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Skin in Rheumatologic Diseases






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JAYPEE

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4

CHAPTER

Immunodiagnostics in Rheumatologic Diseases

Sanat Phatak, Amita Aggarwal

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INTRODUCTION

Laboratory tests play an important role in management of rheumatological diseases. They can be used to confirm or refute a diagnosis and in prediction of prognosis and response to treatment. However, as is true with laboratory tests in any other field of medicine, they must be used in the right clinical context. With unwarranted ordering, the same helpful tests can act as double-edged swords, adding confusion rather than clarity.

Immunodiagnostic tests used in rheumatologic illnesses include:

- Tests to measure inflammation
- Autoantibodies
- Miscellaneous tests: Complement levels, cryoglobulins.

MARKERS OF INFLAMMATION

Connective tissue disorders, especially in their active stages, are characterized by inflammation. The markers of inflammation include normochromic anemia, thrombocytosis, hyperferritinemia, and raised erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). In addition, the levels of albumin may be reduced. Among these ESR and CRP are used most often. The CRP rises quickly and comes down within a week after subsidence of inflammatory stimuli and its level can rise more than 100-fold, thus it is very sensitive to change with treatment. In contrast, ESR rises slowly and comes down over weeks. However, it is easy to do and cheap, thus can be used to monitor chronic inflammation.

AUTOANTIBODIES

Rheumatoid Factor

Rheumatoid factor (RF) is an autoantibody which recognizes Fc component of IgG. While in clinical practice IgM RF is measured, IgG and IgA RF isotypes are also present in patients with RA.

Measurement

Rheumatoid factor can be measured using latex agglutination and nephelometry. While the former is a simpler, less expensive method that can be carried out at the point of care, nephelometric analysis has the advantages of automation and quantification of the antibodies and has thus become standard of care. Rheumatoid factor should be expressed as international unit for ease of comparison across laboratories.

Clinical Utility

Classically, RF is used in the diagnosis of rheumatoid arthritis (RA). It is positive in 70–80% of patients with RA; however, it is not specific. Positive results for RF may be obtained in some healthy subjects as well as a host of other disorders (Table 1).[1]

When should RF be ordered? It should be ordered in a patient with polyarthritis or early arthritis where a diagnosis of RA is being

Table1: Prevalence of rheumatoid factor

Clinical disorder	RF positivity observed
Rheumatoid arthritis	70–80%
Sjögren's syndrome	40–60%
Systemic lupus erythematosus	20%
Mixed cryoglobulinemia	100%
Others: Autoimmune hepatitis Infections: Tuberculosis, infective endocarditis, viral hepatitis, bacterial infections Malignancy: Hematological and solid organ	15–20%
Healthy controls	5–10%

RF, rheumatoid factor.

considered. Presence of RF in such patients (inflammatory arthritis and palindromic rheumatism) increases the probability of progressing to overt rheumatoid arthritis and a need for early treatment. In a patient with RA, presence of RF suggests an aggressive disease with poor prognosis.[2] Rheumatoid factor is also useful in patients with suspected cryoglobulinemia or Sjögren syndrome.

Anticitrullinated Protein Antibodies

A relatively new test for rheumatoid arthritis, measures antibodies to citrullinated peptides and thus called "anticitrullinated peptide antibodies (ACPA)". It can be directed against multiple host antigens like filaggrin, vimentin, etc.

Measurement

It is measured by enzyme-linked immunosorbent assay (ELISA), thus it is easy to do. Second generation ELISA or anti-CCP2 (anti-cyclic citrullinated peptide) antibody test is the standard assay used in clinical practice.

Clinical Utility

Anticitrullinated peptide antibody has the advantage of being more specific (85–95%) for rheumatoid arthritis than RF, while maintaining a similar level of sensitivity. ACPA also predicts a more severe disease phenotype with increased risk of erosions, mortality and extra-articular features.[3] Current guidelines recommend testing for both antibodies in the diagnosis of RA as 5–10% of patients may have either RF or ACPA.

Antinuclear Antibodies

The term "antinuclear antibodies (ANA)" describe antibodies directed against different components of the cell nucleus. These include DNA and associated proteins like histones, RNA associated proteins, nucleolar proteins, centromere, etc.

Measurement

Indirect immunofluorescence (IIF) and ELISA are the most widely used methods. IIF is considered the gold standard and it shows the pattern of nuclear reactivity. Hep2 cells are the most commonly used substrate. These are cultured laryngeal squamous carcinoma cells, which have good sensitivity due to the larger size of their nuclei.[4] Newer Ro-transfected Hep2000 cells have the added advantage of detecting antibodies to Ro which can be missed sometime on Hep2 cells. The report should include the titer as well as the IIF patterns of ANA (Fig. 1) which can provide a clue to the nuclear antigen detected and thereby, the likely disease (Table 2).[5] IIF is inexpensive, sensitive and specific; however, it is time consuming and identification of the different patterns may be difficult for beginners.

In contrast ELISA is automated, quantifiable and easy to perform. ELISA has been shown to have acceptable sensitivity ranging between 70% and 100% when it was compared to IIF; the sensitivity drops at low titer positive ANAs. ELISA also has a high false positivity rate.[6]

Clinical Utility

Antinuclear antibodies should be ordered when clinical features suggest a diagnosis of connective tissue disorder such as SLE (malar rash, polyarthritis, serositis, nephritis, and cytopenias); systemic sclerosis (Raynaud's phenomenon, sclerodactyly, interstitial lung disease); Sjögren's syndrome (dryness of eyes and mouth, polyarthralgias, and renal tubular acidosis). It is also useful in diagnosing autoimmune hepatitis and predicting the risk of uveitis in juvenile idiopathic arthritis (JIA) and progression in isolated Raynaud's phenomenon.

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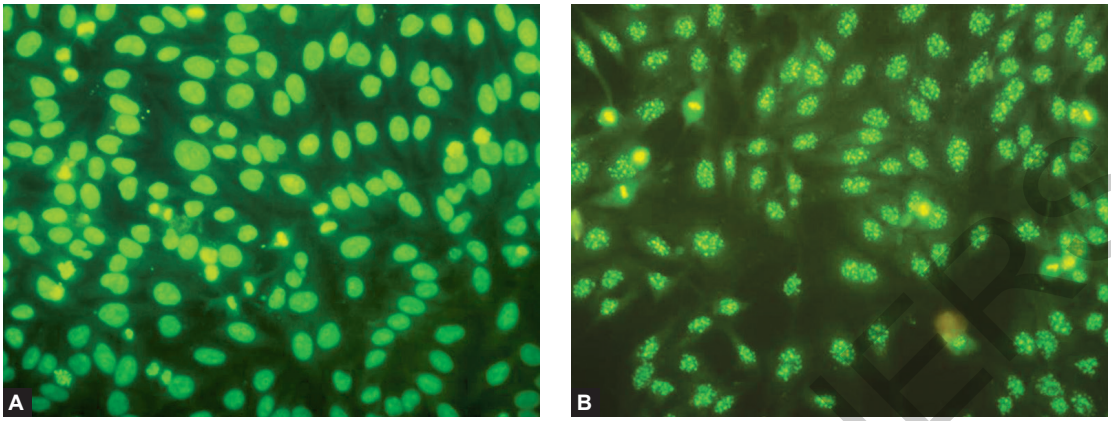


FIGURE 1: Antinuclear antibodies pattern on Hep2 cells. **A,** Homogeneous; **B,** Centromere.

Table 2: Antinuclear antibodies patterns and the probable antigenic targets

ANA pattern	Antigens	Disease associated
Homogenous	Histone, dsDNA	SLE
Speckled	SSA, SSB, ribosomal P Scl-70, RNP Jo-1	SLE, Sjögrens Systemic sclerosis, MCTD Myositis
Centromere	CENP-A to E	Limited systemic sclerosis
Nucleolar	Pm/Scl, RNP	Systemic sclerosis, myositis

ANA, antinuclear antibodies; SLE, systemic lupus erythematosus; SSA, Sjögren's-syndrome-related antigen A; SSB, Sjögren's-syndrome-related antigen B; RNP, ribonucleoproteins; MCTD, mixed connective tissue disease; CENP, centromere proteins; dsDNA, double-stranded DNA.

Antinuclear antibodies are not recommended in the routine evaluation of joint pain, back pain or chronic fatigue. It should be remembered that elderly individuals and patients with infections, thyroiditis and other common diseases might have positive ANA with no clinical significance. Once positive, ANA need not be repeated periodically in patients with connective tissue disorders.

Antinuclear Antibodies Subspecificities

It is possible to characterize the actual nuclear antigens which are the target of antibodies.

- Anti double stranded DNA (dsDNA) antibodies: Antibodies directed against double-stranded DNA are specific for SLE (95%) and should be done in patients with suspected lupus where ANA is positive. ELISA is most commonly used as it is quantitative and cheap method. In contrast IIF using *Crithidia lucillae*, a hemoflagellate whose kinetoplast contains pure double-stranded DNA unadulterated by other nuclear antigens has a high specificity but is not quantitative.[7] The Farr assay is considered the most reliable method but it is cumbersome and carries the hazard of handling radioactivity[8]
- Antibodies to extractable nuclear antigens (ENA): Tissue extracts provided the actual nature of antigens present within the nucleus, Smith being the first to be identified. Now a large number of antigens have been identified, many of which have found their way into laboratory testing. Though not absolute, they display disease specificity and are therefore helpful in diagnosing the type of CTD (Table 3). In fact some diseases have come to be defined by their ENA specificities, the prototype being mixed connective tissue disease with antibodies to U1-RNP.

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Antibodies to ENAs were originally described using gel precipitation assays.[9] ELISA and Western blotting have now replaced these older techniques. Popular assays utilize nitrocellulose paper with purified antigens blotted as dots (dot-blot assay) or as lines on a linear strip (line-blot assay). A blue-colored line produced by their binding to the specific antigen signifies the presence of autoantibodies in a test serum.[10] Usually, ENA is ordered in a patient with a positive ANA to characterize the type of CTD or in patients with Sjögren syndrome.

Table 3: Different extractable nuclear antigens specificities and disease association

ENA	Disease association
Smith, ribosomal-P, nucleosome, histone	SLE
Ro/(SSA), La/(SSB)	SLE (subacute cutaneous lupus, neonatal lupus), Sjögren's syndrome
Scl-70, RNA polymerase III	Diffuse cutaneous systemic sclerosis
RNP	SSc, MCTD
CENP-A to E	Limited cutaneous SSc
Jo-1	Inflammatory myositis

ENA, extractable nuclear antigens; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; CENP, centromere proteinsSSc, systemic sclerosis; RNP, ribonucleoproteins.

Antineutrophil Cytoplasmic Antibodies

The ANCA are antibodies directed against primary granules of neutrophils, described in necrotizing vasculitides—now termed "ANCA associated vasculitis".

Measurement

Antineutrophil cytoplasmic antibodies can be detected using IIF and ELISA. IIF is performed on ethanol fixed neutrophils. Two separate patterns are discerned (Fig. 2): (1) cytoplasmic ANCA (c-ANCA) with fluorescence diffusely spread throughout the cytoplasm; (2) perinuclear ANCA (p-ANCA) shows fluorescence concentrated around the nucleus.[11] Though there are many antigenic targets serine protease proteinase-3 and myeloperoxidase are of clinical use, thus ELISA tests are available for them. IIF is more sensitive and is useful for screening, while ELISA is specific and is used for confirmation. It is recommended to do both IIF and ELISA to increase specificity.

Clinical Utility

The prevalence of ANCA in different disease varies from 30 to 80% (Table 4).[12]

Antineutrophil cytoplasmic antibodies should be ordered when features suggestive of necrotizing vasculitis are present such as pulmonary nodules, hemorrhage, pauci-immune glomerulonephritis, chronic otitis media, and chronic sinusitis, amongst others.[13] Indiscriminate use can lead to

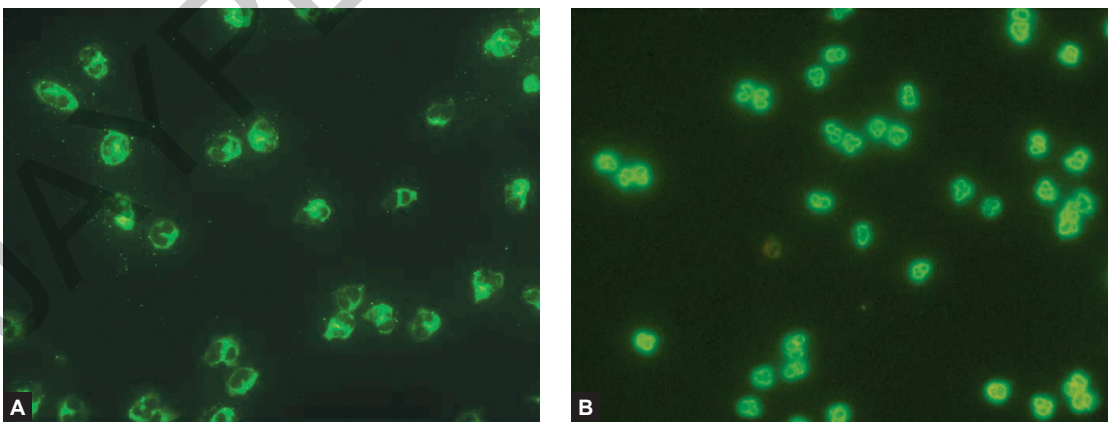


FIGURE 2: Antineutrophil cytoplasmic antibodies (ANCA) patterns on ethanol fixed human neutrophils. **A,** C-ANCA; **B,** P-ANCA.

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Table 4: Prevalence of antineutrophil cytoplasmic antibodies in different diseases

Disease	Salient clinical features	IIF pattern	ELISA	Positivity (%)
Granulomatosis with polyangiitis, previously Wegener's granulomatosis	Upper respiratory involvement, pulmonary nodules, glomerulonephritis	c-ANCA	Proteinase-3 (PR-3)	60–80
Microscopic polyangiitis	Lower respiratory involvement (Alveolar hemorrhage), glomerulonephritis	p-ANCA	MPO	75
Eosinophilic granulomatosis with polyangiitis (EGPA, previously Churg-Strauss syndrome)	Reactive airways disease, peripheral eosinophilia, mononeuritis multiplex	p-ANCA	MPO	30–50

ANCA, antineutrophil cytoplasmic antibodies; IIF, indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay; MPO, myeloperoxidase.

high false positive rates, especially in IIF, due to antibodies to antigens other than MPO and PR3 like Ro, dsDNA, cathepsin, and lactoferrin. Common causes of ANCA positivity apart from AAV include other CTDs such as SLE, RA; infections such as tuberculosis, infective endocarditis and leprosy; inflammatory bowel disease; chronic liver disease-autoimmune hepatitis type 1; and malignancies like lymphoma. A positive ANCA report in these settings has no clinical utility.

MISCELLANEOUS TESTS

Complement Levels

Complement proteins are a highly conserved system of plasma and membrane proteins which play an important role in both innate and adaptive immunity. Its major role lies in effective clearance of immune complexes, opsonization of bacteria, and amplification of inflammatory response. Therefore, disorders characterized by immune complex deposition have reduced levels of complement due to a "mopping up" effect. A useful screening test for complement activation is the CH 50 (or total hemolytic complement assay) which demonstrates reduction in the level of any one of nine complement components. Antigenic assays for C3 and C4 are more commonly used and can be measured by nephelometry or turbidimetry.

Reduced complement levels are useful in SLE, cryoglobulinemia, and few forms of hypocomplementemic vasculitides such as urticarial vasculitis and rheumatoid vasculitis.[14] They are also useful in following up patients with lupus nephritis where low levels may signify flare, and Sjögren's syndrome where new onset fall in complement levels may be a harbinger for the development of lymphoma. Some patients may have inherited deficiencies of complement components and have an increased incidence of autoimmune diseases. The C3 and C4 are acute phase reactants and are raised in infections or inflammatory disorders, thus may be raised in patients with RA, etc.

Cryoglobulins

Cryoglobulins, as the name suggests, refers to proteins that precipitate at low temperature. They may be comprised of monoclonal immunoglobulins alone, or may be mixed with complement proteins. Three types of cryoglobulins have been described (Table 5).[15]

Cryoglobulinemias often present with palpable purpura, digital gangrene, arthritis, myalgias, and glomerulonephritis. Cryoglobulins are detected in sera, cooled to 4°C for 72 hours, and the precipitated proteins can then be quantified and characterized into subtypes.[16] Types II and III are called "mixed" cryoglobulinemia and testing will show presence of RF. Low levels of C4 with normal C3 are typical.

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Table 5: Subtypes of cryoglobulins and their association

Type	Description	Disease associated	Symptomatology
Type I cryoglobulins	Monoclonal IgM or IgG	Monoclonal gammopathies—multiple myeloma, waldenstrom's macroglobulinemia, lymphomas, hepatitis C infection	Hyperviscosity and bland vessel thrombosis
Type II cryoglobulins	Monoclonal IgM with polyclonal IgG		Vasculitic manifestations (Immune complex deposition in blood vessels)
Type III cryoglobulins	Polyclonal IgG and polyclonal IgM	Infective, inflammatory disorders	

CONCLUSION

A large armamentarium of immunological tests is available to aid the physician in diagnosis and follow up of rheumatic diseases. Interpretation of these tests must be bolstered by appropriate clinical context. Inappropriate testing entails burden on laboratories, unnecessary referrals and further testing to confirm or refute diagnoses, thereby increasing healthcare costs.

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Skin in Rheumatologic Diseases

Key Features

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