# LABORATORY TESTS USEFUL IN RHEUMATOLOGICAL EVALUATION

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In the common rheumatic (connective tissue) disease clinical manifestations, e.g., patient's symptoms, the pattern and distribution of joint involvement, extra-articular physical findings and chronicity, offer the best diagnostic clues. No single test or battery of tests can supplant a good history, a thorough physical examination, and pertinent x-rays. The value of laboratory tests, however, cannot be negated. Under some circumstances, certain tests are vital for diagnosis and proper treatment. Septic arthritis and crystal-induced synovitis (gout and pseudogout) are two good examples where laboratory tests are required for absolute diagnosis.

The purpose of the present article is to concisely review the principles, applications and diagnostic significance of the laboratory tests that are widely used in clinical rheumatology.

## Erythrocyte Sedimentation Rate (ESR)

As a result of active inflamation, elevation of "acute phase reactant" proteins in serum is frequently seen. This is reflected by elevation of the ESR, a very simple and still quite reliable test. It is most influenced by the plasma values of fibrinogen and alpha- and gamma-globulins. Any disease which raises the concentration of these blood components will raise the ESR. This test has no diagnostic specificity, but is of value in reinforcing a clinical impression with regard to the presence or absence of inflammation, in estimating the severity of the disease process, and in following the course of the disease in a particular patient. For example, it is still a very useful laboratory test for following the course of rheumatoid arthritis.

ESR is most commonly measured by the Westergren method. The upper limits of normal rise slowly with age. Recently the following upper limits of normal have been suggested: below the age of 50 years, 15 mm/hour for males and 25 for females; above age 50, 20 for males and 30 for females (1). ESR is generally normal in osteoarthritis but in erosive osteoarthritis (a recently described entity), ESR may be increased. In polymyalgia rheumatica, ESR is strikingly elevated, often over 100 mm/hour, and serves as a very useful test in following the course of this disease. It should be stressed that in following any disease, serial changes in ESR are more significant than a single determination.

Although a normal ESR suggests no inflammation, the ESR can sometimes be normal in a person with active disease, e.g., the occasional association of normal ESR with very active and severe systemic lupus erythematosus. Occasionally the ESR can be normal in a person with active disease where the inflammatory activity is suppressed by corticosteroids or other anti-inflammatory drugs.

## C-Reactive Protein (CRP)

The CRP test, like ESR,, is another non-specific but useful test. CRP is an "acute phase reactant" protein synthesized by the liver in response to inflammatory stimuli. CRP test has the advantage of being unaffected by factors like severe anemia, congestive heart failure, hyperglobulinemia, fibrinogen level and sickle cell disease, which tend to influence the ESR. It is somewhat more sensitive than ESR and is detected in the serum within hours after the inflammatory stimulus, unlike the ESR which take a few days to become elevated. Another

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advantage of CRP is that one can use fresh or stored serum for its estimation, while fresh blood is required for ESR determination. Unfortunately, CRP is not as readily quantitated as ESR. In addition, CRP test becomes normal faster than the ESR, e.g., in acute rheumatic fever CRP is very helpful in diagnosis and followup but it will become normal while the ESR is still high. Thus, a patient seen at a later stage of his disease can have a normal CRP. ESR has overall more clinical usefulness than CRP and is a better choice. We routinely do ESR alone and uncommonly need to do the CRP test. CRP test should not be used alone, but may be used to reinforce the result of the ESR.

### Rheumatoid Factor

Rheumatoid factor is an antibody (or group of antibodies) directed against immunoglobulin G (IgG). Human rheumatoid factor will react not only with autologous and homologous IgG, but also with heterologous IgG (e.g. rabbit IgG). Rheumatoid factor is found in the sera of 70 to 80% of patients with rheumatoid arthritis. There is no specific laboratory test for rheumatoid arthritis, but the presence of rheumatoid factor has considerable diagnostic significance, and is included as one of the American Rheumatism Association's eleven criteria for diagnosing rheumatoid arthritis (2). Since rheumatoid factor may be present in other connective tissue diseases, chronic inflammation, or infections like subacute bacterial endocarditis etc., the detection of rheumatoid factor does not establish the presence of rheumatoid arthritis. Nor does its absence exclude this diagnosis since 20 to 30% of rheumatoid arthritis patients lack this factor. Rarely rheumatoid factor may be found in healthy persons, particularly elderly individuals. With regard to the causes of rheumatoid factor in the tropics, see the paper by Gonzalez-Pares et al (3).

Tests used to detect rheumatoid factor depend on this antibody's ability to react specifically with purified IgG which has been heat-aggregated and coated onto sheep erythrocytes or latex or bentonite particles resulting in agglutination. The commonly used test is the "RA-latex test". Although there is no uniform agreement, 1:80 is the lowest titer generally considered to be diagnostically significant. Recent studies show that rheumatoid factor plays an important role in the pathogenesis of rheumatoid arthritis (4). The lack of rheumatoid factor in most patients with juvenile rheumatoid arthritis and 20 to 30% of patients with adult rheumatoid arthritis may be explained by the recent findings that such sera contain rheumatoid factors which are mainly IgG and IgA indetectable by the usual tests like the RA-latex test. The IgM rheumatoid factor, which is particularly responsible for the agglutination reactions of the RA-latex test, is found in very small amounts in such sera.

A positive test for rheumatoid factor, if it is to occur, tends to develop fairly early in the course of the disease — within the first year or two, and is associated with an increased probability of more severe disease, particularly when the titer is high.

### Lupus Erythematosus Cell (LE cell) and Antinuclear Antibodies (ANA)

The LE cell, which was first described by Hargraves in 1948, forms after unclotted blood has been incubated *in vitro* (5). It is an intact polymorphonuclear leukocyte that contains a large homogeneous reddish-purple inclusion in the cytoplasm. The test is time-consuming, requiring an experienced observer to do a thorough microscopical examination of the prepared slides. Although occasionally these cells can be found on direct examination of pleural, pericardial or synovial fluid, or in the bone marrow, they are usually found after the *in vitro* incubation of peripheral blood. LE cells test is positive in up to 80% of patients with active systemic lupus erythematosus (SLE), in drug induced SLE and in some patients with severe rheumatoid arthritis or other connective tissue diseases. Although LE cells may disappear with treatment, the test cannot be used as a guide to the adequacy of therapy because there is little correlation between the number of LE cells and disease activity.

The LE cell test has been supplanted by the antinuclear antibody test (ANA). For screening purposes, ANA is much more sensitive and easier to perform. For practical purposes a negative ANA rules out active SLE. Although ANA is positive in virtually all patients with active SLE, it may also be positive in many other connective tissue disease, e.g., up to 30% of patients with

rheumatoid arthritis. The test can also be positive in other conditions, e.g., in some individuals on long term therapy with certain drugs like procainamide, isoniazid, anti-convulsants, etc., even in the absence of induction of SLE by these drugs. Therefore, in any patient with a positive ANA, a drug history is extremely important. Rarely ANA may be positive in normal people, especially the elderly.

Antinuclear antibodies, as the name indicates, are antibodies (immunoglobulins) that react with the various constituents of cell nuclei *in vitro*. An immunofluorescent technique is usually used to demonstrate the antinuclear antibodies, using as a substrate mammalian tissues rich in nuclei (e.g., mouse liver or rat kidney). A positive ANA test is indicated by the fluorescence of the nuclei when examined by fluorescent microscope. The degree of brightness is usually expressed as 1+ to 4+. Some laboratories do further diluting of the positive sera to determine the titer. In addition, distinct profiles of antinuclear antibodies can be identified in certain connective tissue diseases (6). A number of patterns of nuclear fluorescence have been described (7). Homogeneous pattern is most common and is primarily due to the presence of antinuclear antibodies against nucleoproteins, the antibodies that are responsible for the positive LE cell test. Shaggy (peripheral or rim) pattern is due primarily to the antibody to DNA, and is more specific for SLE, often associated with disease activity, particularly renal disease. Speckled pattern is seen in many connective tissue diseases, particularly mixed connective tissue disease and scleroderma. Other patterns of nuclear fluorescence of uncertain significance have also been described.

The presence of antibodies to double stranded DNA in high titer has been found to be quite specific for SLE (6). However, antibodies to double stranded DNA are not found in all patients with active SLE. They often tend to be associated with renal involvement. At present, reliable anti-DNA antibody assays are not generally available for the practicing physician.

## Cryoglobulins

These are immune complexes or aggregated immunoglobulins which have the property of precipitating on exposure to cold (e.g. when left

at 4°C overnight). This is a reversible phenomenon redissolve on warming at 37°C. Most laboratories report the cryoglobulin result in the form of a cryocrit, which is the percentage of the cryoglobulin precipitate in a tube filled with the serum. To do the test properly, it is extremely important that the blood sample be taken to the laboratory immediately after it is drawn from the patient. In the laboratory the blood sample is allowed to clot at 37°C to separate the serum. If it is allowed to get cold before reaching the laboratory, cryoglobulins may precipitate out in the blood clot and be undetectable in the serum. Cryoglobulins are useful in diagnosis and followup of diseases where circulating immune complexes might be playing an important part in the disease process (e.g., SLE, mixed cryoglobulinemic vasculitis, glomerulonephritis, etc.) (8).

## Complement

Complement (c) is a group of 9 serum proteins (mostly beta-globulins) which interact with each other in sequential manner when activated by antigen-antibody reaction (9). These complement components are designated C1, C2 — C9. Most laboratories perform assays for only a limited number of these components (e.g., C3 and C4), or determine the total hemolytic complement activity ($CH_{50}$). Since some of the complement components are quite labile, certain technical problems have to be considered when evaluating the results.

Disorders of the serum complement system may be congenital or acquired. Congenital deficiences are rare. The acquired complement abnormalities may be divided into conditions with elevated serum complement levels and those with decreased levels. In general, rheumatoid arthritis patients have normal or elevated serum complement levels while SLE patients, especially when the disease is active have decreased levels. Fall in serum complement level suggests its consumption in an immune reaction, and this is seen in some other diseases besides SLE, associated with circulating immune complexes (e.g., acute post-streptococcal glomerulonephritis, vasculitis associated with mixed cryoglobulinemia etc.). Since complement complonents act as acute phase reactants, serum complement levels are generally increased in

most other inflammatory conditions where there is no complement consumption.

Serial serum complement levels are helpful in follow-up of patients with lupus nephritis. Control of disease activity is often accompanied by return of complement level to normal and recurrence of disease activity is often heralded by a decrease in serum complement level.

### Serum Uric Acid

Uric acid is the final product of purine metabolism in man, formed from exogenous dietary purines and from endogenous sources. Presence of hyperuricemia in gouty patients was first demonstrated by Garrod in 1848. Normal concentration of uric acid in adult males is less than 7 mg per 100 ml (uricase method). In females it is 1 mg lower. The local laboratory should be asked to state the values they accept as normal. An acute attack of gout is caused by release of urate crystals into joint cavity (10, 11). One cannot diagnose acute gout on the basis of hyperuricemia alone, even when associated with acute arthritis, because other diseases may mimic acute gout. Presence of urate crystals in the synovial fluid is pathognomonic for gout.

A fair number of people who have hyperuricemia do not have gout. In a recent study, 13.2% of 4, 148 male patients were found to be hyperuricemic at the time of admission to the hospital (12). When 200 patients with hyperuricemia were studied in detail, the most common causes of hyperuricemia were found to be ingestion of diuretics (20%), azotemia (20%) and acidosis (20%). Only 12% had a history of gout. Eighty percent of patients did not require specific therapy for their hyperuricemia. Many drugs besides diuretics can cause hyperuricemia. Aspirin in low doses can cause hyperuricemia but when taken in excess of 3 gm/day causes hyplouricemia. Persons who go on starvation diet will have high uric acid. Hyperuricemia is also seen in disorders with accelerated turnover of nucleoproteins (i.e., leukemia, myeloid metaplasia, chemotherapy or radiation treatment of lymphoma etc.). Thus the evaluation for the significance of hyperuricemia includes a careful history (including drug history), thorough physical examination (looking for synovitis and tophi, in particular) and the examination of synovial fluid for crystals wherever possible.

### Studies of Synovial Fluid

Examination of synovial fluid can be of major importance in the differential diagnosis of the arthritic disorders. The volume, gross appearance (color and clarity), viscosity, mucin clot, cell count (total and differential), culture, Gram stain, and glucose and protein concentrations should be noted. Polarizing microscopy is needed to reliably look for crystals, though larger crystals can be seen under regular light microscope if the condenser is turned down.

The need and priority of these tests of synovial fluid depend upon the volume of synovial fluid obtained and the provisional clinical diagnostic impression. When septic arthritis is suspected, priority should be given to culture, Gram's stain and white cell count in that order. If fluid remains, glucose measurement together with simultaneous blood glucose determination should be done. In a non-diabetic patient with septic arthritis, the difference between the blood and the synovial fluid glucose levels may be over 50 mg/100 ml. In the case of acute crystal-induced synovitis, microscopic examination of synovial fluid with a polarizing microscope will reveal negatively birefringent needle-like urate crystals (in polymorphonuclear cells and some free in fluid) pathognomonic for gout; or positively birefringent rhomboid or rod-like crystals of calcium pyrophosphate diagnostic of pseudogout (13, 14). If the joint has recently been injected with corticosteroids, the synovial fluid may contain numerous crystals of the injected corticosteroid, which must not be confused with urate or calcium pyrophosphate crystals.

The techniques of joint aspiration and synovial fluid analysis are detailed in standard textbooks (15, 16). There is also a well-illustrated monograph (17) that I recommend to readers interested in learning more about synovial fluid analysis. Small booklets dealing with the techniques of arthrocentesis are freely available from pharmaceutical companies like Upjohn and M.S.D. (Merk, Sharpe and Dohme).

### Antistreptolysin "O" (ASO) Titer

Most of the patients with streptococcal infection produce antibodies against streptococcal antigens. The streptococcus produces two types of hemolysins —

streptolysins "O" and "S". The ability of streptolysin "O" to hemolyze sheep red cells is depressed if serum containing antistreptolysin "O" (ASO) is added. Therefore, by using standardized preparation of streptolysin "O" and sheep red cells, one can determine the dilution of the test serum necessary to produce inhibition of hemolysis, and this can be recorded as ASO titer. By convention the test is frequently reported as the reciprocal of the titer, e.g., "1:200" titer becomes "200".

An elevated ASO titer (greater than 200 units in most laboratories) is nonspecific and merely indicates recent streptococcal infection with or without rheumatic fever. The height of the ASO titer bears no relationship to the severity of rheumatic fever. In addition, the rate of fall of the ASO titer has no relationship to the subsequent course of rheumatic fever. Within the first two months of the attack at least 80% of patients have an increased ASO titer. If additional antibodies to streptococcal antigens (e.g., antideoxyribonuclease-B, antihyaluronidase, antistreptokinase, etc.) are measured, almost all patients would show elevation of at least one of these antibodies. Occasionally, when a period of several months elapses between the occurrence of streptococcal infection and the diagnosis of rheumatic fever, these titers may have already declined to normal levels.

Throat cultures are of relatively limited value since only 50% of patients who contract rheumatic fever continue to harbor streptococci during the course of their illness. A history of antecedent pharyngitis is also of limited value in diagnosing rheumatic fever, as only 40 to 50% of patients with the disease give such a history.

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