

ANA PROFILES IN ANA-POSITIVE RHEUMATIC DISEASE

ANTIBODY SPECIFICITY	ACTIVE SLE	MCTD	PSS	CREST	PRIMARY SJOGREN'S	RA	DRUG-INDUCED SLE
ANA	>95%	>95%	70-90%	60-90%	>70%	40-50%	100%
Anti-dsDNA	60%	Negative	Negative	Negative	Rare	Rare	Negative
Anti-Sm	30%	Negative	Negative	Negative	Negative	Negative	Negative
Anti-RNP	30%	>95% (high titer)	Common (low titer)	Negative	Rare (low titer)	Rare	10-20% (low titer)
Anti-Centromere	Occasional	Rare	10-15%	60-90%	Occasional	Negative	Negative
Anti-Ro (SS-A)	30%	Rare	Rare	Negative	70%	10-15%	Negative
Anti-La (SS-B)	15%	Rare	Rare	Negative	60%	Rare	Negative
Anti-Nucleolar	Occasional	Negative	Common	Negative	Occasional	Rare	Negative
Anti-Scl-70	Occasional	Negative	10-20%	Negative	Negative	Negative	Negative
Anti-Histone	24-95%	Occasional	Occasional	Occasional	Occasional	20%	Procainamide: 67-100% Sensitivity Hydralazine: 50-100% Sensitivity
Anti-Chromatin	70%	Occasional	Occasional	Occasional	Occasional	Occasional	Procainamide: >90% Sensitivity Hydralazine: <50% Sensitivity

► GUIDE TO INTERPRETATION

1. A negative **ANA** excludes active Systemic Lupus Erythematosus (SLE) in >95% of cases.
2. False-positive **ANAs** occur in the following frequencies:
 - at 1:40: 32%
 - at 1:80: 13%
 - at 1:320: 3%
 - The number of false-positive **ANAs** increases with age.
3. Positive **ANAs** lack specificity, and can occur in many autoimmune rheumatic diseases, chronic inflammatory and infectious diseases, malignancies, and can also be induced by certain drugs.
4. Although unusual, low titer **ANAs** (1:40, 1:80) can be accompanied by other autoantibodies including Anti-DNA, Anti-Chromatin, Anti-RNP, Anti-Ro and others.
5. **Anti-Centromere Abs** strongly suggest CREST Syndrome and can be seen less commonly in Progressive Systemic Sclerosis (PSS), Raynaud's Phenomenon and Primary Biliary Cirrhosis. They can also occur occasionally in SLE and Sjogren's.
6. **Anti-dsDNA Abs** are essentially restricted to SLE and are seen infrequently in severe Rheumatoid Arthritis (RA). Increases in Anti-dsDNA Ab titers may predict flares in SLE.
7. **Anti-Sm Abs** are 99% specific for SLE. Sensitivity is higher in Blacks and Asians than Caucasians of European descent.
8. High titer **Anti-RNP Abs** (>1:10,000) are characteristic of Mixed Connective Tissue Disease (MCTD), particularly if unaccompanied by other autoantibody specificities.
9. **Anti-RNP Abs**, which are diagnostic for MCTD, especially at high titer, are also commonly seen in SLE, but titers are usually modest. Anti-RNP Abs can also be seen in PSS, Myositis, some RA and Sjogren's in low to modest titers.
10. **Anti-Ro and Anti-La Abs** are most often seen in Primary Sjogren's Syndrome, less frequently in SLE and least frequently in Secondary Sjogren's Syndrome. Anti-Ro and Anti-La Abs are strongly associated with Subacute Cutaneous LE, Neonatal Lupus Dermatitis, Congenital Complete Heart Block and rarely in Lupus Nephritis.

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► **GUIDE TO INTERPRETATION** CONT'D.

11. **Anti-Ro Ab** has also been associated with:
 - Photosensitive skin rash in SLE
 - Homozygous C2 Deficiency in SLE-like illness
 - Congenital Complete Heart Block
 - Interstitial Pneumonitis Disease in SLE
 - Pregnant women with Lupus accompanied by Anti-Ro have a 5% chance of having an infant with Congenital Complete Heart Block
 - Asymptomatic mothers of infants born with Congenital Complete Heart Block are at increased risk of developing a Connective Tissue Disease.
 - Thrombocytopenia (SLE, Sjogren's)
 - Lymphopenia (SLE, Sjogren's)
 - Nephritis, Anti-Ro without Ant-La
12. **Anti-Sci-70 Abs** (Anti-Topoisomerase 1 Abs) are seen in PSS and correlate with Pulmonary Fibrosis. They can occur in SLE occasionally.
13. **Anti-PCNA (Proliferating Cell Nuclear Ag) Abs** are highly specific for SLE, but sensitivity is only ~4%.
14. **Anti-Ribosomal P Protein Abs:**
 - Psychosis/depression in SLE: 45-90% reported (controversial)
 - Highly specific for SLE occurring in 10-20% of patients
 - CNS neuropsychiatric association in children and adolescents is less reliable than in adults.
15. **Anti-Chromatin Abs:**
 - Useful marker for SLE with Nephritis and can be seen in the absence of Anti-DNA
 - Seen in SLE with sensitivity of 70%
 - Seen in Drug Induced LE where it targets H₂A - H₂B linked to DNA which appears to be the major antigen in Drug Induced LE compared to Anti-Histone Abs which are directed against potentially all histone components (H₁, H₂A, H₂B, H₃, H₄ as well as H₂A-H₂B-DNA) in SLE and other disorders.
 - Can help to distinguish Drug Induced LE (Anti-H₂A-H₂B-DNA) compared to Drug Induced ANA.
 - Specificity overall is good for SLE, Drug Induced LE but can be seen in PSS, RA, MCTD and Type I Chronic Autoimmune Hepatitis.
16. **Anti-Mitochondrial Abs** are associated with Primary Biliary Cirrhosis, Scleroderma and CREST Syndrome.
17. **Anti-Thyroid Microsomal (Thyroid Peroxidase) Abs** are associated with Autoimmune Thyroid Disease, are predictive of development of biochemical Hypothyroidism and occur commonly with positive ANAs.
18. **Anti-Histone Abs** may help in confirming a suspicion of Drug Induced LE but cannot distinguish Drug Induced ANA from Drug Induced LE which typically targets H₂A-H₂B-DNA (Anti-Chromatin), particularly in Pronestyl Induced LE.
 - 95% of Drug Induced LE
 - 70-80% of SLE
 - Can be seen occasionally in Scleroderma, RA, Sjogren's, JRA, Felty's Syndrome, MCTD, Vasculitis, Neoplasms and Liver Disease.

AUTOANTIBODIES IN SLE (HIGH SPECIFICITY)

AUTOANTIBODY	SPECIFICITY	PREVALENCE	CLINICAL UTILITY/ CORRELATION	OTHER DISEASE ASSOCIATIONS
1) Anti-DNA (Double Strand)	High	Active SLE (50-60%)	1) Marker for SLE 2) Helpful in assessing activity of nephritis 3) Anti-DNA level modified by immuno-suppressive Rx	Rare in other Rheumatic Diseases
2) Anti-Sm	High	Caucasians (10-20%) Asians (30%) Blacks (40%)	Marker for SLE	Rare in other Rheumatic Diseases
3) Anti-Ribosomal P Protein	High	Random SLE (10%) Active SLE (40%)	1) Apparent correlation with neuro-psychiatric SLE, e.g. psychosis 2) Rising levels in active SLE	Rare in other Rheumatic Diseases
4) Anti-Proliferating Cell Nuclear Ag (PCNA)	High	Less than 5%	Marker antibody	Rare in other Rheumatic Diseases

CLINICAL ASSOCIATIONS OF ANTICARDIOLIPIN ABS REPORTED BY SPECIALTY

IMMUNOLOGY/ RHEUMATOLOGY	OBSTETRICS	HEMATOLOGY	NEUROLOGY	CARDIOLOGY	PULMONARY	DERMATOLOGY	MISCELLANEOUS
<ol style="list-style-type: none"> 1. Antiphospholipid Antibody Syndrome 2. SLE 3. Lupus Anticoagulant 4. Chronic Biologic False Positive Test for Syphilis 5. Rheumatoid Arthritis 6. Sjogren's Syndrome 7. Ulcerative Colitis 8. Behcet's Syndrome 9. Drug Induced LE 10. Other Autoimmune Disorders 	<p style="text-align: center;">Various Pregnancy Morbidities</p>	<ol style="list-style-type: none"> 1. Arterial & Venous Thrombosis 2. Thrombocytopenia 3. Coomb's Positive Hemolytic Anemia 4. Evan's Syndrome 	<ol style="list-style-type: none"> 1. Cerebral Thrombosis 2. TIA 3. Chorea 4. Transverse Myelopathy 5. Epilepsy 6. Migraine 7. Sneddon's Syndrome 8. Cognitive Dysfunction 9. Ischemic Optic Neuritis 10. Retinal Vein & Artery Occlusion 	<ol style="list-style-type: none"> 1. Libman Sacks Endocarditis 2. Premature MI 3. Coronary Thrombosis 	<ol style="list-style-type: none"> 1. Pulmonary Emboli 2. Pulmonary Hypertension 3. Alveolar Hemorrhage 	<ol style="list-style-type: none"> 1. Livedo Reticularis 2. Digital Gangrene 3. Chronic Leg Ulcers 	<ol style="list-style-type: none"> 1. Drugs: Chlorpromazine Pronestyl Others 2. Infections: AIDS Mononucleosis Hepatitis C Others 3. Malignancies

GUIDE TO INTERPRETATION OF ANTIPHOSPHOLIPID ABS

- ▶ Anticardiolipin Abs are seen in:
 - ~40% of SLE
 - Active SLE can increase ACA titer.
- ▶ Prevalence of ACA:
 - 0 - 10% of blood donors and pregnant women
 - 7.5% of healthy women
 - 13.3% of elderly with chronic illness.
- ▶ Antiphospholipid antibodies (APA) may be absent in up to 20-30% of patients as follows:
 - Insufficient removal of platelets during specimen processing for Lupus Anticoagulant (LA) which may cause a false negative result
 - False negative results can occur in Nephrotic Syndrome
 - LA titers can fall during Rx
 - APA can disappear with active thrombotic process
- ▶ Other antibody species can occur in the absence of ACA and LA:
 - Anti-beta₂glycoprotein 1
 - Anti-phosphatidylserine
 - Anti-phosphatidyl ethanolamine and others.
- ▶ IgA (ACA) can be the only isotype present in some cases.
- ▶ Repeat testing is recommended if strong suspicion exists for Antiphospholipid Syndrome (APS) given the above scenarios.
- ▶ LA can diminish with active Rx more so than ACA.
- ▶ LA screening tests are not valid in the presence of heparin therapy.
- ▶ APA can occur in many infectious disorders, usually unassociated with clotting episodes and mainly of the IgM Isotype.
- ▶ APA can occur in many autoimmune diseases (RA, Sjogren's Syndrome, PSS, ITP and many types of vasculitis).
- ▶ APA may be present in various malignancies.

CRITERIA FOR CLASSIFICATION OF THE ANTIPHOSPHOLIPID ABS

Clinical

- Vascular Thrombosis
One or more clinical episodes of arterial, venous or small-vessel thrombosis in any tissue or organ. Thrombosis must be confirmed by imaging, Doppler studies or histopathology, with exception of superficial venous thrombosis. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
- Pregnancy Morbidity
 - One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
 - One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe preeclampsia or eclampsia or severe placental insufficiency, or
 - Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

Laboratory

- Anticardiolipin antibody (ACA) of IgG and/or IgM isotype in blood, present in medium or high titer, on two or more occasions, at least 6 weeks apart, measured by a standard enzyme-linked immunosorbent assay for Beta₂-glycoprotein 1-dependent ACAs.
- Lupus anticoagulant (LA) present in plasma on two or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis.

Patients with the syndrome should have at least one clinical plus one laboratory finding during their disease. The aPL test must be positive on at least two occasions more than 3 months apart.

Source: Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum*1999;42:1309-1311

FREQUENCY OF C-ANCA, P-ANCA, MPO & PR-3 ABS IN VASCULITIS

DISEASE CATEGORY	C-ANCA	P-ANCA	ANTI-MPO	ANTI-PR3
Wegener's Granulomatosis Active - generalized Active - limited	3-4+ 2-3+	1+ Occasionally	1+ <1+	3-4+ 2-3+
Idiopathic Necrotizing and Crescentic Glomerulonephritis without Immune Deposits (Pauci-Immune)	Rare	4+	3-4+	Rare
Microscopic Polyarteritis	1+	2-3+	2-3+	1+
Churg-Strauss Syndrome	1+	2+	2+	1+
Classic Polyarteritis Nodosa	Rare	Rare	Rare	Rare
Polyangitis Overlap Syndrome	1+	1+	1+	Rare
Inflammatory Bowel Disease Ulcerative Colitis Crohn's Disease	Absent Absent	2-4+ 1+	Absent Absent	Absent Absent

GRADING SYSTEM: 1+(15-25%); 2+(26-50%); 3+(51-75%); 4+(76-100%)

See next page for a Guide to Interpretation

Combined ANCA IFA testing with ELISA assays for Anti-PR3 and Anti-MPO are the preferred assays in evaluating these disorders. ANA testing should be performed if P-ANCA is present.

GUIDE TO INTERPRETATION

C-ANCA

- C-ANCA are present in ~90% of patients with active generalized Wegener's Granulomatosis (WG). Sensitivity decreases to 60-67% with inactive or limited WG. A negative C-ANCA, therefore, does not exclude a diagnosis of WG.
- Serial ANCA titers can be useful in monitoring disease activity in modest proportion of patients, but therapeutic decisions based solely on changes in ANCA titers are not generally recommended in the majority of patients.
- C-ANCA are seen in only a minority of patients with Microscopic Polyarteritis and Idiopathic Necrotizing and Crescentic Glomerulonephritis (without immune deposits), which are possibly related to WG.
- Although the majority of WG patients have C-ANCA and most patients with ANCA-associated Glomerulonephritis without systemic manifestations have P-ANCA, there is some overlap.
- C-ANCA can also be seen in SBE and Invasive Amoebiasis.

Anti-Proteinase 3 Antibodies

- C-ANCA in WG are primarily due to anti-Proteinase 3 antibodies (anti-PR-3) [80-90% sensitive; 97% specific], but other target antigens are identified.
- 90% of anti-PR-3 positive sera are also C-ANCA positive.

P-ANCA

- P-ANCA are a useful marker for Vasculitis-associated Crescentic Glomerulonephritis and Idiopathic Pauci-immune Crescentic Glomerulonephritis.
- In patients with ANCA-associated Glomerulonephritis, ~90% of P-ANCA are secondary to anti-MPO.
- P-ANCA can be seen in 60-75% of Microscopic Polyarteritis and 75% of Churg-Strauss Syndrome, mainly secondary to anti-MPO Abs.
- Positive predictive value of ANCA positivity in Rapid Progressive Glomerulonephritis approaches 98%.
- P-ANCA are occasionally present in a minority of patients with WG.
- P-ANCA are present in ~10% of Systemic Lupus Erythematosus (SLE) patients.
- False positive P-ANCA can be caused by ANA's, hence, concurrent ANA testing is performed on P-ANCA positive sera.
- Atypical P-ANCA are commonly seen in Ulcerative Colitis, Sclerosing Cholangitis and Type I Autoimmune Hepatitis, but are unrelated to anti-MPO antibodies.
- P-ANCA can be seen in 10-30% of patients with Goodpastures Syndrome.
- P-ANCA can be seen in many Connective Tissue Diseases, including SLE, RA, DM/PM, Sjogren's Syndrome and others directed against a variety of intracellular neutrophil cytoplasmic antigens.
- Anti-elastase, anti-cathepsin G, anti-lactoferrin and anti-lysozyme antibodies can cause a P-ANCA pattern, but lack clinical utility.

Anti-Myeloperoxidase (Anti-MPO) Antibodies

- Most P-ANCA occurring in Systemic Necrotizing Vasculitis (90%) are due to anti-MPO.

Drug Induced ANCA Associated Vasculitis

- Several drugs have been implicated in ANCA-associated Vasculitis including PTU, minocycline, hydralazine, and are directed against various cytoplasmic neutrophil antigens.

Infections Associated with ANCA

- SBE
- Invasive Amoebiasis
- Cystic Fibrosis
- HIV
- Some Respiratory Infections
- Chromomycosis
- Acute Malaria
- Hepatitis C

IMPORTANT AUTOANTIBODIES IN RHEUMATOID ARTHRITIS

Antibody	Sensitivity	Specificity	Predictor of Erosion	Presence in Early RA (<1 Yr)	Presence in Seroneg. RA	Assay Type	Commercially Available	Clinical Utility	Presence in Other Diseases & Comments
IgM Rheumatoid Factor	~75%	Moderate unless present at high titer	Yes	Variable, but usually absent	No	Nephelometry preferred	Yes	High titers are usually specific for RA and useful prognostically	Yes - Other connective tissue diseases: SLE, PSS, MCTD, Sjorgren's, etc. - Other infectious diseases: EBV, Parvo, Hep C, SBE, others - 1-5% normals; 25% elderly
Anti-Cyclic Citrullinated Peptide Ab (Anti-CCP)	70-80%	96 %	Yes	40-70%	~40%	EIA*	Yes	Specific marker for RA - Presents frequently in early RA - 40% Seronegative RA - Predictor of erosions	Rarely seen in other diseases <5-10% in SLE <5-10% in PSS JRA?
Anti-Keratin Ab*	36-59%	88-99%	Likely	Yes	33%	IFA*	Immco	Highly specific marker for RA - Requires technical skill	Rarely seen in other diseases
Anti-(Pro) Filaggrin*	47-54% by EIA	95-99%	Yes	Yes	45%	IB/EIA*	No	Highly specific marker but antigen preparation can be problematic	Rarely seen in other diseases
Anti-Perinuclear Factor*	49-91%	73-99%	Likely	Yes	Yes	IFA	No	Highly specific but plagued by technical problems	Can be seen in JRA (Pauci Articular) 1° Sjogren's, 1° Mxyedema but titers are low
Anti-Sa**	43%	99%	Yes	Yes	Yes	IB	No	Highly specific for RA	Rarely seen in other diseases
Anti-BIP (P-68)	63%	96%	?	?	?	IB	No	Highly specific for RA	Rare Specificity - Needs confirmation with additional studies
Anti-RA33	36%	Modest unless it occurs without U1-RNP	No	Yes	Yes	IB	No	Potential use in differentiating RA vs. SLE when Ra33 occurs without U1-RNP	- In SLE, Anti-RA33 is associated with U1-RNP/Anti-SM - Occurs in SLE, MCTD, PSS - Absent in AS, PSA

EIA: Enzyme Immuno Assay
IB: Immunoblot
IFA: Indirect Immuno-Flourescence

* Anti-CCP, AKA, Anti-(Pro) Filaggrin and Anti-PNF are closely related antibodies directed against citrullinated peptides.

** Anti-Sa appears to be directed against citrullinated vimentin.

SPECIFIC AUTOANTIBODIES IN AUTOIMMUNE LIVER DISEASE

I. Type I Autoimmune Hepatitis

a. ANA

- ANA is the major antibody seen in autoimmune hepatitis.
- It has extensive heterogeneity (anti-dsDNA, anti-ssDNA, anti-chromatin, anti-histone, anti-nuclear lamins and anti-RNP).

b. Anti-Smooth Muscle

- Is detected by immunofluorescence but not specific unless titers are 1:320 or greater.
- Can be seen in other liver diseases and non-liver diseases as well.

c. Anti-Actin

- Type of anti-smooth muscle antibody with much better specificity for autoimmune hepatitis than anti-smooth muscle antibody.
- Sensitivity reported to be 75% in Type I autoimmune hepatitis.
- Can be seen in 3-15% of other types of chronic hepatitis.

d. Anti-Soluble Liver Antigen (Anti-SLA)

- 100% specific for autoimmune hepatitis, Type I.
- Seen in 30% of cases in conjunction with other autoantibodies.
- Importantly, it can be seen as the sole autoantibody response in 10-15% of patients.
- Some researchers use this antibody as a marker for autoimmune hepatitis Type 3 but these patients are clinically similar to autoimmune hepatitis Type I patients.

e. Atypical P-ANCA

- Seen in 65-95% of Type I patients, usually in high titers.
- Also seen in sclerosing cholangitis.
- It is not seen in Type 2 autoimmune hepatitis.

II. Type 2 Autoimmune Hepatitis

a. Anti-Liver/Kidney Microsome 1 (anti-LKM1)

- Major serologic marker in Type 2 autoimmune hepatitis seen in 95-100% of cases.

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- Directed against cytochrome P4502D6.
- Can be seen in hepatitis C but uncommon.

b. **Anti-Liver Cytosolic Protein**

- 50% of patients with anti-LKM1 antibodies have antibodies to liver cytosolic protein.
- Can be the only autoantibody seen in Type 2 autoimmune hepatitis

III. **Primary Biliary Cirrhosis (PBC)**

a. **Anti-Mitochondrial Antibody (AMA)**

- AMA is the marker antibody for PBC and is seen in 90% of cases.
- 10% of PBC are therefore AMA negative.
- The antigenic target is the E2 subunit common to several mitochondrial enzyme systems.
- Anti-centromere antibodies are present in 10-15% of cases.
- Antinuclear pore antibodies can be seen in AMA-negative PBC.
 - Clinically similar to AMA positive patients.
- An additional nuclear dot staining pattern can be seen on hep 2 substrate.
- The IgM isotype is frequently increased in PBC.

IV. **Sclerosing Cholangitis**

a. **Atypical P-ANCA**

- Atypical P-ANCA is the marker antibody in this disorder occurring in 65-85% of cases with or without ulcerative colitis.
- Atypical P-ANCA can persist after liver transplantation.

b. **Anti-Nuclear Ab (ANA)**

- ANA can be seen occasionally

V. **Autoantibodies Associated with Hepatitis C**

- a. **Rheumatoid factor:** 70% b. **Anti-nuclear ab:** 10-30% c. **Anti-smooth muscle ab:** 60-70% d. **Anti-liver/kidney microsomal ab:** reported
 e. **Anticardiolipin abs:** 22% f. **Antineutrophil cytoplasmic abs:** reported g. **Anti-thyroid abs** h. **Cryoglobulins**
 i. Presence of **HLA-DR4** is associated with a five-fold increase in incidence of autoimmune diseases.