SELECTION AND USE OF LABORATORY TESTS IN THE RHEUMATIC DISEASES

AIMS
On completion of this chapter you should be able to select the appropriate laboratory investigation for the patient’s condition and interpret the results.

1. Introduction

Rheumatic conditions are diagnosed on clinical grounds. The history and physical examination of the patient presenting with rheumatic symptoms are usually diagnostic. Laboratory testing provides supportive evidence.

2. SYNOVIAL FLUID ANALYSIS

Joint fluid analysis can be as important in the evaluation of joint disease as urinalysis is in renal disease.

• Arthrocentesis

Always aspirate the joint with a good aseptic technique.

Advice
Make a bit of a show of the aseptic technique with the patient, using a mask and gloves etc., so that in the very rare case of sepsis; there can be no comebacks about poor technique.

Even a single drop of fluid in the aspirating needle is often enough for examination. Aspirated fluid must be examined promptly, as artefactual crystals may develop within a few hours.

If no fluid is obtained but sepsis of the joint is suspected, the joint can be irrigated with a small amount of water that is then aspirated and sent for culture. Even this all too often does not yield a positive culture, particularly if antibiotics have been given in the past. In these cases where sepsis is strongly suspected and the cultures of the joint fluid are negative, a synovial biopsy is indicated and this is then cultured.

Important: Always use a good aseptic technique when aspirating a joint.
• **Gross examination**

• **Volume**

A swollen joint that is difficult to aspirate even if you are sure you are in the joint, may be due to thick fibrin, rice bodies or other debris blocking the needle point. Often thickened synovial folds will also block the needlepoint.

**Advice:**
Draw the needle back slightly as you aspirate. This often frees the needlepoint from obstructing material.

• **Viscosity**

The viscosity of synovial fluid can be estimated by slowly pushing a few drops from the tip of the syringe. Normal fluid holds together for about 20mm before falling. Low viscosity fluids drip from the syringe like water. Viscosity is generally decreased in inflammation.

• **Colour and clarity**

Normal fluid is clear and slightly straw coloured. If the synovial fluid is cloudy, it suggests inflammation but microscopic examination is needed to determine if the opacity is due to inflammatory cells, crystals, lipids, fibrin or amyloid.

**Advice:**
Use a clear plastic syringe to aspirate or place some of the fluid in a clear tube. Place the syringe on a page of typing and try to read the type through the fluid. If you can, the fluid is non-inflammatory.

• **Leucocyte count**

The leucocyte count is the basis for classification of an effusion as septic, inflammatory or non-inflammatory. Normal joint fluid has 50 to 200 leucocytes/mm\(^3\). Counts of 200 to 2000 are generally considered to be due to non-inflammatory causes, but low-grade infection cannot be excluded.
Counts of over 60,000 should be regarded as very suggestive of septic arthritis, but counts of over 100,000 cells/mm$^3$ can also be found in gout, psoriatic arthritis and Reiter's syndrome.

**Advice:**
The fluid must be examined promptly as clumping of cells and clotting can occur with time. Also, ordinary counting fluid must be replaced with 0.3% saline. This lyases red blood cells and makes counting easier.

- **Wet preparation**

This is probably the most important part of the synovial fluid analysis. Even if only a single drop is available, it should be used for microscopic examination. Erythrocytes and leucocytes can be seen, if present. Fragments of cartilage may be seen, and large lipid droplets appearing as Maltese crosses can be found in traumatic arthritis. Various crystals may be seen. Urate crystals in gout patients are usually small rods or needles, while the other common crystal, calcium pyrophosphate, is smaller and can be thick or thin and rod shaped. Calcium apatite crystals are very small and form round clumps. Cholesterol crystals are flat plates with notched corners. Normally, none of these should be present.

- **Polarized microscopy**

Normally a special polarizing microscope is needed, but a cheap modification to a standard microscope can be used. Polarizing filter material can be obtained from a photographic shop. One piece is placed over the light source. Light is now oriented in a single plane. A second piece of polarizing material is placed over one eyepiece. Note that only one eyepiece can be used. If the eyepiece is rotated 90 degrees, almost all light is blocked. If material containing crystals is placed between the two polaroid filters, some of the light is deflected by the crystals and is visible on the dark field (a phenomenon known as birefringence). The two most important crystals you will encounter are urate crystals in gout and calcium pyrophosphate in chondrocalcinosis or pseudo-gout.

Urate crystals are brightly birefringent, while pyrophosphate crystals are weakly birefringent. Further identification of the crystals does require a special polarizing microscope.
• **Dry preparation**

If sepsis is suspected, a Gram stain can be done on a dry slide. Bacteria can be classified into Gram positive or Gram negative organisms, but frequently no bacteria are seen.

**Advice:**
If you strongly decolour the slide with acetone, the intracellular diplococci of gonorrhoea can often be seen; they are not easily seen with the usual Gram technique.

3. **SEROLOGY**

Never ask for the so-called arthritis panel of blood tests. This group of tests may confuse more than clarify. Once you have a clinical approach to your patient, ask for the relevant tests.

Example
Many diseases besides rheumatoid arthritis (RA) are associated with the presence of rheumatoid factor (see later).

4. **ERYTHROCYTE SEDIMENTATION RATE**

**Definition**
The Westergren erythrocyte sedimentation rate (ESR) is a simple marker for inflammatory activity in a variety of conditions. This test measures metabolic changes that the liver undergoes in response to systemic inflammation.

The ESR level is, to a large extent, an indirect index of the level of acute-phase reactants and mainly of the fibrinogen level. It is believed to correlate with the severity of inflammatory disease, but occasionally some patients with active rheumatic diseases may have a normal ESR level. However, the ESR can also be elevated by conditions unrelated to rheumatic disease, such as aging, anaemia, infection, pregnancy, trauma, malignancy and stress. It is also higher in women than in men.
5. **C-REACTIVE PROTEIN**

A large number of systemic and metabolic changes, collectively called the acute-phase response, begin to occur within hours after an inflammatory stimulus. Many represent defensive mechanisms that precede the immune response. This response occurs in association with a wide variety of stimuli, including bacterial infection, trauma, various immunological stimuli, crystal-induced inflammation and various neoplasms. The main acute-phase proteins are C-reactive protein (CRP), serum amyloid-A, haptoglobin and fibrinogen and serve as markers for disease activity. For clinical purposes, and in the rheumatological context, CRP is the most important. It rises steeply and declines rapidly with the course of the inflammatory process, which makes it another useful clinical marker of active inflammation and its resolution and ideal for following responses to anti-inflammatory treatment.

**Important:** The CRP test is not as inexpensive as the ESR and takes time to perform. The CRP level can be elevated in pregnancy, trauma, and stress, but does not vary with the patient's age or sex, unlike the ESR.

The CRP level correlates better than the ESR with pain, stiffness and grip strength. Serial CRP levels also correlate with radiographic progression of rheumatoid arthritis.

**Advice:**
The CRP is often normal in systemic lupus erythematosus. A raised CRP in lupus patients requires a thorough search for a possible septic focus.

6. **RHEUMATOID FACTOR**

**Definition**
Rheumatoid factors are autoantibodies against the patient's own immunoglobulin G (IgG) molecules.

These autoantibodies are either IgG or IgM molecules against IgG. They are generally associated with rheumatoid arthritis, but rheumatoid factors may also be present in some normal persons as well as in a variety of other...
diseases. Positive rheumatoid factor is often found in many black people, particularly those living in rural areas, which are otherwise quite healthy and with no evidence of rheumatoid arthritis.

Some diseases associated with rheumatoid factor:

- **Rheumatic diseases:**
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
  - Scleroderma
  - Mixed connective tissue disease
  - Sjögrens syndrome
- **Viral infections:**
  - Hepatitis
  - Influenza
  - AIDS
  - Mononucleosis
- **Parasitic infections:**
  - Malaria
  - Schistosomiasis
- **Chronic bacterial infections:**
  - Tuberculosis
  - Salmonellosis
  - Brucellosis
  - Subacute bacterial endocarditis
- **Neoplasms:**
  - After radiation or chemotherapy
- **Other hyperglobulinemic states:**
  - Chronic liver disease
  - Sarcoid
  - Some chronic pulmonary diseases.

About 20% of patients with definite rheumatoid arthritis will be rheumatoid factor negative, so the test is not very specific for the disease.
Despite its lack of specificity for rheumatoid arthritis, a positive rheumatoid factor test has been found to predict more severe disease such as progression of joint erosions, rheumatoid nodules and vasculitis.

**Important:** Do not use the rheumatoid factor test to diagnose rheumatoid arthritis. Its main use is a guide to prognosis.

7. **ANTINUCLEAR ANTIBODIES (ANAs)**

**Definition**
Antinuclear antibodies (ANAs) are a diverse group of antibodies found most prominently in systemic lupus erythematosus (SLE), systemic sclerosis, mixed connective tissue disease and Sjögren's syndrome.

Such autoantibodies, however, may appear in a variety of other diseases, including infections, inflammatory diseases and neoplastic diseases, and in some normal individuals. These ANAs are immunoglobulins directed against intracellular antigens including DNA, ribonuclear proteins, histones and centromeres.

A general sifting test for ANAs is done first, and if this is positive, further tests for the more specific antibody are required. The test is nonspecific, but it is valuable because of its sensitivity. If it is negative, it is unlikely that the patient has an active autoimmune disease. At least 95% of patients with SLE will test positive for ANA, but almost all of the other systemic autoimmune diseases are associated with a positive ANA, as are a number of other conditions as listed above.

**Important:** A negative ANA makes an active autoimmune disease unlikely. A positive test requires further investigation.

The ANA titre can be useful. Most patients with SLE have very high titres of ANA. The presence of active lupus in a woman with an ANA titre of about 1:40 and even 1:160 is highly unlikely. Mixed connective tissue
disease, drug-induced lupus and rheumatoid arthritis also are associated with high ANA titres.

Because different ANAs have different intracellular targets, observing the fluorescence pattern within the cell can give useful information.

A positive ANA test may show a homogeneous, speckled, nucleolar or peripheral (rim) pattern, depending on which specific antibodies are present. Unfortunately, however, these tests frequently do not give clear-cut diagnostic information, and although regularly reported with the ANA test, they have been largely replaced with testing for the specific antibodies against extractable nuclear antigens (ENA).

- **Anti-DNA Antibodies**

  Antibodies against ssDNA are antibodies against single-strand DNA, hence the name ssDNA. These are denatured fragments of DNA. They appear in several diseases, including SLE, drug-induced lupus, chronic active hepatitis, infectious mononucleosis and rheumatoid arthritis. Although regularly reported in blood reports, ssDNA is not specific enough to be of much clinical value.

  Antibodies against double-strand DNA (dsDNA) are against the native DNA helix and of much more specific value, as a high titre is diagnostic of SLE. Even a moderately or slightly elevated titre is specific for lupus, except for occasional cases.

  The anti-dsDNA test is also useful therapeutically because it reveals active disease that is not necessarily symptomatic. On follow-up visits, therefore, it can detect flare-ups before they become clinically significant. Immune complexes containing anti-DNA antibody are deposited in the glomerular basement membrane and have been correlated with the development of active lupus nephritis.

- **Anti-RNP and anti-Smith antibodies**

  In the late 1960s, Sharp described a connective tissue disease with features of sclerodactyly, myositis, a nonerosive arthritis, Raynaud's phenomenon
and swollen hands. The patients with this "mixed" connective tissue disease all had very high titres of antibodies to ribonuclear protein (RNP). These patients respond well to corticosteroids and did not develop renal or neural disease. Almost all patients with mixed connective tissue disease have anti-RNP antibodies, and a positive test provides a very high index of suspicion that the patient with Raynaud's will progress to this disease.

The Smith antibody is another antibody to extractable nuclear antigen. Its presence is essentially diagnostic for SLE, that is if present it is very specific. However, it is not very sensitive, as only about 30% of patients with SLE have the antibody. Thus many patients with lupus may have a negative anti-Smith antibody test.

- **Anticentromere and anti-Sci-70 antibody**

Anticentromere antibodies are frequently found in a group of patients with a limited form of scleroderma known as CREST (calcinosis, Raynaud's phenomenon, oesophageal involvement, sclerodactyly, telangiectasia). A positive test with a high anticentromere antibody titre appears in 80-90% of CREST patients and is indicative of a good prognosis.

The anti-Scl-70 antibody is specific of the more diffuse, systemic form of scleroderma, but has low sensitivity. Only 10-20% of patients with this type of scleroderma have the anti-Scl-70 antibody.

- **Antihistone antibody**

The antihistone antibody is found in at least 95% of patients with drug-induced lupus syndrome. In patients taking such drugs as procainamide, quinidine, hydralazine or phenytoin and have fever, arthritis and pulmonary symptoms, a negative antihistone antibody test essentially rules out drug-induced lupus as the cause of symptoms. These patients may have a positive ANA test, but it has no clinical significance. An ever-increasing list of drugs can on rare occasions cause a lupus-like reaction.

**Advice:**
Unexplained fever and arthralgia in patients on drug therapy require an antihistone antibody test to exclude drug-induced lupus.
• **Anti-Ro(SSA) and anti-La(SSB) antibody**

Both the anti-SSA (or anti-Ro) and the anti-SSB (anti-LA) antibody appear in up to 55% of patients with Sjögren's syndrome, and particularly in patients with extra-glandular involvement. They may also occur in 15% of patients with lupus.

**Advice:**
The very rare lupus patient that is negative for antinuclear factor may well be positive for anti-SSA.

A pregnant woman with lupus should always be tested for anti-SSA and SSB. If these are present, she may give birth to a baby with the neonatal lupus syndrome of a rash, haemolytic anaemia and complete heart block.

• **Antineutrophil cytoplasmic antibody**

Antineutrophil cytoplasmic antibody (ANCA) reacts with cytoplasm antigens of polymorphonuclear (PMN) leucocytes. Two types of ANCA are known: C-ANCA and P-ANCA. Both are associated with vasculitis, especially Wegener's granulomatosis, certain kinds of periarteritis nodosa and crescentic glomerulonephritis.

Tests for these antibodies are especially valuable in that they provide a way to diagnose vasculitis without the need for organ tissue. C-ANCA is highly specific of Wegener's syndrome, but its sensitivity depends on the stage of disease activity; the sensitivity may be as high as 90% during systemic activity but as low as 30% during remission. Because the titre parallels disease activity, serial C-ANCA testing is useful for monitoring patients with Wegener's granulomatosis.

P-ANCA has been described in a wide variety of diseases, most of them autoimmune. However, the lack of sensitivity and disease specificity has diminished the clinical value of indiscriminate P-ANCA testing. It is also associated with crescentic glomerulonephritis and occurs in 70% of patients with Churg-Strauss syndrome and 90% with sclerosing cholangitis with ulcerative colitis.
8. **ANTIPHOSPHOLIPID SYNDROME**

Antiphospholipid antibodies are associated with a syndrome comprising a coagulopathy, thrombocytopenia, recurrent fetal loss and a cutaneous vascular pattern known as livedo reticularis. This syndrome may occur in patients with SLE and as a primary form on its own, without association with an autoimmune disease.

Three methods are used to detect these antibodies, namely:

- Lupus anticoagulant test
- Anticardiolipin antibody (ACA),
- False-positive Venereal Disease Research Lab (VDRL) test result.

Moderate to high titres correlate with active antiphospholipid syndrome. A low titre should be followed up in 3 months with a second test. If the elevation is still low, the patient has a low risk of developing the complications of this syndrome. Anticardiolipin antibodies may occasionally be seen in patients after various infections, including malaria.

9. **CRYOGLOBULINS**

**Definition**

Cryoglobulins are immunoglobulins that precipitate in the cold and then dissolve again when warmed.

Clinical features include Raynaud's syndrome, arthralgias, palpable purpura, glomerulonephritis and neuropathy. Other clinical associations include lymphoproliferative disorders, hepatitis A, B and C, as well as the connective-tissue disorders.

10. **COMPLEMENT CONSUMPTION**

Complement consumption, as measured by levels of C3 and C4 components and CH50 activity, remain useful guides to activity in lupus nephritis. Complement levels correlate much less closely with other organ involvement in SLE.