Synovial Fluid Workshop

• Introduction
  • Gross examination
  • Save sterile fluid for cultures or research
  • Microscopic examination
    – Wet preparations
      • Regular light
      • Polarized light
    – Stained smears. May not be needed
  • Leukocyte count. Not always needed.

No disclosures
REFERENCES
Evidence-base

## Normal Synovial Fluid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee joint volume (cc)</td>
<td>0.18 – 3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>WBC/mm³</td>
<td>13 – 180</td>
<td>63</td>
</tr>
<tr>
<td>%PMN</td>
<td>0 – 25</td>
<td>6.5</td>
</tr>
<tr>
<td>Albumin g/100cc</td>
<td></td>
<td>1.02</td>
</tr>
<tr>
<td>Globulin g/100cc</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrolytes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Approximately same as plasma
<table>
<thead>
<tr>
<th>Initial Most Likely Diagnosis</th>
<th>Same Final Most Likely Diagnosis</th>
<th>Different Final Most Likely Diagnosis</th>
<th>Changed %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoarthritis</td>
<td>31</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>24</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Gout</td>
<td>25</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Infectious arthritis</td>
<td>11</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>Pseudogout</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Traumatic arthritis</td>
<td>7</td>
<td>2</td>
<td>22</td>
</tr>
</tbody>
</table>
Synovial Fluid Workshop

- Introduction

- **Gross examination**

- **Cultures**

- Microscopic examination
  - Wet preparations
    - Regular light
    - Polarized light
  - Stained smears

- Leukocyte count
<table>
<thead>
<tr>
<th>Normal</th>
<th>Non Inflammatory</th>
<th>Inflammatory</th>
<th>Purulent</th>
<th>Bloody</th>
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</table>

The image shows samples under different conditions, including normal, non-inflammatory, inflammatory, purulent, and bloody stages.
## Joint Fluid Characteristics

<table>
<thead>
<tr>
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<th>Group I (Non-Inflammatory)</th>
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<tr>
<td><strong>Volume</strong> (knee, in rat)</td>
<td>&lt;3.5</td>
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*Rapid accumulation of fluid will lower viscosity

\(^t\)2000 is an approximation. Usually less than 500

\(^tt\) may be lower with partially treated or low-virulence organisms
What do you think of this opaque, creamy fluid?
What can cause this “cream of tomato soup” synovial fluid?
Fat on the surface after centrifugation of bloody effusion due to intra-articular fracture
Opaque synovial fluid not due to cells but due to amyloid
“Gold paint” synovial fluid loaded with cholesterol crystals
Rice Bodies
Very viscous knee synovial fluid due to myxedema. Can also be seen in ganglia and cysts on Heberden nodes.
Clumps of urate crystals in 1st MTP joint fluid
If infection is being considered send unadulterated fluid to the laboratory with instructions as to which infections are concerns.
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If synovial fluid is not obtained, maintain suction on the syringe as you withdraw.
Drop of joint fluid

Syringe

Glass slide

Drop covered with coverslip

Fig. 1. The technique of making a wet preparation of joint fluid.
Synovial fluid cells examined first under regular light.
Synovial fluid leukocytes with cytoplasmic inclusions
Synovial fluid neutral fat droplets
Fat droplets stained with Sudan black
Fragment of synovial villus containing ochronotic shards found floating in synovial fluid.
Ochronotic Joint Fluid

Osteoarthritic Joint Fluid
What do you think of this joint fluid?
Regular light microscopy
Apatite crystal clumps by regular light microscopy
Alizarin red S stain for calcium must be passed through a millipore filter
Alizarin red S stained apatite clumps
Individual apatite crystals are seen only by electron microscopy.
Calcific periarthritis due to apatite at 2nd MCP joint
Synovial fluid fibrils often seen in osteoarthritis. Regular light
Amorphous clump of synovial fluid amyloid
Congo red positive amyloid
Apple green birefringence of amyloid with plain polarized light
Calcium oxalate crystal
Calcium oxalate crystal stained with Alizarin red S
Charcot-Leyden crystals in eosinophilic laden synovial fluid
Negatively birefringent MSU crystal
Polarized Light

- Polarizing discs
- Rotate until dark field
- Crystal will appear white
- First order red plate
- Background red
- Crystal yellow or blue
A. Ocular
B. Analyzer
C. Compensator
D. Polarizer
E. Condenser
MSU Crystal

Plain Polarized Light

Compensated Polarized Light
POLARIZED LIGHT MICROSCOPY
(Urate crystal)

Orienting Line,
First Order Red Plate
COMPENSATOR

Analyzer
Polarizer
MICROSCOPIC FIELD
MSU crystals can vary widely in size
Lower magnification intracellular MSU crystal and unidentified dot-like fragment
Centrifuged synovial fluid pellet to concentrate MSU crystals
Weakly positively birefringent CPPD crystal in WBC vacuole
CPPD crystals may be more brightly birefringent
CPPD Crystals Can Be Rhomboid or Rod Shaped
Intracellular CPPD

CPPD may sometimes be seen more easily with regular light.
Faintly positively birefringent CPPD can be very small
CPPD May Be Non-birefringent
CPPD concentrated in a cartilage fragment
Single cell containing MSU and CPPD crystals
Cholesterol and lipid liquid crystals
Lipid Liquid Crystals

- Appear as maltese crosses
- Positively birefringent
- Associated with some acute otherwise unexplained arthritis
- Can be phagocytized
- Seen as membranous arrays by EM
- Possibly derived from RBC or other cell membranes.
- Don’t confuse with urate microspherules (negatively birefringent) or talc.
Membranous arrays of phospholipid in lipid liquid crystals by EM
Massive positively birefringent lipid liquid crystals
Negatively birefringent MSU crystal overlying positively birefringent Maltese cross lipid liquid crystal
What do you see here?
Cryoglobulin and other protein crystals stain with toluidine blue.
Pyramidal aspect of oxalate crystals are accentuated by polarized light
Artefacts that May Be Seen on Polarized Light Examination of Joint Fluid

- Depot corticosteroids
- Anticoagulant crystals
  - Oxalate
  - EDTA
- Drying artefact
- Glass fragments
- Fibrils from lens paper
- Corn starch from sterile gloves
- Lipids from degenerated cells
- Birefringent nail polish used to seal coverslips
Depot medrol is very bright and irregular
Celestone soluspan can mimic CPPD or cholesterol
Glass fragments from broken coverslips can mimic MSU crystals
Lens paper is brightly birefringent
What do you see here?
These negatively birefringent lipid crystals can form in neutral lipid droplets in specimens left over night
Lipid crystals forming in neutral fat droplet
Corn starch from gloves
Green fragment from tube stopper found in synovial fluid. Nail polish used to seal coverslip can seep into specimen.
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- Introduction
- Gross examination
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- Leukocyte count
If leucocyte differential or gram stain may be needed make several thin smears for later staining
Cells seen in synovial fluid

- PMN
- Small lymphocytes
- Activated lymphocytes
- Large granular lymphocytes
- Monocytes
- Large mononuclears
- Synovial lining cells (synthetic type)
- Eosinophils
- Plasma cells
- Mast cells
- Others
Wright stain of synovial fluid showing lymphocytes, monocytes and PMN as often seen in RA
Occasional synovial fluids may have predominantly lymphocytes
One lymphocyte, two monocytes and the large cell is a synovial lining cell
A large cell with a nucleus filling most of the cytoplasm is an activated lymphocyte as may be seen in RA or SLE.
This large cell is an LE cell
Synovial lining cell with phagocytized MSU crystal
“Reiter cell” typical of spondyloarthritis
World champion “Reiter cell”
Metastatic adenocarcinoma cells
Bacteria can be suspected on Wright stain
Gram stain showing gram positive cocci
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Leukocyte Counts on Joint Fluids

- Use heparin or EDTA tubes
- Leukocyte counts fall with time so test best done promptly
- Use 0.3N saline as diluent to lyse red blood cells.
- Automated counters may become clogged and may count material other than cells so should be avoided
- With clear fluids estimated counts can be made. 0-2 WBC/HPF means that actual counts will virtually always be less than 2000/mm³
Abnormally high leukocyte count reported on an automated counter
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Sequential changes in synovial fluid leukocyte counts over a 6 hour time period at room temperature

<table>
<thead>
<tr>
<th>Synovial fluids</th>
<th>Immediate exam</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>6 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Borderline inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>4,700</td>
<td>4,200</td>
<td>3,750</td>
<td>3,550</td>
<td>1,850</td>
</tr>
<tr>
<td>#2</td>
<td>6,200</td>
<td>6,000</td>
<td>4,800</td>
<td>3,500</td>
<td>1,950</td>
</tr>
<tr>
<td>#3</td>
<td>4,850</td>
<td>3,660</td>
<td>2,450</td>
<td>2,200</td>
<td>1,800</td>
</tr>
<tr>
<td>#4</td>
<td>3,150</td>
<td>2,250</td>
<td>1,950</td>
<td>1,600</td>
<td>1,300</td>
</tr>
<tr>
<td><strong>Marked inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>17,550</td>
<td>16,400</td>
<td>13,350</td>
<td>12,800</td>
<td>10,500</td>
</tr>
<tr>
<td>#2</td>
<td>45,000</td>
<td>42,880</td>
<td>38,650</td>
<td>35,600</td>
<td>30,440</td>
</tr>
<tr>
<td>#3</td>
<td>16,600</td>
<td>15,550</td>
<td>12,600</td>
<td>8,700</td>
<td>7,950</td>
</tr>
</tbody>
</table>

Borderline inflammatory SFs >2,000 WBC/mm³ had decreased into a non-inflammatory range <2,000 WBC/mm³ after 6 hours
<table>
<thead>
<tr>
<th>Estimated WBC/hpf</th>
<th>0-1000</th>
<th>1050-2000</th>
<th>2050-10,000</th>
<th>10,050-20,000</th>
<th>20,000-50,000</th>
<th>&gt;50,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>25</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3-4</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5-10</td>
<td>2</td>
<td>11</td>
<td>3</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>10-25</td>
<td>---</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>2</td>
<td>---</td>
</tr>
<tr>
<td>25-50</td>
<td>---</td>
<td>---</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>&gt;50</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
CAUSES OF NON-INFLAMMATORY JOINT FLUIDS

Osteoarthritis
Traumatic arthritis
Acromegaly
Gaucher’s disease
Hemochromatosis
Hyperparathyroidism
Ochronosis
Paget’s disease
Jaccoud’s arthritis
Hemarthrosis, hemophilia
Mechanical derangement
Fractures
Osteochondritis dessecans
Epiphyseal dysplasias

Primary tumors
Metastatic tumors
Pigmented villonodular synovitis
Aseptic necrosis
Ehlers-Danlos syndrome
Sickle cell disease
Amyloidosis
Hypertrophic pulmonary osteoarthropathy
Pancreatitis
Charcot joints
Wilson’s disease
Other Tests are Rarely Useful

- Rheumatoid factor is not needed and can mislead
- Cytokines, cell surface markers, enzymes, etc. are still mostly for research
- PCR may be an important test in the near future for difficult to identify infections
- Consider synovial biopsies if synovial fluid is not diagnostic. Decide if your question can be better answered by examining tissue.
PCR demonstration of chlamydial nucleic acid in reactive arthritis synovial fluid
Chlamydia Identification by PCR is More Often Positive in Synovium than Synovial Fluid

- Total of Patients: 37
  - (+) Synovium: 24 (64.8%)
  - (+) Synovial fluid: 13 (35.1%)
  - (+) On both: 14 (37.9%)
  - (-) On both: 11 (29.7%)
  - (+) Syn (-) Sf: 10 (27.0%)
  - (-) Sf (+) Syn: 2 (5.4%)
TB granuloma detected in synovium despite negative synovial fluid culture
Other less common diseases like multicentric reticulohistiocytosis may also be detected by synovial biopsy.
Conclusions

• Examining synovial fluid may be the only way to determine the process involving a given joint
• Gross appearance and wet drop examination are most helpful
• Your examination is important and worth documenting on a SF report form