

# Interpretation of Immunological Tests in Rheumatology

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Onlineမှာပြထားသောပုံ



ရောက်လာသောပုံ



# Disclosure

**No actual or potential conflicts of interest**



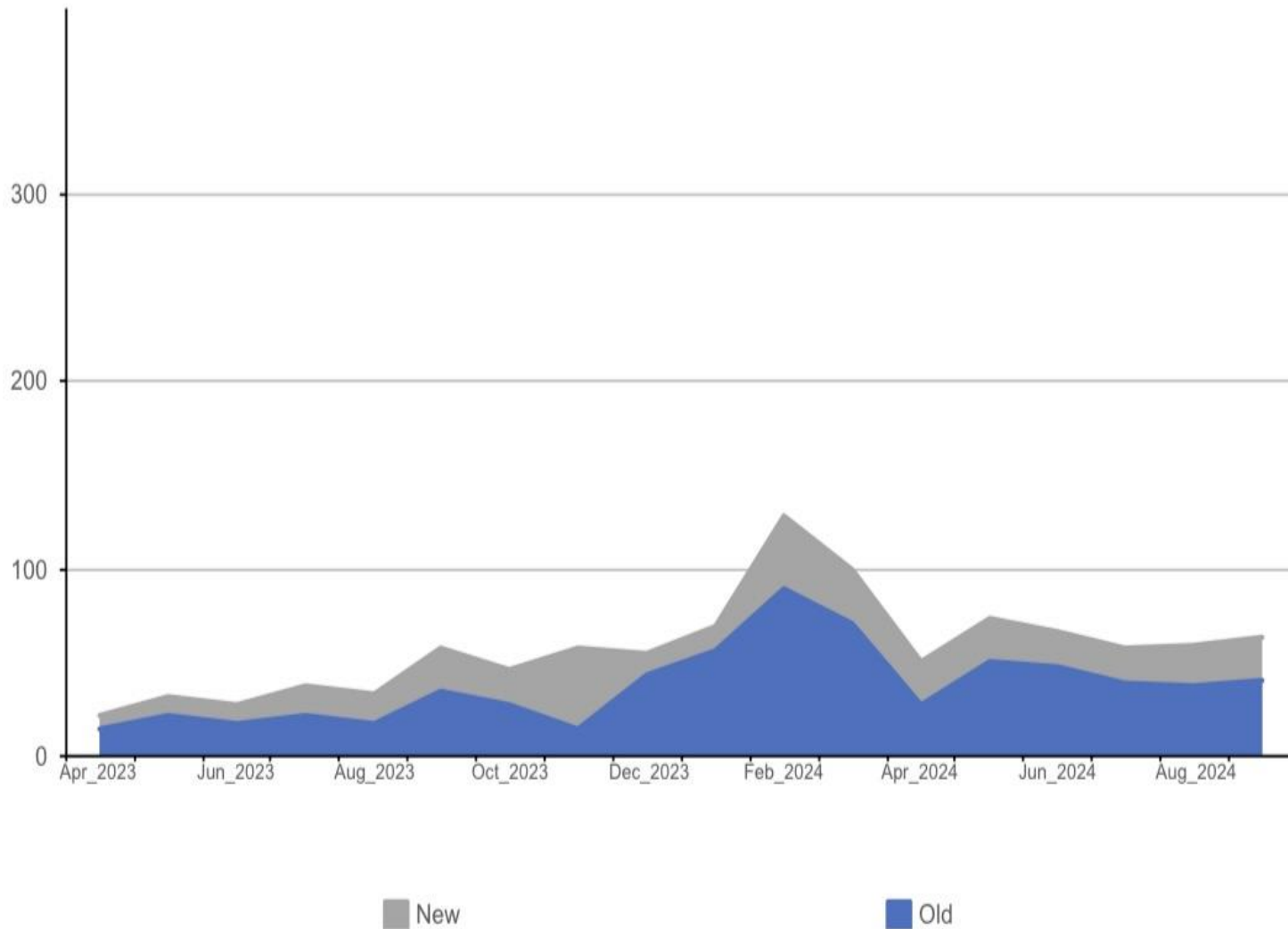




ကိုယ်တိုင် SLE ရောဂါဖြစ်တော့မှ မသက်မွန်ကို ကိုယ်ချင်း  
တမိတယ်



Old vs New Cases in Rheumatology Clinic



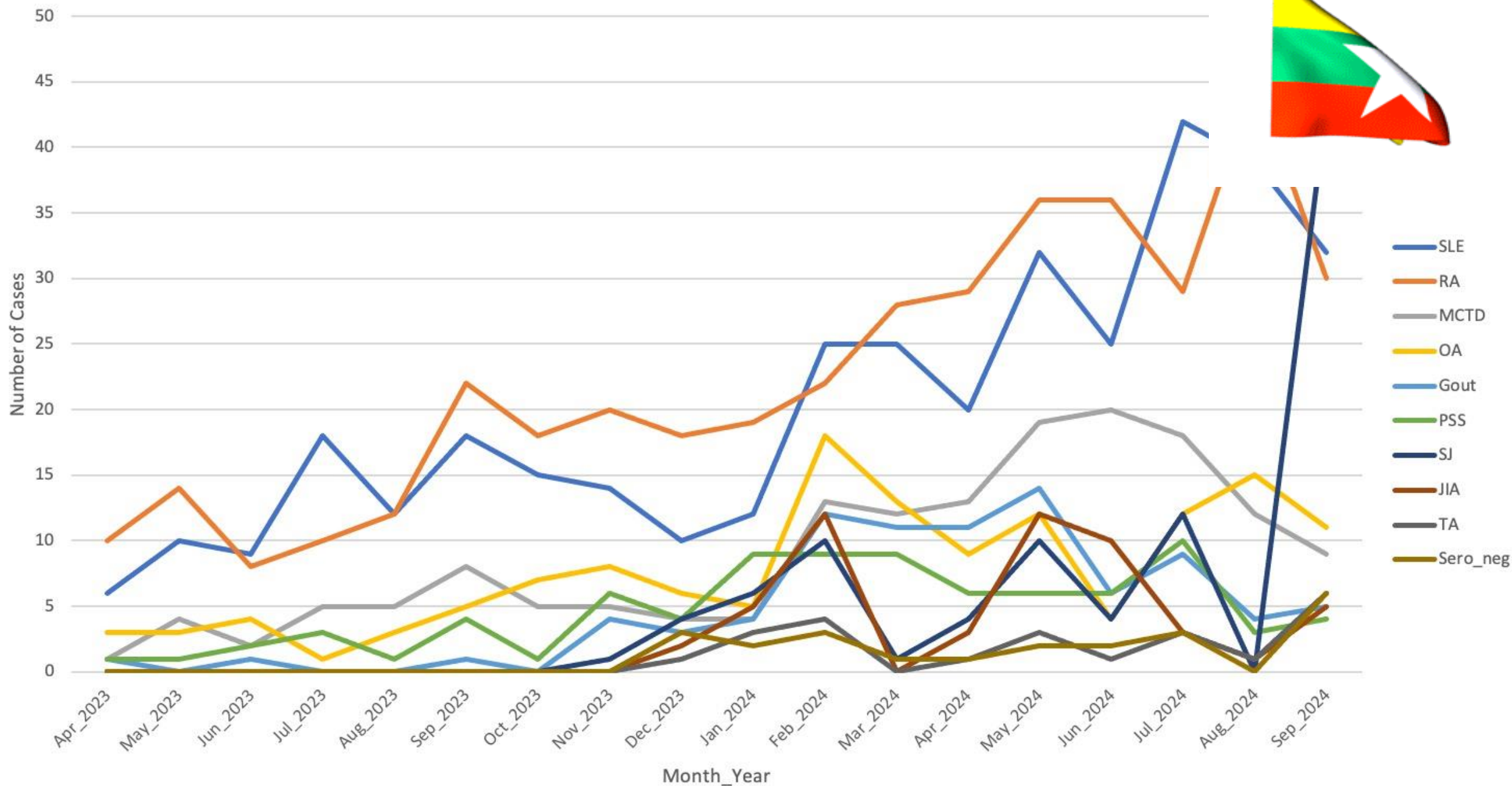
April 2023 to Sept 2024

NPT General Hospital  
(1000-bedded)  
Rheumatology OPD

Total – 1035  
Old cases– 669  
New cases - 366

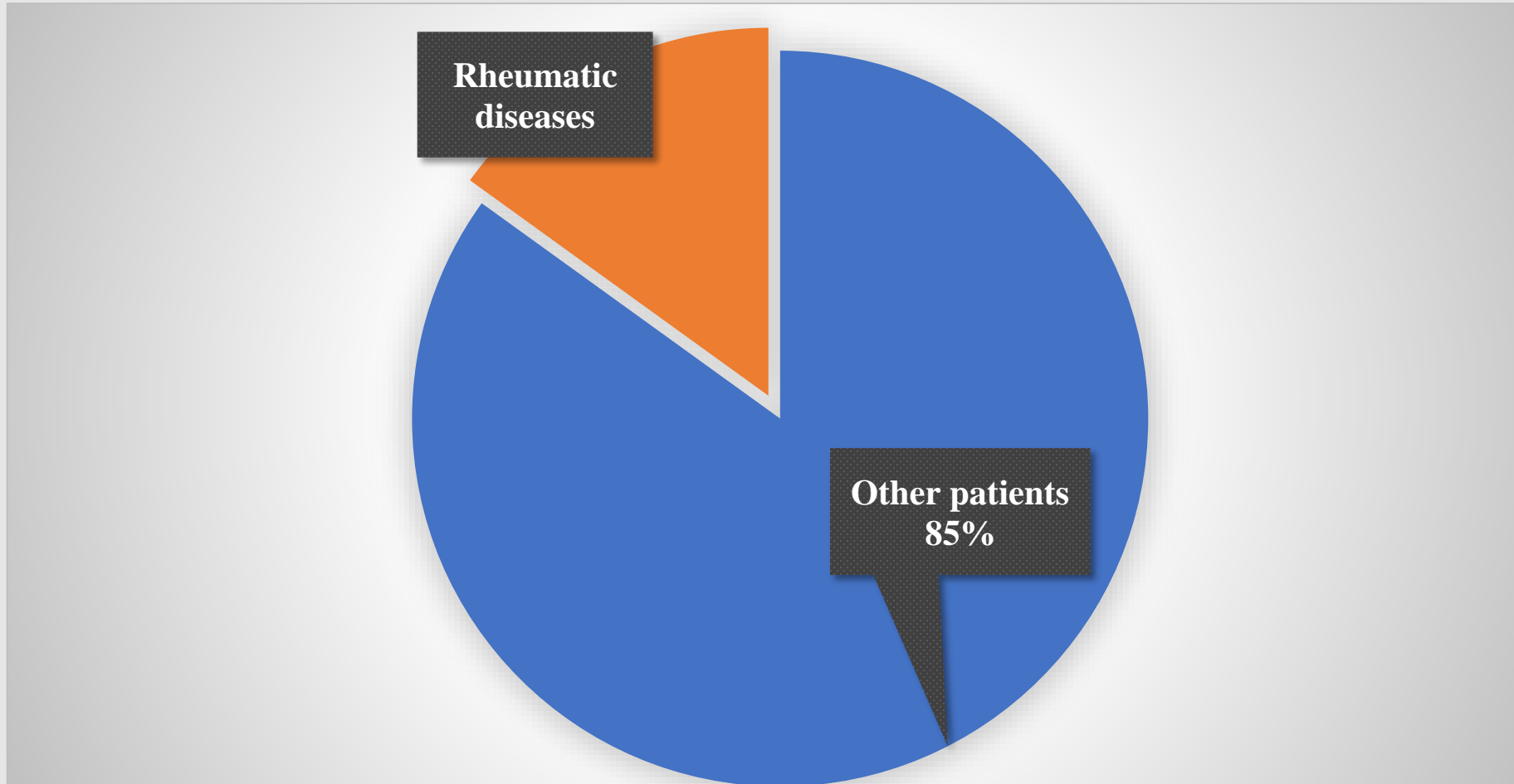


Different types of cases in Rheumatology Clinic





Gen Med OPD (March 2018 to September 2019)  
(total admission-1158) at 3/300 MH



# Contents

- Overview and principles of immunological tests
- Rheumatoid Arthritis
- SLE and auto-antibodies
- ANA/ ENA positive Dilemma
- Others...
- Take Home Message



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# Autoimmune Serology

- Immunological testing is primarily for diagnostic purposes and may help sub-setting patients.
- useful adjunct to clinical evaluation in the diagnosis of rheumatic diseases
- false positive results are common.
- The absence of auto-antibody doesn't exclude a disease

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### LABORATORY REPORT

<u>TEST</u>	<u>RESULT</u>	<u>UNIT</u>	<u>REFERENCE RANGE</u>	<u>REMARK</u>
Uric Acid	2.7	mg/dL	2.4 - 5.7	
ASO(Quantitative)	303.22	IU/mL	<200	Positive
RA(Quantitative)	<10.0	IU/ml	<18	Negative

# Rheumatoid arthritis



- *#with Patient's consent#*

# Rheumatoid factor (RF)

- antibodies with specificity against C-terminal domain of the constant region of the heavy chain in human IgG
- role in both diagnostic and prognosis
- higher levels indicate more severe disease



# Rheumatoid factor (RF) (Cont;)

- can be detected in other systemic diseases, such as Sjogren's syndrome and systemic infections, and in about 10% of healthy people.



## 26.7 Conditions associated with a positive rheumatoid factor\*

Condition	Approximate frequency (%)
Rheumatoid arthritis with nodules and extra-articular manifestations	100
Rheumatoid arthritis (overall)	70
Primary Sjögren syndrome	90
Mixed essential cryoglobulinaemia	90
Primary biliary cholangitis	50
Infective endocarditis	40
Systemic lupus erythematosus	30
Tuberculosis	15
Age > 65 years	20

**sensitivity 69%      specificity 85%**

\*Normal healthy people can be positive for rheumatoid factor.

# Anti-bodies to Citrullinated Peptides (Anti-CCP)

- ◆ a complementary marker

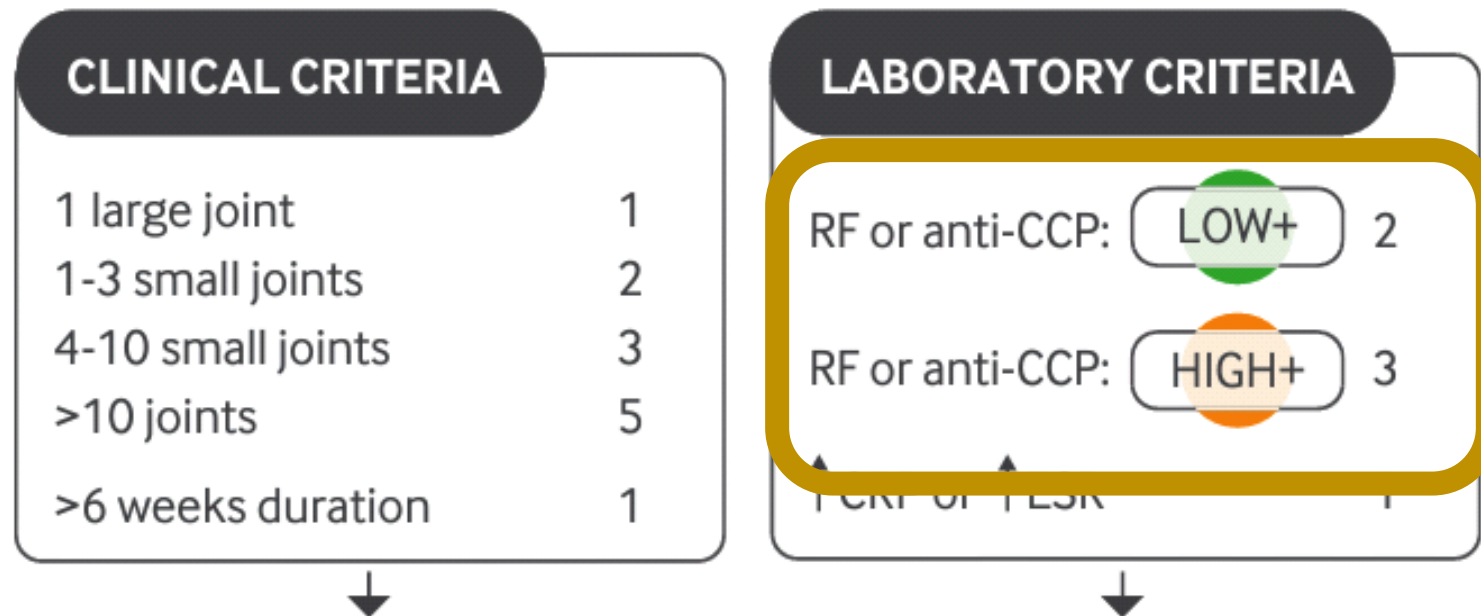


# Anti-CCP (Cont;)

- ◆ dual positive in more severe disease
- ◆ emerge as early as 10-14 years before the onset of symptoms.
- ◆ independently associated with development of IHD

**sensitivity 67%      specificity 95%**

## 2010 CLASSIFICATION CRITERIA FOR RHEUMATOID ARTHRITIS



**Fig 1 | American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) 2010 classification criteria for rheumatoid arthritis**

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# Systemic Lupus Erythematosus (SLE)



- *#with Patient's consent#*



# Antinuclear Antibodies (ANA)

- Heterogenous group of autoAb directed against components of nucleus
- Indirect immunofluorescence (IIF) assay using Hep2 cells
- Reported in titers

Peripheral  
(rim)



anti-DNA (not  
seen on HEp-2)

SLE

Homogeneous  
(diffuse)



anti-DNA  
anti-histone  
anti-DNP  
(nucleosomes)

RA & SLE  
Misc. Disorders  
(anti-ssDNA)

Speckled



anti-Sm & RNP  
anti-Ro & La  
anti-Jo-1 & Mi-2  
anti-Scl-70

SLE & SS  
PM/DM  
PSS (Systemic)

Centromere



anti-centromere

PSS (CREST)

Nucleolar

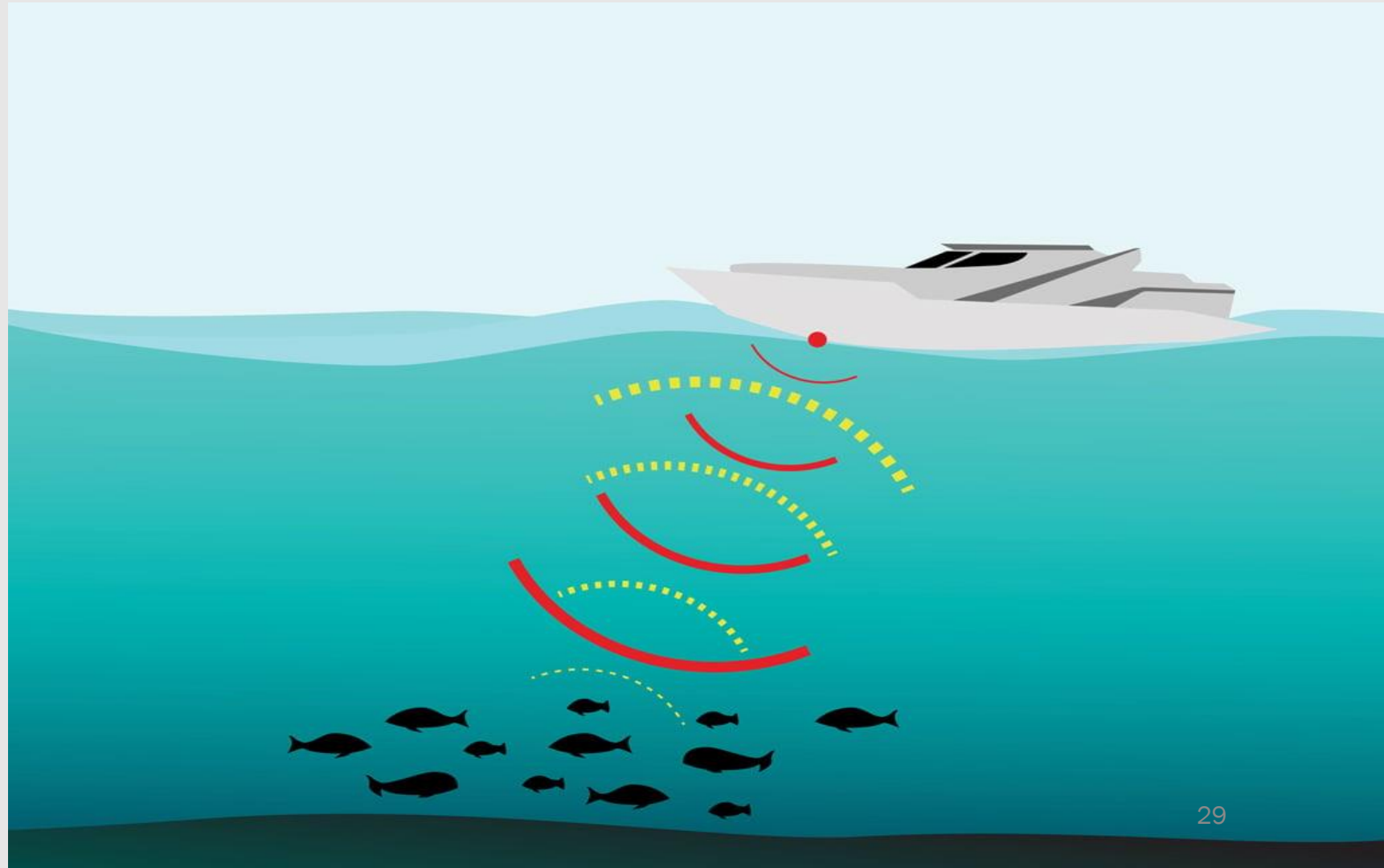


anti-nucleolar

SLE & PSS

# ANA

- sensitivity > 98% for SLE and lupus nephritis
- lacks of specificity (other autoimmune diseases, thyroid, hepatic diseases, cancers, chronic infection and elderly)
- Low positive predictive value of ANA testing
- Negative test has less than a 3% chance of having SLE
- In the presence of typical features of lupus a negative ANA test does not exclude the diagnosis



# SLE

AMERICAN COLLEGE  
of RHEUMATOLOGY  
*Empowering Rheumatology Professionals*

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## SPECIAL ARTICLE

# 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus

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**Entry criterion**

Antinuclear antibodies (ANA) at a titer of  $\geq 1:80$  on HEp-2 cells or an equivalent positive test (ever)

If absent, do not classify as SLE  
If present, apply additive criteria

↓

**Additive criteria**

Do not count a criterion if there is a more likely explanation than SLE.  
Occurrence of a criterion on at least one occasion is sufficient.  
SLE classification requires at least one clinical criterion and  $\geq 10$  points.  
Criteria need not occur simultaneously.

Within each domain, only the highest weighted criterion is counted toward the total score.

Clinical domains and criteria	Weight	Immunology domains and criteria	Weight
<b>Constitutional</b>		<b>Anticardiolipin antibodies</b>	
Fever	2	Anti-cardiolipin antibodies OR	
<b>Hematologic</b>		Anti- $\beta 2$ GP1 antibodies OR	
Leukopenia	3	Lupus anticoagulant	2
Thrombocytopenia	4	<b>Complement proteins</b>	
Autoimmune hemolysis	4	Low C3 OR low C4	3
<b>Neuropsychiatric</b>		Low C3 AND low C4	4
Delirium	2	<b>SLE-specific antibodies</b>	
Psychosis	3	Anti-dsDNA antibody* OR	
Seizure	5	Anti-Smith antibody	6
<b>Mucocutaneous</b>			
Non-scarring alopecia	2		
Oral ulcers	2		
Subacute cutaneous OR discoid lupus	4		
Acute cutaneous lupus	6		
<b>Serosal</b>			
Pleural or pericardial effusion	5		
Acute pericarditis	6		
<b>Musculoskeletal</b>			
Joint involvement	6		
<b>Renal</b>			
Proteinuria $>0.5\text{g}/24\text{h}$	4		
Renal biopsy Class II or V lupus nephritis	8		
Renal biopsy Class III or IV lupus nephritis	10		

**Total score:**

↓

**Classify as Systemic Lupus Erythematosus with a score of 10 or more if entry criterion fulfilled.**

# Systemic sclerosis

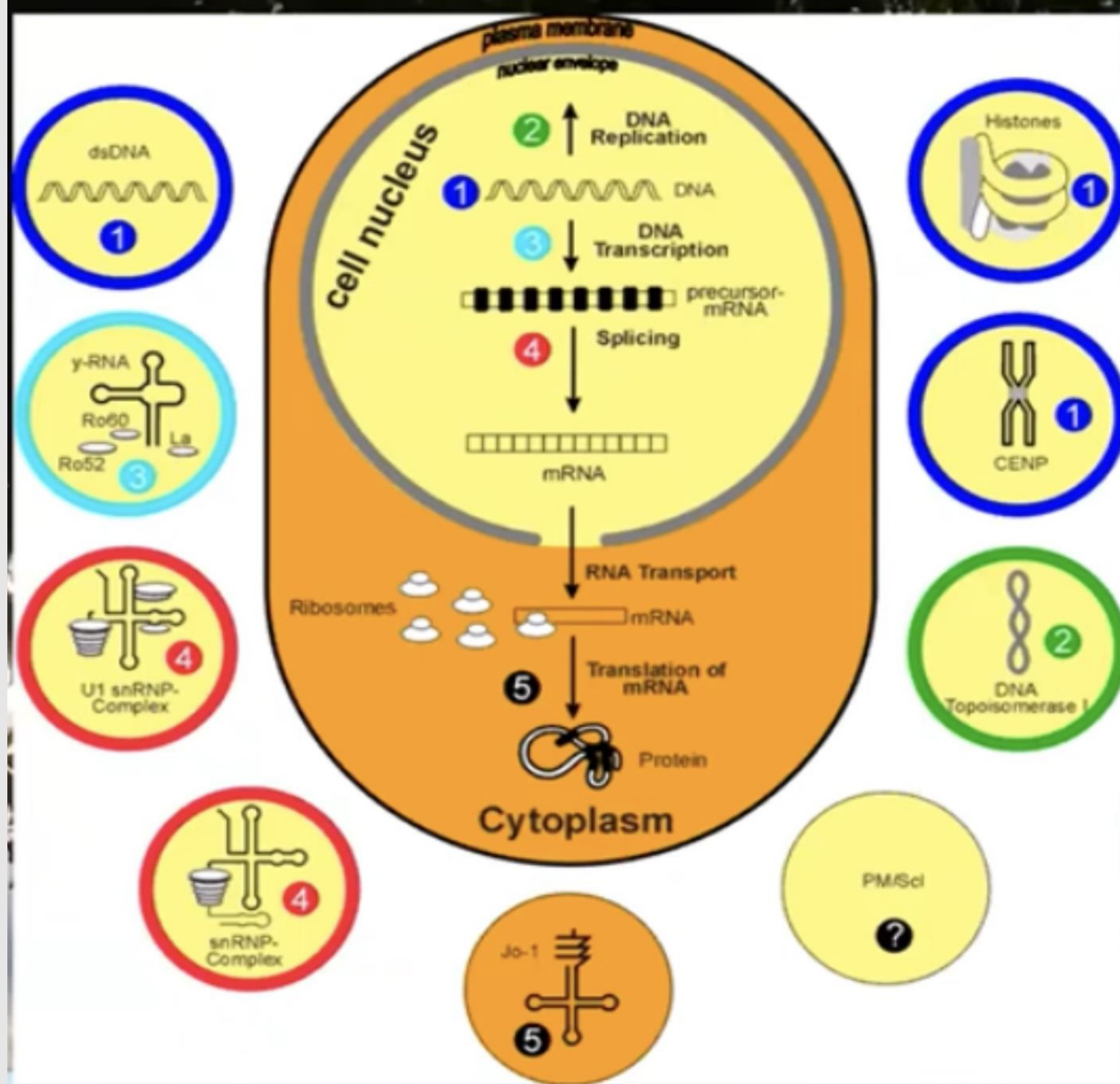


- *#with Patient's consent#*

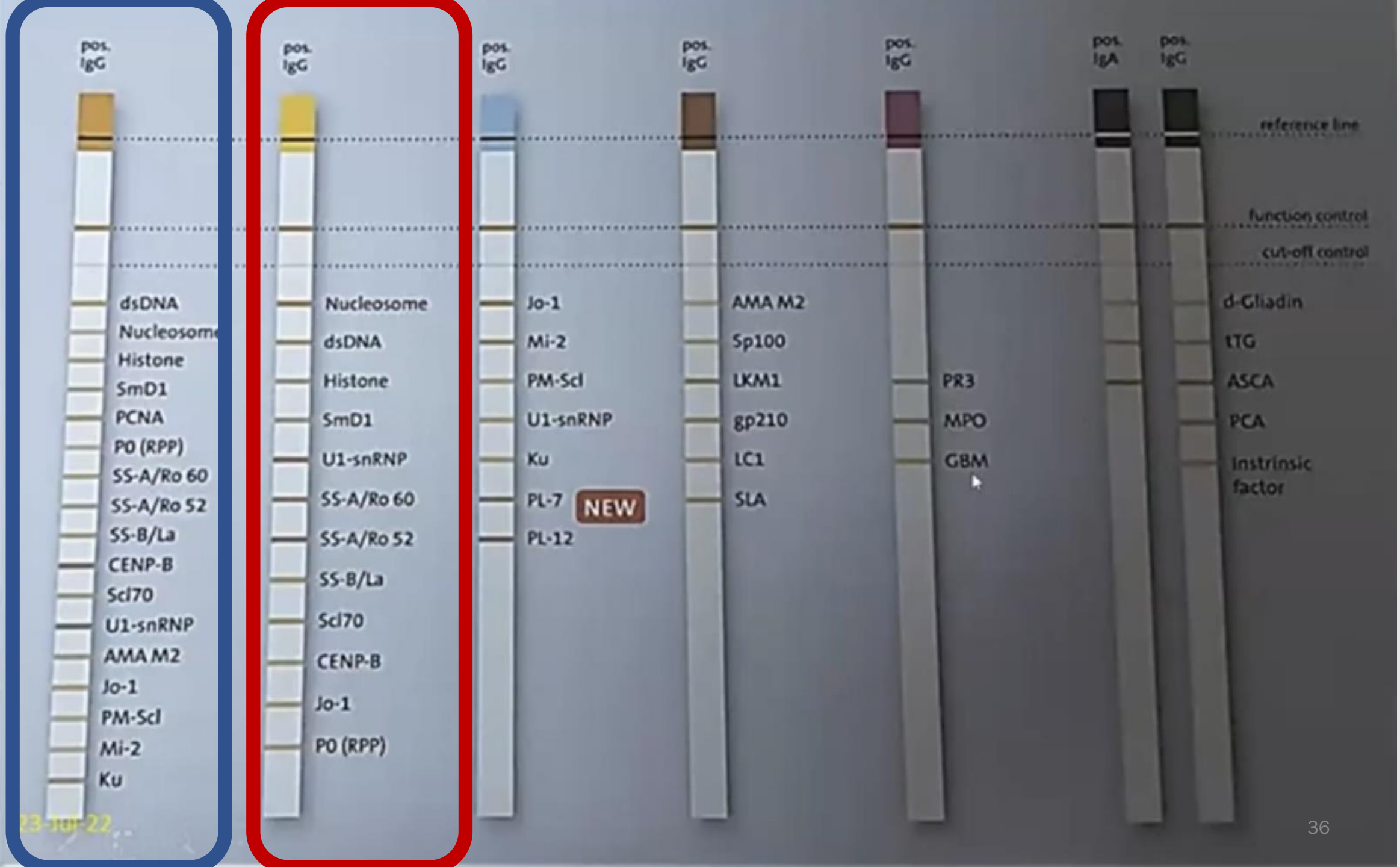
## 2013 ACR/EULAR classification criteria for systemic sclerosis

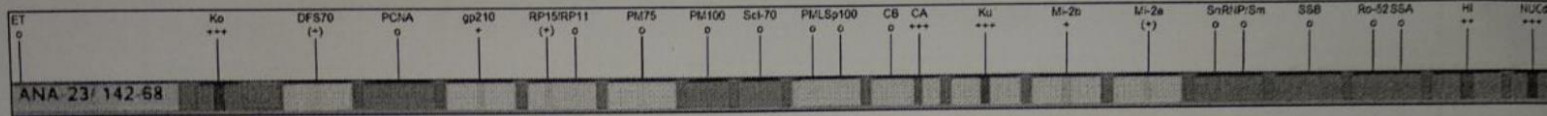
The ACR/EULAR criteria for the classification of SSc		
Category	Subitems	Weight
Skin <sup>a</sup>	Skin thickening of the fingers of both hands extending proximal to the MCPs <sup>b</sup>	9
	Puffy fingers	2
	Whole finger, distal to MCP	4
Fingertip lesions <sup>a</sup>	Digital tip ulcers	2
	Pitting scars	3
Telangiectasia	—	2
Abnormal nail fold capillaries	—	2
PAH and/or interstitial lung disease	—	2
Raynaud's Phenomenon (RP)	—	3
Scleroderma-related antibodies (any of anticentromere, anti-topoisomerase-I [anti-Scl-70], anti-RNA polymerase-3)	—	3

# **Antibodies to Extractable Nuclear Antigens (ANA/ENA profiles)**









Antigen	Intensity	Class	o (+) + ++ +++
dsDNA [AC-1] (dsDNA)	37	++	
Nucleosomes [AC-1] (NUC)	54	+++	
Histones [AC-1] (HI)	39	++	
SS-A [AC-4] (SSA)	1	o	
Ro-52 (Ro-52)	3	o	
SS-B [AC-4] (SSB)	1	o	
RNP/Sm [AC-5] (RNP/Sm)	4	o	
Sm [AC-5] (Sm)	3	o	
Mi-2alpha [AC-4] (Mi-2a)	10	(+)	
Mi-2beta [AC-4] (Mi-2b)	18	+	
Ku [AC-4] (Ku)	118	+++	
Centromere A [AC-3] (CA)	96	+++	
Centromere B [AC-3] (CB)	1	o	
Sp100 [AC-6] (Sp100)	1	o	
PML [AC-6] (PML)	1	o	
Scl-70 [AC-29] (Scl-70)	1	o	
PM-Scl100 [AC-8] (PM100)	1	o	
PM-Scl75 [AC-8] (PM75)	3	o	
RP11 [AC-10] (RP11)	2	o	
RP155 [AC-10] (RP155)	10	(+)	
gp210 [AC-11] (gp210)	11	+	
PCNA [AC-13] (PCNA)	1	o	
DFS70 [AC-2] (DFS70)	7	(+)	

Intensity	Class	Explanation
0	o	Negative
6-10	(+)	Borderline
11-25	+	Positive
26-50	++	Positive
51-256	+++	Strong positive

# Antibodies in nuclear target antigens

	Antibody	Diseases	Prevalence
1	Anti-Sm	SLE	30%
2	Anti-nRNP	SLE	40%
		Mixed connective tissue disease	100%
		Rheumatoid arthritis	20%
3	Anti-Ro	Primary Sjogren Syndrome	60%
		SLE	30%
4	Anti-La	Primary Sjogren Syndrome	30%
		SLE	15%
5	Anti-centromere	Limited Systemic Sclerosis	50%
6	Anti-Scl 70	Diffuse Systemic Sclerosis	45%

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# **ANA/ ENA positive Dilemma**

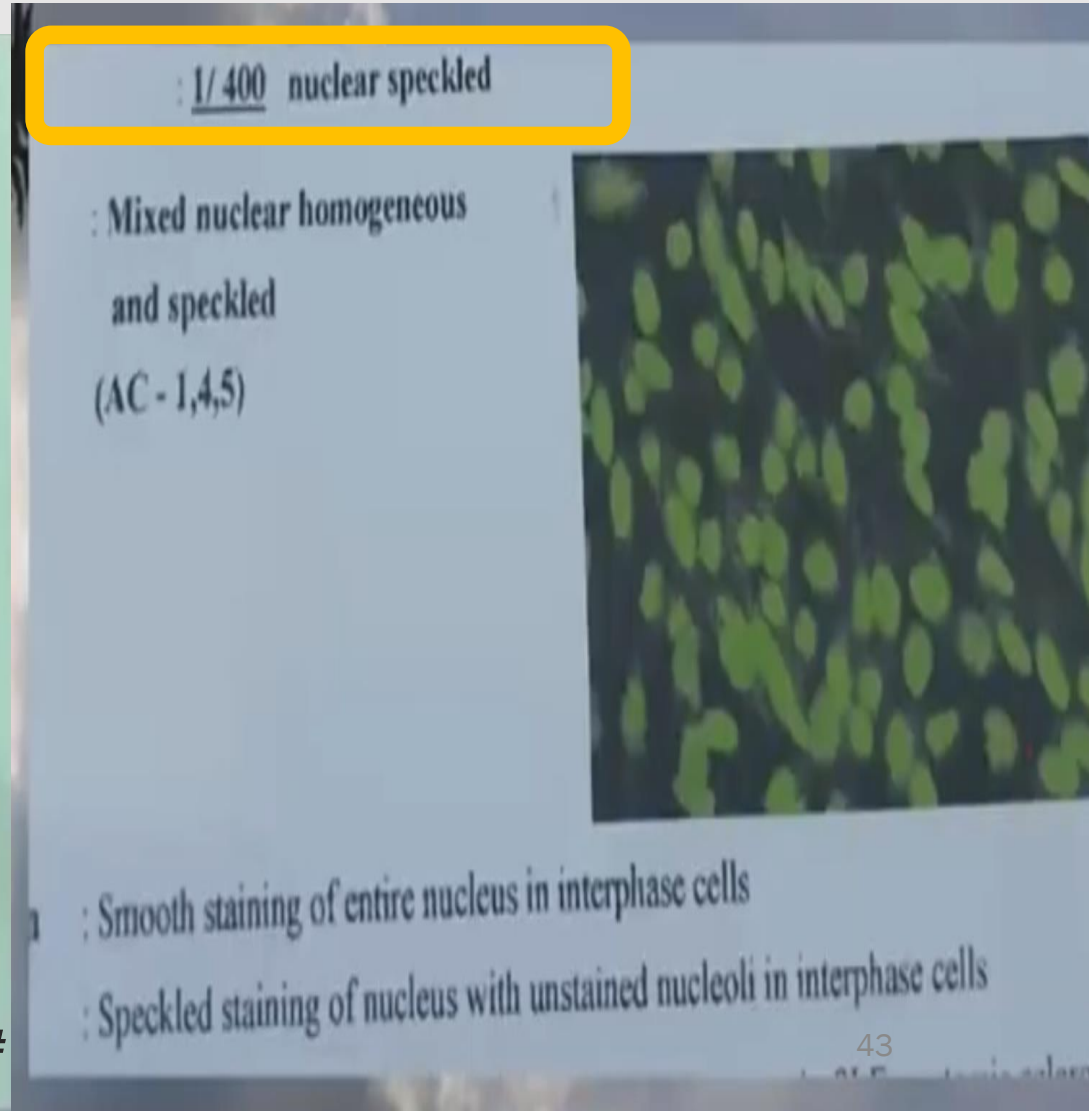
- ❖ ANA - positive, ENA – negative
- ❖ ANA – negative, ENA – positive
- ❖ ANA – positive, ENA – positive with typical S/S of SLE
- ❖ ANA– positive, ENA – positive with no symptoms

# ANA/ ENA positive Dilemma

- ❖ **ANA - positive, ENA – negative**
- ❖ ANA – negative, ENA – positive
- ❖ ANA – positive, ENA – positive with typical S/S of SLE
- ❖ ANA– positive, ENA – positive with no symptoms



• #with Patient's consent#



: 1/400 nuclear speckled




: Mixed nuclear homogeneous  
and speckled  
(AC - 1,4,5)

: Smooth staining of entire nucleus in interphase cells

: Speckled staining of nucleus with unstained nucleoli in interphase cells

**i**

## 26.8 Conditions associated with a positive antinuclear antibody\*

Condition	Approximate frequency (%)
Systemic lupus erythematosus	100 
Systemic sclerosis	90–95
Sjögren syndrome	40–70
Dermatomyositis or polymyositis	30–80
Mixed connective tissue disease	100 
Autoimmune hepatitis	100 
Rheumatoid arthritis	30–50
Autoimmune thyroid disease	30–50
Malignancy	Varies widely
Infectious diseases	Varies widely

\*Low-titre positive antinuclear antibody can occur in people without autoimmune disease, without obvious clinical consequences, particularly in older adults.

- Other auto-immune diseases
- Early course of the disease (Unclassified connective tissue disease)



# **ANA/ ENA positive Dilemma**

- ❖ ANA - positive, ENA – negative
- ❖ ANA – negative, ENA – positive
- ❖ ANA – positive, ENA – positive with typical S/S of SLE
- ❖ ANA– positive, ENA – positive with no symptoms

# ANA/ ENA positive Dilemma

- ❖ ANA - positive, ENA – negative
- ❖ **ANA – negative, ENA – positive**
- ❖ ANA – positive, ENA – positive with typical S/S of SLE
- ❖ ANA– positive, ENA – positive with no symptoms

# ANA – negative, ENA – positive

	004				
	005				
007					
	005				
	006				
	069				
008					
002					
<b>Renal P</b>					
011					
010					

<b>Autoimmune</b>					
072					Anti ds DNA / SLE
073					ANF (Latex Agglutination)
074					ANA (IFA)
075					ANA Profile (ENA 15 Panel)
114					Autoimmune Liver Profile
	076				Autoimmune Liver Panel
	116				ASMA
	080				ANCA (pANCA, cANCA) (IFA)
077					ANCA (Anti-MPO,PR3, GBM) (IgG)
078					Food Allergy (20 Panel)
079					Inhalation Allergy (20 Panel)

# ANA – negative, ENA – positive

	004				
	005				
007					
	005				
	006				
	069				
008					
002					
<b>Renal P</b>					
011					
010					

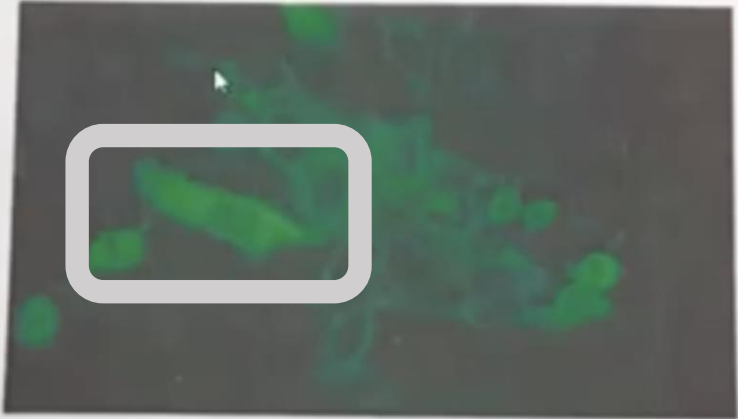
<b>Autoimmune</b>			
072			Anti ds DNA / SLE
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077			ANCA (Anti-MPO, PR3, GBM) (IgG)
078			Food Allergy (20 Panel)
079			Inhalation Allergy (20 Panel)

# ANA – negative, ENA – positive

EMALE  
OSP: AR YU  
Reported Date : 27.12.2020

Result	Interpretation
ANA) : <u>1/400</u>	See below

: Cytoplasmic patterns  
(AC 15 to 23)



fine speckled to homogenous cytoplasmic fluorescence with Hep-2 cell.

positive cases ( $\geq 1/400$ ) can be seen in Primary biliary cirrhosis, SLE, myositis ,  
immune hepatitis, Sjögren syndrome, Systemic sclerosis, Mixed connective tissue



# **ANA/ ENA positive Dilemma**

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- ❖ ANA– positive, ENA – positive with no symptoms

# Distribution of new cases consultation

New Cases	Frequency	Percentage
Joint pain	80	51.9
Low back pain	38	24.8
Skin lesion	12	7.8
Raynaud	9	5.8
Oral ulcer	8	5.2
Alopecia/ hair falling	5	3.2
Oedema	2	1.3
Total	154	100



Test

ANA

Result

1/400

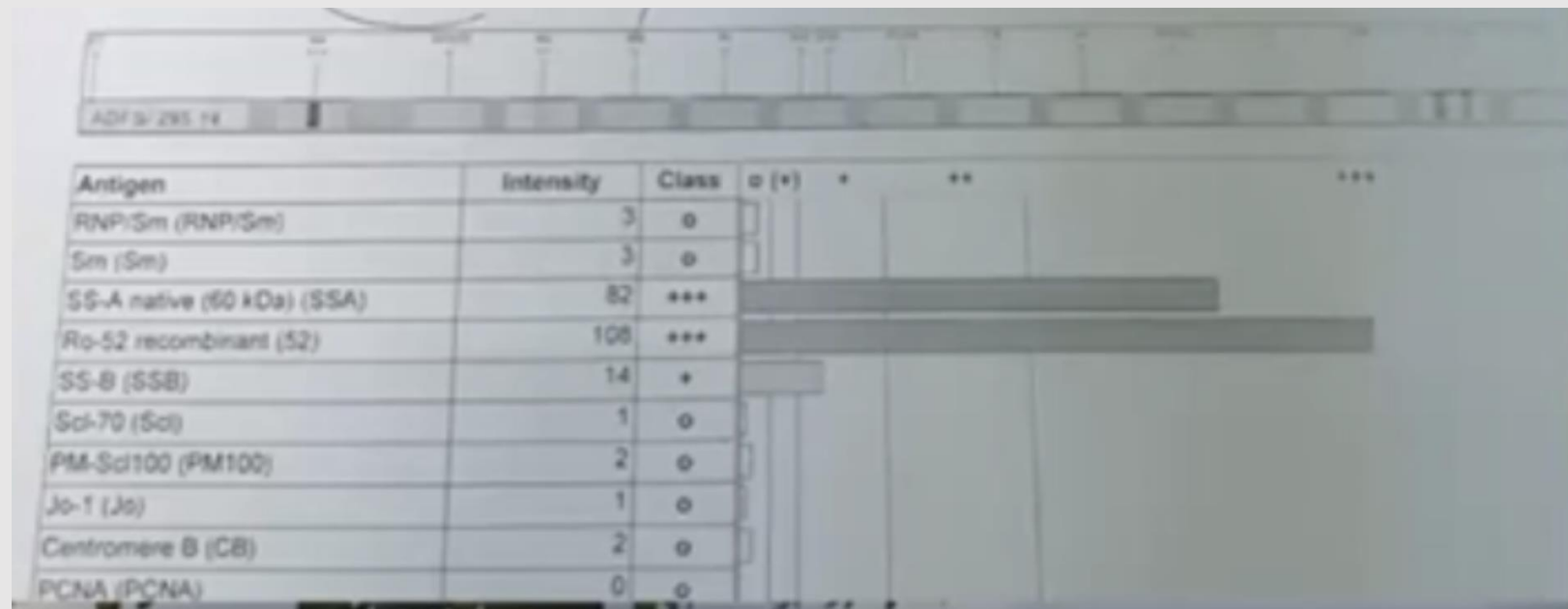
Remarks

See Below

Pattern : ANA (Dense Fine Speckled Pattern & Speckled Pattern)

Interpretation :

1/100	Dilution : Trace
1/200	Dilution : Weakly Positive
1/400	Dilution : Positive
1/800	Dilution : Strong Positive





# ANA/ ENA positive Dilemma

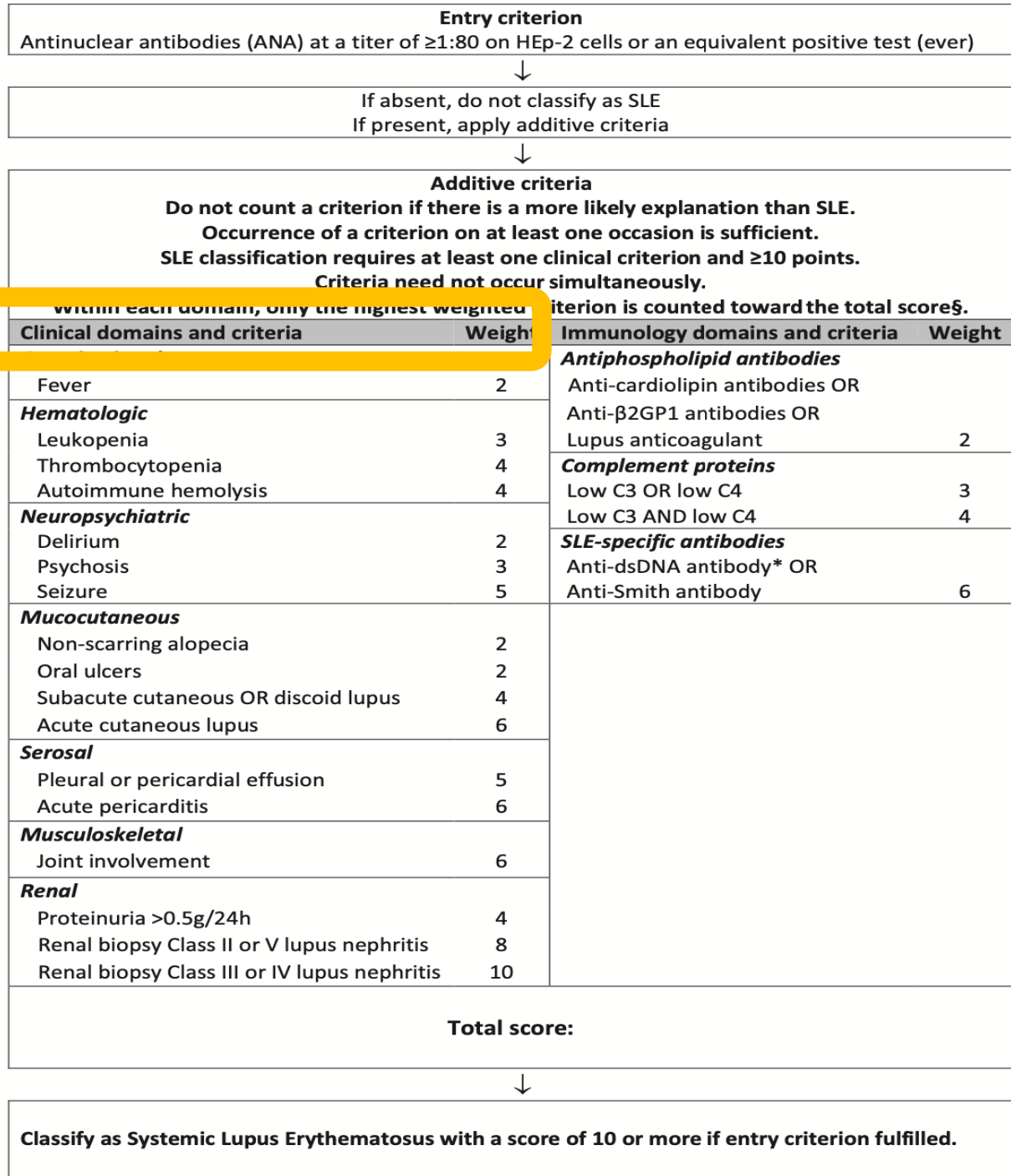
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# Others....

- Antiphospholipid antibodies (APLS)
- Antineutrophil cytoplasmic antibodies (ANCA)
- Anti dsDNA
- Complements

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- ✓ Auto-immune tests should not be included in routine yearly medical check up for healthy population
- ✓ Routine re-testing of auto-antibody profiles is seldom helpful unless the clinical features of the disease change or evolve with time



- ❑ Not all ANA + are auto-immune patients
- ❑ Not all ANA+ are SLE
- ❑ If indicated, even with the result coming back as ANA+, need to proceed to ENA/ANA profiles



- Need to use clinical acumen for interpretation of the autoimmune tests

# ACKNOWLEDGEMENTS





