

# What's the meaning of a positive ANA test? What to do next?



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***Why the hell  
has an ANA test  
been ordered  
for this patient?***

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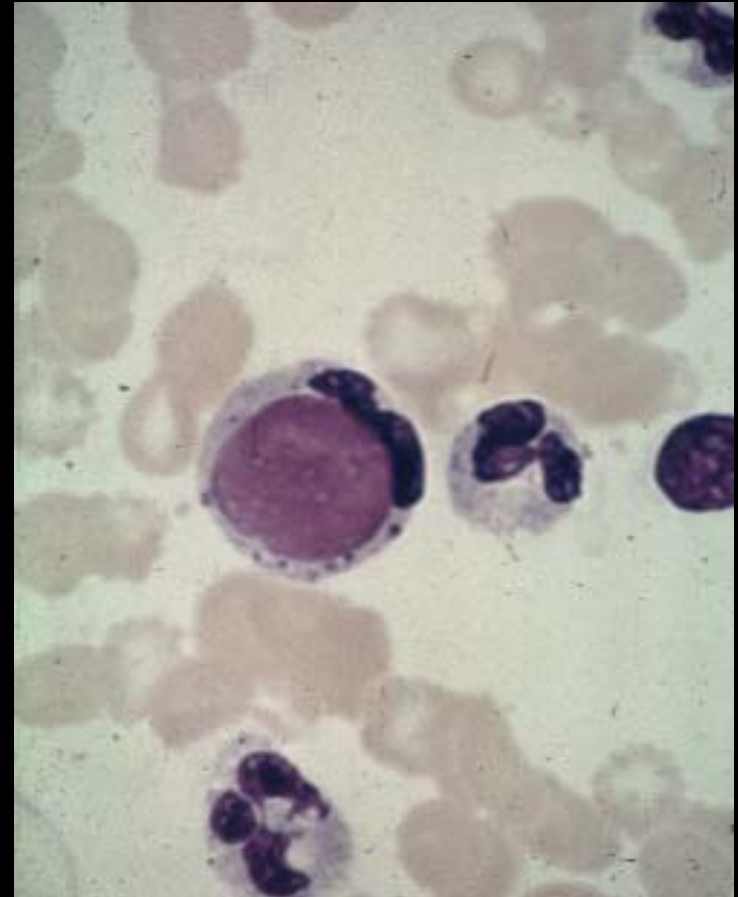
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- **Brief history of ANA testing**
  - When should an ANA test be ordered?
  - Frequently asked questions about ANA
-

# ANA: the LE cells

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- *in vitro* damaged white cells are coated by the 'LE factor'
- the nuclei of damaged cells are ingested by neutrophils
- the cytoplasm and nucleus of the neutrophil are stretched around a homogeneous mass (ingested material)



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*Hargraves et al., 1948*

# ANA: the LE-cell test

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- **Important support for the diagnosis of SLE (previously only tissue biopsies)**
  - **Low sensitivity and specificity**
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# ANA: the discovery

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- **LE factor: a family of antibodies to nuclear constituents**
  - **Late 1950s: many *in vitro* immunofluorescence-based tests developed (rodent kidney/liver sections)**
  - **Increase in sensitivity, further reduction in specificity**
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# ANA: the refinement

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- **Titers ( $\geq 1:40$ ): increase in specificity**  
*--> ANA+ became a classificative criteria for SLE*
  - **Patterns of immunostaining**  
*--> homogeneous/diffuse, rim/peripheral, nucleolar, centromeric*
  - **Change of substrate (HEp-2)**  
*--> increase in sensitivity (human)*  
*--> pos only if  $\geq 1:80 \rightarrow$  increase in specificity*
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# ANA: the refinement

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## Change of substrate (HEp-2)

*low sensitivity for anti-Ro/SS-A antibodies*

*--> Use of a Ro/SS-A-transfected HEp-2 cell line*

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# ANA: a refinement (?)

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- **EIA/ELISA testing**

*--> greater ease of performance*

*--> lower cost of the test*

For generic ANA:

*- not subject to widespread population testing*

*- lower sensibility*

*- no possibility to establish the ANA pattern*

**→ Useful for specific autoAbs to nuclear Ags**

**(SSA, SSB, dsDNA)**

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# ANA: quality assurance

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- **EIA/ELISA generic ANA testing**

*not advised*

- **IF generic ANA testing**

- *on HEp-2 cells (not on rodent tissues)*

- *human-IgG specific secondary Ab*

- *FITC:protein ratio  $\approx 3$*

- *Ab:protein ratio  $\geq 0.1$*

- *specific Ab content 30-60  $\mu\text{g/mL}$*

- *working dilutions determined by regular test on reference sera*

- *skilled and referenced personnel*

# What's the meaning of a positive ANA test? What to do next?

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- Brief history of ANA testing
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  - Frequently asked questions about ANA
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***“No test for ANA and for specific autoantibodies to nuclear antigens should be performed without a clinical evaluation that leads to a presumptive diagnosis”***

Kavanaugh et al. *Guidelines for clinical use of the ANA test and tests for specific autoantibodies to nuclear antigens.* Arch Pathol Lab Med. 2000;124:71-81.

***Why the hell  
has an ANA test  
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for this patient?***

*Dagna L. et al., unpublished*

**When should  
an ANA test  
be ordered?**

# When is an ANA test useful?

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- **To help establishing a diagnosis in a patient with clinical features suggestive of an autoimmune or connective tissue disorder**
- **To exclude such disorders in patients with few or uncertain clinical findings**
- **To subclassify a patient with an established diagnosis of an autoimmune or connective tissue disease**

**To monitor disease activity**



# Conditions associated with positive IF-ANA test results

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- **Diseases for which an ANA test is very useful for diagnosis**

- SLE 95-100%
- systemic sclerosis (scleroderma) 60-90%

- **Diseases for which an ANA test is somewhat useful for diagnosis**

- Sjögren syndrome 40-70%
- idiopathic inflammatory myositis (dermato/polymyositis) 30-80%

- **Diseases for which an ANA test is useful for monitoring/prognosis**

- Juvenile chronic oligoarticular arthritis with uveitis 20-50%
- Raynaud phenomenon 20-60%

- **Diseases for which a positive ANA test is an intrinsic part of diagnostic criteria**

- drug-induced SLE ≈ 100%
  - MCTD ≈ 100%
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# Conditions associated with positive IF-ANA test results

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- **Diseases for which an ANA test is not useful in diagnosis**
  - *rheumatoid arthritis* 30-50%
  - *multiple sclerosis* ≈ 25%
  - *idiopathic thrombocytopenic purpura* 10-30%
  - *thyroid disease* 30-50%
  - *discoid lupus* 5-25%
  - *infectious diseases* wide variations
  - *malignancies* wide variations
  - *patients with silicone breast implants* 15-25%
  - *fibromyalgia* 15-25%
  - *healthy relatives of pts with SLE o scleroderma* 5-25%
- **Normal young persons\***
  - *ANA ≥ 1 : 40* 20-30%
  - *ANA ≥ 1 : 80* 10-12%
  - *ANA ≥ 1 : 160* ≈ 5%
  - *ANA ≥ 1 : 320* ≈ 3%

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\* Frequency increases with female sex and increasing age

# Conditions associated with positive IF-ANA test results

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- **Infections classically associated with a positive ANA test**

- *infectious mononucleosis*
- *HCV infection*
- *subacute bacterial endocarditis*
- *tuberculosis*
- *HIV infection*

- **Drugs**

- *procainamide*
  - *hydralazine*
  - *minocycline*
  - *diltiazem*
  - *penicillamine*
  - *isoniazid*
  - *TNF- $\alpha$  blockers*
  - *IFN- $\alpha$*
  - *anticonvulsants (phenytoin)*
  - *quinidine*
  - *anti-thyroid drugs*
  - *rifampin*
  - *beta blockers*
  - *lithium*
-

***“No test for ANA and for specific autoantibodies to nuclear antigens should be performed without a clinical evaluation that leads to a presumptive diagnosis”***

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# Systemic lupus erythematosus

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**Sensitivity: 95-100%**

**PPV general population: 11-13%**

**Acceptable specificity and positive predictive value**

**ONLY IF**

**there is a reasonable *pre test* clinical suspicion of SLE**

**→ *should not be used for screening for SLE***

# Systemic sclerosis (scleroderma)

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**Sensitivity: 60-90%**

- a positive ANA test supports the diagnosis in the presence of clinical signs and symptoms
- a negative ANA test should lead the physician to consider other fibrosing illnesses (linear/local scleroderma, eosinophilic fasciitis, scleredema)

# Sjögren syndrome

## Idiopathic inflammatory myopathies

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**Sensitivity: 40-70% (SS) / 30-80% (IIM)**

- a positive ANA test supports the diagnosis in the presence of the specific clinical signs and symptoms
- a negative ANA test does not rule out the diagnosis

# Pauciarticular juvenile chronic arthritis (PJCA)

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- ANA test is not useful for diagnosing PJCA
- 30-40% of patients with JCA and a positive ANA test will develop uveitis

**A positive ANA test in patients with PJCA may predict the development of uveitis**

→ Screen patients known to have PJCA for ANA

→ If positive, screen for uveitis

# Raynaud phenomenon (RP)

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- primary RP (81%): patients who will never develop a systemic rheumatic disease
- secondary RP (19%): patients who will develop a rheumatic disease (SLE, RA, SScI, ...)

## An ANA test in patients with RP

if positive, increases the likelihood of a secondary RP (19% → 30%)

if negative, reduces the likelihood of a secondary RP (19% → 7%)

# Drug-induced LE, MCTD

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**ANA positivity is required for the diagnosis**

*→ it is impossible to assess sens & spec*

- a **positive ANA test** supports the diagnosis in the presence of the specific clinical signs and symptoms
- a **negative ANA test** formally excludes the diagnosis

# What's the meaning of a positive ANA test? What to do next?

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- Brief history of ANA testing
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-

# ANA: FAQs

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***Q: If the ANA result is negative, should be the test repeated or should other tests be done (ENA) ?***

**A: No, if errors in testing are not suspected**

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# ANA: FAQs

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***Q: What other testing should be done following a positive ANA test result?***

**A: Different tests, according to the suspected diagnosis**

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# ANA: FAQs

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**Q: What other testing should be after a diagnosis of SLE is made?**

**To confirm the diagnosis:**

dsDNA spec 90-100% (↑↑ titers)  
sens 25-85%

Sm spec ≈ 100%  
sens 15-30%

RNP not indicated: spec low  
sens 30-40%

**For prognostic information:**

dsDNA high titers correlate with:

- flares of disease activity
- presence of lupus nephritis
- activity of lupus nephritis

Ro/SSA, La/SSB correlates with:

- neonatal lupus
- photosensitivity
- sicca syndrome
- thrombocytopenia
- subacute cutaneous LE rash

**Both issues:** aPL, anti- $\beta$  2-gp-I, LLAC

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# ANA: FAQs

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***Q: What other testing should be after a diagnosis of Sjögren syndrome is made?***

## **To confirm the diagnosis:**

**Ro/SSA** sens 50-90%  
spec low for differential

**La/SSB** almost only in pts w/ Ro/SSA  
spec low for differential

## **For prognostic information:**

**Ro/SSA, La/SSB** correlates with  
extraglandular disease:

- vasculitis
  - purpura
  - lymphadenopathy
  - leukocytopenia
  - thrombocytopenia
  - hypergammaglobulinemia
  - presence of rheumatoid factor
-

# ANA: FAQs

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***Q: What other testing should be after a diagnosis of DM/PM is made?***

***A: Patients with anti-aminoacyl-tRNA synthetases (Jo1)***

Abs more frequently have a specific syndrome (anti-synthetase syndrome) with:

- pulmonary involvement
  - arthritis (in some reports)
-

# ANA: FAQs

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***Q: What other testing should be after a diagnosis of systemic sclerosis is made?***

**Patients with limited SSc (CREST syndrome) more often have:**

**Centromeric ANA pattern**

**Anti-centromere Abs**  
(sens 60%, spec 95%)

**Patients with diffuse SSc more often have:**

**Anti topoisomerase- I (Scl-70) Abs**  
(sens 40%, spec 98%)

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# ANA: FAQs

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***Q: What other testing should be after a diagnosis of drug-induced SLE is made?***

***A:*** drug-induced LE is associated (90-100%) with the development of **anti-histone (H2A-H2B) Abs**. Those Abs are also present in:

- 80% of pts with idiopathic SLE
- RA, JCA, SSc, vasculitis, autoimmune hepatitis

→ insufficient data to support the use of anti-histone for differential diagnosis of drug-induced SLE

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# ANA: FAQs

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***Q: What other testing should be after a diagnosis of MCTD is made?***

***A:*** Patients with **MCTD** characteristically have high titers of **anti-RNP Abs**. Anti-RNP Abs have been used to categorize those patients.

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# ANA: FAQs

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**Q: What tests should be performed in young women with symmetric arthralgias?**

**A: 1) arthralgias (not arthritis)  $\leq$  6 wks: nothing**

**Viruses frequently: HBV, HCV, rubella (also vaccine), parvo  
occasionally: EBV, HIV, mumps, HAV, coxsackie,  
echo, adeno, VZV, HSV, CMV**

**2) arthralgias  $\geq$  6 wks or arthritis:**

**→ further investigation warranted**

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# ANA: FAQs

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**Q: What tests should be performed in young women with symmetric arthralgias  $\geq$  6 wk or arthritis?**

**A: RF & ANA**

- if RF positive, consider RA also in the presence of a positive ANA
  - a positive ANA with negative RF **does not rule out RA**  
(7.5% of pts with RA are RF-/ANA+ [typically anti-histones])
  - If ANA strongly pos & RF neg/low titer:  
anti-ds-DNA, anti-Sm, anti-RNP, anti-centromere, anti-Scl-70,  
anti-Ro/SSA, La-SSB
  - consider autoimmune hepatitis, Hashimoto's thyroiditis,  
Lyme disease
-

# ANA: FAQs

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***Q: My patient has anti-phospholipid syndrome (APS). Should an ANA test be performed?***

**A:** - ANA test is not useful for diagnosing APS  
- 40-50% of patients with APS have ANA

**A positive ANA test in patients with APS increases the likelihood that APS is secondary to SLE**

# ANA: FAQs

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***Q: What other testing should be after in an ANA positive asymptomatic patient?***

**A: Nothing more.....**

**but.....**

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# Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus

Melissa R. Arbuckle, M.D., Ph.D., Micah T. McClain, Ph.D., Mark V. Rubertone, M.D., R. Hal Scofield, M.D., Gregory J. Dennis, M.D., Judith A. James, M.D., Ph.D. and John B. Harley, M.D., Ph.D.

N Engl J Med  
Volume 349;16:1526-1533  
October 16, 2003



The NEW ENGLAND  
JOURNAL of MEDICINE

## Detection of Autoantibodies before Diagnosis and before the Onset of Symptoms in 130 Patients with Systemic Lupus Erythematosus

**Table 1.** Detection of Autoantibodies before Diagnosis and before the Onset of Symptoms in 130 Patients with Systemic Lupus Erythematosus.\*

Autoantibody	Positive Test before Diagnosis	Time from First Detection to Diagnosis	Positive Test in First Serum Sample	Total Patients with Positive Test	Interval between Positive Test and Diagnosis	Positive Test before Onset of Symptoms†	Interval between Positive Test and Onset of Symptoms
	<i>no. (%)</i>	<i>yr</i>	<i>%</i>	<i>no. (%)</i>	<i>yr</i>	<i>no. (%)</i>	<i>yr</i>
Antinuclear antibodies	101 (78)	9.2	50	109 (84)	3.01±0.25	89 (77)	2.25±0.27
Anti-Ro antibodies	61 (47)	9.4	64	64 (49)	3.68±0.34	55 (48)	2.97±0.30
Anti-La antibodies	44 (34)	8.1	62	45 (35)	3.61±0.38	39 (34)	2.83±0.43
Antiphospholipid antibodies	24 (18)	7.6	67	27 (21)	2.94±0.50	19 (17)	2.29±0.56
Anti-double-stranded DNA antibodies	72 (55)	9.3	48	80 (62)	2.24±0.31	54 (47)	1.24±0.31
Anti-Sm antibodies	41 (32)	8.1	31	49 (38)	1.47±0.34	28 (24)	0.47±0.44
Anti-nuclear ribonucleo-protein antibodies	34 (26)	7.2	23	43 (33)	0.88±0.32	23 (20)	0.20±0.47

\* Plus-minus values are means ±SE.

† The percentages are based on data from the 115 patients who had serum samples available from before the onset of symptoms.

Arbuckle, M. et al. N Engl J Med 2003;349:1526-1533



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# Autoantibodies appear years before the onset of autoimmune diseases

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- **Systemic lupus erythematosus** (*dsDNA, SSA/SSB, Sm*)
  - **Scleroderma** (*centromere, Scl-70*)
  - **Sjögren syndrome** (*Ro/SSA, La/SSB*)
  - **Rheumatoid arthritis** (*CCP*)
  - **Autoimmune myositis** (*tRNA synthetases*)
  - **Primary biliary cirrhosis** (*mitochondria E2*)
  - **T1DM, autoimmune thyroiditides, celiac disease, pemphigus, multiple sclerosis, vitiligo, ...**
-

# Conditions associated with positive IF-ANA test results

## ● Diseases for which an ANA test is not useful in diagnosis

- rheumatoid arthritis	30-50%
- multiple sclerosis	≈ 25%
- idiopathic thrombocytopenic purpura	10-30%
- thyroid disease	30-50%
- discoid lupus	5-25%
- infectious diseases	wide variations
- malignancies	wide variations
- patients with silicone breast implants	15-25%
- fibromyalgia	15-25%
- healthy relatives of pts with SLE o scleroderma	5-25%

## ● Normal young persons\*

- ANA ≥ 1 : 40	20-30%
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- ANA ≥ 1 : 160	≈ 5%
- ANA ≥ 1 : 320	≈ 3%

**So...**

***what to do?***

# Pattern of ANA fluorescence

	Homo geneous	Speckled nuclear	Centromere	Nucleolar	Cyto plasmic
SLE	dsDNA nucleosomes Ku	Sm U1RNP SS-A/SS-B	rare	nucleolin ASE-1	P-proteins SS-56
SSc	Scl70 Ku	U1RNP U2RNP	CENP-A CENP-B CENP-C	PM/Scl	rare
PM/DM	Ku	U1RNP Mi-2 SS-A	rare	PM/Scl	Jo-1
Sjögren	rare	SS-A SS-B SL	rare	nucleolin	SS-56
MCTD	rare	U1RNP hnRNPs SS-A	rare	rare	rare

***Always consider  
history, physical and  
simple lab tests***

Patient with a significantly positive ANA test

evaluate

Skin  $\pm$  joint involvement

drug exposure

Raynaud, sclerodactyly, myositis, telangiectasis esophageal & lung involvement

Sicca symptoms

dsDNA, RNP, Sm, SSA/SSB, aPL

histones

ScI-70, PM/ScI centromere, RNP, Jo-1

SSA/SSB

SLE

drug induced LE

SSc

MCTD

DM/PM

Sjogren

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\* Frequency increases with female sex and increasing age

# Take home messages

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- No test for ANA should be performed without a clinical evaluation that leads to a presumptive diagnosis (SLE, SSc, Sjögren, PM/DM, JCOA, Raynaud, drug-induced SLE, autoimmune hepatic disease, MCTD)

ANA testing have an extremely low specificity and

- PPV in the general population.

ANA and ENA are different tests (ANA more sensitive,

- anti-ENA more specific)
-

# Take home messages (2)

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- Many diseases may cause ANA positivity; many healthy individuals have a positive ANA test.
  - Some ANA patterns (nucleolar, centromeric) can be more specific than others (diffuse, homogeneous, speckled).
  - Patient referred for a positive ANA should be evaluated considering for signs and symptoms of the above mentioned disease. If those are absent, no further investigations may be warranted.
-