Oh no, the ANA is positive!!
How to use autoantibody test results with sophistication

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Outline

- Brief overview of most *common autoantibody tests* used for rheumatic diseases
- Discussion of use of autoantibodies for the *diagnosis* of rheumatic diseases
- Discussion of use of autoantibodies for the *prognosis* following diagnosis of rheumatic diseases

Autoantibody tests: Alphabet soup of tests

Some associate with systemic autoimmune diseases

- Systemic lupus erythematosus (SLE): ANA, anti-dsDNA, anti-Sm
- Rheumatoid arthritis (RA): RF, anti-CCP
- Antiphospholipid syndrome (APLS): Anti-GpI, Anti-cardiolipin
- ANCA-associated vasculitis: ANCA confirmed by ELISA for PR3 or MPO
- Sjögren's: Ro (SS-A), La (SS-B)
- Scleroderma: Anti-Scl70, Anti-RNA pol III
- Mixed connective tissue disease (MCTD): Anti-RNP
- Myopathies: *Way* too many to list….

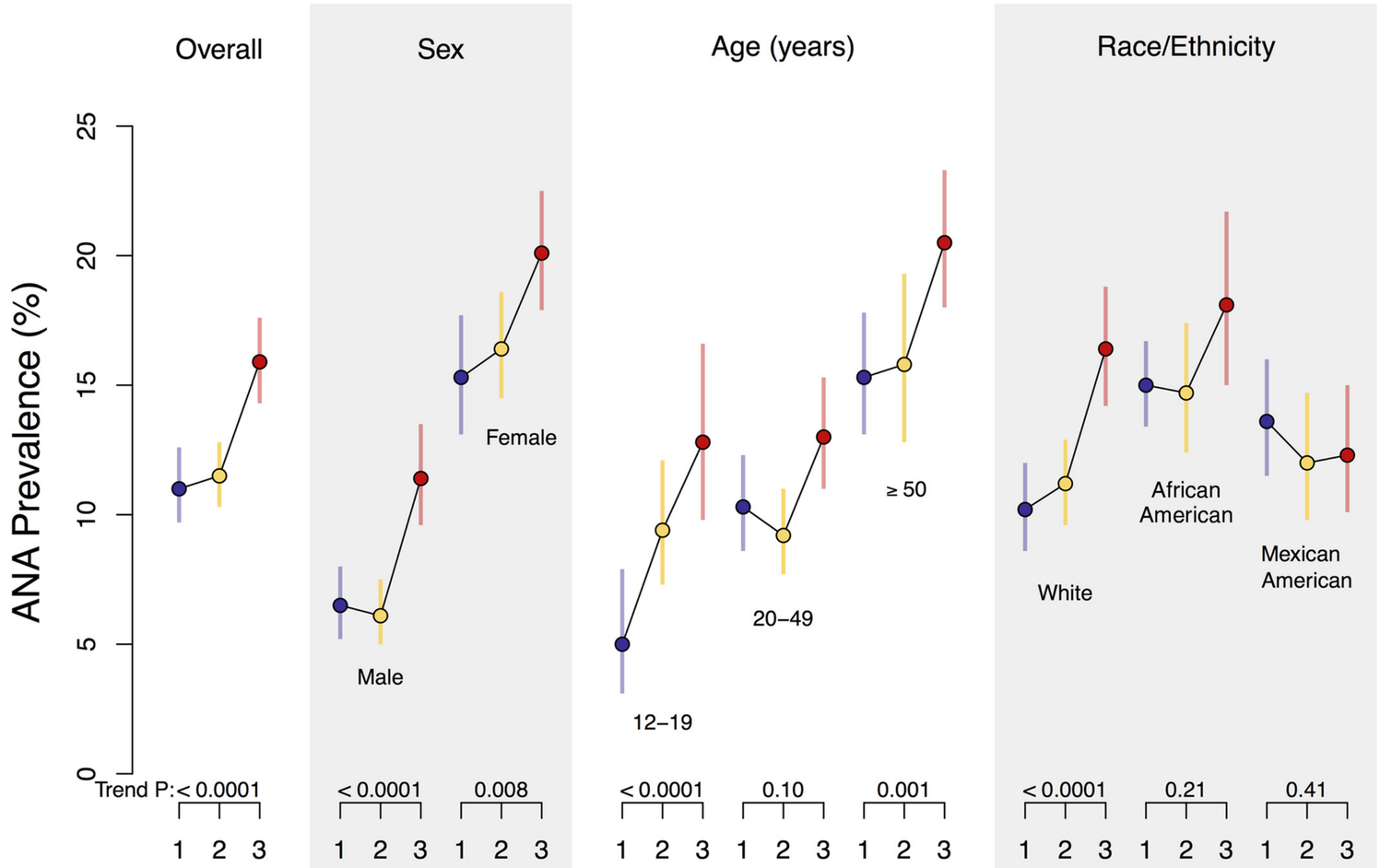
ANA: Antinuclear antibody
dsDNA: Double-stranded DNA
Sm: Smith
RF: Rheumatoid factor
CCP: Cyclic citrullinated peptide
GpI: Glycoprotein-1
ANCA: Anti-neutrophilic cytoplasmic antigen
ELISA: Enzyme-linked immunosorbent assay
PR3: Proteinase 3
MPO: Myeloperoxidase
SS-A/B: Sjögren's syndrome A/B
Scl70: Scleroderma 70 (topoisomerase 1)
RNA pol III: RNA polymerase 3
RNP: Ribonucleoprotein

Take-home lesson

- Unfortunately, autoantibody labs for rheumatic disease still do NOT inclusively diagnose our diseases
- Rather, signs and symptoms collectively form a subjective “pre-test probability”
- Test results, in turn, a post-test probability
- We never use the test to dictate what disease to think about
- Rheumatology is very similar to psych, except in psych, they don’t have tests

ANA prevalence is increasing

41 million positive ANA results in 2011-2 = ~16% of US population



1: 1998-1991
2: 1999-2004
3: 2011-2012

Dinse *et al*, *Arthritis Rheumatol*, 2020, PMID: 32266792

ANA testing: “no better than a vital sign”

Only 1 of every 10 positive ANAs have SLE; similar for RF and RA

Antinuclear Antibody Testing

A Study of Clinical Utility

Cindi A. Slater, MD; Roger B. Davis, ScD; Robert H. Shmerling, MD

Arch Intern Med. 1996;156:1421-1425

	(+) ARD	(-) ARD
(+) ANA	33	120
(-) ANA	22	804

ARD=Any Rheumatic Disease
ANA=Antinuclear Antibody

For any rheumatic disease, PPV=21.5%, NPV=97.3%

For SLE, PPV=11% but has NPV=100%

PPV=positive predictive value
NPV=negative predictive value

Slater *et al*, *Arch Intern Med*, 1996, PMID: 8678710

Other autoantibody tests

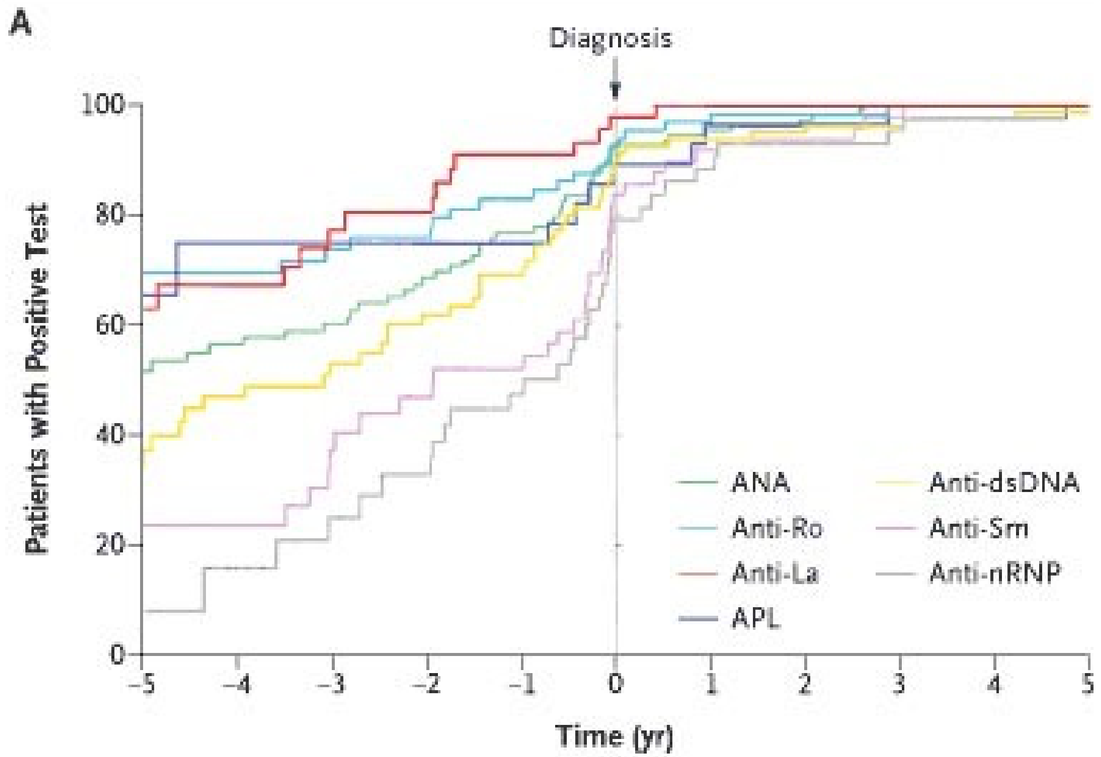
Associations somewhat strong, but not 100% diagnostic

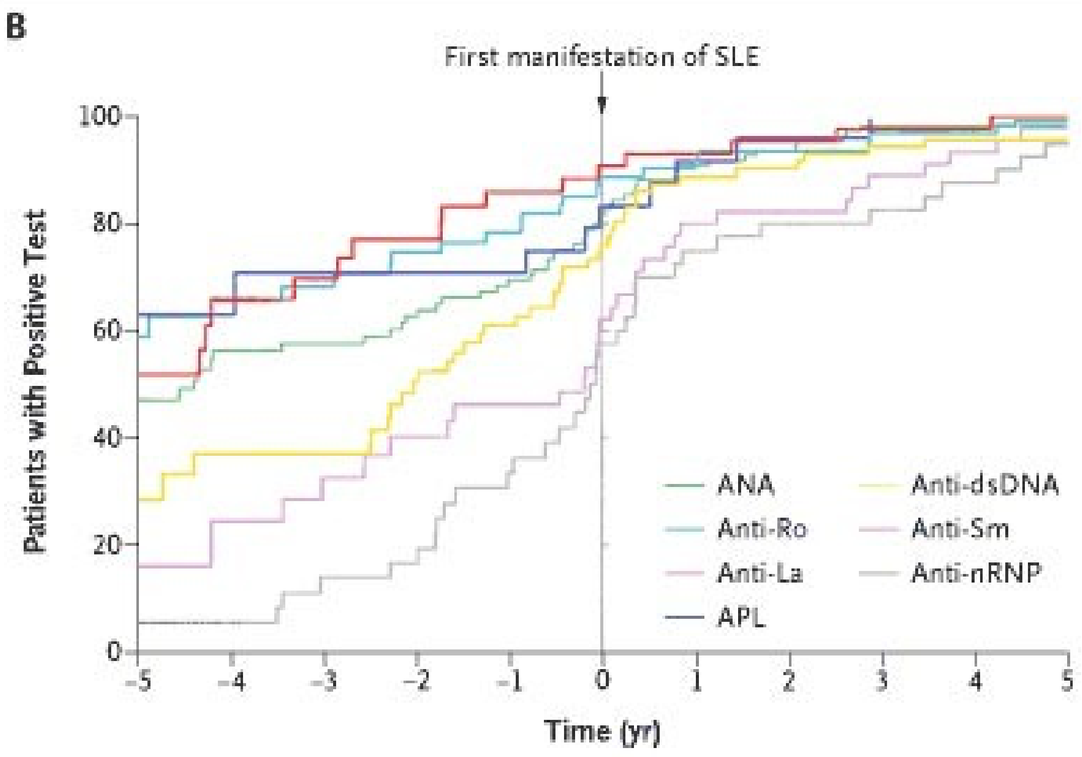
- SLE
  - anti-dsDNA: 97% specificity vs 33% sensitivity
  - anti-Sm: 98% vs 14%
- RA
  - anti-CCP: 90.4% vs 66.0%
- ANCA-associated vasculitis
  - ANCA (with confirmed ELISA): 98% vs 81%
- Sjögren’s
  - Ro (SS-A): 87% vs ~50%
  - La (SS-B): 94% vs ~35%
- Scleroderma
  - anti-Scl70, anti-RNA pol III: ~95% vs 25%

dsDNA/Sm & SLE: Putterman *et al, Lupus Sci Med*, 2014, PMID: 25396070
CCP & RA: Lee & Schur, *Ann Rheum Dis*, 2003, PMID: 12922961
ANCA: Mandl *et al, Arch Intern Med*, 2002, PMID: 12090888
SSA/B: Solomon *et al*, *Arthritis Rheum*, 2002, PMID: 12209492

Presence of autoantibodies precede SLE diagnosis

Autoantibodies appear YEARS prior to diagnosis, also true for RA





SLE: Arbuckle *et al*, *New Engl J Med*, 2003, PMID: 14561795
RA: Aho *et al, J Rheumatol*, 1991, PMID: 1757925

Other autoantibody tests

Associations somewhat strong, but certainly NOT 100% diagnostic

Table 2. Pathogenic Autoantibodies in Systemic Lupus Erythematosus.*

Antigen Specificity	Prevalence†	Main Clinical Effects	Source of Evidence		
			Clinical Studies	Studies of Tissues from Patients with Lupus	Animal Models
	%				
Anti–double-stranded DNA	70–80	Kidney disease, skin disease	ter Borg et al.,[23] Bootsma et al.,[31] Tseng et al.[32]	Koffler et al.[20]	Ravirajan et al.,[33] Ehrenstein et al.,[34] Madaio et al.[35]
Nucleosomes	60–90	Kidney disease, skin disease	Amoura et al.[26]	Grootscholten et al.,[36] Kalaaji et al.,[37] Kalaaji et al.[38]	Kramers et al.,[39] van Bruggen et al.[40]
Ro	30–40	Skin disease, kidney disease, fetal heart problems	Buyon and Clancy,[41] Sontheimer et al.[42]	Mannik et al.,[25] Clancy et al.,[43] Maddison and Reichlin[44]	
La	15–20	Fetal heart problems	Buyon and Clancy[41]	Mannik et al.[25]	
Sm	10–30	Kidney disease	McCarty et al.[45]	Mannik et al.[25]	
NMDA receptor	33–50	Brain disease	Yoshio et al.,[46] Lapteva et al.[47]	Kowal et al.[27]	Kowal et al.[27]
Phospholipids	20–30	Thrombosis, pregnancy loss	Alarcón-Segovia et al.[48]		Girardi et al.,[49] Pierangeli et al.[50]
α-Actinin	20	Kidney disease	Mason et al.,[51] Becker-Merok et al.[28]		Mostoslavsky et al.,[52] Deocharan et al.[53]
C1q	40–50	Kidney disease	Siegert et al.[29]	Mannik et al.[25]	

* NMDA denotes *N*-methyl-D-aspartate.

† Prevalence data were obtained from a number of sources, including Amoura et al.,[26] Kowal et al.,[27] Becker-Merok et al.,[28] Siegert et al.,[29] and Ehrenstein and Isenberg.[30]

Rahman & Isenberg, *New Engl J Med*, 2008, PMID: 18305268

Autoantibody tests: Can be helpful for prognosis

Only a handful have demonstrated durability over time

- SLE:
  - Anti-dsDNA, anti-C1q → lupus nephritis
- RA:
  - High-titer RF, anti-CCP → erosive disease
  - RF → interstitial lung disease (which is an independent risk factor for mortality in RA)
- Scleroderma:
  - Anti-Scl70 → interstitial lung disease
  - Anti-RNA pol III → scleroderma renal crisis, rapid progressive skin problems
- SS-A/La → Fetal heart block

LN: Mannik *et al*, *J Rheumatol*, 2003, PMID: 12858447
Yin *et al*, *Lupus*, 2012, PMID: 22777943

RF & ILD: Mori *et al*, *Respir Med,* 2012, PMID: 22867979
Natalini *et al, Ann Am Thorac Soc*, 2021, PMID: 33026891

SRC & Anti-RNA pol III: Hamaguchi *et al, Arthritis Rheumatol*, 2015, PMID: 25512203

Conclusions

- Many more people without autoimmune disease have autoantibodies compared to those with autoimmune disease
  - Alternatively, virtually all patients with an autoimmune disease possess autoantibodies

- Diagnosis of autoimmune disease (the world most of you live in): Autoantibodies only helpful ONCE CLINICAL SUSPICION IS ESTABLISHED
  - Caveat: autoantibodies likely present years prior to symptoms
  - Repeat testing usually not impactful (Lee *et al, Pathology*, 2016, PMID: 27600602)

- Certain autoantibodies can be very helpful in prognosis of established disease (unfortunately, usually in the hands of a specialist at this stage)

What’s in the future…

- “Functional” tests evaluating immunologic activity
  - “Next-generation” complement testing assessing complement activation products rather than C3 or C4
  - Gene signatures of immune pathways: Interferons for example
  - Cytokine panels