Cleveland Clinic

# The Power of the ANA

April 2018

Emily Littlejohn, DO MPH

# Question 1:
# the ANA test is:

A) A screening test with high specificity to diagnose an autoimmune disease
B) A screening test that can help determine the etiology of arthralgia
C) A test to be ordered in the setting of high clinical suspicion for an autoimmune disease
D) A diagnostic test for lupus

# Question 1:
# the ANA test is:

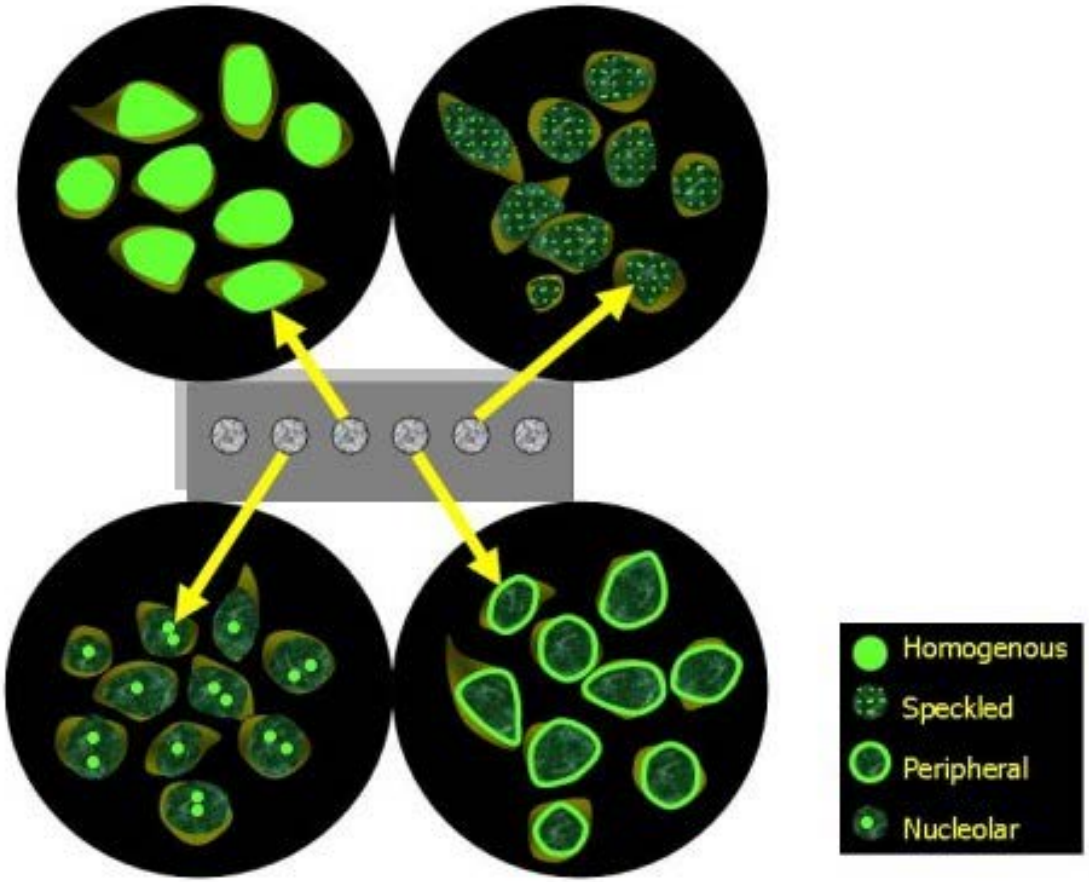
A) A screening test with high specificity to diagnose an autoimmune disease
B) A screening test that can help determine the etiology of arthralgia
**C) A test to be ordered in the setting of high clinical suspicion for an autoimmune disease**
D) A diagnostic test for lupus

# What is an ANA?

# Anti nuclear antibodies

- ANA are a specific class of autoantibodies that have the capability of binding certain structures within the nucleus of the cells

- Anti-dsDNA
- Anti-U1RNP
- Anti-ribosomal P
- Anti- RNA polymerase III
- Anti-Smith
- Anti-centromere
- Anti-histone
- Anti-SSA/Ro
- Anti Scl-70
- Anti-U3 RNP
- Anti-SSB/La
- Anti-Jo-1
- Anti-PM-Scl


Homogenous
Speckled
Peripheral
Nucleolar

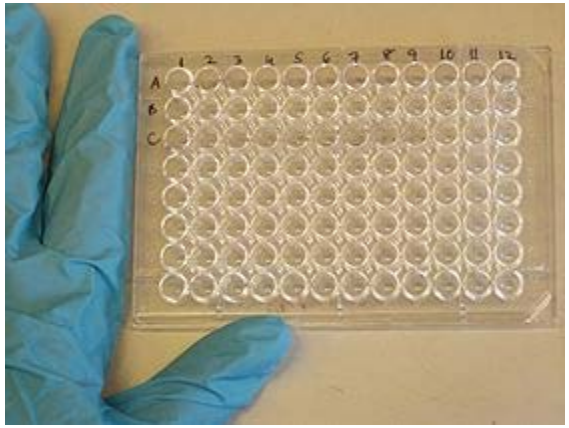
Common IF-ANA patterns associated with specific diseases

| ANA pattern | Antigen | Associated diseases |
|---|---|---|
| Speckled | ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1, ribosomal-P | SLE, Mixed CTD, SS, Primary Sjogren's syndrome, PM |
| Homogenous | dsDNA, Histones | SLE, Drug induced SLE |
| Peripheral (rim) | RNP, Sm, SSA/Ro | SLE, SS |
| Nucleolar | Anti-PM-Scl, anti-RNA polymerase I-III, anti-U3-RNP, To RNP | SS, PM |
| Centromere | CENP A-E | Limited SS |

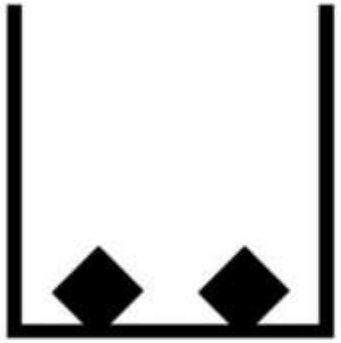
# ANA Testing

# ANA by Enzyme Immunoassay (EIA) testing

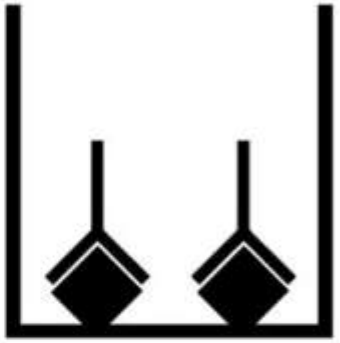
- Multiple antigens are coated on to microtitre plates; ***usually*** dsDNA, SSA/Ro, SSA/La, histone, Jo-1, Sm, Scl-70, U1-RNP
- Incubated with patient's serum. Enzyme labeled antibody added.
- The optical density of the substrate reaction is graded against a cutoff that is specific to each assay system to give a positive/negative result.
- The concentration and specific preparation of each antigen is not standardized (determined by manufacturer.)
- Can eliminate non-specific false positives that can provide confusing results.
- Good screening test?



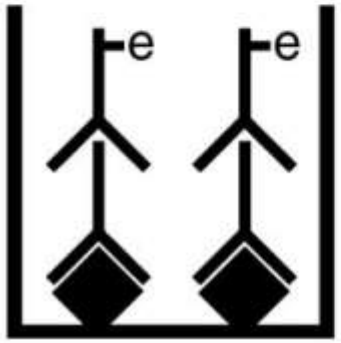
Step 1
Specific antigen is attached to a solid-phase surface



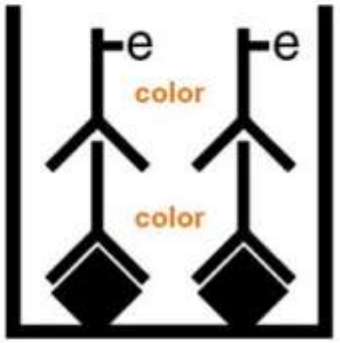
Step 2
Test specimen is added, which may or may not contain the antibody



Step 3
An enzyme-labeled antibody specific to the test antibody is added (conjugate)




Step 4
Chromogenic substrate is added, which in the presence of the enzyme, changes color.

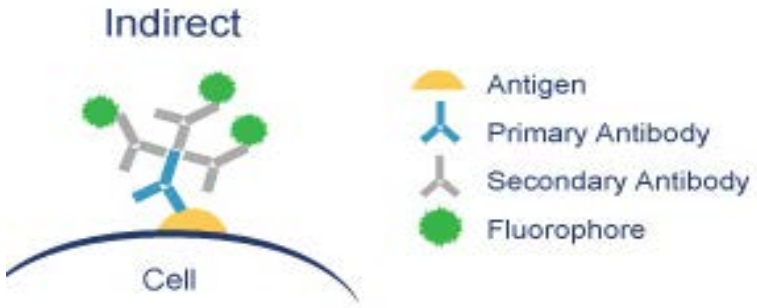


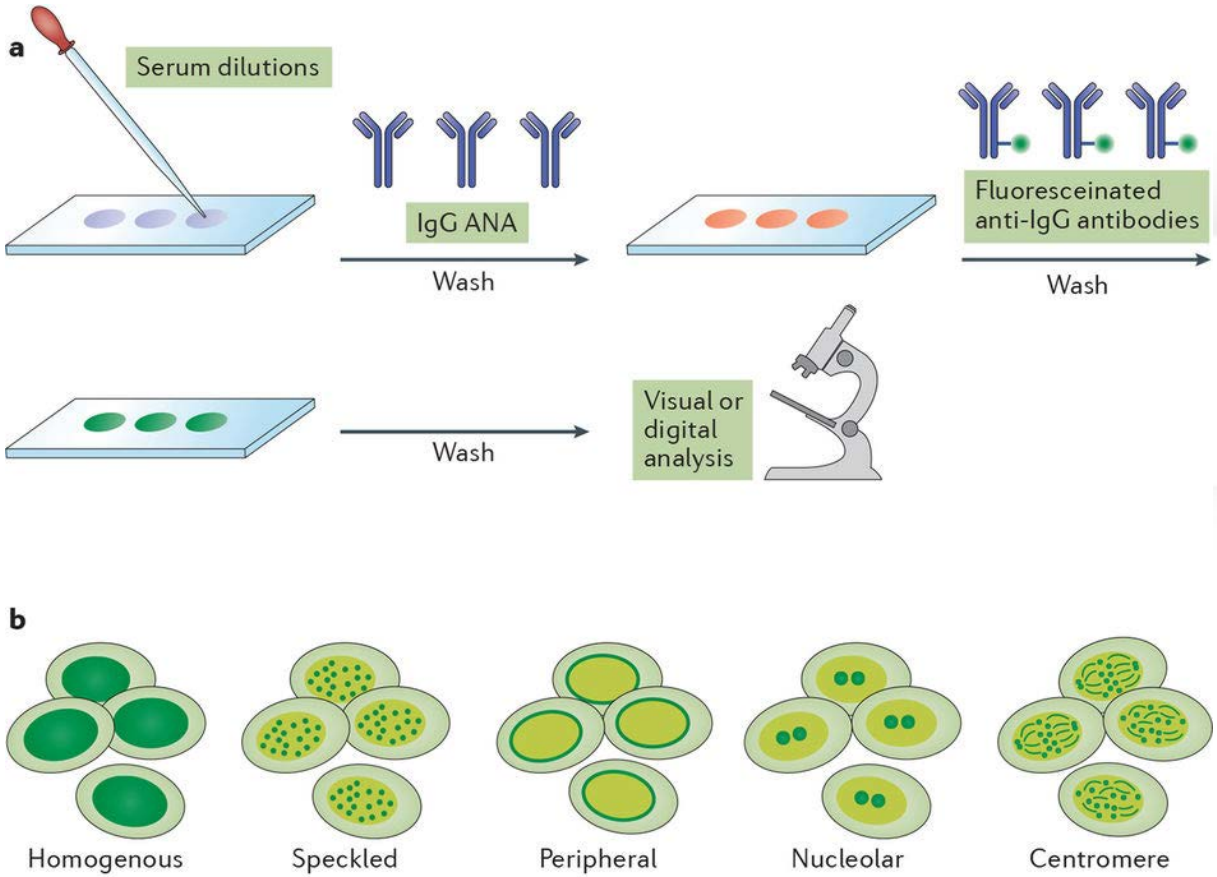
# ANA by Enzyme Immunoassay (EIA) testing

- Issues:
  - The concentration and specific preparation of each antigen is not standardized (determined by manufacturer.)
  - Can eliminate non-specific false positives that can provide confusing results.
  - Good screening test?

# ANA by immunofluorescence (ANA by IFA)

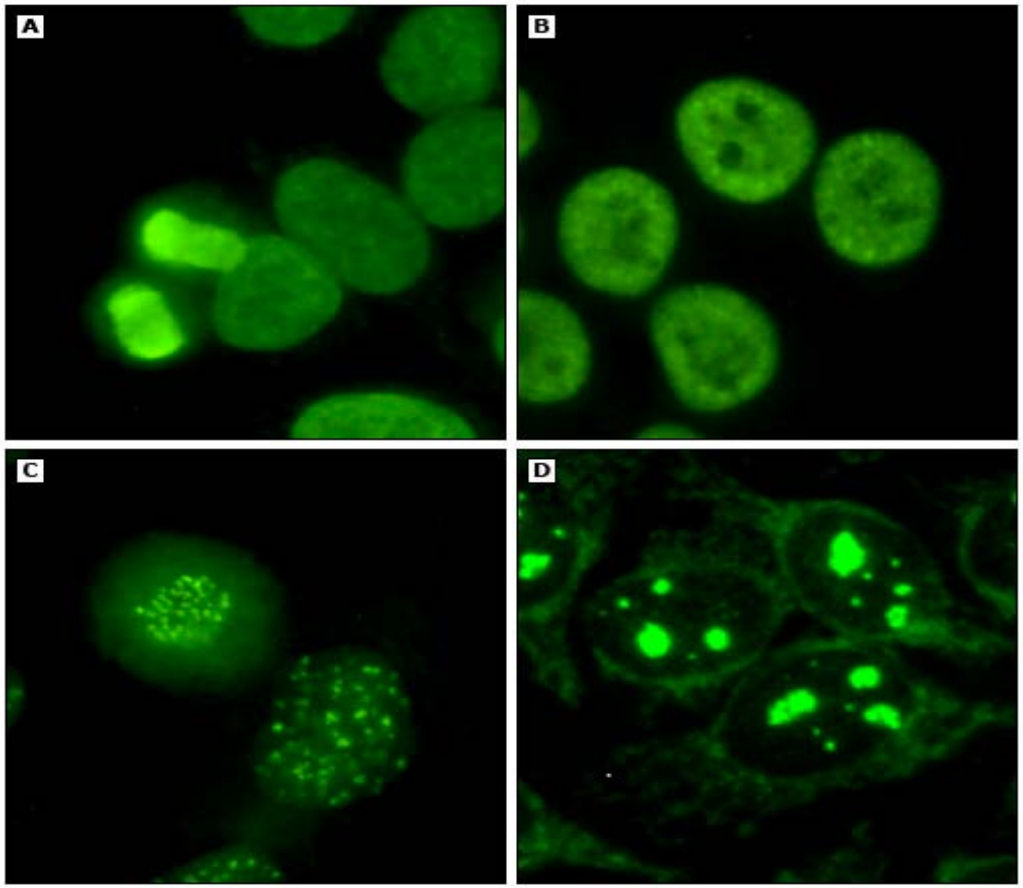
- Slides prepared from human epithelioid cells (HEp-2 cells) as a substrate are incubated with diluted serum.
- The presence of autoantibodies is detected by fluorescent anti-immunoglobulin antibody, and characteristic morphologic patterns of fluorescent staining are observed
- Detects a large number of autoantibodies




a
Serum dilutions
IgG ANA
Wash
Fluoresceinated anti-IgG antibodies
Wash
Wash
Visual or digital analysis
b
Homogenous
Speckled
Peripheral
Nucleolar
Centromere

# ANA by IFA
# Indirect immunofluorescence

- Human epithelial type **2** (**HEp**-**2**) **cells**, considered to originate from a human laryngeal carcinoma
- Hep -2 cells have a large nuclei and divide rapidly
- Allow recognition of over 30 different nuclear and cytoplasmic patterns that are given by upwards of 50 different autoantibodies are associated with various autoimmune conditions.




(A) In the homogeneous pattern, the entire nucleus is diffusely stained.
(B) In the speckled pattern, very small, uniform, fluorescent dots are seen throughout the nucleus.
(C) The centromere pattern is characterized by the presence of 30 to 60 dots distributed throughout the nucleus in resting cells.
(D) The nucleolar staining pattern

# ANA Testing
# EIA versus IFA

- Because of its high negative predictive value, EIA can be used reliably to detect ANA-negative samples; however, the low positive predictive value indicates that EIA-positive specimens should be retested by an IFA to determine the final result.

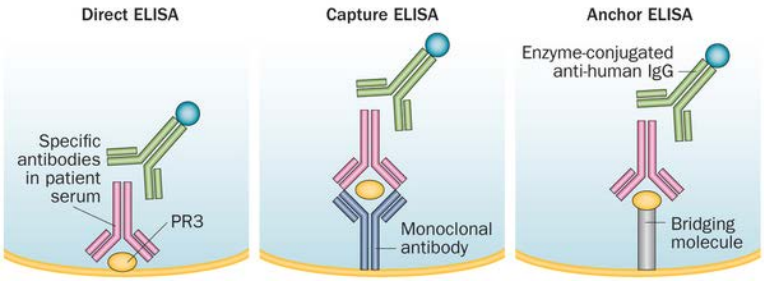
Comparison of ANA-EIA with ANA-IFA

| ANA-EIA Result | ANA-IFA ≥1:160 | ANA-IFA <1:160 |
|---|---|---|
| Positive | 52 | 91 |
| Negative | 3 | 561 |
| Borderline | 7 | 94 |

*Data from* Reisner BS, D. Blasi J, Goel N. Comparison on an enzyme immunoassay to an indirect fluorescent immunoassay for the detection of antinuclear antibodies. Am J Clin Pathol 1999;111:503–6.

# Extractable Nuclear Antigen (ENA) panel

**Solid phase assays**:
ELISA: uses antibodies and color change to identify a substance.

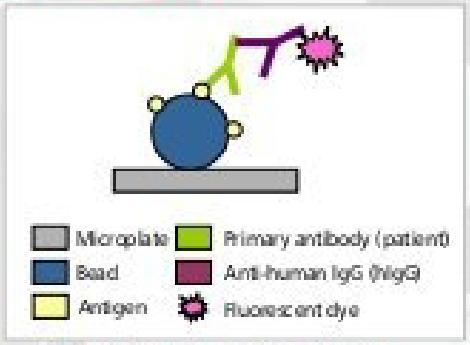



- **Less sensitivity as it uses a limited number of antigens**

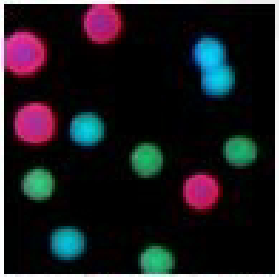
# ENA panel

**attomol® ANA-Bead Assay**
Multiplex Detection on *BioResponse*
Technology

## Technology




Indirect immunobead assay



Fluorescent bead populations

Different extractable nuclear antigens (ENA) were immobilised each on an individually fluorescence encoded bead population. Coated beads were then fixed onto the bottom of the cavities of 8-well ELISA strip and used in immunobead assay. Processing in routine labs is possible using ELISA automation. Using FI 100 Fluores-cence Imaging Analyser, 96 Bead Assays (10-plex) can be measured in less than 1h.

Attomol GmbH
Schulweg 6, OT Lüpten
03205 Bronkow
Germany

tel: +49 35329 5806-0
fax: +49 35329 5806-19
e-mail: info@attomol.de
web: www.attomol.de

# What to make of the positive ANA test?

# Is ANA positivity specific for SLE or an autoimmune disease?

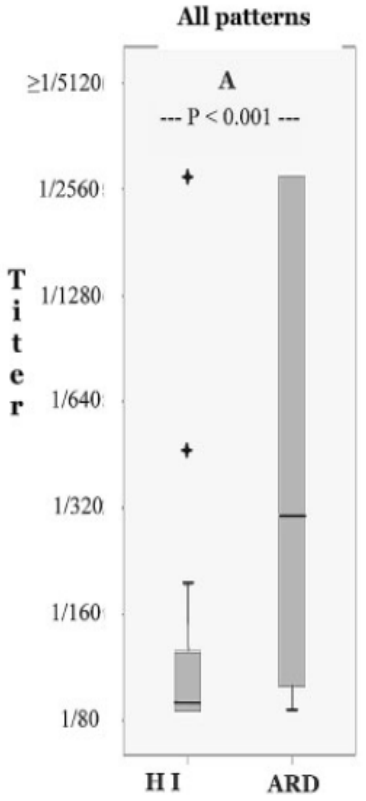
- Answer NO
  - By IFA, cutoff 1:80, **15-18%** of U.S. population is ANA positive
    - Women are more likely to be ANA positive than men
    - ANA increasing with age (> 50)
  - By EIA, 25% healthy controls are ANA positive
  - Prevalence of SLE in US is 0.1%, and autoimmune diseases 5-7%
  - **The usefulness of ANA testing depends on the pretest probability the patient has an ANA associated disorder ; if a test is ordered indiscriminately, looking for a disease with a 1% prevalence in population, 5% of patients will have a positive test**

Arbuckle M. Development of autoantibodies before the clinical osent of SLE. NEJM 2003349:1526-33;Li QZ Risk factors for ANA positivity in healthy persons. Arthr Res Ther 2011:13: R38; Slight-Webb S. Autoantibody Positive healthy individuals display unique immune profiles that may regulate autoimmunity. Arthritis and Rheum 2016 : Abeles AM The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126:342-8: Selmi C. Serum antinuclear and extractable nuclear antigen antibody prevalence over 15 years. Autoimmunity Rev, 2016; 15:162-166

# Is the titer of ANA significant and of diagnostic value?

- ANSWER:
  - The likelihood of autoimmune disease increases with increased ANA titer, but high titers can be seen in healthy controls
  - 918 healthy controls and 153 pts with autoimmune rheumatic diseases

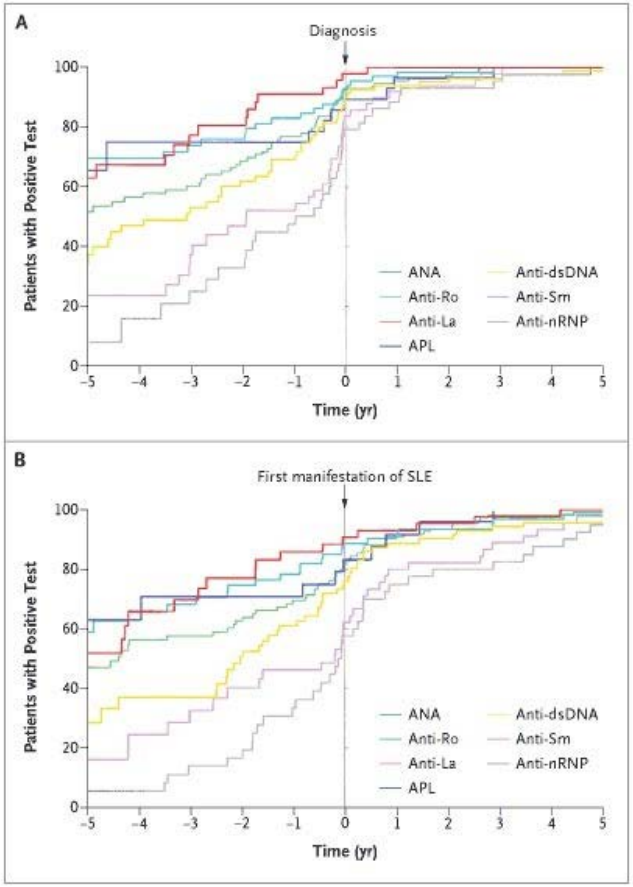
| | All patterns | | |
|---|---|---|---|
| Titer | Healthy individuals (n = 118) | Patients with ARDs (n = 138) | *P*† |
| 1:80 | 54 (45.8) | 10 (7.2) | <0.001 |
| 1:160 | 9 (7.6) | 5 (3.6) | 0.259 |
| 1:320 | [illegible] | [illegible] | 0.477 |
| [illegible] | 21 (17.8) | 0 (0) | [illegible] |
| 1:1,280 | 9 (7.6) | 31 (22.5) | 0.002 |
| 1:2,560 | 3 (2.5) | 1 (0.7) | 0.507 |
| ≥1:5,120 | 7 (5.9) | 68 (49.3) | <0.001 |




Mariz HA. Pattern of antinuclear antibody-Hep 2 test is ca critical parameter for discriminating antinuclear antibody positive healthy individuals and patients with autoimmune diseases. Arhtritis and Rheum 2011;63:191-200

# Is the titer of ANA significant and of diagnostic value?

- ANA positivity precedes the onset of systemic lupus erythematosus




Arbuckle M. Development of autoantibodies before the clinical onset of SLE. NEJM 2003349:1526-33

# Is the ANA pattern significant and of diagnostic value?

- Answer: Yes, some ANA patterns are more likely to be seen in patients with AI
- Dense, fine speckled nuclear pattern not autoimmune associated

**Table 2.** Distribution of 118 ANA-positive healthy individuals and 138 ANA-positive patients with ARDs according to the pattern on the ANA–HEp-2 test*

| Pattern on ANA–HEp-2 test | ANA-positive healthy individuals† | ANA-positive patients with ARDs† | *P*‡ |
|---|---|---|---|
| Nuclear fine speckled | 54 (45.8) | 58 (42.0) | 0.636 |
| Nuclear dense fine speckled | 39 (33.1) | 0 (0) | <0.001 |
| Nuclear coarse speckled | 0 (0) | 36 (26.1) | <0.001 |
| Nuclear homogeneous | 0 (0) | 10 (7.2) | 0.008 |
| Nuclear centromeric | 0 (0) | 11 (8.0) | 0.005 |
| Nuclear quasihomogeneous speckled | 5 (4.2) | 19 (13.8) | 0.017 |
| Nucleolar | 8 (6.8) | 18 (13.0) | 0.148 |
| Cytoplasmic | 12 (10.2)§ | 5 (3.6)¶ | 0.065 |
| Other | 8 (6.8) | 7 (5.1) | 0.604 |

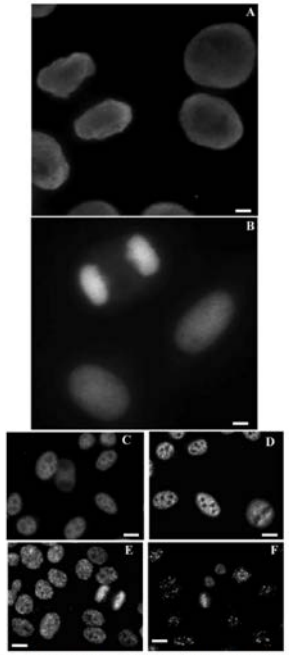



Figure 2. Morphologic characteristics of the most relevant nuclear patterns found on indirect immunofluorescence assay on HEp-2 cells, performed on samples from healthy individuals and patients with autoimmune rheumatic diseases. Patterns are as follows: A, homogeneous; B, quasihomogeneous; C, nuclear fine speckled; D, nuclear coarse speckled; E, nuclear dense fine speckled; and F, nuclear centromeric. Sera were diluted 1:80. Bars = 5 μm in A and B; 13 μm in C–F.

Mariz HA. Pattern of antinuclear antibody-Hep 2 test is ca critical parameter for discriminating antinuclear antibody positive healthy individuals and patients with autoimmune diseases. Arhtritis and Rheum 2011;63:191-200

# Utility of ANA testing outside of the rheumatology setting

- Analysis of 232 patients referred for a positive ANA between 2007-2009
- No ANA associated rheumatic disease was found for ANA titers <1:160
- The most common reason for ordering and ANA was widespread pain
- **CONCLUSION: >90% of positive ANA referrals to rheumatology had NO evidence for ANA associated rheumatic disease**

**CLINICAL SIGNIFICANCE**

- A positive antinuclear antibody (ANA) test result was rarely clinically meaningful.
- The positive predictive value of an ANA test was less than 10% for any connective tissue disease and less than 3% for lupus.
- Most patients were sent for ANA testing despite low pretest likelihood of connective tissue disease.
- ANA testing often was ordered indiscriminately, which can lead to adverse consequences.

Abeles AM The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126:342-8:

# ANA takeaway

- The usefulness of ANA depends on the pretest probability the patient has an ANA associated disorder
- Having high clinical suspicion for an autoimmune disease should prompt ANA testing and referral to Rheumatology

# What is clinical suspicion?

# Question 2:
# Which scenario clinically suggests a ANA disease?

A) 60 yo male with new onset focal weakness, weight loss and fatigue
B) 30 yo female with recurrent late miscarriages and photosensitivity
C) 45 yo female with symmetric small joint pain and swelling, family history of rheumatoid arthritis
D) 55 yo female with dry eyes, dry mouth and lymphadenopathy
E) All of the above

# Which scenario is clinically suspicious?

A) 60 yo male with new onset focal weakness, weight loss and fatigue
B) 30 yo female with recurrent late miscarriages and photosensitivity
C) 45 yo female with symmetric small joint pain and swelling, family history of rheumatoid arthritis
D) 55 yo female with dry eyes, dry mouth and lymphadenopathy
**E) All of the above**

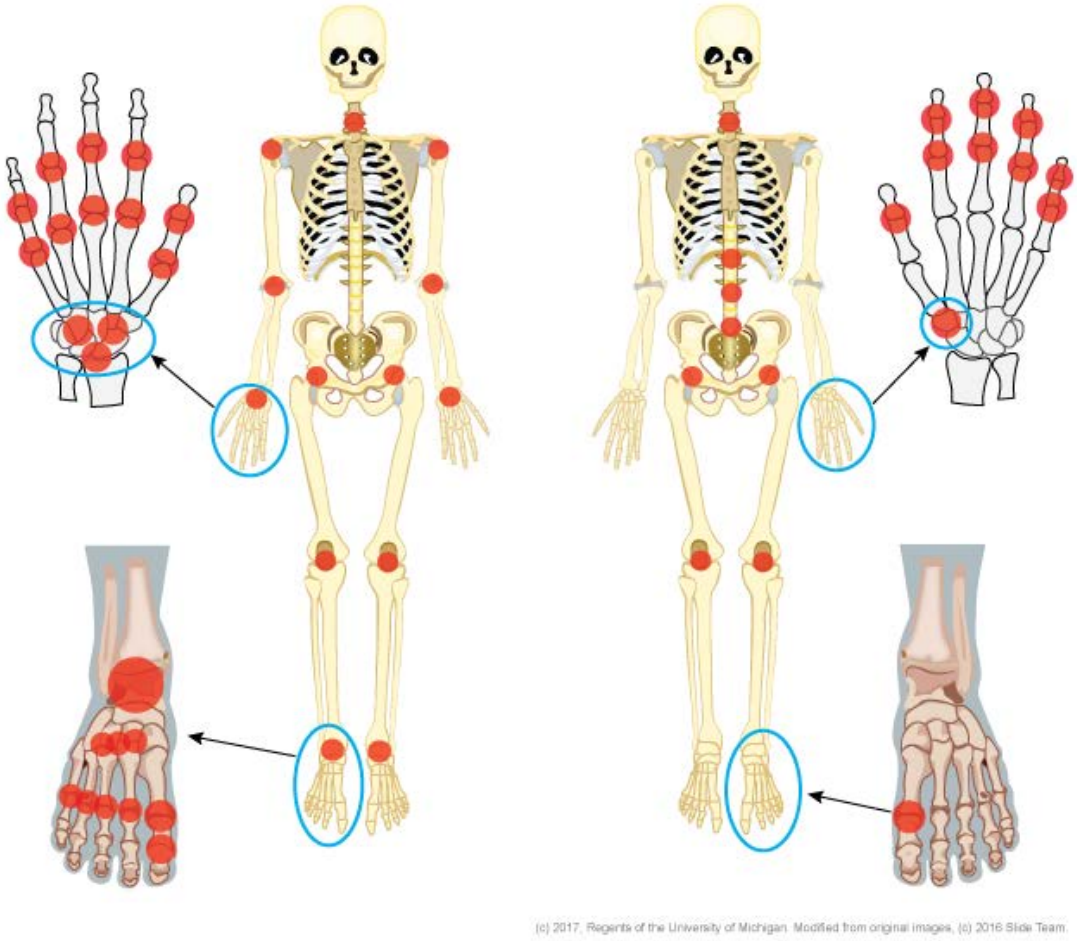
# Inflammatory arthritis

- Presence of heat, swelling, pain
- Worse in the morning, with morning stiffness > 30 minutes
- “Boggy” sensation on physical exam
- Responsive to prednisone

# Osteoarthritis

- No heat, minimal swelling
- Worse with exertion or activity
- Presence of Heberden’s or Bouchard’s nodes
- Less responsive prednisone **

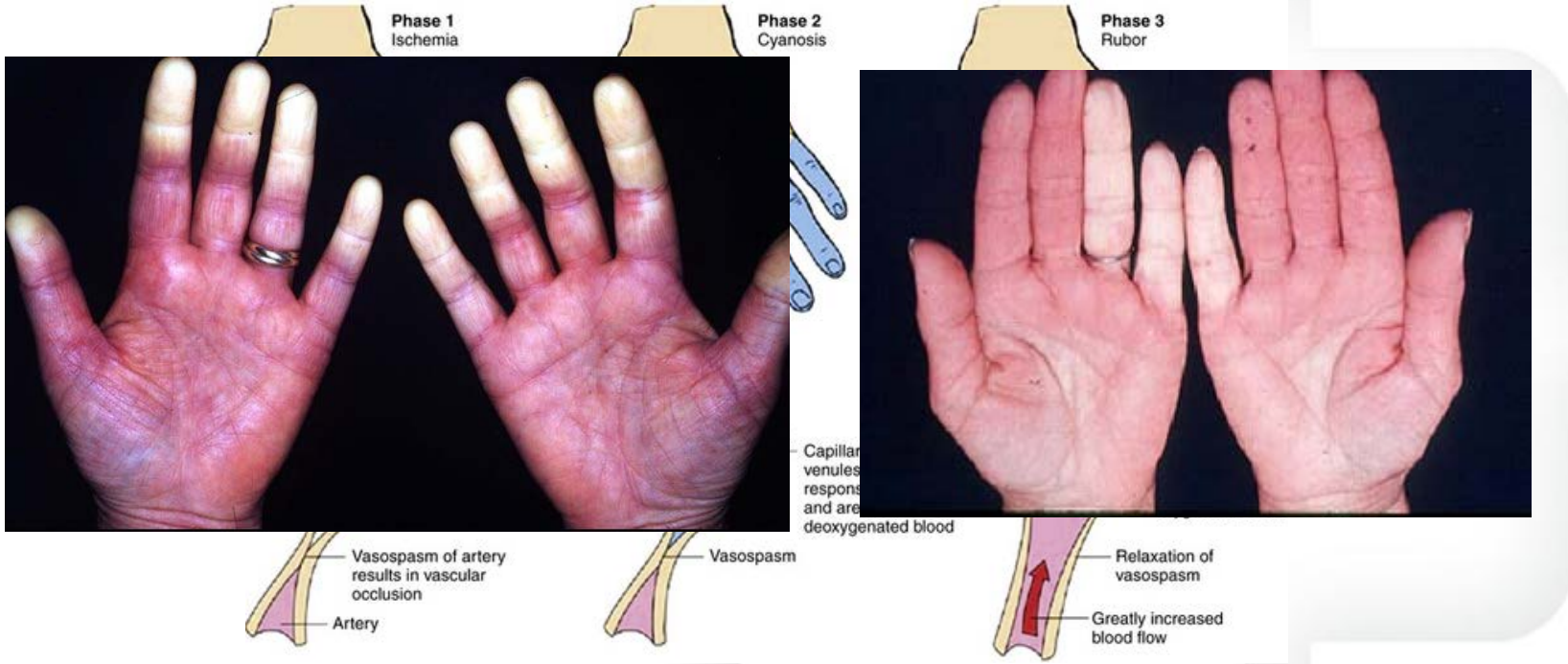
# Inflammatory arthritis versus osteoarthritis



(c) 2017, Regents of the University of Michigan. Modified from original images, (c) 2016 Slide Team.

# Raynaud's phenomenon

- **Tri-phasic**
- **Capillary abnormalities**
- **Can be fingers, toes, nipples**



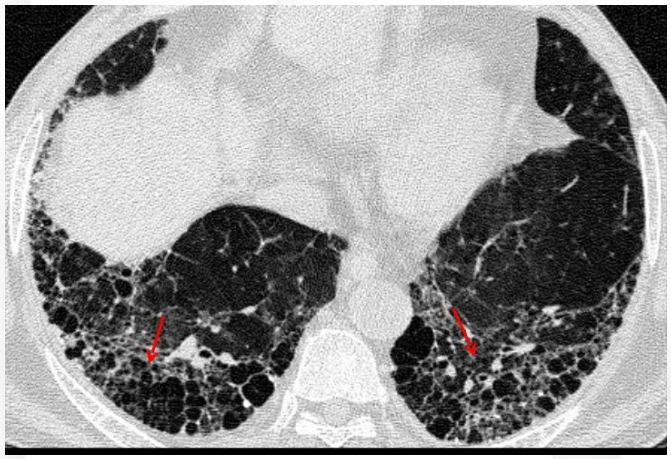
# Inflammatory rash

- **Inflammatory process**
- **Take hours days to develop and remit**
- **Raised, palpable**
- **Associated with systemic symptoms; fatigue, other “flare” symptoms**

# Hair loss

# Respiratory symptoms/Lung disease

- Shortness of breath with suspicion for elevated PAP
  - Elevated RVSP, decreased DLCO
- Pleuritis
- Interstitial lung disease; restrictive disease
  - NSIP
  - UIP
  - LIP



# Other clinical sx/sx

- **Hematuria/proteinuria, elevated blood pressures NOS**
- **Oral, nasal ulcers**
- **Extreme dry eye, extreme dry mouth**
- **Photosensitivity**
  - **Extreme fatigue**
  - **Precipitation of other symptoms with prolonged UV exposure**
- **Recurrent miscarriages, after first trimester**
- **History of blood clots**
- **Focal muscle weakness**
- **Dysphagia**
- **Pericarditis, pericardial effusion**
- **Skin tightening/thickening, mechanic's hands**

# Mechanic’s hands

# Question 3:
# Which of the following is true?

A) Headaches are a manifestation of neurologic lupus.
B) Chronic fatigue is a symptom of lupus.
C) Lupus causes widespread pain.
D) All of the above.
E) None of the above.

# Question 3:
# Which of the following is true?

A) Headaches are a manifestation of neurologic lupus.
B) Chronic fatigue is a symptom of lupus.
C) Lupus causes widespread pain.
D) All of the above.
**E) None of the above.**

# Question 3 explanations:

- Migraines are just as common in lupus patients as the general population. And although certain headaches can be an indication of lupus cerebritis, far more commonly they are migraines.

- SLE flares can cause transient fatigue. But chronic fatigue in an inactive lupus state is more likely fibromyalgia.
- Pure fatigue without any other signs/symptoms of lupus is almost NEVER lupus. Much more likely this is due to fibromyalgia.

- Same for widespread pain. Lupus never causes generalized widespread pain.
- Up to10-30% of SLE patients will also have fibromyalgia.

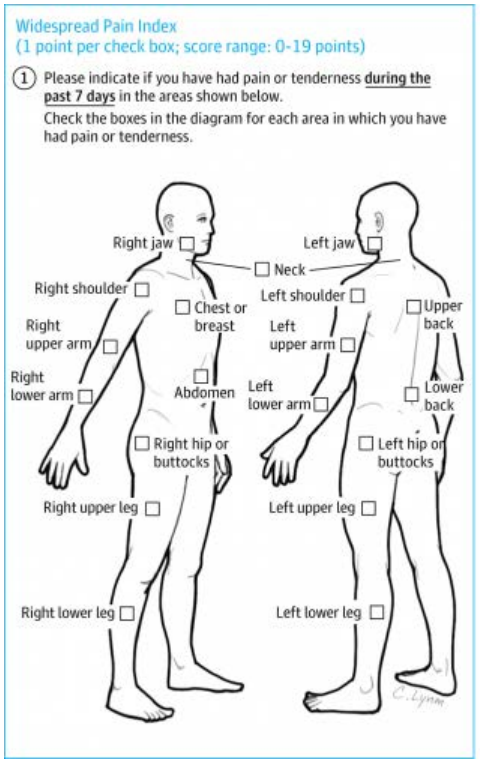
# Fibromyalgia

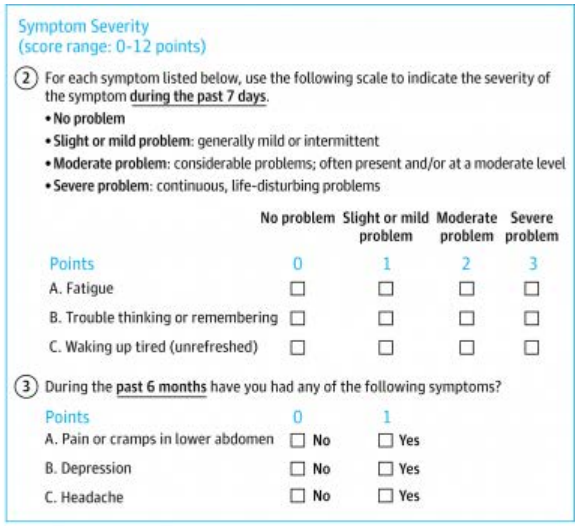
- We know that fibromyalgia is more common in autoimmune diseases (SLE and PsA studies)

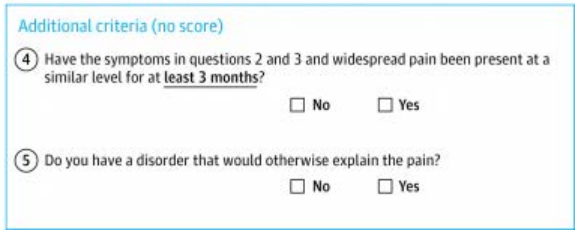
- Central pain syndrome= hypersensitive nerves
  - **Bright lights**
  - **Loud noises**
  - **Widespread pain**

Fan, A et al. Frequency of concomitant fibromyalgia in rheumatic diseases: Monocentric study of 691 patients. Semin Arthritis Rheum. 2017 Aug;47(1):129-132. doi: 10.1016/j.semarthrit.2017.01.005. Epub 2017 Jan 18. Magrey MN et al. High frequency of fibromyalgia in patients with psoriatic arthritis: a pilot study. Arthritis. 2013;2013:762921. doi: 10.1155/2013/762921. Epub 2013 Feb 14.

# ACR 2011 FMS criteria




Widespread pain index: 17
Symptoms severity: 12

Fibromyalgianess scale 29

Fibromyalgianess scale: WPI + SS (0-31, 13 FM cutoff)

# 2016 Criteria

**Table 3**
Fibromyalgia criteria—2016 revision

***Criteria***

A patient satisfies modified 2016 fibromyalgia criteria if the following 3 conditions are met:
(1) Widespread pain index (WPI) ≥ 7 and symptom severity scale (SSS) score ≥ 5 OR WPI of 4–6 and SSS score ≥ 9.
(2) Generalized pain, defined as pain in at least 4 of 5 regions, must be present. Jaw, chest, and abdominal pain are not included in generalized pain definition.
(3) Symptoms have been generally present for at least 3 months.
(4) A diagnosis of fibromyalgia is valid irrespective of other diagnoses. A diagnosis of fibromyalgia does not exclude the presence of other clinically important illnesses.

***Ascertainment***

**(1) WPI**: note the number of areas in which the patient has had pain over the last week. In how many areas has the patient had pain? Score will be between 0 and 19

| *Left upper region (Region 1)* | *Right upper region (Region 2)* | *Axial region (Region 5)* |
|---|---|---|
| Jaw, left[a] | Jaw, right[a] | Neck |
| Shoulder girdle, left | Shoulder girdle, right | Upper back |
| Upper arm, left | Upper arm, right | Lower back |
| Lower arm, left | Lower arm, right | Chest[a] |
| | | Abdomen[a] |
| *Left lower region (region 3)* | *Right lower region (Region 4)* | |
| Hip (buttock, trochanter), left | Hip (buttock, trochanter), right | |
| Upper leg, left | Upper leg, right | |
| Lower leg, left | Lower leg, right | |

**(2) Symptom severity scale (SSS) score**
Fatigue
Waking unrefreshed
Cognitive symptoms
For the each of the 3 symptoms above, indicate the level of severity over the past week using the following scale:
0 = No problem
1 = Slight or mild problems, generally mild or intermittent
2 = Moderate, considerable problems, often present and/or at a moderate level
3 = Severe: pervasive, continuous, life-disturbing problems
**The symptom severity scale (SSS) score:** is the sum of the severity scores of the 3 symptoms (fatigue, waking unrefreshed, and cognitive symptoms) (0–9) plus the sum (0–3) of the number of the following symptoms the patient has been bothered by that occurred during the previous 6 months:
(1) Headaches (0–1)
(2) Pain or cramps in lower abdomen (0–1)
(3) And depression (0–1)

The final symptom severity score is between 0 and 12
**The fibromyalgia severity (FS) scale** is the sum of the WPI and SSS

The FS scale is also known as the polysymptomatic distress (PSD) scale.

[a] Not included in generalized pain definition.

# ANA takeaway

- The usefulness of ANA depends on the pretest probability the patient has an ANA associated disorder
- Having high clinical suspicion for an autoimmune disease should prompt ANA testing and referral to Rheumatology

# Questions?

# Cleveland Clinic

Every life deserves world class care.