INVITED ARTICLE

LABORATORY TESTS IN RHEUMATIC DISEASES: A GUIDE FOR CLINICIANS

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ABSTRACT

In the practice of rheumatology, just as in other disciplines of medicine, laboratory tests are meant to supplement a thorough history and physical examination. The clinician should have a purpose for ordering each test; to screen for a disorder, confirm a diagnosis, exclude a possible diagnosis, monitor therapy or determine prognosis. Many of the rheumatic diseases have in common processes and features that are inflammatory and many of the tests merely establish the presence of an inflammatory process. The measurement of ESR and C-reactive protein are two such tests most widely used. The systemic rheumatic diseases also manifest serologically with the continual production of non-organ specific autoantibodies. Few of these antibodies are specific for a particular disease. The majority of the autoantibodies are positive in various disease states and are only useful as adjuncts to the diagnosis of rheumatic diseases. This discussion deals with commonly ordered tests in rheumatic diseases; how to use the tests appropriately while keeping in mind their limitations.

Keywords: rheumatic diseases; laboratory tests

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The basic "tools" of clinical rheumatology are a good history and thorough clinical examination. These form the basis of a differential diagnosis and a suspicion of whether a specific disease is present. Only after these findings are evaluated should laboratory investigations be considered. Laboratory tests when ordered judiciously can be helpful in the diagnosis and management of rheumatic diseases particularly in the patient with few clinical criteria and thus little probability of disease but in whom the disease cannot be ruled out on clinical grounds. However, too much emphasis should not be placed on laboratory tests and rarely is a single test absolutely required to make a diagnosis.

To be able to select the appropriate tests, the clinician must have a general understanding of the test techniques, the predictive values and the limitations of the tests. This discussion deals with commonly ordered tests in rheumatology; how to use the tests appropriately while keeping their limitations in mind.

ERYTHROCYTE SEDIMENTATION RATE

During inflammation, the liver produces increased amounts of approximately 30 plasma proteins called acute phase reactants. These proteins include C-reactive protein (CRP), complements and other proteins, including those that increase the erythrocyte sedimentation rate (ESR)[1].

The ESR is a measure of the rate at which red blood cells sediment in a column of blood. It is the most common and widely used measure of the acute phase reaction because it is elevated in many inflammatory diseases. Two methods are generally used in measuring ESR: the Wintrobe and the Westergren techniques. The latter is more reproducible and is considered the standard by the World Health Organisation.

The ESR is a useful screening test although it is non-specific and of no diagnostic value. It may also be used in the assessment of disease activity in rheumatic diseases and the response to treatment. A very high ESR in elderly patients is a common manifestation of polymyagia rheumatica or giant cell arteritis. A rapid fall in ESR following treatment is reassurance that the diagnosis is correct and may provide a sensitive indicator of disease activity. In the management of the young man with back pain, a high ESR is helpful indicator of an inflammatory disorder such as ankylosing spondylitis rather than traumatic musculoskeletal process.

It should be remembered that the ESR may uncommonly be elevated in the absence of detectable disease or normal in the presence of inflammation. ESR is affected by red cell morphology, anaemia, polycythemia, estrogen, glucocorticoids, fibrinogen, immunoglobulins, and other factors[2]. It is important to note that with age, the ESR is often mildly to moderately elevated in apparently healthy persons.

C-REACTIVE PROTEIN

The C-reactive protein (CRP) was originally described by Tillet and Francis during work on serological response to pneumococcal infection. It is now known that a variety of infectious and inflammatory conditions stimulate an elevation of the CRP with two notable exceptions: systemic lupus erythematosus (SLE) and ulcerative colitis[3]. In these two diseases, the CRP may only show a modest rise or remain normal in the face of active disease. As bacterial infection is the most potent stimulant of CRP production, a high CRP in a patient with active SLE and fever, is a useful pointer to intercurrent sepsis as a cause of the fever[4].

Like the ESR, the CRP is of little use in differentiating rheumatoid arthritis from the other inflammatory arthropathies but in individual patients are good indicators of disease activity and response to treatment. Marked and continued elevation of CRP indicates a poor prognosis in rheumatoid arthritis[5].

It is extremely difficult to assess systemic vasculitis clinically. Unfortunately the ESR is highly variable. In these diseases the CRP may be a useful guide to disease activity.

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COMPLETE BLOOD COUNT

A complete blood count is a cheap and useful investigation in the assessment of a patient for rheumatic diseases. Anaemia is a common feature of chronic inflammation, especially if it is normochromic and normocytic. In a patient on nonsteroidal anti-inflammatory agents, a microcytic anaemia indicates possible iatrogenic gastrointestinal bleeding. A profound macrocytic anaemia with reticulocytosis suggests hemolytic anaemia and SLE. One may then proceed to a Direct Coomb's test.

A low total white count suggests one of the connective tissue disorders, notably SLE in which lymphopenia is often also present[6]. Leucopenia with neutropenia in a patient with long standing rheumatoid disease may be a manifestation of Felty's syndrome and is associated with splenomegaly[7]. Eosinophilia with thrombocytosis are evidence of disease activity in rheumatoid arthritis[8]. Eosinophilia may also be one of the earliest features of adverse drug reaction in rheumatoid arthritis patients treated with sodium aurothiomalate (myocrisin) or penicillamine.

Finally the platelet count may be low in active SLE[9]. Low platelet count is a complication of therapy of connective tissue diseases with immunosuppressive agents such as azathioprine and cyclophosphamide and therapy of rheumatoid arthritis with penicillamine and gold.

Most of the systemic vasculitides are accompanied by a mild anaemia, leucocytosis and thrombocytosis. Eosinophilia may be present in polyarteritis nodosa or Churg Strauss vasculitis.

COMPLEMENTS

The levels of complement components in the serum reflect a balance between synthesis, consumption and catabolism. Many of the components behave as acute phase reactants; for example, total hemolytic complement (CH50) is often elevated in a variety of acute inflammatory diseases. Low levels of complements suggest an immune mediated consumption unless there is underlying complement deficiency due to an inherited disorder or impaired synthesis. In active SLE, particularly in active nephritis, complements are generally low[10]. Complements however are not an infallible guide to disease activity and should not be used as the sole criteria for modifying therapy. In rheumatoid arthritis, serum complement levels are usually normal or elevated but may be low in patients with severe systemic disease.

RHEUMATOID FACTOR

Connective tissue disorders such as SLE and rheumatoid arthritis manifest serologically with the continual production of autoantibodies of the non-organ specific category. These autoantibodies are directed at antigenic determinants that are not exclusively located in joints, skin, kidneys or other tissues. There are two main groups of such antibodies; rheumatoid factors (RF) and antinuclear antibodies (ANA).

The rheumatoid factor is an autoantibody directed against antigenic determinant present in the Fc portion of IgG. It is so named because it was initially found in the sera of patients with rheumatoid arthritis. Although IgM, IgG, IgA, and IgE rheumatoid factors have been described, IgM RF is the one measured routinely in clinical laboratories. The easiest method of IgM RF measurement is by the agglutination technique. Typically latex of sheep red blood cells are the particles agglutinated.

The latex test is more sensitive with about 75% of patients with clinical rheumatoid arthritis having a positive test. On the other hand it is not specific. A positive latex test may be present in other rheumatic diseases including Sjogren's syndrome, SLE, systemic sclerosis, and mixed connective tissues disease. Non-rheumatic diseases that are positive include chronic infections such as bacterial endocarditis, tuberculosis, leprosy and syphilis and acute viral infection. Non-infectious "immunologic" diseases such as diffuse interstitial pulmonary fibrosis, chronic active hepatitis, sarcoidosis, and cryoglobulinaemia may have RFs. In normal healthy individuals, the prevalence of a positive latex test rises with age[11]. Thus, a diagnosis of rheumatoid arthritis based mainly or solely on test findings may be erroneous and lead to inappropriate therapy. The diagnosis of rheumatoid arthritis should be based on clinical evidence with laboratory tests serving a confirmatory function.

The sheep cell agglutination test (SCAT) is also known as the Rose-Waaler test. It is highly specific; about 90% of patients with a positive result will in fact have rheumatoid arthritis. On the other hand, this test is positive in only 50% of rheumatoid arthritis patients being far less sensitive than the latex test. Therefore, using current tests available, a negative result for rheumatoid factor does not exclude the diagnosis of rheumatoid arthritis.

Patients with rheumatoid nodules, vasculitis and more severe erosive disease tend to be seropositive and with high titre (1:640 or higher)[12]. A high titre alone however is not an indication of the severity of the disease.

Most patients with rheumatoid arthritis are consistently seropositive or sero-negative but titres may fluctuate over months or years. Most NSAIDs do not affect RF levels. In contrast, both gold and penicillamine often produce a slow fall in the titre of IgM RF and patients may become seronegative after prolonged treatment.

A negative RF is useful in pointing towards other rheumatic diseases. Most patients with ankylosing spondylitis, Reiter's syndrome, enteropathy associated arthritis, psoriatic arthropathy, gout, chondrocalcinosis and pyogenic arthritis are negative for RF. In juvenile chronic arthritis too, only a minority (often older girls) with a disease resembling adult rheumatoid arthritis are seropositive. Patients with Still's disease are mainly seronegative.

LE CELL TEST

The LE cell test described by Hargraves et al, was the first reliable laboratory test for the diagnosis of SLE. The LE cell is an invitro morphologic phenomenon caused by an antibody to nuclear DNA-histone complex. The test is done by traumatising leucocytes to release their nuclear materials. Antibodies present in the serum then bind to this material and the resulting DNA-histone-antibody complex is phagocytosed by neutrophils. These neutrophils with the ingested intracytoplasmic inclusions are called LE cells. The accuracy of the test depends on the technical expertise of the laboratory performing and reading the test. It is not useful as a screening test because the procedure is time consuming. It is positive in 50% to 70% of SLE patients. However it is not specific for SLE and may also be positive in a variety of systemic rheumatic diseases including rheumatoid arthritis. Patients with chronic active hepatitis may show positive LE cell test[13].

FLUORESCENT ANTINUCLEAR ANTIBODY TEST

FANAs or ANAs are autoantibodies directed against various components of cell nuclei, including deoxyribonucleoprotein, single stranded DNA, double stranded DNA, extractable nuclear components, histones and the centromere. ANAs are detected by incubating patient's serum with a substrate that consists of exposed nuclear components. These substrates are either organ sections (eg. rat liver) or tissue culture cells (eg. Hep2 cells). If present, ANAs bind the nuclear components and an anti-

human antibody that is labelled with fluorescent marker is added. The result is read under a fluorescent microscope. It is important to know at what titre a laboratory considers the test to be significant, since the values vary from 1:10 to 1:80 or 1:120[14].

A positive ANA by itself does not necessarily indicate disease as it may be present in a small percentage of normal individuals particularly the elderly and pregnant women[15].

These antibodies are present in a wide variety of rheumatic diseases and are not diagnostic of SLE. As a screening test for SLE, it is very sensitive being positive in more than 90% of SLE patients. A negative test however makes the diagnosis of SLE very unlikely, although it cannot be categorically excluded as 2% to 7% of SLE patients are persistently FANA-negative[16]. High titres are the rule in active, untreated cases of SLE.

In rheumatoid arthritis, 20% to 35% of patients with classic or definite disease (ARA criteria 1963) have positive ANAs, the highest titres being in those with nodules and vasculitis. The test is more important in pediatric rheumatology. Schaller noted an association between a positive FANA result in pauciarticular juvenile chronic arthritis and the presence of clinically silent iridocyclitis[17]. These children should be referred to an ophthalmologist for regular review to prevent blindness.

Table I shows the conditions in which ANAs have been reported. In addition to rheumatic diseases, positive FANA tests have been reported in drug induced lupus, patients on gold and penicillamine therapy, organ specific autoimmune disorders, infections, and neoplastic diseases.

Table I : Conditions in which anti-nuclear antibodies have been reported

1. Rheumatic diseases : systemic lupus erythematosus, progressive systemic sclerosis, rheumatoid arthritis, juvenile chronic arthritis, polymyositis/dermatomyositis, Sjogren's syndrome, polyarteritis nodosa, discoid LE
2. Organ specific autoimmune diseases : Myasthenia gravis, Hashimoto's thyroiditis, pernicious anaemia, dermatitis herpetiformis, cryptogenic fibrosing alveolitis
3. Lupoid hepatitis and other autoimmune liver disease
4. Drug induced lupus
5. Infections : Tuberculosis, leprosy, infectious mononucleosis
6. Neoplasm : Lymphoma, melanoma, thymoma
7. 2% of normal population but in 20% of persons over 90 years old
8. Pregnancy

Different patterns of fluorescence are observed in the FANA test. The pattern observed with a given serum often varies with the dilution used and immunofluorescent patterns overlap disease entities. These patterns include the homogenous pattern produced by antibodies to DNA-histone, rim pattern most often seen with anti-dsDNA antibodies and the nucleolar pattern most characteristic of systemic sclerosis. Speckled patterns are associated with antibodies to extractable nuclear antigens. Additional clues provided by these patterns are usually of marginal benefit to the clinician although of interest to investigators.

ANTI-DNA ANTIBODIES

Antibodies to pure double-stranded DNA (nDNA) are generally held to be relatively specific for SLE[18]. The specificity of the test in the diagnosis of SLE is dependent on the quality of the DNA used in testing for the antibody. Pure nDNA is difficult to prepare and most tests measure antibodies against both double and single stranded DNA. Anti-nDNA antibodies are measured by using the kinetoplast of the flagellate Crithidia luciliae or by more quantitative immunochemical assays.

Although the presence of anti-nDNA antibodies is not absolutely specific for SLE, the test is useful as an adjunct to the history and clinical examination. The prevalence of this antibody in SLE is about 60% compare with about 90% prevalence of FANA in SLE. The anti-nDNA test is therefore not useful as a screening test for SLE. The titre of the antibody may be useful as a guide to therapy. High titres of the antibodies are often associated with active SLE particularly nephritis[18]. Borderline elevation of DNA binding may be seen in other disease states especially in Sjogren's syndrome and chronic active hepatitis.

ANTI-Sm ANTIBODIES

This antibody is highly specific for SLE and its presence is virtually diagnostic of the disease[19]. Anti-Sm antibodies however are present in only 25% to 30% of patients with SLE. The antibody is most commonly determined by immunodiffusion and counter immunoelectrophoresis.

ANTI-RNP ANTIBODIES

These antibodies are also commonly measured by immunodiffusion or counter immunoelectrophoresis. High titres of the antibody are found almost exclusively in patients with mixed connective disease where synovitis and Raynaud's phenomenon are the main clinical features[20]. The anti-RNP (ribonucleoprotein) antibody may also be present in SLE, systemic sclerosis and rheumatoid arthritis. It is not a useful diagnostic test.

ANTI-Ro AND ANTI-La ANTIBODIES

The anti-Ro antibody (also known as anti-SSA) has been described in a number of ANA-negative SLE[16]. A hallmark of this condition appears to be an annular photosensitive rash termed subacute cutaneous LE (SCLE). Anti-Ro antibody is also associated with neonatal lupus and it should be determined in the SLE patient planning a family. Transplacental transfer of this antibody from mother to fetus may result in neonatal lupus, a transient lupus-like syndrome characterised by a photosensitive annular rash, thrombocytopenia, and damage to the conducting system of the heart[21]. Scarring results in complete heart block which may require pacing.

Anti-La (anti-SSB) antibodies are found in 75% of patients with Sjogren's syndrome but also in rheumatoid arthritis, SLE and systemic sclerosis.

OTHER ANTINUCLEAR ANTIBODIES

Many other antibodies have been described in systemic rheumatic diseases. One such antibody is the anti-Jo 1 antibody directed against histidyl-tRNA synthetase. It is relatively specific for adult polymyositis and identifies a subset of polymyositis patients with an increased frequency of interstitial lung disease[22]. Anti-centromere antibody is determined by indirect immunofluorescence on tissue culture cell substrates as discrete speckled staining of metaphase and interphase cells. It is found in high frequency (57% - 96%) in patients with CREST (calcinosis, raynaud's, esophagitis, sclerodactyl, and telangiectasia) syndrome[23]. The antibody is also found in patients with idiopathic Raynaud's phenomenon but rarely in other connective tissue diseases. In systemic sclerosis, the presence of anti-Scl-70 antibody is highly diagnostic[24]. This antibody is directed against DNA topoisomerase I and is detected by immunodiffusion.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)

Antineutrophil cytoplasmic antibodies (ANCAs) are antibodies directed against cytoplasmic antigens of neutrophils and monocytes. In 1985, Van de Woude et al demonstrated the high sensitivity of ANCA in the diagnosis of Wegener's granulomatosis (WG)[25]. The titre of the antibody fluctuates with clinical activity of the disease and falls or disappears with treatment. Thus it is very useful in the management of patients with WG. ANCAs however are not specific for Wegener's granulomatosis and have been found in other disorders where vasculitis is either a constant or frequent pathological feature. It has been reported in microscopic polyarteritis nodosa, Kawasaki's disease, necrotising and crescentic glomerulonephritis, Churg Strauss' vasculitis, Takayasu's disease, SLE, relapsing polychondritis, Behcet's syndrome, and ulcerative colitis[26].

Using the indirect immunofluorescence technique, 2 patterns of ANCA have been identified: (1) classical ANCA which produces a coarsely granular immunofluorescence and (2) perinuclear ANCA which gives a perinuclear pattern. ANCA may also be determined by enzyme linked immunosorbent assay (ELISA) using purified primary granules of neutrophils (and recently, purified antigens) which is more objective but is not at present readily available. The ANCA pattern seen in WG is the granular type.

THE LUPUS ANTICOAGULANT

This was first described in SLE in 1952. The antibody acts as an inhibitor of the prothrombinase complex prolonging invitro clotting tests for both intrinsic and extrinsic pathways. Paradoxically, it is associated with thrombosis (arterial and venous), recurrent abortion and thrombocytopenia[27]. The lupus anticoagulant is an antiphospholipid antibody and accounts for the frequent association with false positive serological tests for syphilis in patients with SLE.

It is now known that antiphospholipid antibodies are not specific for SLE. It has been found in other autoimmune, neoplastic and drug-induced diseases as well as in healthy individuals. The titre of the antibody does not predict the occurrence of a clinical event or a remission.

HLA TYPING

The major histocompatibility complex called HLA in humans, is associated with specific rheumatic diseases. It may aid in the understanding of pathogenic processes, but is of limited clinical value in the management of patients. The presence of HLA-B27 for instance does not absolutely confirm a diagnosis of spondyloarthropathy despite its expense.

SERUM URIC ACID

Appropriate interpretation of the significance of serum uric acid level is important. An elevated serum uric acid does not in itself indicate gout even in the presence of joint symptoms. Gout is diagnosed unequivocally only by the demonstration of urate crystals in the joint fluid.

CONCLUSION

Laboratory tests are meant to supplement a good history and clinical examination. Inappropriately used, laboratory tests can lead to confusion and unnecessary expense. In the practice of rheumatology, it is not uncommon that even after a thorough history, physical examination and investigations, a diagnostic label cannot be given to the patient's problem. In such an instance it is best to observe the patient rather than to give non-specific laboratory tests more significance than they deserve.

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