

# Autoantibodies in Patients with Autoimmune Connective Tissue Diseases

# Introduction

- Autoimmune CTDs are a group of autoimmune disorders that have overlapping clinical features.
- The hallmark of these diseases is the production of aAb.
- Accurate diagnosis depends on four parameters
  - Clinical findings
  - Histopathology
  - Tissue immunofluorescence
  - Serologic testing
- This lecture will concentrate on serologic testing

- If used appropriately serological testing can be useful in the diagnosis and management of CTD.
- The clinician should be familiar with the serologic tests currently used as well as the disease association of the various aAbs.

## **Table 1.** Autoimmune CTDs

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1. LE
    - A. Systemic LE
    - B. Discoid LE
    - C. Subacute cutaneous LE
    - D. Neonatal LE
    - E. Overlap of two or more LE subsets
    - F. Overlap of LE with other CTDs
  2. Scleroderma
    - A. Cutaneous scleroderma (morphea)
    - B. Systemic scleroderma
      1. Limited disease (acrosclerosis, CREST syndrome)
      2. Diffuse disease (SSc)
  3. Dermatomyositis
  4. Sjögren's syndrome (primary and secondary)
  5. MCTD
  6. Overlap and undifferentiated CTD
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## **Table II.** Antibodies in autoimmune CTDs

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1. Antibodies to DNA
    - A. Antibodies to nDNA (dsDNA)
    - B. Antibodies to ssDNA
  2. Antibodies to small ribonucleoproteins
    - A. Antibodies to Ro(SS-A)
    - B. Antibodies to La(SS-B)
    - C. Antibodies to U<sub>1</sub>RNP
    - D. Antibodies to Sm
  3. Antibodies to histones
  4. Antibodies to centromere
  5. Antibodies to phospholipid (cardiolipin)
  6. Antibodies to other cellular components
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# Biology of the Antibody Specificity

- Those with CTD have antibodies against several self antigens.
- They are directed against nuclear, cytoplasmic, and cell membrane molecules.
- The role the antibodies play in the pathogenesis of the clinical manifestations is suspected, but not confirmed.

- When evaluating autoantibodies be aware of two findings:
  - Some of the antibodies are not unique to CTD and can be present in normal people
  - The specificity of each of the antibodies for the various CTD varies
    - Sm and dsDNA highly specific for SLE
    - ssDNA are of low diagnostic value

# Techniques for Serologic Testing

- Radial immunodiffusion and immunofluorescence remain of important value, but are slowly being replaced.
- ELISA is the newest technique replacing most of the older methods.



# ELISA

- The principle of ELISA and immunofluorescence is similar.
- An antigen is placed on a plastic plate.
- The patients serum is then added at different titers with positive and negative controls and incubated.
- If the patient has antibodies it will bind the substrate.

- The plates are then washed.
- Anti-human antibodies are then added with a linked enzyme.
- The plates are again washed.
- A substrate is then added and if enzyme linked anti-human antibodies are present the reaction will change colors.
- The higher the titer the more antibody that is present.

# Comparison to immunofluorescence

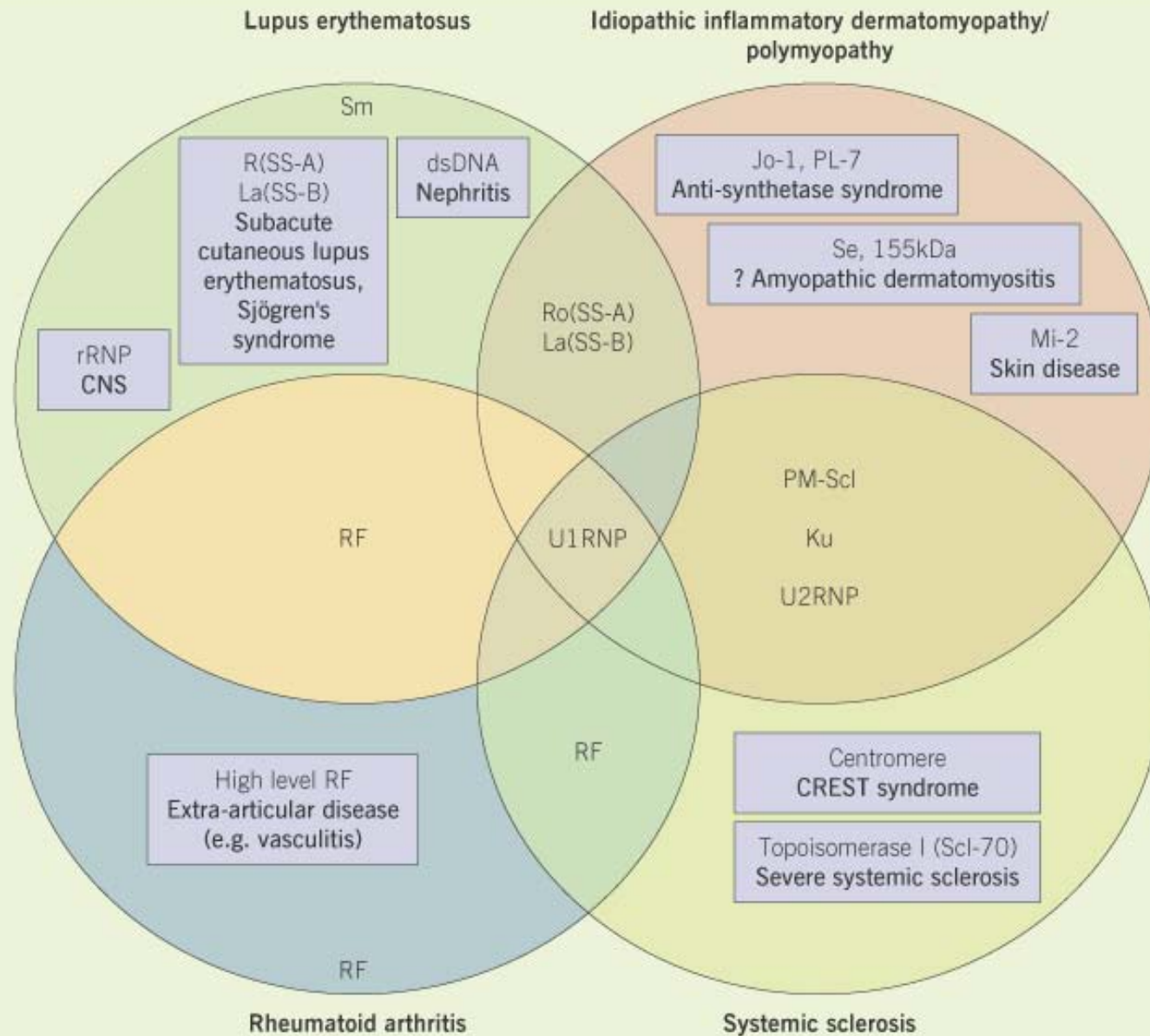
- Advantages

- Cheaper
- Less labor intensive
- Can be used to screen large numbers of sera
- Less subjective
- More sensitive

- Disadvantages

- Less specific
- Results need to be interpreted with caution
- [http://www.biology.arizona.edu/immunology/activities/elisa/neg\\_ELISA.html?](http://www.biology.arizona.edu/immunology/activities/elisa/neg_ELISA.html?)
- [http://www.biology.arizona.edu/immunology/activities/elisa/pos\\_ELISA.html?](http://www.biology.arizona.edu/immunology/activities/elisa/pos_ELISA.html?)

# CLINICOSEROLOGIC CORRELATIONS OF AUTOIMMUNE CONNECTIVE TISSUE DISEASES



# The classic ANA assay

- The ANA test is an IIF that utilizes substrate rich nuclear material.
- The classic ANA IIF assay is the most efficient screening test for CTD.
- One must interpret these tests cautiously.

Table 42.1 Major issues related to the interpretation of results of antinuclear antibody assays.

MAJOR ISSUES RELATED TO THE INTERPRETATION OF RESULTS OF ANTINUCLEAR ANTIBODY ASSAYS
<b>Pitfalls</b>
<ul style="list-style-type: none"><li>• Differences in ANA substrates</li><li>• Subjective nature of ANA assay endpoint</li><li>• 'Normal' versus 'abnormal' ANA levels</li><li>• Patient age</li><li>• Drug-induced ANA</li><li>• Other ANA-Related disorders</li><li>• Sontheimer's corollary to Greenwald's Law of Lupus</li></ul>
<b>Questions frequently asked</b>
<ul style="list-style-type: none"><li>• What is the significance of a positive ANA in a young healthy adult?</li><li>• Do ANA titers reflect systemic disease activity?</li><li>• What is the significance of ANA immunofluorescence patterns?</li><li>• Is it possible to have SLE if the ANA is negative?</li><li>• What is the role of the ANA assay in phototherapy?</li><li>• What does it mean when ANA results are reported in international units?</li><li>• What is the appropriate evaluation of a patient with a positive ANA result who is suspected of having a rheumatic disease with cutaneous expression?</li></ul>

- A positive ANA does not indicate the specific type of Ab.
- Examination of the pattern can be helpful in suggesting the specific Ab.

# When should you order on ANA?

- Work up of photosensitivity eruption that is undiagnosed.
- Diagnosed with extensive DLE patient to get baseline level in case patient develops SLE.
- Suspicion of CTD associated with autoantibodies.
- Baseline for phototherapy?
- Chronic vasculitis of unknown etiology
  - SLE
  - Sjogrens

# Interpretation of ANA results

- Look at 3 parameters
  - Substrate used
    - Sensitivity varies with substrate used.
    - Animal substrates are not very sensitive.
  - Titer of a positive test
    - Indirect measure of amount of antibodies present.
  - Pattern of fluorescence
    - Can give clues about the type of Ab present.



# ANA substrate

- Two major types
  - Animal such as mouse kidney and rat liver
  - Cultured human cells
- Some sera of patients with SLE tested negative on animal substrates.
- Animal substrates lacked some of the autoantigens present in human cell nuclei (eg. Ro).
- This created the category of ANA negative SLE.
- This occurred in up to 15%.
- Only 1-2% of SLE patients are ANA negative.

- Therefore, human substrates are more sensitive.
- The SLE sera that tested negative by animal substrate was found to be positive on human substrate.
- ANA negative SLE is predominantly a historical phenomenon.
- Most labs now use cultured human esophageal squamous cell carcinoma cells.
- These are referred to as HEp-2 cells.
- Occasional labs are still using animal substrates.
  - Thus, if suspicion is high retest on HEp-2 cells.

- Young and healthy people can have low titers of ANA.
- Up to 15% of individuals over 55 years of age have significantly elevated ANA titer of no apparent consequence.
- Those with high titers and clinical suspicion most likely have CTD.
- A good cutoff for clinically relevant values is 1:160 to 1:320.
- 5% of healthy young adults will have 1:160 or higher.
- Some healthy people have titers >1:320.

**Table VIII.** Positive fluorescent ANA test in healthy persons (on HEp-2 cells)<sup>9</sup>

Titer	Prevalence
1:40	32%
1:80	13%
1:160	5%
1:320	3%

- The intermediate titers are seen in some with CTD and in some with other conditions.
- The diagnosis of CTD should not be made on ANA alone.

**Table VII.** Conditions other than autoimmune CTDs with positive ANA

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Elderly persons<sup>12,153</sup>

Pregnant women<sup>154,155</sup>

Relatives of patients with CTD<sup>12,156</sup>

Other autoimmune diseases (eg, primary biliary cirrhosis, autoimmune thyroiditis)<sup>14,196</sup>

Drugs (eg, procainamide, hydralazine)<sup>68-83,157</sup>

Chronic infections<sup>10,14</sup>

Neoplasms<sup>10,14</sup>

Healthy persons<sup>9,11,12,153</sup>

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# Should ANA and Ro be ordered before phototherapy?

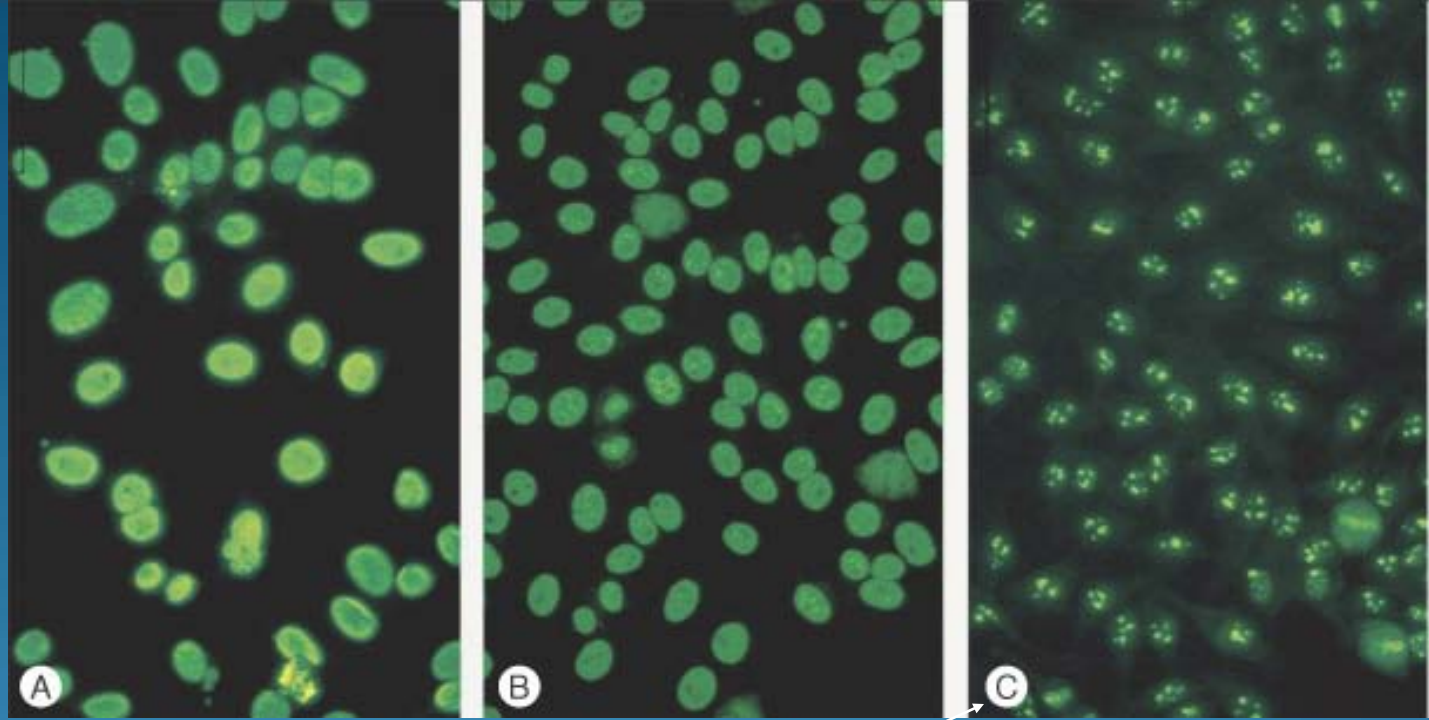
- It is rare that a diagnosis of photosensitive systemic autoimmune disorders is uncovered in the absence of other clinical signs.
- A good rule to remember when screening patients is:
  - Patients having any degree of clinically significant SLE are virtually never asymptomatic.
- Studies have shown that phototherapy does not significantly induce appearance of ANA or other aAbs.

# ANA Patterns

- The patterns of fluorescence is usually associated with specific ANAs.
  - Homogenous (diffuse)
  - Peripheral (rim)
  - Nucleolar
  - Centromere (discrete speckled)
  - Speckled (particulate)
    - Not very specific for disease

**Table IX.** ANA patterns and their antigen and disease associations

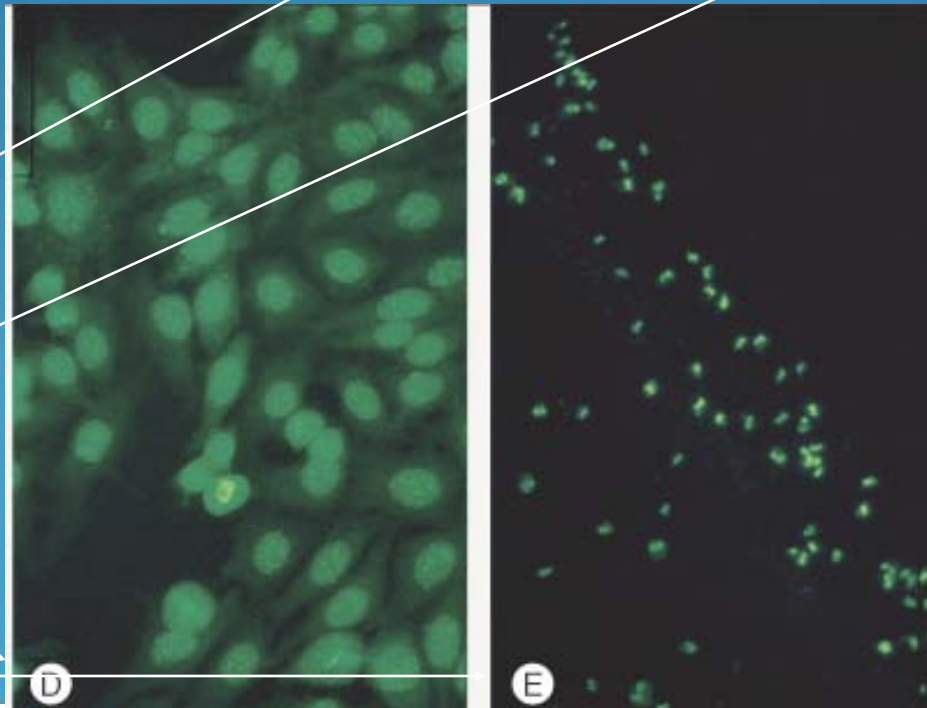
ANA	Predominant antigen	Disease	Reference Nos.
Peripheral	nDNA	SLE	10,14,161
Homogeneous	nDNA, histones	SLE	14,161
Nucleolar	Nucleolar RNA	SSc, SLE	14,158,161
Centromere	Kinetochores	CREST	14,159
Speckled	Various ribo-nucleo-proteins	MCTD, SLE, SSc, Sjögren's Syndrome	14,161



A

B

C



D

E

Homogenous

Speckled

Nucleolar

Centromere

dsDNA to flagellate  
*Crithidia luciliae* by IIF

# nDNA antibodies

- Presently ELISA is used more frequently than IF.
- The IF is performed on *Crithidia luciliae*.
- *Crithidia* is a hemoflagellate organism that possesses a giant mitochondrion
- The concentrated mitochondrial DNA is called the kinetoplast.
- It contains primarily nDNA (and histones) without ssDNA.



- ELISA for nDNA uses calf thymus extract and is more sensitive than IF.
- nDNA is highly characteristic of SLE.
- It is usually associated with:
  - Positive DIF (lupus band)
  - Low circulating complement levels
  - Lupus nephritis
  - Poor prognosis

# Interpretation of Results

- Positive IF or ELISA value higher than 2-3 SD above the mean is diagnostic of SLE.
- A significantly positive test confirms SLE, but a negative test does not exclude it.
- It is positive in only 50-80% of SLE patients
- Low levels of nDNA may be seen in
  - RA
  - Hashimoto's disease
  - Graves' disease
  - Waldenstrom's macroglobulinemia
  - MCTD
  - SSc
  - Autoimmune liver disease
  - Sjogren's

# Indications for ordering nDNA

- In the setting of patient with clinical suspicion for SLE



# ssDNA antibodies

- ssDNA antibodies are detected by ELISA.
- The extracted nDNA molecules must be further denatured to produce ssDNA.
- The most common source is calf thymus.
- They have very low diagnostic value.
- They have been detected in:
  - LE (70% prevalence)
  - DM (40%)
  - Morphea (10%)
  - Sjogrens
  - Linear morphea in children

# Interpretation of results

- Low levels may be detected in patients without CTD.
- Levels should be 3 SD above mean to be of value in CTD.
- There diagnostic value in the work-up of patients with CTD is low since ssDNA is nonspecific.

# Histone antibodies

- Histones are basic proteins that bind the DNA helical structure.
- Histones contribute to supercoil formation.
- Histone antibodies are characteristic of drug-induced SLE.
- They can be detected by
  - IF
  - Complement fixation
  - Radioimmunoassay
  - ELISA

- ELISA uses commercially available histones.
- IF assay utilizes animal substrates such as rat liver.
- The majority (approximately 90%) of patients with drug induced SLE have antihistone Ab to the exclusion of other Ab.
- Approximately 30% of patients with idiopathic SLE with also have antihistone antibodies with other antinuclear antibodies.

- Histone antibodies should be ordered in those suspected of having drug induced SLE.
- Their presence strongly supports the diagnosis.
- Idiopathic SLE, however, cannot be excluded on the basis of antihistone Ab.
- Rarely see skin disease.
- Arthralgia, arthritis, myalgia, and serositis.



# RNP antibodies

- Autoantibodies directed to the small ribonucleoproteins (sRNP).
- This type is the smallest portion of cellular RNA (<1%).
- sRNP contains several molecules that contain RNA and an associated protein.
- The protein has enzymatic activity and help in processing RNA molecules.

- Antibodies to sRNP are directed against epitopes in the protein component.
- Examples of sRNP are Ro(SS-A), La(SS-B), U<sub>1</sub>RNP, and Sm.
- The diagnostic specificity of each of these antibodies is variable.
- Sm antibodies are characteristic of SLE.
- Ro(SS-A) in subsets of lupus and other CTD's.

- The major technique for detection of sRNP are
  - ELISA
    - High sensitivity
    - Low specificity
- The mere presence of Ab is of less diagnostic value than the total amount.
- Look for an ELISA titer of more than 2-3 SD above mean of normal range.

# Anti-Ro(SS-A) and anti-La(SS-B)

- Ro is characteristic of two diseases
  - Lupus (SCLE 75-90% and neonatal (99%))
  - Annular erythema of Sjogren's syndrome
- They are strongly associated with photosensitivity especially in patients with SCLE (both idiopathic and drug induced).
- Ro may be associated with a higher incidence of vasculitis.

- There appears to be a genetic predisposition.
  - HLA-DR<sub>3</sub>
  - DQ<sub>2</sub>
  - DRw<sub>52</sub>
- Of those with La 90% will have Ro.
- La is associated mainly with SLE and Sjogren's.
- The incidence in these diseases is about half that of Ro.

# Indications for ordering

- Occasionally helpful in the work-up of a patient with photosensitivity.
- Helpful in the initial baseline evaluation of cutaneous LE with features of photosensitivity.
- Helpful in confirming clinical diagnosis of diseases highly associated with these Ab
  - SCLE
  - Neonatal lupus
  - Sjogren's

- An occasional patient with chronic idiopathic vasculitis may be revealed to have undiagnosed Sjogren's.
- Useful for clinical manifestations of SLE or SCLE if the fluorescent ANA is negative.

# U1RNP and Sm

- U1RNP is found in patients with MCTD and SLE.
- U1RNP are detected in 100% of those with MCTD and 30% with SLE.
- It is seen rarely in neonatal lupus.
- MCTD will only have U1RNP.
- If LE has U1RNP it will usually have other Ab.



- U<sub>1</sub>RNP is rarely seen in patients with SSc.
- Since SLE incidence is much higher than MCTD the majority of patients with U<sub>1</sub>RNP will have SLE.
- U<sub>1</sub>RNP is usually associated with:
  - Sclerodactyly
  - Raynaud's
  - Esophageal dysmotility
  - Low incidence of renal disease
  - Pulmonary dysfunction
  - Arthritis
  - myositis

- Sm is a spliceosome RNP (involved in splicing pre-mRNA).
- Sm is diagnostic of SLE.
- They have not been reported in patients with other CTDs.
- The incidence in SLE is 15-40%.
- Most with Sm will also have U<sub>1</sub>RNP
- Most with U<sub>1</sub>RNP will not have Sm.
- Obtaining Sm and U<sub>1</sub>RNP is indicated when confirming SLE and MCTD.

# Other Ab

- Scl-70 is directed against topoisomerase I.
- Topoisomerase-I is a 100kd basic protein that affects the tertiary structure of DNA (unwinds DNA).
- Scl-70 is characteristic for diffuse SSc (60%).
- It is helpful in differentiating diffuse from limited disease.

- Anti-centromere is the marker for CREST (80%).
- Fibrillin-1 has been seen in patient (30-50%) with localized scleroderma.
- Jo-1 is seen in DM/PM (20%)
- Ab against histidyl tRNA synthetase (150kd).
  - Seen with antisynthetase syndrome (arthritis, Raynaud's, and interstitial lung disease).
  - Associated with mechanics hand.
- Mi-2 (15%) in DM/PM is a helicase nuclear protein classically seen with
  - Gottrons papules, shawl sign, periungal telangiectasias, and cuticular dystrophy
- These are detected in a small number with dermatomyositis and polymyositis and have low clinical utility.

- Amyopathic DM is associated with 155kDa/Se polypeptide.
- It has a higher prevalence (80%) of aAb.
- Anti-SRP (5%) is associated with fulminant DM/PM with cardiac involvement and high mortality.

**AUTOANTIBODIES ASSOCIATED WITH LUPUS ERYTHEMATOSUS**

Autoantibody	Median prevalence*	Molecular specificity	Clinical associations
<b>High specificity for SLE</b>			
dsDNA**	60%	Double-stranded (native) DNA	LE nephritis; monitoring activity of SLE
Sm	30%	Spliceosome RNP (ribonucleoprotein particles involved in splicing pre-mRNA)	–
rRNP	7%	Ribosomal P proteins (proteins involved in ribosome function)	Neuropsychiatric LE
<b>Low specificity for SLE</b>			
ANA (most common IF patterns: homogeneous, peripheral)	99%		
ssDNA	70%	Denatured DNA	Possible risk for SLE in DLE patients; also seen in RA, DM/PM, MCTD, SSc, SSj
C1q	60%	C1q component of complement	Severe SLE, hypocomplementemic urticarial vasculitis syndrome
PCNA	50%	A component of multiprotein complexes involved in cell proliferation	–
U1RNP	50%	Spliceosome RNP	Overlapping features with other CTD; MCTD (100%)
Ro (SS-A)	50%	hYRNP	SCLE (75–90%), neonatal LE/congenital heart block (99%), SCLE-SSj overlap, SSj
Cardiolipin	50%	Cardiolipin, a negatively charged phospholipid	Recurrent spontaneous abortions, thrombocytopenia, and hypercoagulable state in SLE (cutaneous manifestations include livedo reticularis, leg ulcers, acral infarction/ulceration, hemorrhagic cutaneous necrosis); similar associations in primary antiphospholipid antibody syndrome; clinical associations strongest with IgG class of anticardiolipin
Histones	40%	Histones	Drug-induced SLE, RA
β2 glycoprotein I	25%	An important cofactor for cardiolipin in cardiolipin autoantibody assays	Relatively high risk of thrombosis in SLE and primary antiphospholipid antibody syndrome
Rheumatoid factor	25%	Fc portion of IgG	
La (SS-B)	20%	hYRNP	SCLE (30–40%), SCLE-SSj overlap, primary SSj (20%)
Ku	10%	DNA end-binding repair protein complex	Overlap with other CTD such as DM/PM, SSc
Alpha-fodrin	10%	An actin-binding protein found at the periphery of chromaffin cells that may be involved in secretion	SSj

## AUTOANTIBODIES ENCOUNTERED IN THE IDIOPATHIC INFLAMMATORY DERMATOMYOPATHIES (DM/PM)

Autoantibody	Median prevalence*	Molecular specificity	Clinical association
<b>High specificity for DM/PM</b>			
155 kDa and/or Se	80%	Uncharacterized polypeptides (nuclear?)	Clinically amyopathic DM
Jo-1	20%	Histidyl tRNA synthetase	Antisynthetase syndrome
Mi-2	15%	Helicase nuclear proteins	Gottron's papules/sign, shawl sign, periungual telangiectasias, cuticular overgrowth/dystrophy
SRP	5%	Signal recognition particle	Fulminant DM/PM, cardiac involvement
PL-7	3%	Threonyl tRNA synthetase	Antisynthetase syndrome
PL-12	3%	Alanyl tRNA synthetase	Antisynthetase syndrome
OJ	Rare	Isoleucyl tRNA synthetase	Antisynthetase syndrome
EJ	Rare	Glycyl tRNA synthetase	Antisynthetase syndrome, possibly increased frequency of skin changes
Fer	Rare	Elongation factor 1- $\alpha$	-
Mas	Rare	Small RNA	-
KJ	Rare	Translation factor	-
<b>Low specificity for DM/PM</b>			
ANA (most common IF patterns: speckled, nucleolar)	40%		Clinically amyopathic DM (80%)
ssDNA	40%	Single-stranded DNA	SLE, SSc
PM-Scl (PM-1)	10%	Ribosomal RNA processing enzyme	Overlap with scleroderma
Ro (especially 52 kDa Ro)	15%	hYRNP	Overlap with SSj, SCLE, neonatal LE/CHB, SLE
U1RNP	10%	spliceosome RNP	Overlap connective tissue diseases
Ku	3%	DNA end-binding repair protein complex	Overlap with scleroderma
U2RNP	1%	U2RNP	Overlap with scleroderma



**AUTOANTIBODIES ASSOCIATED WITH SSc AND LOCALIZED SCLERODERMA (MORPHEA)**

Autoantibody	SSc, All	SSc with diffuse cutaneous scleroderma	SSc with limited cutaneous scleroderma (CREST syndrome)	Localized scleroderma (linear scleroderma; morphea, localized and generalized)
ANA (most common IF patterns: speckled, nucleolar, centromere)	95%*			40%
Centromere (CENP-B)		30%	80%	
Scl-70 (DNA topoisomerase I, which unwinds DNA)		60%	15%	
Fibrillin 1 (major component of microfibrils in the extracellular matrix)		5%	10%	30%
Histones	40%			35%**
Rheumatoid factor	25%			25%
ssDNA	10%			50%
Fibrillarin (U3RNP)	5%			
PM-Scl	5%			
RNA polymerase I	5%			
Th/To RNP (mitochondrial enzyme)		11%	19%	5%
Calpastatin	25%			
HMG (a non-histone nucleosomal protein)		30%	40%	



**Table X.** Serologic profiles in CTDs<sup>27</sup>

Profile	nDNA	Sm	U <sub>1</sub> RNP	Ro(SS-A)	La(SS-B)	Centromere	Scl-70	Histone	Disease
A	+	+							SLE
B	-	-	+						MCTD
C	-	-	-	+	+				SS, SCLE
D	-	-	-	-	-	+	+		SSc, CREST
E	-	-	-	-	-	-	-	+	Drug-SLE

SS, Sjögren's syndrome; SCLE, subacute cutaneous lupus erythematosus.

# Antiphospholipid antibodies

- APAs are directed against negatively charged phospholipids in cell membranes.
- They cause false positive VDRLs.
- They have negative FTA-abs.
- They are detected by various techniques
  - Lupus anticoagulant
  - Most frequently ELISA using bovine cardiolipin
    - Thus, the term anticardiolipin antibodies
- The sensitivity for both is 75-90%

- Sera containing APAs delay the coagulation pathway of normal blood in vitro.
- This is the way the functional lupus anticoagulant test works.
- Clinically patients have thrombosis.
- Some sera may be positive by one assay and negative by the other.
- Each assay detects approximately 90% of those patients with APA's.
- If ELISA negative and strong clinical suspicion then order lupus anticoagulant.

# Disease associations

- APAs are most prevalent in patients with SLE (approximately 50%).
- Other CTDs have a lower prevalence.
- They can be seen in patients who
  - Are taking medications (cocaine, IFN, procainamide, hydralazine, phenothiazines, quinine, quinidine, phenytoin, fansidar)
  - Have chronic infections (syphilis, mono, TB, leprosy, malaria, CMV, HIV, HCV, etc.)
  - Primary APA syndrome
- Patients have arterial and venous thrombosis in various organs including heart, CNS, and skin.

- Young women with recurrent miscarriages has been associated with APAs
- Dermatologically one sees livedo reticularis, purpura, necrosis, and ulcers.
- Low levels of APAs are of no clinical relevance.

# When should APAs be ordered?

## **Table XI.** Indications for APA testing\*

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Livedo reticularis

Purpura and necrosis

Ulcers

Internal organ thrombosis

Recurrent miscarriages

Screening in patients with SLE

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# Antineutrophilic cytoplasmic antibodies

- cANCA
  - Proteinase-3 or PR<sub>3</sub>-ANCA
  - Wegeners
- pANCA
  - Myeloperoxidase or MPO-ANCA
  - Microscopic PAN