INTERPRETATION OF LABORATORY TESTS IN RHEUMATIC DISEASE

Laboratory tests are an important adjunct in the clinical diagnosis of rheumatic diseases and are sometimes helpful in monitoring the activity of a disease.

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Interpretation of laboratory tests is not always straightforward, as the presence of a laboratory abnormality does not always imply the presence of disease, and a single test generally does not confirm (or exclude) a diagnosis.

This means that understanding the sensitivity (the proportion of patients with the disease who have a positive test) and the specificity (the proportion of patients without the disease who have a negative test) of tests is critically important. A test with both a high sensitivity and specificity is useful in diagnosis. Positive and negative predictive values are often more helpful in decision-making. The positive predictive value of a particular test reflects the proportion of patients with a positive test who truly have the disease, and a negative predictive value reflects the proportion of patients with a negative test who truly do not have the disease. Predictive values are dependent on the background prevalence of the disease and are disease specific (Table I).

Table I. **Sensitivity, specificity and predictive value**

	Disease present	Disease absent
Positive test	a (true positive)	b (false positive)
Negative test	c (false negative)	d (true negative)

Sensitivity = a/a+c
Specificity = d/b+d
Positive predictive value = a/a+b
Negative predictive value = d/c+d

GENERAL TESTS OF INFLAMMATION

General tests of inflammation are not diagnostically specific, but are helpful in determining the presence and intensity of an inflammatory process. They are useful for monitoring disease activity.

The acute phase response is determined by proteins produced by the liver in response to the pro-inflammatory cytokines IL-1, IL-6 and TNF-alpha. The most commonly measured protein is C-reactive protein (CRP). The platelet count is also often raised in the presence of inflammation due to stimulation of megakaryocytes by the pro-inflammatory cytokines. The level of some plasma proteins may even be reduced in inflammatory states, e.g. albumin. The acute phase response is seen in a wide variety of acute and chronic disorders including infection, infarction, inflammatory arthritides and certain neoplastic disorders.

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ERYTHROCYTE SEDIMENTATION RATE (ESR)

The ESR has been used for many years as an indicator of nonspecific inflammation. It is a measure of rouleaux formation, which is dependent on the concentration of fibrinogen and immunoglobulins. It is simple and cheap to perform. Limitations of the test are:

- it is an indirect measurement of plasma acute phase response and can be influenced by conditions such as anaemia
- it rises and falls slowly compared with the CRP
- values increase with age
- values are slightly higher in women
- the range of abnormal values is less than that for the CRP.

CRP

The CRP is a sensitive and early indicator of inflammation. The level is not affected by age, sex or anaemia. This test is valuable in monitoring disease activity, particularly in rheumatoid arthritis (RA) and polymyalgia rheumatica. Some rheumatologists feel that the CRP levels correlate somewhat better with activity than does the ESR, and that the test is more sensitive. However the higher cost limits its popularity in some areas.[1]

Although helpful in determining the presence or absence of inflammation, 10% of patients with mild RA may have CRP values in the normal range. The level of acute phase reactants may have prognostic implications in RA, with time-integrated values (i.e. the elevation of CRP over a period of time) correlating with radiographic progression.

ANTINUCLEAR ANTIBODIES

Antinuclear antibodies (ANAs) are autoantibodies directed against various nuclear antigens. Immunofluore-scent techniques are used for the detection of ANAs in general, and enzyme-linked immunosorbent assays (ELISA) for the detection of specific antinuclear antibodies.

The immunofluorescent ANA test is useful as a first screening test in patients with suspected connective tissue disease. The results are expressed as the highest titre at which fluorescence is detected. Although titres of 1:20 or 1:40 are commonly reported as positive, titres of 1:320 or higher are usually considered more clinically meaningful. Patterns of staining by ANAs can be seen (homogenous, speckled, nucleolar), and may provide a clue as to the specific autoantibody present, e.g. double-stranded DNA antibodies give rise to a homogenous pattern. However, interpretation of these patterns requires considerable experience and skill and has largely been replaced by the determination of specific antinuclear antibodies using ELISA in the evaluation of specific disorders.

The ANA test is very sensitive for the diagnosis of systemic lupus erythematosus (SLE) (sensitivity 98%, specificity 90%) in an unselected population. This means that most people with SLE will have a positive result, and a negative result will make SLE very unlikely (negative predictive value 99%). The positive predictive value of the ANA test for SLE is low (30 - 40%) and is limited by the fact that the ANA is positive in a number of other conditions (rheumatoid arthritis 40%; scleroderma 90%; Sjögrens syndrome 70%). This means that two-thirds of patients with a positive ANA test may have a disease other than SLE.[2]

False positive ANAs are commonly found in the normal population, including family members of patients with rheumatic disorders. These are usually low in titre (1:40; 1:80). Higher titres are more likely to be associated with underlying illness.

The ANA test should be performed when evaluating patients with photosensitive skin rashes, inflammatory polyarthritis, nephritis or cytopenias. The ANA is not a sensitive way of following disease activity and fluctuating titres do not correlate well with a change in clinical status.

ANTI-DNA ANTIBODIES

Only antibodies to double-stranded DNA are clinically useful. They are

Table II. **Conditions other than RA associated with a positive RF test**

	Prevalence of RF (%)
Young healthy individuals	5
Age > 60 years	5 - 25
Other rheumatic diseases	
SLE	20 - 30
Sjögrens syndrome	75 - 90
Mixed connective tissue disease	50 - 60
Scleroderma	20 - 30
Polymyositis	5 - 10
Infections	
Bacterial endocarditis	25 - 50
Tuberculosis	10 - 20
Syphilis	10
Viral infections (including HIV)	15 - 65
Miscellaneous	
Interstitial pulmonary fibrosis	10 - 50
Primary biliary cirrhosis	50 - 70
Sarcoidosis	5 - 33
Malignancy	5 - 25

Table III. **Association of spondylarthropathies with HLA B27 in white persons (%)**

Ankylosing spondylitis	90
Reactive arthritis	40 - 80
Enteropathic spondylarthritis	35 - 75
Psoriatic arthritis	40 - 50
Undifferentiated spondylarthropathy	70
Acute anterior uveitis	50

highly specific for SLE (specificity > 99%), i.e. very useful for diagnosis when positive. However, dsDNA antibodies are only present in 60% of patients with SLE, and their absence would not exclude the diagnosis of SLE. Anti-DNA testing should be reserved for patients with a positive ANA. Some studies have shown a modest correlation between the titre of dsDNA antibodies and clinical activity, particularly in patients with renal disease. This suggests that the test may be useful in monitoring as well as diagnosis.[3] Therapeutic decisions should always be considered within the clinical context, rather than based solely on the change in titre.

ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS

Antibodies to extractable nuclear antigens (ENA) include ANAs directed at Sm, RNP, SS-A (Ro) and SS-B (La) antigens. These are often present, and may co-exist, in SLE and other connective tissue diseases.

Antibodies to Sm antigens have a high specificity for SLE (99%) but only occur in 25% of patients with SLE. It is diagnostically useful to test for Sm antibodies when dsDNA antibodies are negative, as a positive result strongly suggests a diagnosis of SLE.

Antibodies to ribonuclear proteins (RNP) bind to antigens that are different from, but related to, Sm. In SLE, RNP antibodies usually accompany a positive Sm antibody. However, patients who are negative for Sm antibodies, but positive for RNP antibodies, would generally have U1 RNP antibodies. These are more specific for mixed connective tissue disease, which is a syndrome of arthritis, myositis, Raynaud's phenomenon and sclerodactyly.[4]

ANTI-Ro/La

Antibodies to SS-A (Ro) and SS-B (La) usually co-exist and are found in approximately 50% of SLE patients and 75% of patients with primary Sjögren's syndrome. Anti-Ro antibodies are specifically associated with subacute cutaneous lupus and mothers of infants with neonatal lupus. Similarly, they may be positive in a small subset of patients with Sjögren's syndrome with a negative ANA.

Antibodies to ENA should only be looked for in patients who have a positive ANA screening test, except for isolated clinical conditions where determination of anti-Ro antibody positivity may have diagnostic implications. Patients tend to maintain the same antibody profile over the course of their illness, thus testing for these antibodies on a single occasion is usually sufficient.

ANTI-CENTROMERE AND ANTI-TOPOISOMERASE 1 ANTIBODIES

The fluorescent patterns of ANA staining (centromeric, nucleolar) may suggest the possibility of scleroderma. Anti-centromere antibodies are found almost exclusively in patients with the limited cutaneous forms of the disease or CREST (calcinosis, Raynaud's, oesophageal dysmotility, sclerodactyly and telangectasia) syndrome. They occur in 50 - 60% of patients.

Anti-topoisomerase 1 antibodies (previously known as Scl-70) are associated with the more diffuse form of scleroderma and are seen in 15 - 20% of patients. The presence of these antibodies is associated with a higher risk of pulmonary fibrosis. It is important to note that the diagnosis of all forms of systemic sclerosis is still dependent on clinical evaluation, with the specific autoantibodies supporting the diagnosis and allowing distinction of clinical subgroups.

ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)

ANCAs target antigens in the cytoplasm of the neutrophils. Two subtypes are recognised, namely pANCA (perinuclear) and cANCA (cytoplasmic) based on their patterns of staining on immunofluorescence. The specific antigens recognised by these antibodies are myeloperoxidase (MPO) and proteinase-3 (PR-3) respectively.

cANCA are associated with Wegener's granulomatosis with a sensitivity of 60 - 80% and specificity of 98%. However, as Wegener's is a relatively rare disease in the general population, the positive predictive value is low.[2] The diagnosis is usually made on clinical grounds, assisted by biopsy, with a positive cANCA supporting the diagnosis. The titre of cANCA may help in following disease activity in these patients. However, the correlation of titre and disease activity is weak and should not be used as the sole parameter to justify immunosuppressive therapy.[5]

pANCA are associated with microscopic polyangiitis and other forms of pauci-immune glomerulonephritis, including polyarteritis nodosa. These antibodies are also found in other disorders, including SLE, rheumatoid arthritis, and inflammatory bowel disease. For this reason their specificity is poor (60%) and sensitivity ranges from 60% to 90%.

To improve diagnostic utility, these tests should only be requested when the level of suspicion of the associated disease (the pre-test probability) is high, e.g. in unexplained nephritis or pulmonary-renal syndromes.

RHEUMATOID FACTOR

Rheumatoid factors (RF) are autoantibodies directed at the Fc portion of IgG molecules. Currently used tests measure IgM RF, but IgG and IgA subtypes exist. The presence of RF can be detected by agglutination of IgG-sensitised sheep red

Table IV. **Classification of synovial fluid findings**

	Normal	Non-inflammatory	Inflammatory	Septic
Clarity	Transparent	Transparent	Translucent/opaque	Opaque
Colour	Colourless	Yellow/straw	Yellow	Yellow
Viscosity	High	High	Low	Variable
WBC (/mm^3)	< 200	200 - 2 000	2 000 - 50 000	> 50 000
Polymorphs (%)	< 25%	< 25%	> 50%	> 75%
Culture	Sterile	Sterile	Sterile	Positive

cells (SCAT) or latex particles coated with human IgG (Rose Waaler), ELISA and nephelometry. No one technique has a clear advantage over another, however the ELISA and nephelometry are slightly more sensitive. Most laboratories will screen sera with a more sensitive technique and confirm the presence of an RF with a more specific technique.

In an unselected population, the RF is 80% sensitive and 95% specific for rheumatoid arthritis. However, as the disease is relatively uncommon, the positive predictive value is low.[6] Only 20 - 30% of unselected patients who have a positive test will actually have RA. Most of the positive tests will be false positive as there are a large number of other conditions that can give rise to a positive RF (Table II). In addition, the presence of a (false) positive RF test rises with age. These patients are more likely to have osteoarthritis, thus emphasising the need for careful clinical evaluation.

When used in a group of patients with rheumatic disease, the positive predictive value of the RF test increases to 80%. However, up to 50% of patients with RA will test negative for RF at disease onset, with some of these patients converting to a positive test over the first 2 years of disease. A persistently negative test is seen in 20% of patients with RA. The RF has been shown to be of no value in monitoring disease activity and serial monitoring of RFs is not indicated.

ANTI-CCP ANTIBODIES

Anti-cyclic citrullinated peptide (CCP) antibodies are more recently discovered RA-specific antibodies targeting the modified amino acid, citrulline. The second-generation tests have sensitivity similar to IgM RF and a very good specificity of up to 97%. This test is valuable in confirming the diagnosis of early RA when the diagnostic criteria are not yet fulfilled. Anti-CCP antibodies are of additional diagnostic value in the RF-negative patient, being positive in up to a third of these patients. In other words, a positive test supports the diagnosis in ambiguous cases. The presence of this antibody has also been found to be associated with a more aggressive disease course. The IgM RF is still the most useful test for screening patients with suspected RA.[9]

HLA B27

HLA B27 is a class I MHC allele strongly associated with the spondylarthropathies. This association varies among the different forms of spondylarthopathies (Table III).

The HLA B27 test is not a routine diagnostic or screening test for ankylosing spondylitis (AS) in patients presenting with back pain or arthritis. The prevalence of AS correlates with the prevalence of HLA B27 in a given population. In South Africa, the prevalence of HLA B27 varies according to ethnic origins, and ranges from 8% to 11% in Caucasians to less than 1% in black Africans.[8]

In general, epidemiological surveys indicate that 4 - 7% of HLA B27-positive people will have the disease. This means 93 - 96% of patients who are HLA B27-positive will not have the disease.[4]

The predictive value of the test depends on the pre-test probability of AS. In patients in whom history and physical examination suggest AS (e.g. inflammatory back pain, other features of spondylarthropathies), but whose radiographic findings do not permit this diagnosis to be made, the HLA B27 test may minimise uncertainty. In patients with nonspecific back pain, with no other features suggestive of an inflammatory spondylitic disorder, HLA B27 testing is inappropriate, as a positive test would still not allow a diagnosis of AS. Routine screening of family members of an HLA B27-positive patient with AS is of limited practical value as it is not entirely predictive of the disease (20% of HLA-B27 positive 1st degree relatives develop the disease) and no preventive therapy is available. It does, however, define which family members are at risk.

HLA B27-positivity in patients with reactive arthritis is associated with more severe and prolonged disease as well as the development of sacro-iliitis and spondylitis. Knowledge of HLA B27 status in these patients may predict long-term prognosis.

SYNOVIAL FLUID ANALYSIS

Synovial fluid analysis is inexpensive and very important in confirming or excluding septic arthritis in a patient with a monoarthritis, or in the febrile patient with an acute flare of established arthritis. It can help to distinguish inflammatory from non-inflammatory arthritis and is diagnostic for the crystal-associated arthritides. Fluid should be sent for cell count, crystals, Gram stain and culture. Fluid can also be assessed visually for colour, clarity and viscosity. Even a drop of fluid (in fluid phase) can be examined for the presence of crystals, and Gram stain can then be performed. In this scenario, communication with laboratory staff often facilitates a satisfactory result. Synovial fluid is cate-

gorised as normal, non-inflammatory, inflammatory, septic or haemorrhagic based on clinical and laboratory analysis (Table IV).

CONCLUSION

It must be stressed that the diagnosis of rheumatic diseases is still largely dependent on a thorough clinical evaluation, with specific tests that may support the diagnosis. Tests should be ordered selectively, depending on the clinical picture. Autoantibodies are not uncommon in healthy individuals, and unselective testing of autoantibodies by 'autoimmune screening panels' leads to a high number of false positive results with the resultant anxiety, expense of further evaluation, and inappropriate treatment. Results should always be interpreted in conjunction with the clinical scenario.

References available on request.

IN A NUTSHELL

The presence of a laboratory abnormality does not always imply the presence of disease.

The CRP is a sensitive and early indicator of inflammation.

A negative ANA virtually excludes a diagnosis of SLE.

Low-titre false positive ANAs are common in the normal population.

A positive dsDNA or Sm antibody strongly suggests a diagnosis of SLE.

Only 20 - 30% of unselected patients with a positive RF test will actually have RA.

20% of patients with RA will remain seronegative throughout their disease course.

Anti-CCP antibodies are virtually diagnostic of RA.

The HLA B27 test cannot be thought of as a routine, diagnostic or screening test for ankylosing spondylitis.

Synovial fluid analysis is of utmost importance in excluding septic arthritis in patients with a monoarthritis.