory</td>
<td>Infectious joint disorders; including Ankylosing spondylitis or ulcerative arthritis</td>
</tr>
<tr>
<td>III. Septic</td>
<td>Septic arthritis; including Staphylococcal or Streptococcal arthritis</td>
</tr>
<tr>
<td>IV. Traumatic</td>
<td>Traumatic arthritis; including Fracture or dislocation arthritis</td>
</tr>
</tbody>
</table>

Laboratory Finding in Joints

<table>
<thead>
<tr>
<th>Group Classification</th>
<th>Laboratory Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Noninflammatory</td>
<td>Bloody yellow fluid</td>
</tr>
<tr>
<td></td>
<td>White blood cell count &lt; 10,000</td>
</tr>
<tr>
<td></td>
<td>Neutrophils &lt; 50%</td>
</tr>
<tr>
<td>II. Inflammatory</td>
<td>Bloody yellow fluid</td>
</tr>
<tr>
<td></td>
<td>White blood cell count 10,000-20,000</td>
</tr>
<tr>
<td></td>
<td>Neutrophils &gt; 50%</td>
</tr>
<tr>
<td></td>
<td>Decreased glucose level</td>
</tr>
<tr>
<td></td>
<td>Enzyme levels increased</td>
</tr>
<tr>
<td>III. Septic</td>
<td>Bloody yellow-green fluid</td>
</tr>
<tr>
<td></td>
<td>White blood cell count &gt; 20,000</td>
</tr>
<tr>
<td></td>
<td>Neutrophils &gt; 50%</td>
</tr>
<tr>
<td></td>
<td>Decreased glucose level</td>
</tr>
<tr>
<td>IV. Traumatic</td>
<td>Bloody yellow-green fluid</td>
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</tr>
<tr>
<td></td>
<td>Enzyme levels increased</td>
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</table>
Microscopic Examination

- **Cell Count**
- **WBC**
  - Method
    1. Use Neubauer counting chamber
    2. May pretreat viscous fluids with hyaluronidase and incubate at 37°C for 5 min
    3. Dilution with hypotonic saline is used to lyse any RBC or
    4. Dilute with normal saline/methylene blue mixture to differentiate WBCs from RBC
  - Normal = < 200/µL

Microscopic Examination, Cont’d

- **Standard Neubauer Calculation**
  Formula used for blood cell counts

\[
\text{Number of cells} = \frac{\text{# of cells counted} \times \text{dilution}}{\text{# of squares counted} \times \text{volume of 1 square}} = \frac{\text{cells}}{\mu L}
\]
Microscopic Examination, Cont’d

- **Differential Count**
  1. Cytocentrifuge specimen and prepare typical blood smear
  2. Normal: 60% monocytes, macrophages
     - Neutrophils: <20%
     - Lymphocytes: <15%
     (* values vary between texts)

3. Increased neutrophils: possible septic condition
4. Increased lymphocytes: indicate nonspecific inflammation
Microscopic Examination, Cont’d

• Other Cell Abnormalities
  1. Increased eosinophils: rheumatic fever, parasitic infections, metastatic carcinoma, post radiation therapy or arthrography
  2. LE cells: patients with lupus erythematosus
  3. Reiter cells: macrophages with ingested neutrophils
  4. RA cells (ragocytes): precipitated rheumatoid factor appearing as cytoplasmic granules in neutrophils

Microscopic Examination, Cont’d

  5. Hemosiderin granules: due to hemorrhagic process or cases of pigmented villonodular synovitis
  6. Cartilaginous cells: observed in cases of osteoarthritis
  7. Rice bodies: found in septic and rheumatoid arthritis and Tuberculosis
  8. Fat droplets: indicate traumatic injury
Cells and Inclusions in Synovial Fluid

<table>
<thead>
<tr>
<th>Cell/Inclusion</th>
<th>Description</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>Polymorphonuclear</td>
<td>Bacterial sepsis</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Leukocytic</td>
<td>Crystal-induced inflammation</td>
</tr>
<tr>
<td>Macrophage/nodecct</td>
<td>Mononuclear</td>
<td>Nonseptic inflammation</td>
</tr>
<tr>
<td>Synovial lining cell</td>
<td>Similar to macrophage, but may be multinucleated, resembling a mononuclear cell</td>
<td>Normal</td>
</tr>
<tr>
<td>IL cell</td>
<td>Neutrophil containing characteristic of normal neutrophil.</td>
<td>Normal</td>
</tr>
<tr>
<td>Resin cell</td>
<td>Neutrophil with dark cytoplasm</td>
<td>Lupus erythematosus</td>
</tr>
<tr>
<td>RA cell (macrophage)</td>
<td>Reactive synovial cell</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Cartilage cells</td>
<td>Large, multinucleated cells</td>
<td>Synovial inflammation</td>
</tr>
<tr>
<td>Rice bodies</td>
<td>Macrophage-like, goblet-like</td>
<td>Tuberculous, septic and rheumatoid arthritis</td>
</tr>
<tr>
<td>Fat droplets</td>
<td>Stains with Sudan stains</td>
<td>Traumatic injury</td>
</tr>
<tr>
<td>Hemosiderin</td>
<td>Iron within plasma cells</td>
<td>Pigmented cells with inclusion</td>
</tr>
</tbody>
</table>

Synovial Lining Cell
Lymphs in Synovial Fluid

Microscopic Examination, Cont’d

- **Crystals**
  1. Microscopic examination of synovial fluid for the presence of crystals is an important diagnostic test in the evaluation of arthritis
  2. Crystal formation in a joint frequently results in an acute, painful inflammation
Microscopic Examination, Cont’d

- **Crystals Formation**
  - Crystal formation may be due to:
    1. Metabolic disorders
    2. Decreased renal excretion
    3. Cartilage and bone degeneration
    4. Medicinal injection (ex: corticosteroids)

Microscopic Examination, Cont’d

- **Fluid is Examined Using the Wet Preparation Technique**
  1. ASAP examination as pH and temperature affect observation
  2. Ideally examined prior to WBC disintegration
  3. Examine under both direct and compensated polarizing light
  4. May also be observed in Wright stain preparations
Microscopic Examination, Cont’d

• Under Polarizing Light (Direct Polarization)
  • Birefringent substances appear as bright objects on a black background

Microscopic Examination, Cont’d

• Under Compensated Polarizing Light
  1. A red compensator plate is placed between the crystal and slide
  2. Crystals aligned parallel to the compensator appear yellow (negative birefringence)
  3. Crystals aligned perpendicular to the compensator appear blue (positive birefringence)
Polarized Light

Synovial Crystals

- **Monosodium Urate Crystals (MSU)**
  1. Indicate gouty arthritis due to:
     - Increased serum uric acid
     - Decreased renal excretion of uric acid
     - Impaired metabolism of nucleic acid
  2. Exhibit negative birefringence
  3. Intracellular (acute stages) and extracellular location
  4. Polarized light: strongly birefringent
  5. Compensated polarized light: **yellow** when **parallel**
  6. blue when perpendicular
  7. Needle shaped
Synovial Crystals, Cont’d

- **Calcium pyrophosphate (CCPD)**
  1. Indicates pseudogout due to:
     - Degenerative arthritis
     - Endocrine disorders with increased serum calcium
     - Calcification of cartilage
  2. Exhibit positive birefringence
  3. Seen intracellular and extracellularly
  4. Polarized light: weakly birefringent
  5. Compensated polarized light: **blue** when parallel (yellow when perpendicular)
  6. Blunt rods or rhombic shapes

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**Negative and Positive Birefringence**

![Diagram](image-url)

*Figure 12-9 Diagram of negative and positive birefringence in MSU and CCPD crystals. (A) MSU crystal with grains running parallel to the long axis. The slow ray passes with the grain producing negative (yellow) birefringence. (B) CCPD crystal with grains running perpendicular to the long axis. The slow ray passes against the grain and is retarded producing positive (blue) birefringence.*
Synovial Crystals, Cont’d
Acute Gout (Uric Acid Crystals)

Synovial Crystals, Cont’d

- **Cholesterol Crystal**
  1. Nonspecific indications
     - Associated with chronic inflammation
  2. Exhibit negative birefringence (compensated polarized light)
  3. Usually seen extracellularly
  4. Polarized light: strongly birefringence
  5. Rhombic plates
**Synovial Crystals, Cont’d**

- **Hydroxyapatite (HA) (Calcium Phosphate) Crystals**
  1. Associated with calcific deposition conditions
  2. May produce an acute inflammatory reaction
  3. Intracellular
  4. Not birefringent
  5. Require an electron microscope to examine
  6. Small, needle shaped

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**Synovial Crystals, Cont’d**

- **Corticosteroid Crystals**
  1. Associated with intra-articular injections; NO clinical significance
  2. Primarily intracellular
  3. Exhibit positive and negative birefringence
    - Can closely resemble MSU and CCPD
  4. Flat, variable shaped plates
Corticosteroid in Synovial Fluid

Synovial Crystals, Cont’d

- **Calcium Oxalate Crystals**
  - Following renal dialysis

- **Birefringent Artifacts**
  1. Anticoagulant crystals (calcium oxalate, lithium heparin)
  2. Starch granules
  3. Prosthesis fragments
  4. Collagen fibers
  5. Fibrin
  6. Dust particles
Biochemistry Tests

1. Because synovial fluid is biochemically an ultrafiltrate of plasma, biochemistry test values are approximately the same as serum values.
2. Few biochemistry tests are considered clinically important.
3. Most frequently requested test is the glucose determination because markedly decreased values are indicative of inflammatory or septic disorders.

Biochemistry Tests, Cont’d

- **Glucose**
  1. Done simultaneously with blood sample (prefer 8 hour fast)
  2. Should be run within 1 hour of collection
  3. Draw in sodium fluoride – prevents glycolysis
Biochemistry Tests, Cont’d

5. Difference between blood and synovial glucose values is evaluated
   • Normal = < 10 mg/dL
   • Inflammatory conditions = > 25 mg/dL
   • Sepsis = > 40 mg/dL
   Considered low if < ½ serum plasma glucose value

Biochemistry Tests, Cont’d

• **Total protein**
  1. Not routinely performed
  2. Normal = < 1/3 of serum value (~3 g/dL)
     • Large molecule, not easily filtered by membrane
  3. Increased protein
     • Changes in membrane permeability
     • Increased joint synthesis
     • Indicates an inflammatory process
**Biochemistry Tests, Cont’d**

- **Uric Acid**
  1. Alone, not diagnostic
     - May determine gout in conjunction with plasma uric acid, esp. when crystals are undetectable
  2. Normal = serum level

**Biochemistry Tests, Cont’d**

- **Lactate**
  1. May differentiate between inflammatory and septic arthritis
  2. Septic arthritis = >250 mg/dL
  3. Gonococcal arthritis = normal to low levels
  4. Production results from:
     - Increased demand for energy
     - Tissue hypoxia
     - Severe inflammatory conditions
Microbiology Tests

1. Infections may occur as a secondary complication of inflammation
2. Gram stains and cultures are two of the most important tests performed on synovial fluid

Microbiology Tests, Cont’d

- **Gram Stain**
  1. Performed on all specimens
  2. Most infections are bacterial:
     - *Staphylococcus*
     - *Streptococcus*
     - *S. pyogenes*
     - *S. pneumoniae*
     - *Hemophilus*
     - *Neisseria gonorrhea*
  3. Fungal, viral and tubercular agents may also be observed
Microbiology Tests, Cont’d

• Cultures
  1. Routine culture
  2. Enrichment medium (chocolate agar)
  3. Specialty media depending on clinician orders and indications

Serologic Tests

1. Because of the association of the immune system to the inflammation process, serological testing plays an important role in the diagnosis of joint disorders
2. Majority of the tests are performed on serum with actual analysis of synovial fluid serving as a confirmatory measure in cases that are difficult to diagnose
Serologic Tests, Cont’d

- **Autoantibody Detection**
  (same as found in serum)
  1. Rheumatoid arthritis (RA)
  2. Lupus erythematosus (LE)
- **Antibody detection in patient’s serum**
  1. *Borrelia burgdorferi*
    - Causative agent of Lyme disease
    - Cause of arthritis

Joint Disorder Classification

<table>
<thead>
<tr>
<th>Group Classification</th>
<th>Pathological Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Noninflammatory</td>
<td>Degenerative joint disorders</td>
</tr>
<tr>
<td>2 Inflammatory</td>
<td>Immunologic problems (RA, LE)</td>
</tr>
<tr>
<td></td>
<td>Gout &amp; pseudogout (rystal induced)</td>
</tr>
<tr>
<td>3 Specific</td>
<td>Microbial infection</td>
</tr>
<tr>
<td>4 Hemorrhagic</td>
<td>Traumatic injury</td>
</tr>
<tr>
<td></td>
<td>Coagulation deficiency</td>
</tr>
</tbody>
</table>

Note:
* categories overlap
* multiple conditions can occur simultaneously
* disease stage can vary laboratory results
* see text for details of associated abnormal laboratory findings (pages 179-185)
THE END

Any questions?