

Synovial Fluid Analyses, Synovial Biopsy,
and Synovial Pathology
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Synovial Fluid Analyses, Synovial Biopsy, and Synovial Pathology

- Analysis of synovial fluid samples by leukocyte count, cytology, polarized microscopy, Gram stain, and culture provides key diagnostic information, particularly in acute monoarthritis.
- Synovial biopsy performed by using closed needle techniques or arthroscopy may provide valuable diagnostic information, particularly in persistent monoarthritis.

Synovial Fluid Analyses, Synovial Biopsy, and Synovial Patholog

- Analysis of synovial tissue using immunohistology and other molecular techniques has increased understanding of the mechanisms of synovitis.
- Sequential analysis of synovial tissue samples in the context of therapeutic trials provides unique information regarding the effects of treatment on the target organ.

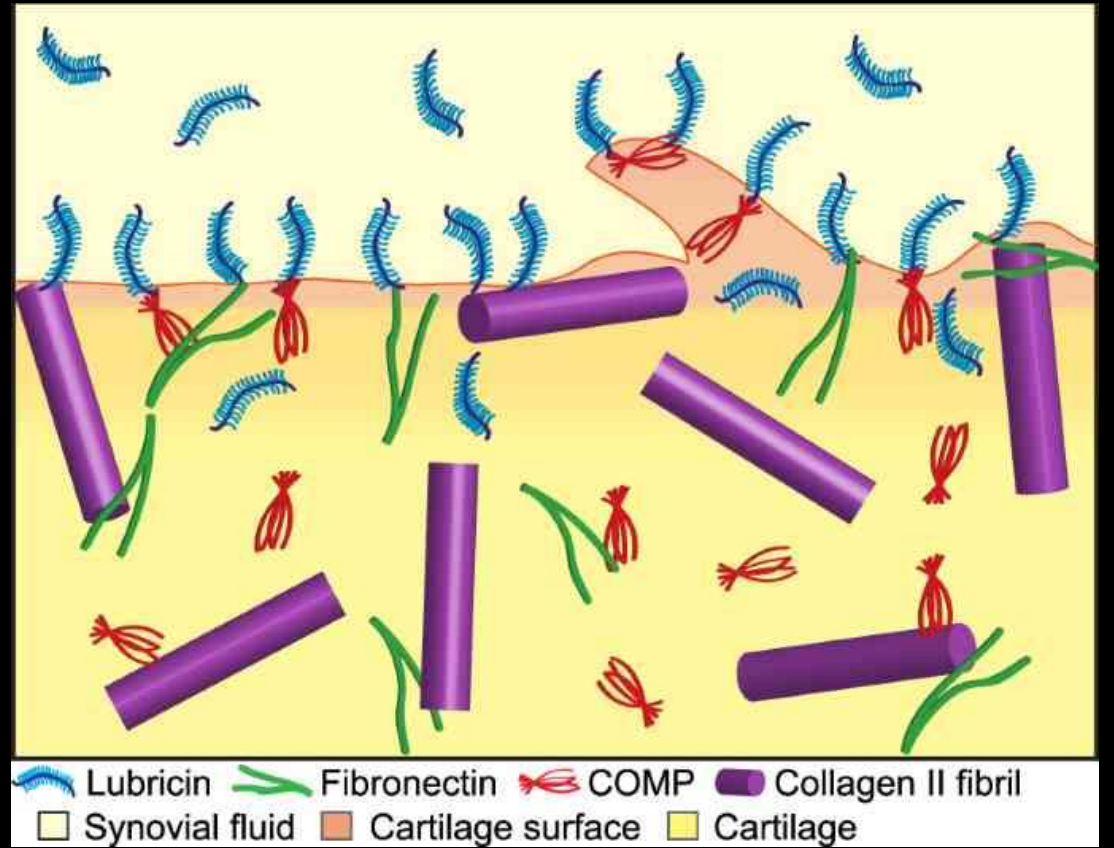
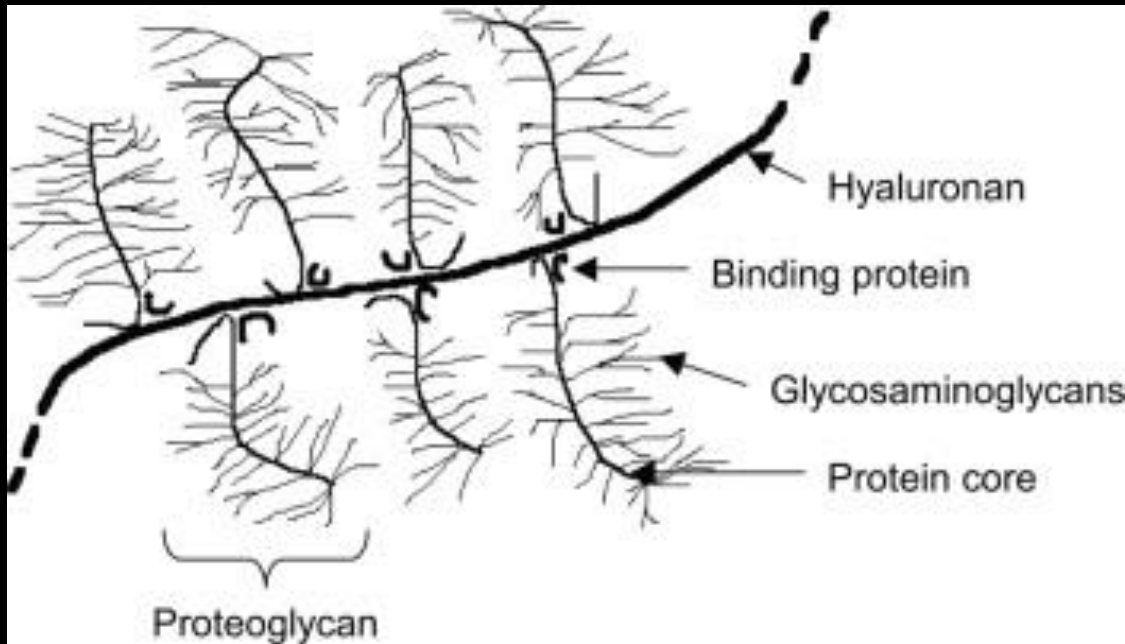
Synovial Fluid Analysis

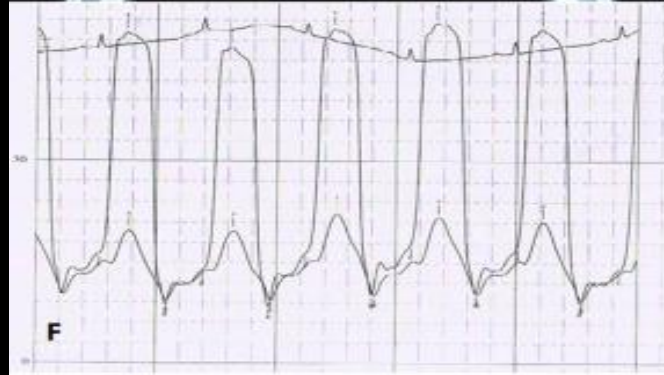
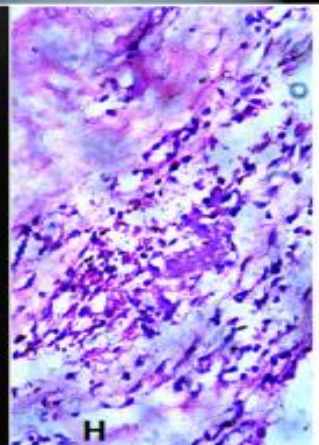
Synovial Fluid in Health

- Under normal conditions, a small volume of synovial fluid is present in each joint, forming a thin interface between the surfaces of the articular cartilage and providing for friction-free movement of these surfaces. In a large joint such as the knee, the volume of synovial fluid is estimated to be less than 5 ml

Hyaluronan is the major proteoglycan synthesized by synovial cells and secreted into synovial fluid. Hyaluronan is highly polymerized and reaches molecular weights exceeding one million Daltons, which gives this fluid its characteristic viscosity

The lubricating capacity of the synovial fluid is attributed to a glycoprotein called **lubricin**. This molecule has been fully characterized on the basis of the study of individuals with mutations of the **PRG4 gene**, which encodes for its production. These mutations result in an autosomal recessive loss-of-function disorder called the **camptodactyly–arthropathy–coxa vara–pericarditis syndrome**, which features a progressive, non-inflammatory arthropathy characterized by severe cartilage destruction associated with proliferation of synovial lining cells





Accumulation of Synovial Effusions

- non-inflammatory
- inflammatory
- septic disorders.
- traumatic
- nontraumatic disorders

Arthrocentesis

- Difficulty in aspiration of synovial fluid may stem from a number of intra-articular factors, including **viscosity**, the **presence of debris** such as rice bodies, and **loculation** of fluid into inaccessible areas
- It is important to analyze aspirated synovial fluid samples as quickly as possible to avoid spurious results. Ideally, leukocyte count and differential should be performed on **fresh specimens**

Gross Examination

- Viscosity
- Color
- hemarthrosis

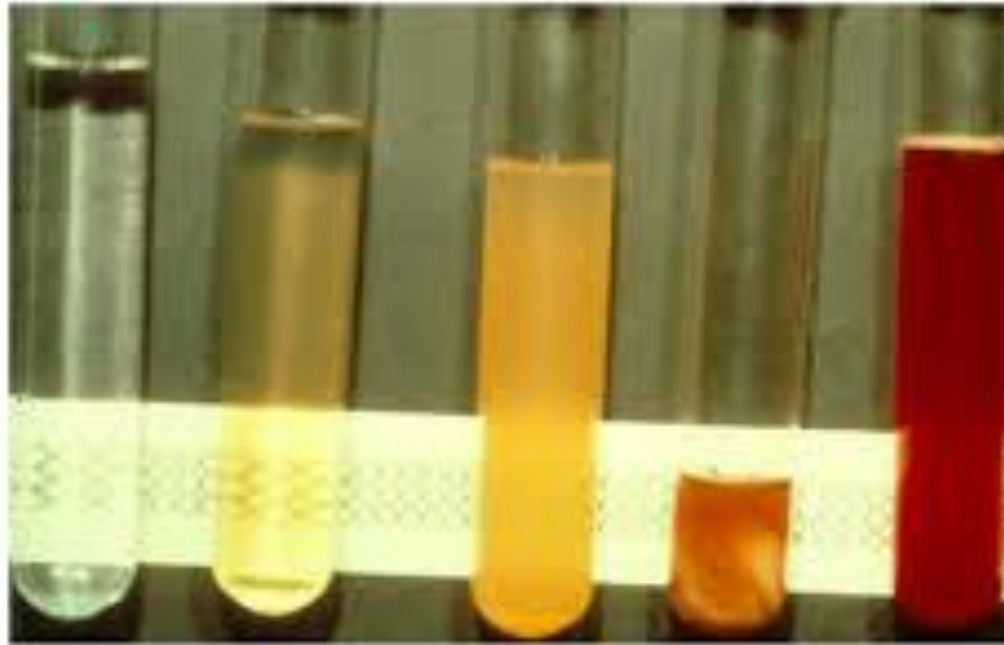
Macroscopic Analysis: Viscosity

- Evaluated using “String test”
- Normal = 5cm long before breaking
- Low viscosity indicates inflammation

String test showing normal synovial fluid viscosity.



Synovial Fluid Color and Clarity



Normal

Non-
Inflammatory

Inflammatory

Septic

Hemorrhage

Leukocyte Count

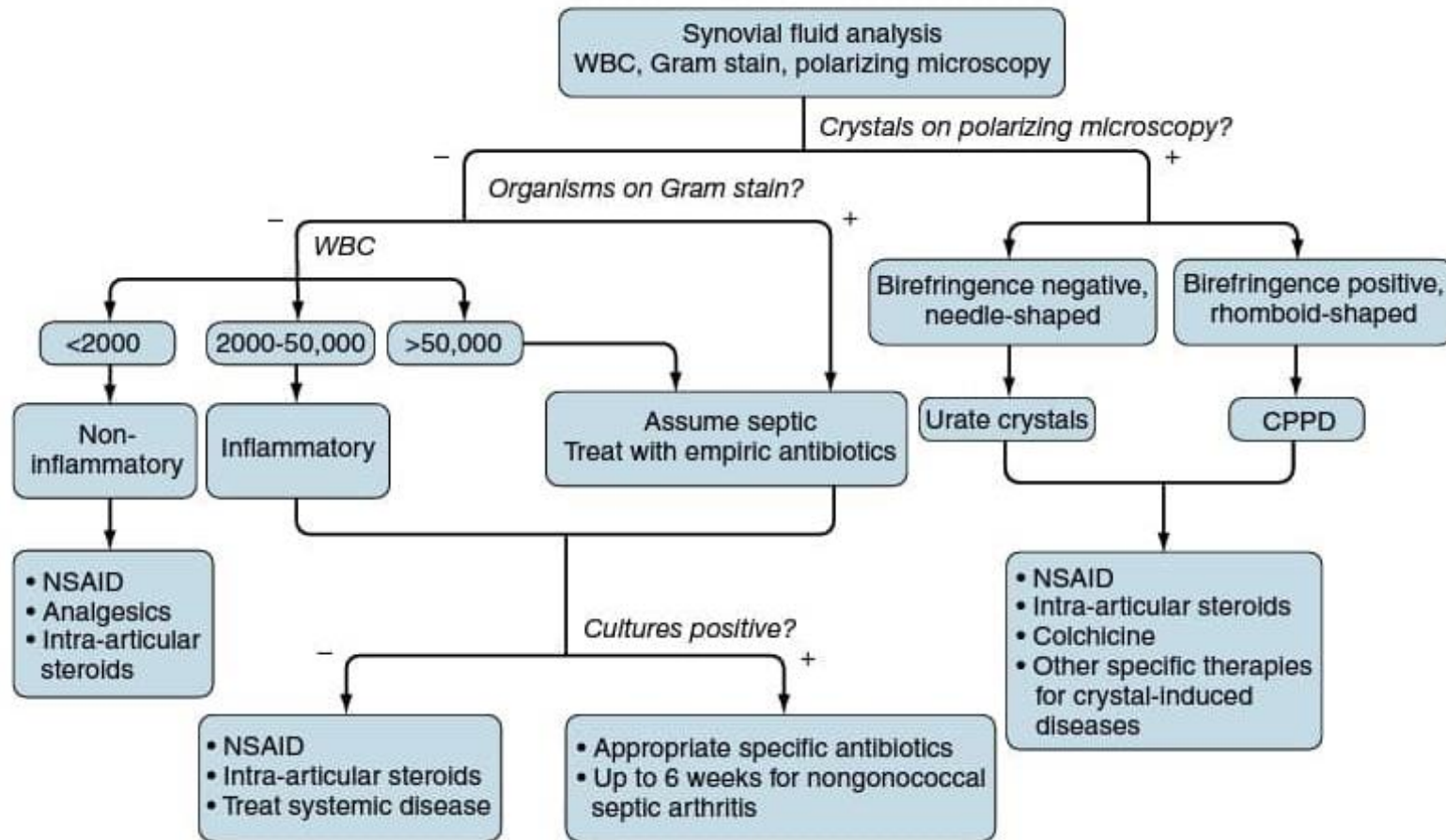
- Normal synovial fluid contains fewer than 180 nucleated cells/mm³
- non-inflammatory (<2000 cells/mm³)
- inflammatory (2000 to 50,000 cells/mm³)
- septic (>50,000 cells/mm³).

**TABLE
56.1**

Characteristics of Synovial Fluid

	Appearance	Viscosity	Cells per mm ³	% PMNs	Crystals	Culture
Normal	Transparent	High	<200	<10%	Negative	Negative
Osteoarthritis	Transparent	High	200-2000	<10%	Occasional calcium pyrophosphate and hydroxyapatite crystals	Negative
Rheumatoid arthritis	Translucent	Low	2000-50,000	Variable	Negative	Negative
Psoriatic arthritis	Translucent	Low	2000-50,000	Variable	Negative	Negative
Reactive arthritis	Translucent	Low	2000-50,000	Variable	Negative	Negative
Gout	Translucent to cloudy	Low	200->50,000	>90%	Needle-shaped, negatively birefringent monosodium urate monohydrate crystals	Negative
Pseudogout	Translucent to cloudy	Low	200-50,000	>90%	Rhomboid, positively birefringent calcium pyrophosphate crystals	Negative
Bacterial arthritis	Cloudy	Variable	2000->50,000	>90%	Negative	Positive
PVNS	Hemorrhagic or brown	Low	—	—	Negative	Negative
Hemarthrosis	Hemorrhagic	Low	—	—	Negative	Negative

PMNs, Polymorphonuclear neutrophils; PVNS, pigmented villonodular synovitis.



• **Fig. 56.1** A simplified algorithm for analyzing synovial fluid samples and initiating a plan of management. *CPPD*, Calcium pyrophosphate dihydrate; *NSAID*, nonsteroidal anti-inflammatory drug; *WBC*, white blood cell; -, negative test; +, positive test.

Synovial Fluid Cytology

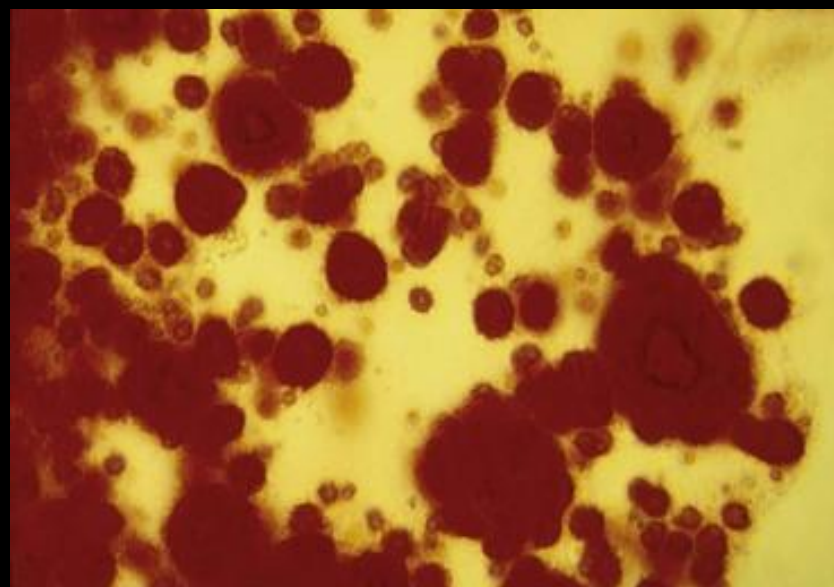
- In addition to leukocytes, and in the case of traumatic taps or hemarthroses, or large numbers of erythrocytes, wet mount may reveal the presence of **clumps of fibrin** and **crystals, cartilage** and **synovium fragments**, and **lipid droplets**
- **Septic-range** synovial fluid containing more than 50,000 cells/mm³ is almost always associated with a high preponderance of **polymorphonuclear** leukocytes, often greater than 90%.

Synovial Fluid Cytology





- **Monocytes and lymphocytes** predominate in the synovial fluid of patients with viral arthritis, lupus, and other connective tissue diseases
- The presence of large numbers of “**ragocytes**,” which are granulocytes that have engulfed immune complexes, is associated with active RA, and their presence may indicate an unfavorable prognosis in this disease
- **Reiter’s cells** represent cytophagocytic mononuclear cells that have phagocytized apoptotic polymorphonuclear leukocytes.

Wet Smear Analysis by Polarized Microscopy

- **MSU** crystals appear as strongly negatively birefringent needle-shaped objects, many of which are intra-cellular, having been phagocytized by synovial fluid leukocytes
- **CPPD** crystals seen during attacks of pseudogout tend to be smaller, rhomboidshaped objects that are weakly positively birefringent
- **Hydroxyapatite** can be detected in synovial fluid, but because these crystals are generally nonbirefringent, it is not possible to detect them by polarized microscopy



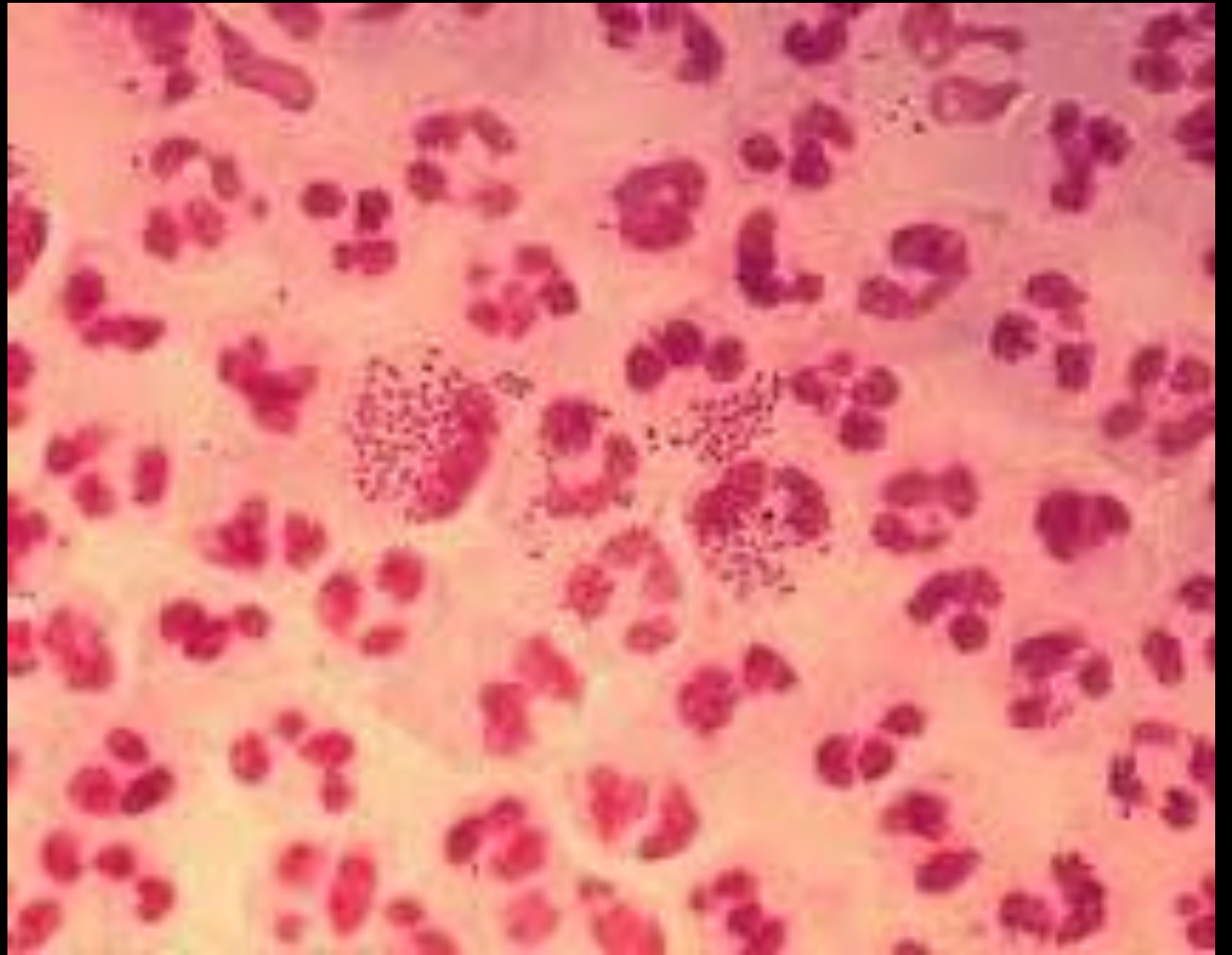
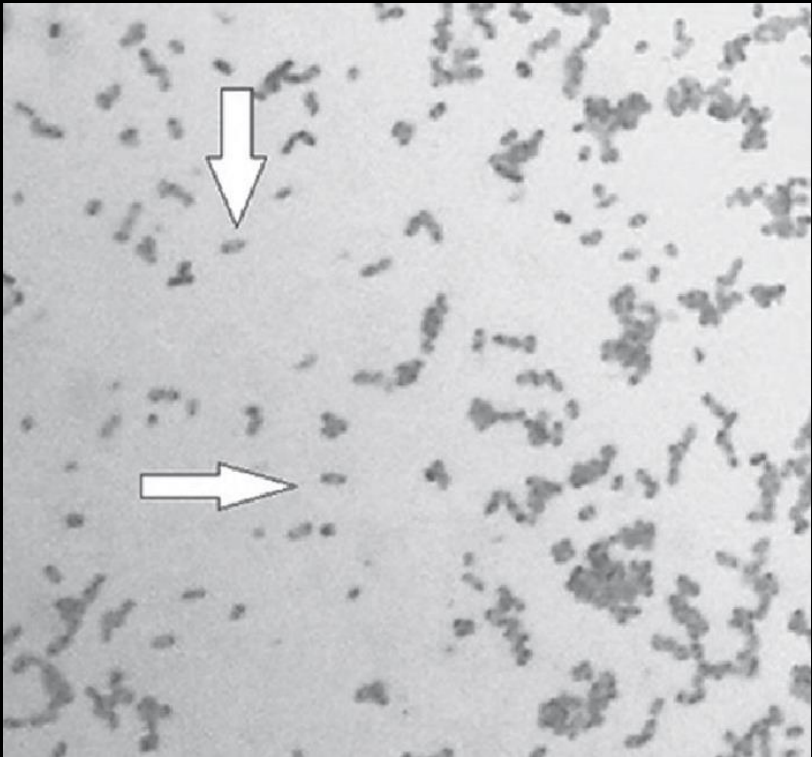
MSU
CPPD

Characteristic	Gout	Pseudogout
Composition	Uric acid	Calcium pyrophosphate
		
Shape	Needle-like	Rhomboid
Stain	Negative	Weakly positive
Common joint	1st MTP	Knee
Radiography	"Rat-bite" erosions	White lines of chondrocalcinosis
		
Treatment	NSAID	NSAID

- Synovial **cholesterol crystals** appear as flat, plate-like structures with notched corners and lipid crystals have the appearance of Maltese crosses. Both can be strongly birefringent, both negatively and positively
- **Corticosteroid crystals** can be highly birefringent and mimic urate or CPPD crystals.

Detection of Microorganisms by Gram Stain, Culture, and Polymerase Chain Reaction Analysis of Synovial Fluid

- A **Gram stain** performed on fresh synovial fluid will identify an organism in an estimated 50% of cases of septic arthritis, with the highest sensitivity for Gram-positive organisms. Moreover, the **specificity** of a positive Gram stain approaches 100%
- The **gold standard** for diagnosing septic arthritis is still bacteriologic culture, which has a sensitivity of 75% to 95% and a specificity of 90% in cases of nongonococcal septic arthritis



- In the case of **gonococcal arthritis**, the sensitivity of bacteriologic culture, even if performed on a sample collected by using appropriate media, is low, with an estimate of less than 10%.
- **Polymerase chain reaction (PCR)** carries a high degree of sensitivity and specificity for the detection of microorganisms in synovial fluid and tissue, even in individuals who are culture negative

Biochemical Analysis of Synovial Fluid

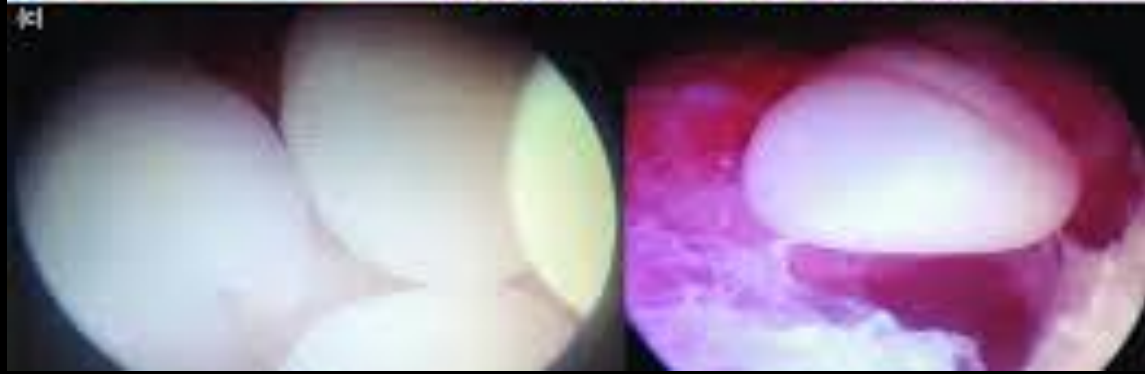
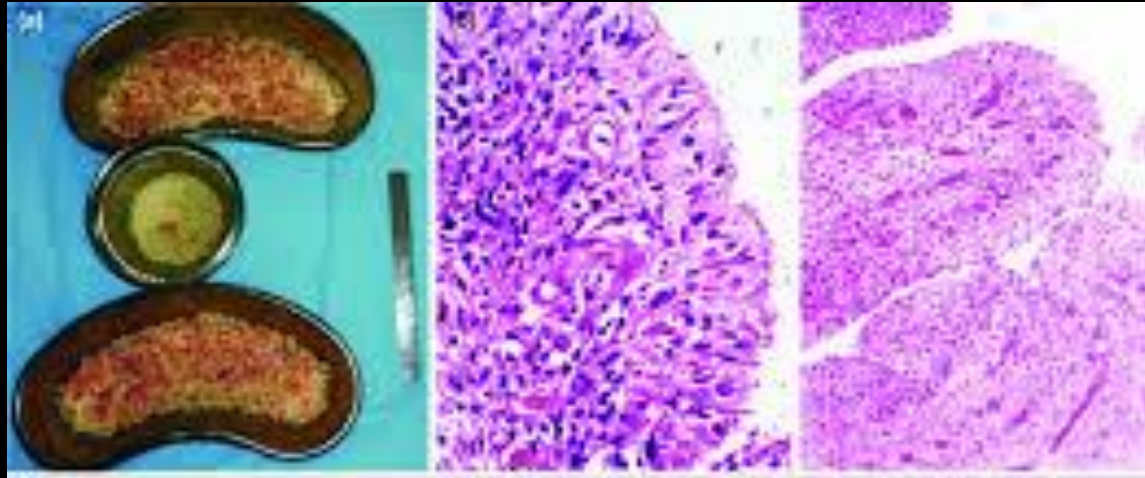
- Testing for synovial fluid **glucose, protein, and lactate dehydrogenase (LDH)** has long been included in routine practice, and values obtained should be compared with serum values
- **Serologic testing** of synovial fluid to detect rheumatoid factor, anti-nuclear antibodies, and complement levels has been suggested as a method that can be used to confirm a diagnosis of RA or other connective tissue disease

Synovial Biopsy

1. It can be particularly valuable in evaluating an **undiagnosed persistent monoarthritis** when other investigations, including synovial fluid analysis, have failed to provide a specific diagnosis
2. In **research settings**, analysis of synovial tissue samples has dramatically improved our understanding of the pathogenetic mechanisms underlying RA, spondyloarthropathies, and other chronic articular disorders.
3. synovial biopsy has been explored as a method for defining the **target tissue response** to therapeutic agents

Synovial Biopsy

- Blind Percutaneous Synovial Biopsy
- Arthroscopically Guided Synovial Biopsy
- Ultrasound Guided Synovial Biopsy
- Processing Synovial Tissue Samples

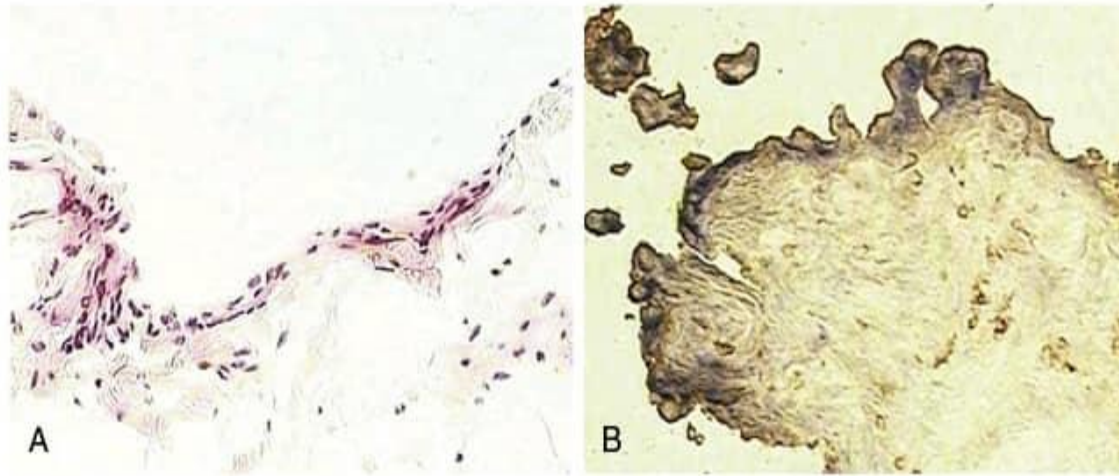


Normal Synovial Pathology

- the normal synovial lining layer is one to three cells thick and is composed of closely associated **macrophage-like (type A)** and **fibroblast-like synoviocytes (type B)** that are not separated from the underlying stroma by a basement membrane
- Macrophage-like synoviocytes are **myeloid in origin**, as they exhibit the morphologic characteristics of phagocytic cells and express macrophage markers such as **CD68, CD14, and FcγRIIIa**
- Fibroblast-like synoviocytes are **synthetic cells** of mesenchymal origin that are the primary source of **hyaluronan** and other **proteoglycans** found in normal synovial fluid. express **CD55, (VCAM)-1, UDPGD**

Normal synovial Pathology

- Rich network of capillaries with fenestrated endothelium
- Loose connective tissue
- The synovium of completely asymptomatic individuals commonly exhibits a modest infiltrate of T lymphocytes that are occasionally organized in perivascular aggregates, although B cells are not seen



• **Fig. 56.7** Normal synovium. (A) A lining layer one to two cells deep that is composed of macrophage-like synoviocytes (type A) and fibroblast-like synoviocytes (type B). (B) Normal synovium stained for the enzyme uridine diphosphoglucose dehydrogenase, an indicator of hyaluronan synthesis by fibroblast-like synoviocytes.

Synovial Histopathology in the Evaluation of Monoarthritis

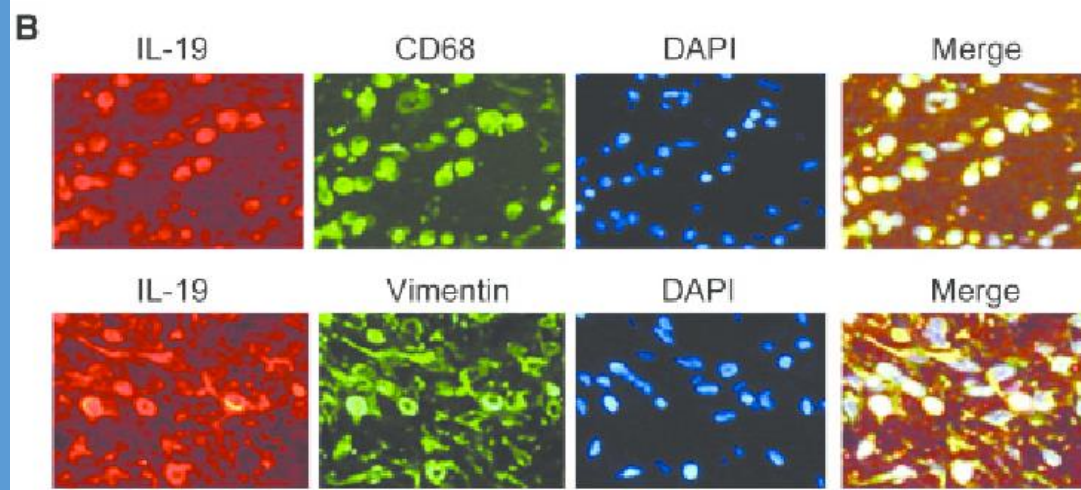
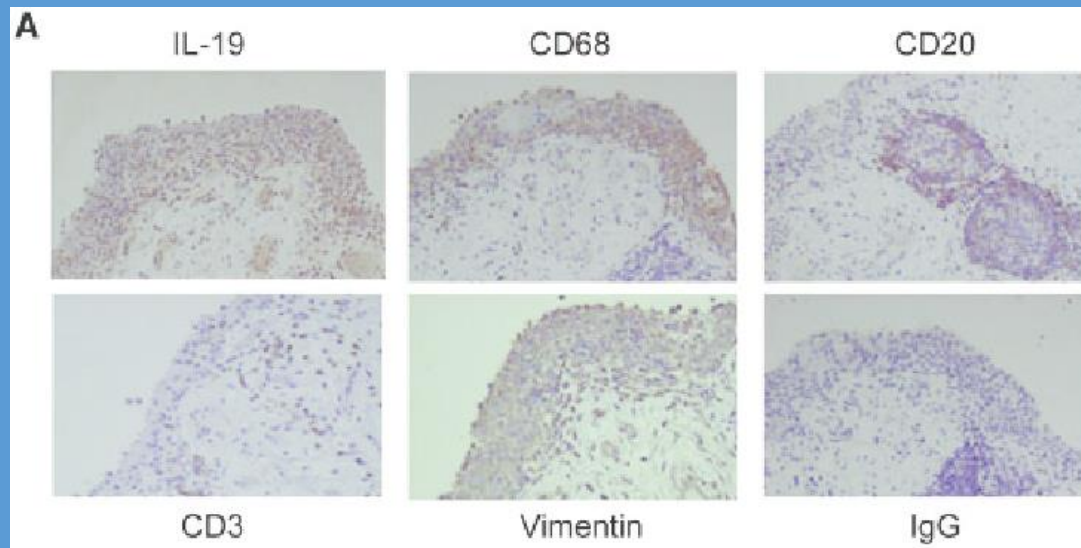
- The presence of large numbers of **neutrophils** in the synovial tissue stroma is highly suggestive of septic arthritis
- A **mononuclear cell infiltrate**, on the other hand, is more consistent with a chronic inflammatory process and has a wide differential diagnosis
- The presence of **granulomas** supports a diagnosis of tuberculous arthritis or sarcoidosis
- **Pigmented villonodular synovitis** . diffusely hypervascular proliferative lesion with mononuclear cells of the monocyte/macrophage lineage, foamy multinucleated cells resembling osteoclasts, and hemosiderin deposits

Synovial Histopathology in the Evaluation of Polyarthrititis

- research studies
- The two characteristic features seen in RA synovitis are **hyperplasia** of the lining layer and **infiltration of the sublining stroma** with mononuclear cells, **lymphoid aggregates**, **Multinucleated giant cells**
- Comparative studies have suggested that synovial lesions in psoriatic arthritis are **more vascular** than those of RA, with **more tortuosity** of the synovial microvasculature
- The synovium of lupus patients showed **synovial hyperplasia**, **inflammatory infiltrates**, **vascular proliferation**, **edema and congestion**, **fibrinoid necrosis** and **intimal fibrous hyperplasia** of blood vessels, and **superficial fibrin deposits**

Synovial Immunohistology

- Immunohistology utilizes specific **monoclonal or polyclonal antibodies** with well-defined molecular targets and is an effective tool for analyzing the cellular and molecular features of the synovium
- The easiest method is to generate **semiquantitative scores** of staining intensity (e.g., on a 0 to 3 scale) from multiple areas of the tissue, and on the basis of these to obtain an **average score** for the entire tissue



Synovial Lining Cell Layer

- Lining layer in RA and other forms of chronic inflammatory arthritis is often **hyperplastic**, resulting from an increase in both type A and type B cells, as indicated by an increase in CD68 and CD55 staining, respectively
- Expression of **adhesion molecules**, expression of **CD55, VCAM-1, cadherin-11** , principal source of **cartilage-degrading proteases**, particularly matrix metalloproteinase (MMP)-1 and MMP-3
- Human leukocyte antigen **(HLA)DR** is highly expressed, particularly by macrophage-like cell

Synovial Lymphocytes and Plasma Cells

- A predominance of **CD3+ T cells** is found in the synovial tissues of patients with RA and spondyloarthropathies,
- CD4 T cells expressing **CD25** and the gene **FoxP3**, so-called Tregs, are now known to play a regulatory role in antigen-specific T cell expansion
- B cells are identified by expression of **CD19** and **CD20** and are particularly abundant in tissues that exhibit large lymphoid aggregates with germinal centers.
- The areas surrounding the lymphoid aggregates are often densely infiltrated with sheets of **CD38+ plasma cells**

- The areas immediately adjacent to the dense lymphoid aggregates, which comprise primarily CD4+ T cells and B cells, have been called **transitional zones, lower CD4/CD8 ratio, rich in macrophages and interdigitating dendritic cells,**
- Natural killer cells can be identified by cell surface markers, expression of granzymes, and functional assays

Synovial Sublining Macrophages and Dendritic Cells

- **CD163+ macrophages**, which have recently been called M2 macrophages, were found to be more abundant in spondylarthropathy than in RA synovium
- **M1 macrophages**, which produce TNF and IL-1 β , are more abundant in RA and are under-represented in psoriatic arthritis and other spondyloarthropathies in which M2 macrophages are more abundant
- Mature **dendritic cells** are the most efficient and potent of the **antigen-presenting cells**, and are found abundantly in inflamed synovium in close contact with T lymphocytes.

Synovial Microvasculature, Endothelium, and Stromal Mesenchymal Cells

- The stromal elements in RA are often expanded in parallel with the inflammatory cell infiltration.
- Increased microvasculature due to local stimulation of angiogenesis
- vessels immediately adjacent to the lining layer is actually reduced compared with normal tissue. ↑ Increased HIF 1 α

Synovium-Cartilage-Bone Interface

- The interface between inflamed synovium and adjacent cartilage and bone
- That synovial macrophages and fibroblasts are abundant at the pannus-cartilage interface, and that high levels of proteases are expressed by these cells.

Conclusion

- Analysis of synovial fluid and tissue samples provides valuable diagnostic information in specific clinical settings
- In cases of **undiagnosed chronic monoarthritis**, synovial biopsy may provide definitive evidence of conditions such as TB, sarcoidosis, and pigmented villonodular synovitis.
- Systematic analysis of synovial tissue in RA and other forms of inflammatory arthritis, particularly with the use of immunohistology, has provided a wealth of information concerning the cellular and molecular mechanisms that sustain synovial lesions.