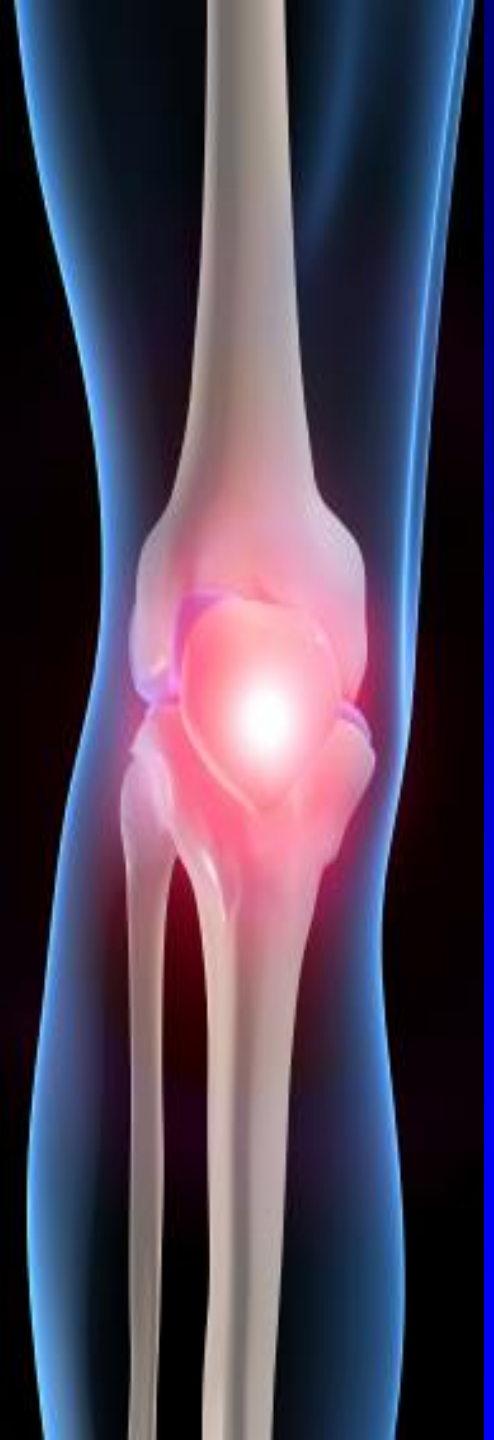

Testing for Rheumatologic Diseases:

Finding The Signal Through The Noise

Ari Weinreb M.D./Ph.D.

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Associate Professor of Medicine

David Geffen School of Medicine at UCLA

When a patient with arthritis comes in my front door, I try to go out the back door.

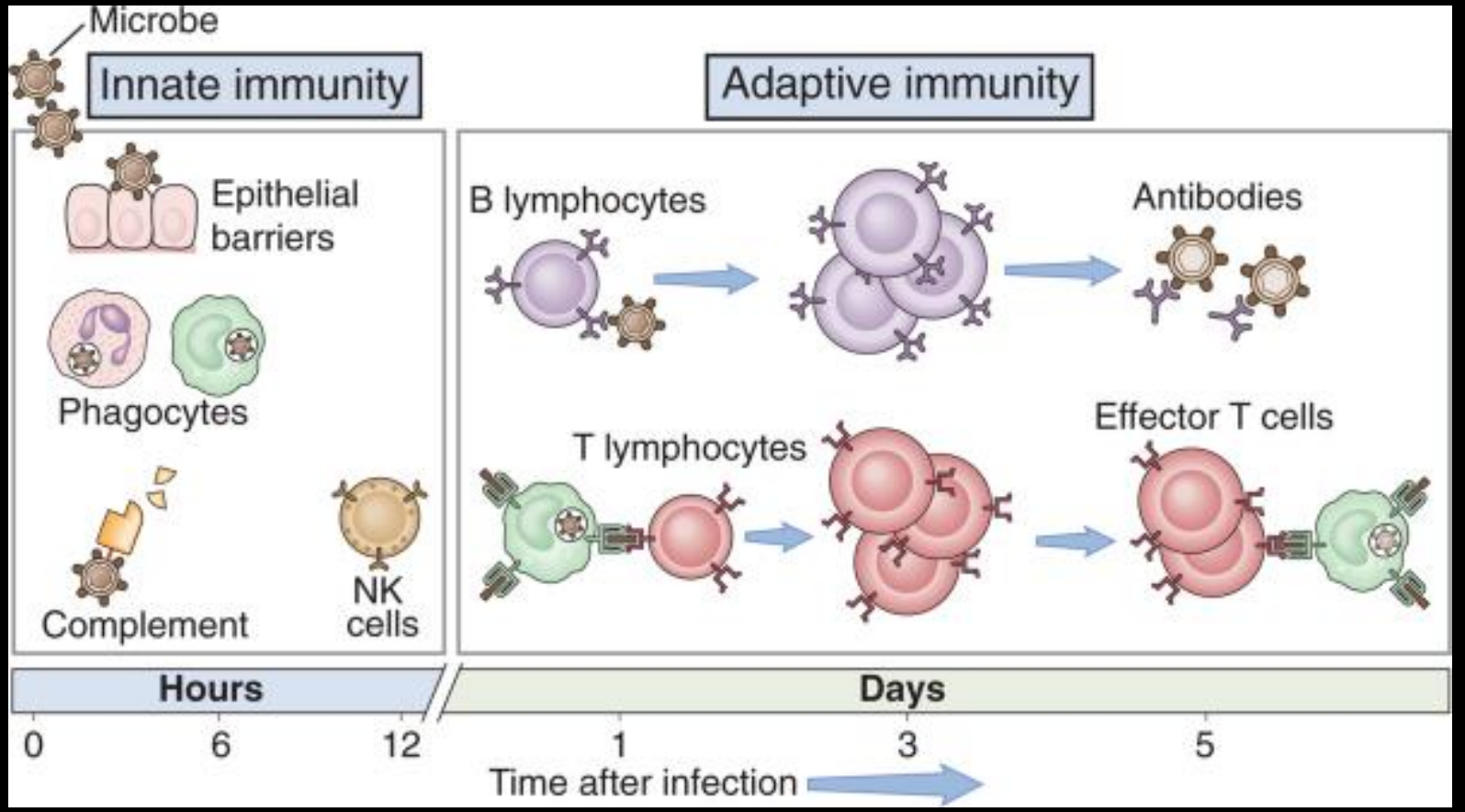
-Sir William Osler

Topics to Cover

1. **Autoimmunity**
   - -How does the development of autoimmunity relate to immunologic test interpretation?
2. **Bedside Test Statistics**
   - -Why is it important to know what you are really testing for?
3. **Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide Antibody (ACPA)**
   - -Is that joint pain really due to rheumatoid arthritis?
4. **Antinuclear Antibody (ANA)**
   - -It must be lupus, SSRD (Some Sort of Rheumatologic Disease), or maybe something else?

Autoimmunity

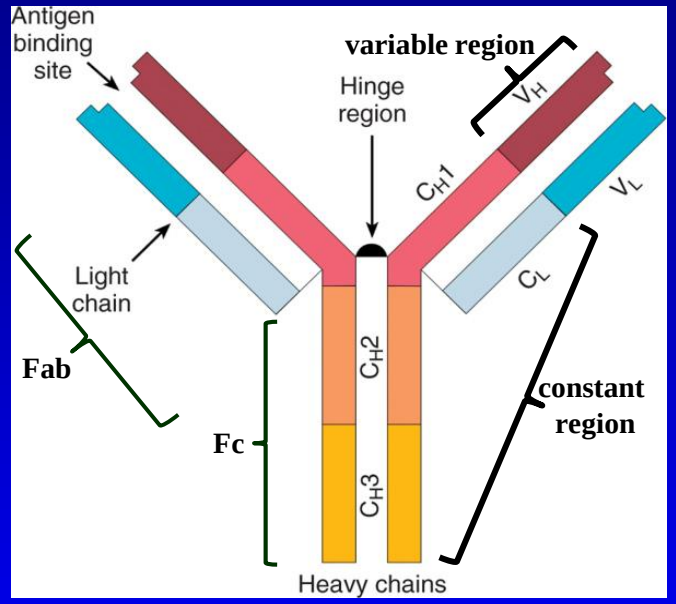
The Immune System

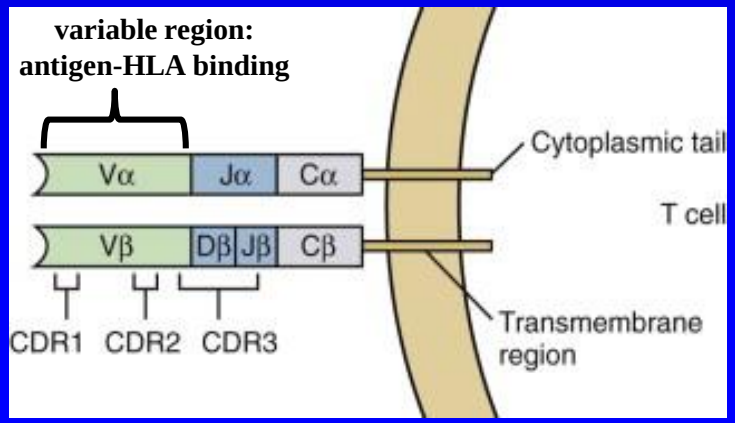


a non-specific and rapid 1º line of defense; effector of autoimmunity (and autoinflammatory diseases)

specific recognition of antigens by antibodies and T-cell receptors confer a more specific and effective 2º line of defense; specific self-reactive antibodies and T-cells define the presence of an autoimmune process

Adaptive Immunity: Immunoglobulins and T-Cell Receptors

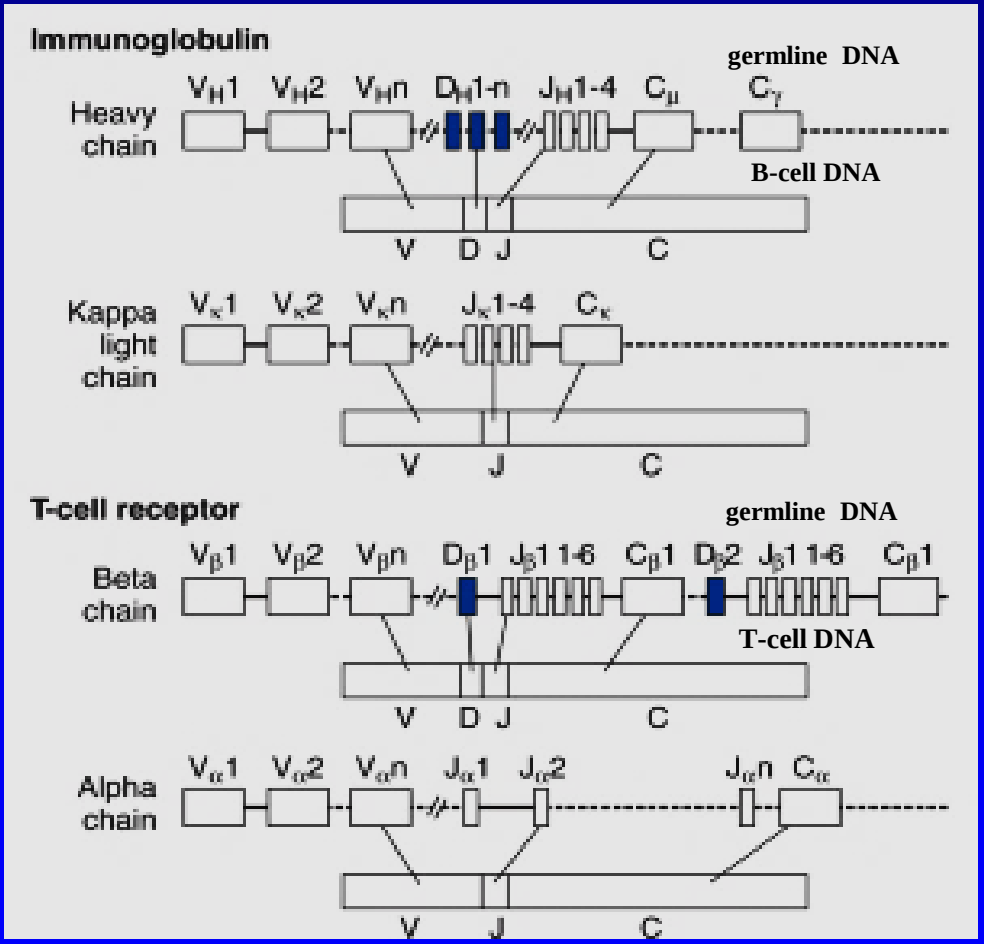




-<u>diversity</u> of the adaptive immune response is determined by the <u>variable regions</u> of the immunoglobulin and T-cell receptor molecules

-the <u>constant regions</u> determine other antibody and T-cell receptor related functions

Adaptive Immunity: Diversity



V, D, J Chromosomal Gene Rearrangement

-occurs during development of the immune system

-creates the diversity of the variable region antigen binding sites of antibodies (IGs) and T cell receptors

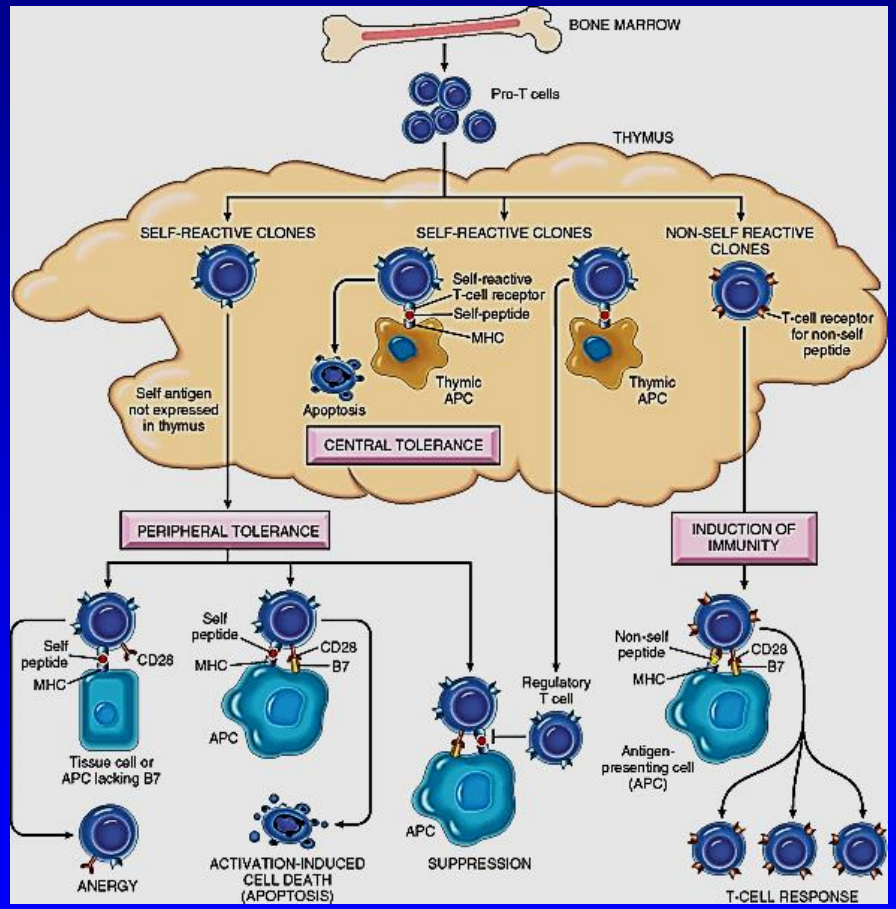
-random process

·generates diverse B cell and T cell clones with antibody molecules and T-cell receptors reactive to both foreign antigens and self-antigens

Tolerance vs. Autoimmunity

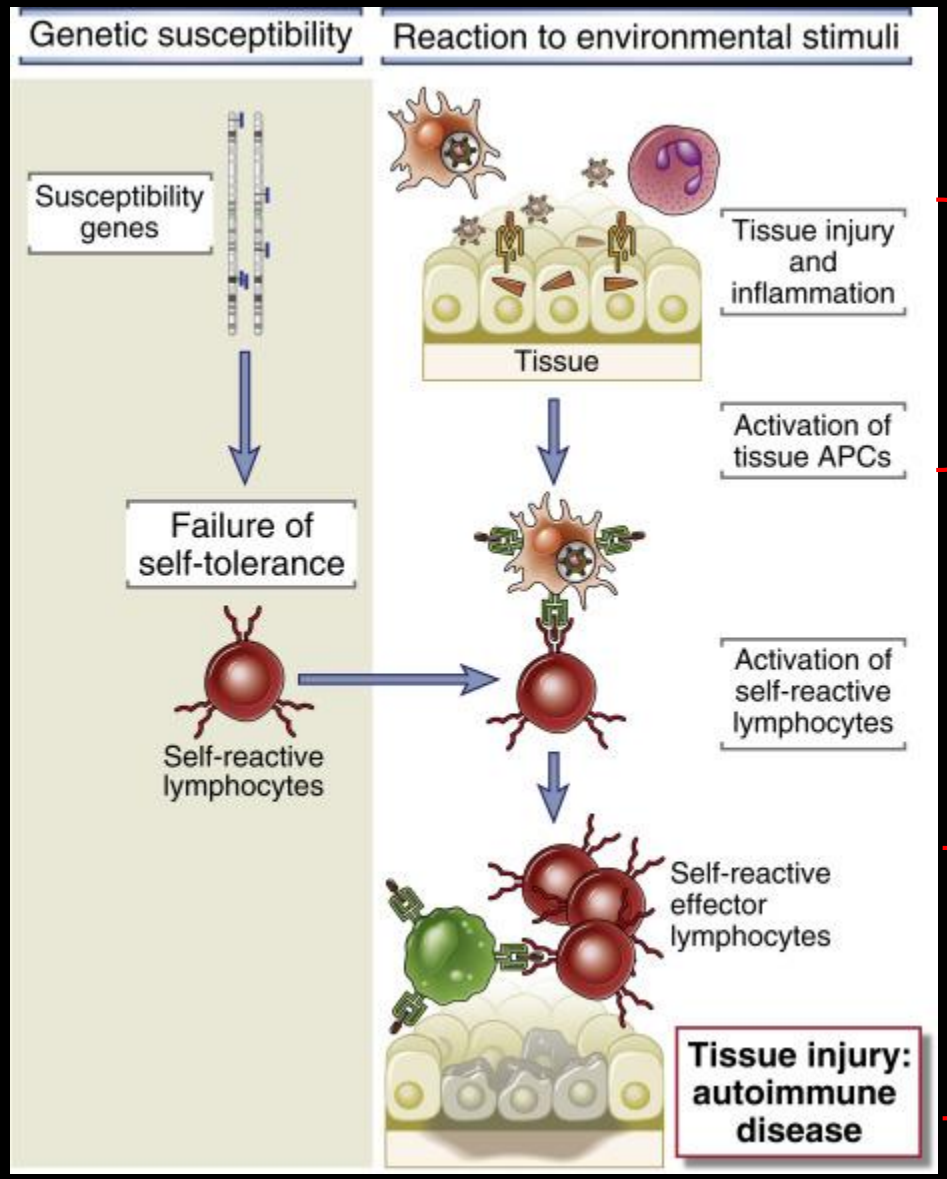
-tolerance
- ·elimination or inactivation of autoreactive lymphocytes
- ·<u>not perfect</u>: a low level of non-pathogenic autoreactive lymphocytes persist
- ·possible physiologic role for autoreactivity (e.g. clearance of cellular debris and immune complexes)

-autoimmunity
- ·results from failure of tolerance mechanisms and activation, expansion, and evolution of pathologic autoreactive lymphocytes



Autoimmunity: The Current Pathophysiologic Model

Genetics:
predisposing alleles that alter cytokines, signaling apoptosis, and other immune related functions



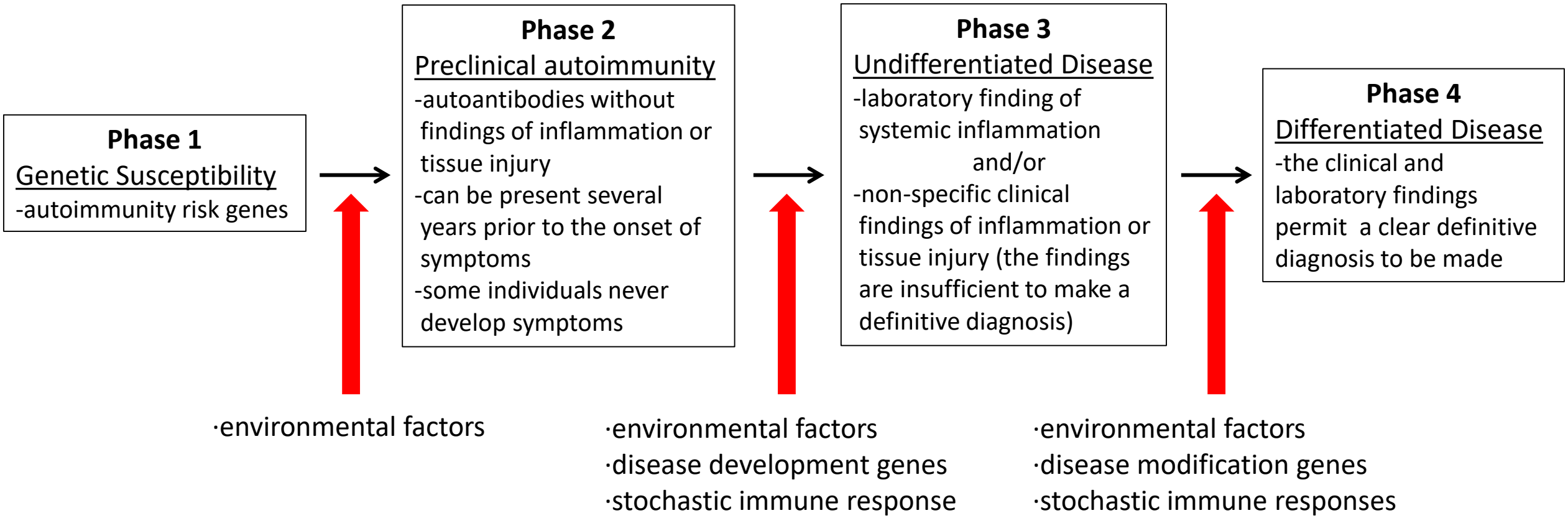
Environment:
cross-reactive infectious antigens or exposure of hidden self-antigens

Altered Immune Responses:
stochastic or random immune system responses to the various antigens, cytokines, and other signals create an inflammatory state

Autoimmunity:
self-reactive lymphocytes in the setting of autoimmune driving genes and the appropriate inflammatory environment result in auotimmunity

Cellular and Molecular Immunology.
Abbas AK, et. al. 2015. Ch. 15:315.

Autoimmune Disease Development: The Current Clinical Model



Autoimmune disease development results from the interaction of an individual's genetics, environmental exposures, and the unpredictable response of the immune system. We are still at the early stage identifying these factors and how they intereact.

How Does Autoimmunity Present Clinically in a Patient?

-serum autoimmunity
- ·presence of autoantibodies with or without clinical findings (clinical testing for autoreactive T-cells not available)

-clinical autoimmunity
- ·non-specific constitutional symptoms, single sites of inflammation, and/or systemic inflammation (multiple organ systems)
- ·caveat: nonautoimmune processes can stimulate an inflammatory response (injury, infection, neoplasm, etc.)

-ROS: disease specific autoimmune findings are most helpful in determining the presence of an autoimmune process
- ·e.g. palpable purpura, mucocutaneous ulcers, pleuritic chest pain, hemoptysis, hematuria/proteinuria, inflammatory arthritis, hemolytic anemia, etc.)

Statistics at the Bedside

Types of Tests

-assessment of organ function

-diagnosis of a disease

-screening for a disease

-prognostication

-assessing disease activity

-disease risk determination

The reason for performing a test impacts on the interpretation of the results.

Some Issues With Rheumatologic Tests That Complicate Interpretation

1. **Crossreactivity**

   -rheumatologic and non-rheumatologic conditions can share positive autoantibody reactivities:

   autoantibody tests should not the sole means of making a diagnosis

2. **Interlaboratory Variability**

   -variability in reagents, assay methods, measurement, and interpretation:

   may make low titer positive results less certain

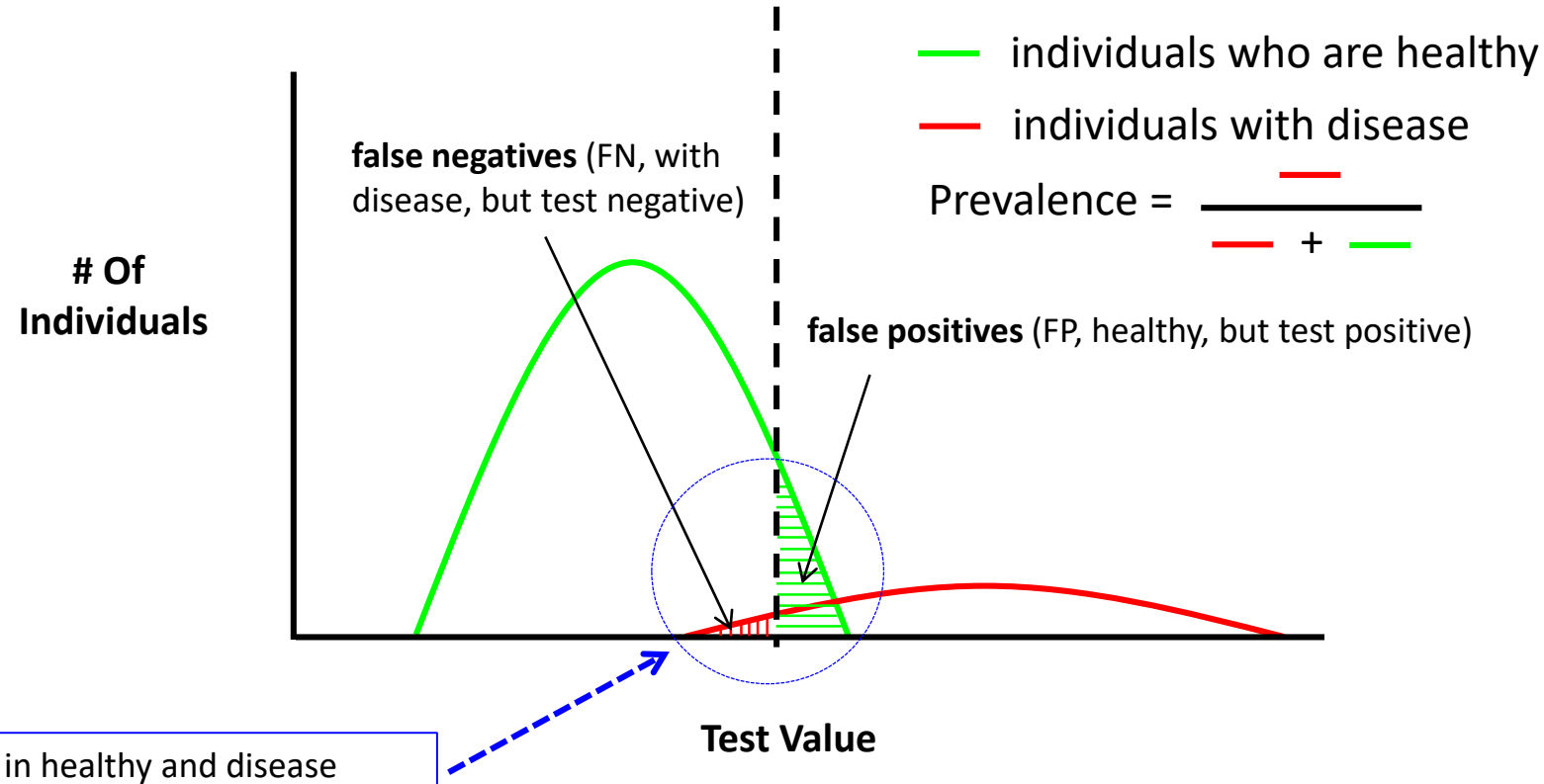
3. **Bias and Generalizability**

   -the population characteristics used to design a test may differ from the actual test population

   know who you are testing

Test Cutoffs and Overlap: FPs and FNs

Cutoff Value for Test: determined by the desired sensitivity and specificity for the test.
(< value=normal, > value=abnormal)



-overlap of test results in healthy and disease populations leads to FP and FN results

-the more tests ordered, the more likely a positive result will occur by chance (=FP).

Sensitivity, Specificity, and Prevalence

	Disease Present	Disease Absent	Total
Test Positive	a	b (FP)	a+b
Test Negative	c (FN)	d	c+d
Total	a+c	b+d	a+b+c+d

Disease **Prevalence**: percent of population with the disease= **a+c/a+b+c+d x100**

Sensitivity: percent of patients with disease who are correctly identified with a positive test result = **a/a+c**

Specificity: percent of patients without disease who are correctly identified by a negative test result = **d/b+d**

The Key Points on Sensitivity and Specificity:
1. determined by the test: population, methodology, cutoffs
2. not affected by disease prevalence/pretest probability
3. do not predict disease given a test result.

Positive and Negative Predictive Values (PPV and NPV)

	Disease Present	Disease Absent	Total
Test Positive	a	b	a+b
Test Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

Positive Predictive Value: % of patients with a positive test with the disease **= a/a+b**

Negative Predictive Value: % of patients with a negative test result without the disease **= d/c+d**

The Key Points to Know

1. determined by the sensitivity, specificity, and disease prevalence/pretest probability
2. addresses the clinical question for which the test was ordered

What Effect Does Disease Prevalence/Pretest Probability Have on the PPV and NPV Of The Test?

Uncommon Disease:
Low Pretest Probability (**0-20%**)

	Disease Present	Disease Absent	Total
Test Positive	90	90	180
Test Negative	10	810	820
Total	100	900	1000

Population Size=1000
Sensitivity=90% Specificity=90%
Prevalence/Pretest Probability=10%

Positive Predictive Value=90/180x100=**50%**
Negative Predictive Value=810/820x100=**98.8%**

FPR=90/180x100=**50%**

More Common Disease:
Midrange Pretest Probability (**21-70%**)

	Disease Present	Disease Absent	Total
Test Positive	450	50	500
Test Negative	50	450	500
Total	500	500	1000

Population Size=1000
Sensitivity=90% Specificity=90%
Prevalence/Pretest Probability=50%

Positive Predictive Value=450/500x100=**90%**
Negative Predictive Value=450/500x100=**90%**

FPR-50/500x100=**10%**

Very Common Disease:
High Pretest Probability (**71-100%**)

	Disease Present	Disease Absent	Total
Test Positive	720	20	740
Test Negative	80	180	260
Total	800	200	1000

Population Size=1000
Sensitivity=90% Specificity=90%
Prevalence/Pretest Probability=80%

Positive Predictive Value=720/740x100=**97.3%**
Negative Predictive Value=180/260x100=**69.2%**

FPR=20/740x100=**2.7%**

The Key Points to Know:
1. the pretest probability determines the strength of the test result in ruling in or ruling out the disease
2. the history and exam are the most important determinants of the pretest probability

Rheumatologic Disease Prevalences

Rheumatoid Arthritis:

Caucasians: ~1%
rural Africans: 0.01%
Pima, Blackfeet, and Chippewa Indians: 5%

Lupus (U.S.):

overall: 0.02-0.15%
Women: white- 0.164% AA- 0.406%
increased prevalence in Asian, African-American, African-Caribbean, Hispanic American

Sjogren's:

0.01-0.09%

Example of the problem of "screening" for rheumatologic diseases with serologic tests:

for an AA woman w/o symptoms on screening for SLE with a +ANA (sens 90%, spec 90%), the PPV would be 3.54%

Systemic Sclerosis:

North American- 0.0276-0.0443%
Global- 0.0038-0.066%

Psoriatic Arthritis:

overall- 0.3-1%
with psoriasis- 7-42%

AAV:

GPA- 0.0065-0.016%
MPA- 0.0039-0.0094%
EGPA- 0.0011-0.0046%

Gout:

US- 2007-2008: 3.9% (8.3 million),
>60 yrs 9.3% (4.7 million)
UK- 1.4%
Taiwan- 3.4%

Bayes' post-test probability calculator

View More by This Develope

By Keiji Matsui

This app is only available on the App Store for iOS devices.

Offers Apple Watch App for iPhone

Description

Bayes' post-test probability calculator, an iPhone / iPod touch application, calculates the post-test probability of diseases from pre-test probability and sensitivity of the test and specificity of the test.

Keiji Matsui Web Site ▸ Bayes' post-test probability calculator Support ▸ ...Mor

What's New in Version 3.0

It can be used on an Apple Watch.

Free

Category: Medical
Updated: Feb 09, 2016
Version: 3.0
Size: 17.5 MB
Apple Watch: Yes
Language: English
Seller: Keiji Matsui
© Keiji Matsui
Rated 12+ for the following:
Infrequent/Mild Medical/Treatment Information

Compatibility: Requires iOS 9.0 or later. Compatible with iPhone, iPad, and iPod touch.

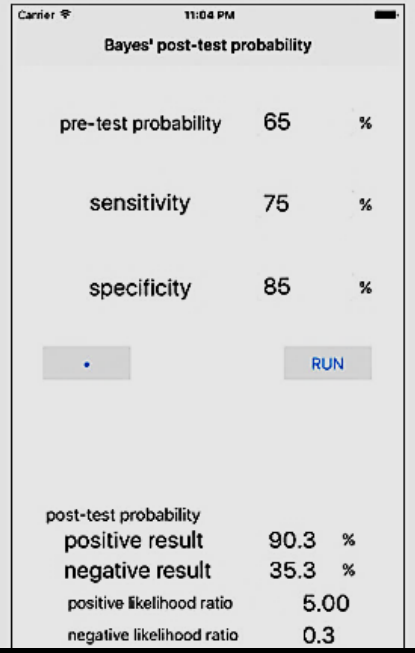
Customer Ratings

This application hasn't received enough ratings to display a summary.

More iPhone Apps by Keiji Matsui

Screenshots

iPhone | Apple Watch



Arthritis Classification and Patterns of Joint Involvement

modified from John Brown

Is the joint pain articular or periarticular?

- periarticular → bone, soft tissue, nerve

Monoarticular (1 joint)

septic arthritis
crystal arthropathy
other monoarthritis

Polyarticular (>1 joint)

Autoimmune +/- Systemic Inflammation

RF/anti-CCP
ANA

Symmetric/Small Joint

rheumatoid arthritis
SLE
systemic sclerosis/scleroderma
Sjogren's syndrome
other collagen-vascular diseases

Asymmetric/Large Joint

seronegative spondyloartropathy (oligoarticular, 2-4 joints):

ankylosing spondylitis
reactive arthritis (Reiter's syndrome)
psoriatic arthritis
inflammatory bowel disease arthritis

* **Exceptions to keep in mind:**

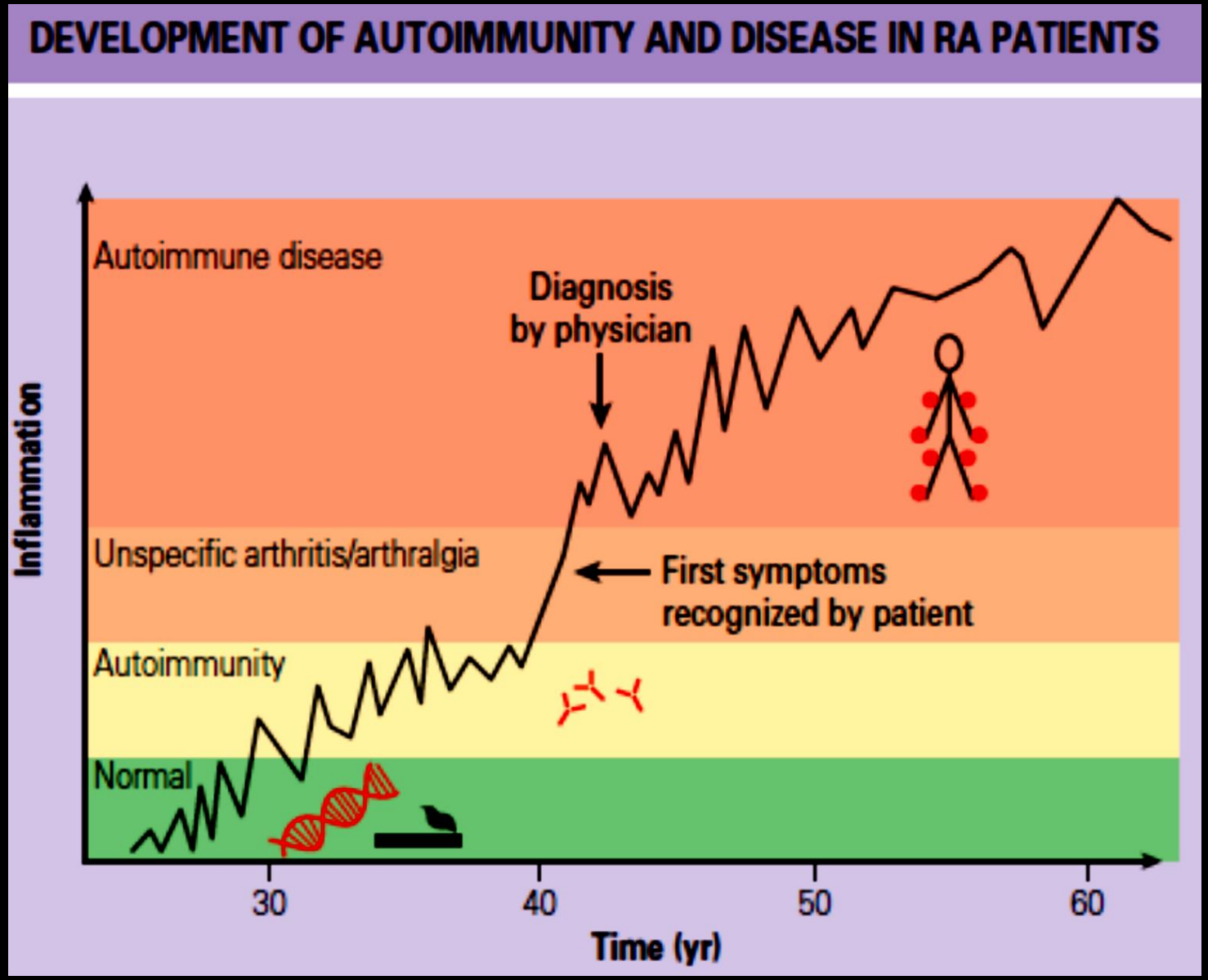
1. **early presentations**
2. **chronic presentations**
3. **undifferentiated forms**
4. **variable presentations**

Nonautoimmune +/- Local Inflammation

degenerative/osteoarthritis

Diagnosing Rheumatoid Arthritis…
and Other Things:

The Rheumatoid Factor (RF)
and
Anti-Cyclic Citrullinated Peptide Antibody (ACPA)



adapted from Rheumatology. Ed. Hochberg M, et. al. Ch. 91 Hoffmann M et. al. Pp.750. Philadelphia, PA. Mosby. 2015.

RA Classification Criteria

Table 3 The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)*	
2) with the synovitis not better explained by another disease†	
Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement§	
1 large joint¶	0
2–10 large joints	1
1–3 small joints (with or without involvement of large joints)**	2
4–10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)††	5
B. Serology (at least 1 test result is needed for classification)‡‡	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)§§	
Normal CRP *and* normal ESR 0	0
Abnormal CRP *or* normal ESR 1	1
D. Duration of symptoms¶¶	
<6 weeks	0
≥6 weeks	1

Ann Rheum Dis. 2010. 69:1580.

Some Rheumatoid Factor History

-1922

Kurt Meyer:

hemagglutinating factor in human sera that strongly agglutinated sheep RBCs (found in patients with cirrhosis and chronic bronchitis)

-1940

Erik Waaler:

antibody against gamma-globulins that agglutinated antibody sensitized sheep RBCs (Waaler Rose test)

-1948

H. M. Rose:

modified Waaler's test and identified these agglutinatng antibodies in patients with rheumatoid arthritis

-1952

these agglutinating antibodies were named rheumatoid factors due to their association with rheumatoid arthritis

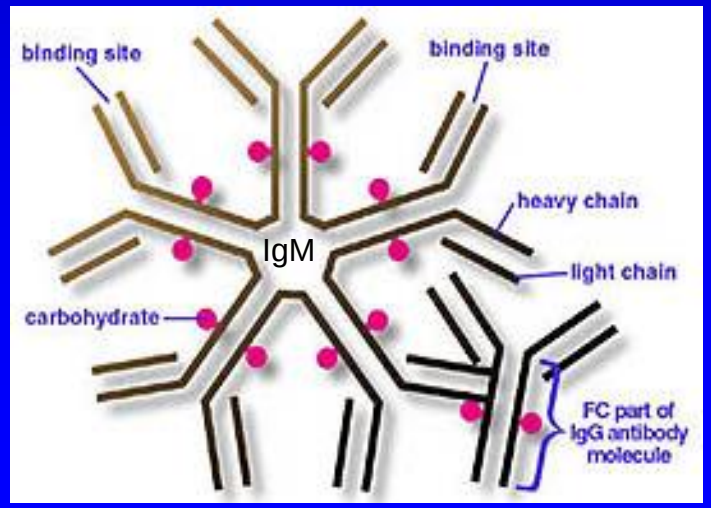
What Is The Rheumatoid Factor (RF)?

-an IgM, IgG, or IgA antibody which binds to the Fc portion of an IgG immunoglobulin; the IgM RFs are clinically measured

-not specific for any condition: reflects a state of chronic immune activation

-an increased RF titer can occur in older individuals, with chronic infection, and in other autoimmune diseases (despite its name, an isolated positive RF is not diagnostic of RA)

-RFs can be detected by agglutination, nephelometry, or ELISA; cost ~$22-$399



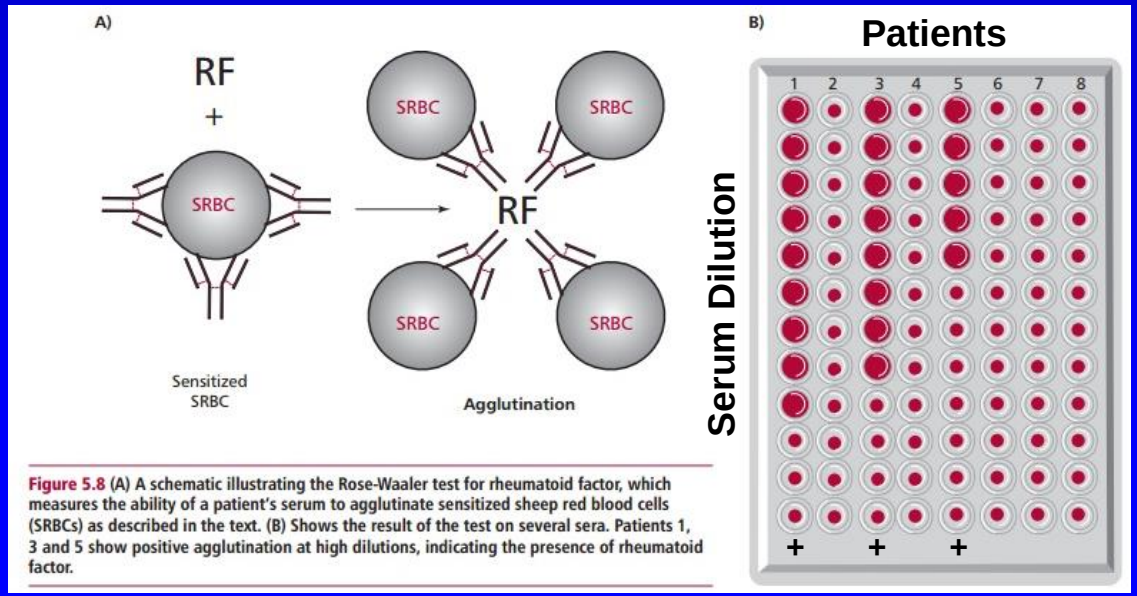


Figure 5.8 (A) A schematic illustrating the Rose-Waaler test for rheumatoid factor, which measures the ability of a patient's serum to agglutinate sensitized sheep red blood cells (SRBCs) as described in the text. (B) Shows the result of the test on several sera. Patients 1, 3 and 5 show positive agglutination at high dilutions, indicating the presence of rheumatoid factor.

RF Associated Diseases and Conditions

Arthritis	
Rheumatoid arthritis	70–90
Juvenile idiopahtic arthritis	5
Psoriatic arthritis	<15
Reactive arthritis	<5
Other connective tissue diseases	
Primary Sjögren's syndrome	75–95
Mixed connective tissue disease	50–60
Systemic lupus erythematosus	15–35
Systemic sclerosis	20–30
Dermato-/polymyositis	20
Systemic vasculitides (panarteritis nodosa, Wegener's granulomatosis)	5–20

Other diseases	
Mixed cryoglobulinemia type II	100*
Liver cirrhosis	25
Primary biliary cirrhosis	45–70
Malignancy	5–25
After multiple immunisations	10–15
Chronic sarcoidosis	5–30
Healthy 50-year olds	5
Healthy 70-year olds	10–25

Interstitial pulmonary fibrosis (10% to 50%)
Silicosis (30% to 50%)
Asbestosis (30%)

Infectious diseases	
Bacterial infections	
Subacute bacterial endocarditis	40
Chlamydia pneumoniae infection	
Klebsiella pneumoniae infection	
Syphilis primary-tertiary	8–37
Tuberculosis	15
Viral infections /Leprosy	
Coxsackie B virus infection	15
Dengue virus infection	10
EBV and CMV infections	20
Hepatitis A, B and C virus infection	25
HCV infection	40–76
Herpes virus infection	10–15
HIV infection	10–20
Measles	8–15
Parvovirus infection	10
Rubella	15
Parasitic	
Chagas	15–25
Malaria	15–18
Onchocerciasis	10
Toxoplasmosis	10–12

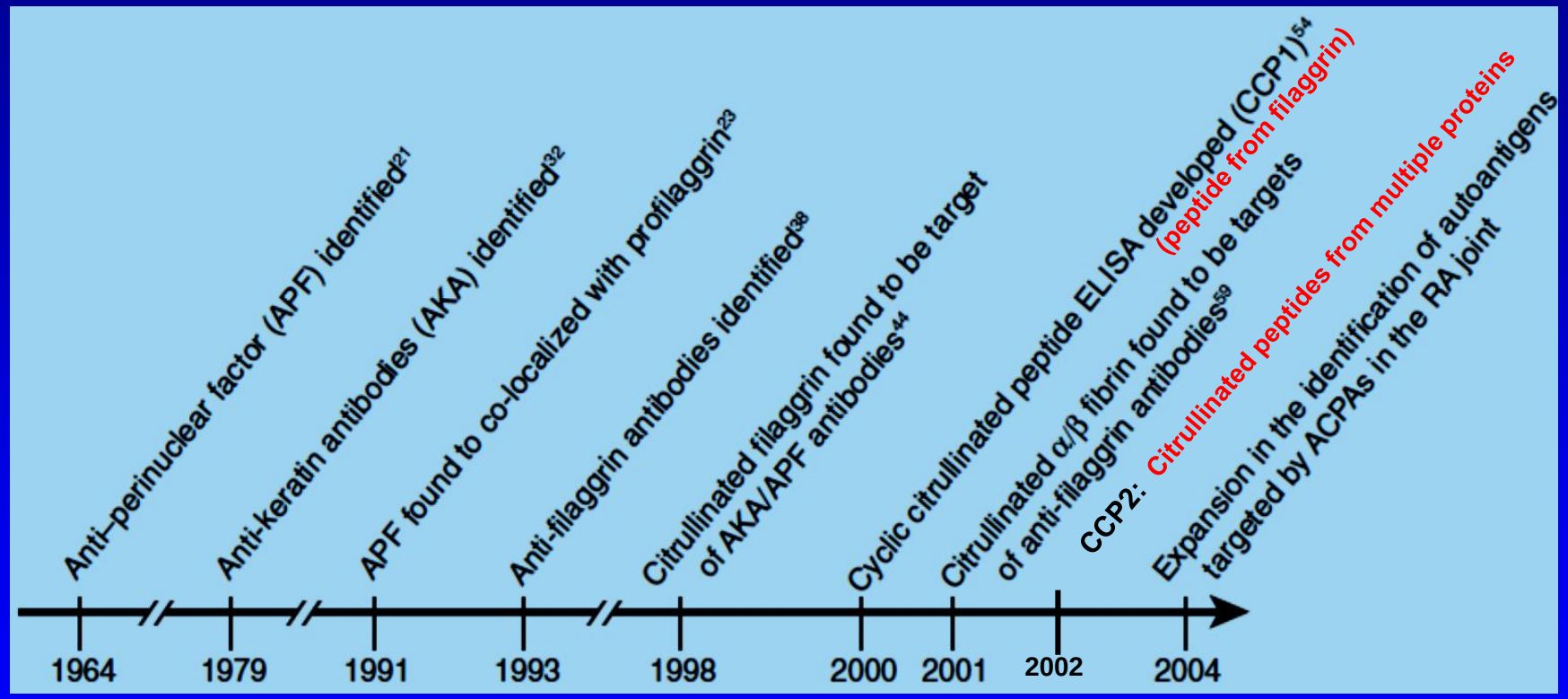
-chronic immune stimulation is a driver for the production of rheumatoid factor

-CHRONIC

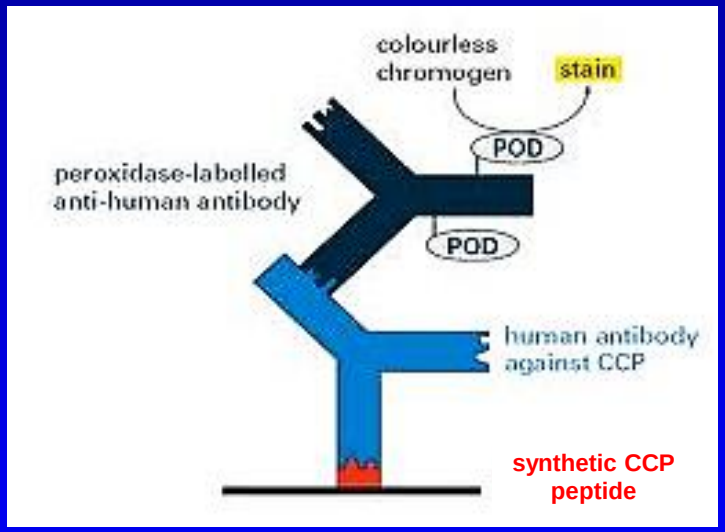
- **·CH: CHronic disease**
- **·R: Rheumatoid arthritis**
- **·O: Other rheumatic diseases**
- **·N: Neoplastic**
- **·I: Infection (esp. chronic infections, HIV)**
- **·C: Cryoglobulinemia (esp. hep C)**

Adapted from Disease Markers. Ingegnoli F et. al. 2013. 35:727.

Some Anti-Cyclic Citrullinated Peptide Antibody (ACPA) History



Anti-CCP/ACPA Assay



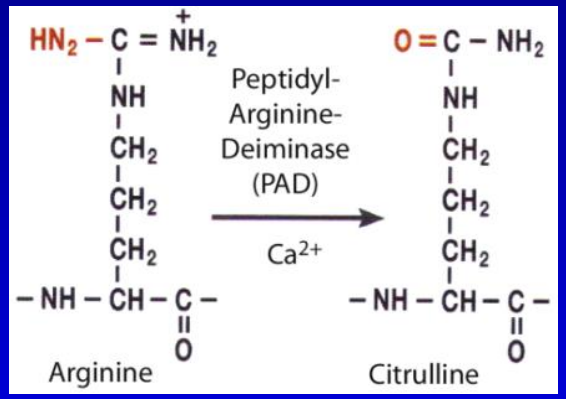
-the anti-CCP/ACPa assay detects binding to peptides containing the citrullinated epitope from several different proteins

adapted from Kelley and Firestein's Textbook of Rheumatology. Ed. Firestein GS, et. al. Ch. 56 Andrade Fet. al.: 831. Philadelphia, PA. Elsevier. 2017.

ACPAs and RA

-antibodies that recognize a citrullinated epitope on several different proteins

-epitope generated by posttranslational deamination of arginine residues by the enzyme peptidylarginine deiminase (PAD: 5 human isotypes)



-PAD4 associated with RA susceptibility in certain ethnic groups

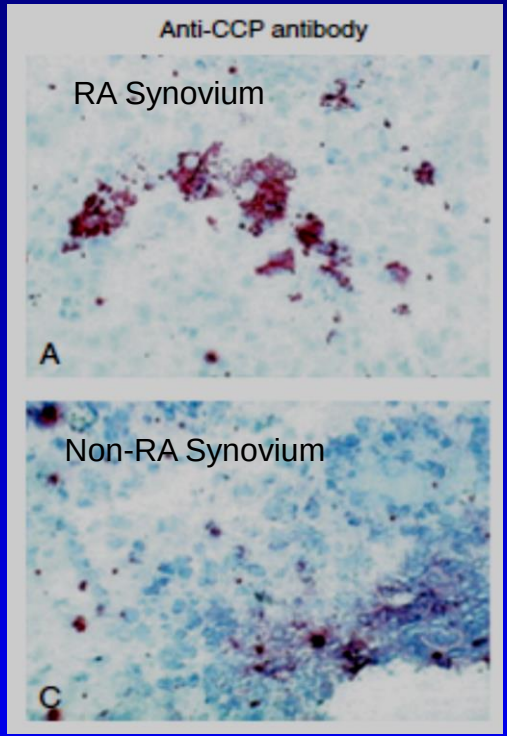
-subset of RA patients with severe disease have activating anti-PAD4 antibodies

-<u>periodontitis (RA risk factor)</u>: the periodontitis associated oral gram negative bacterium *Porphyromonas gingivalis* expresses a unique prokaryotic PAD enzyme

-<u>smoking (RA risk factor)</u>: associated with increased pulmonary PAD2 and PAD4 expression and protein citrullination in the lung

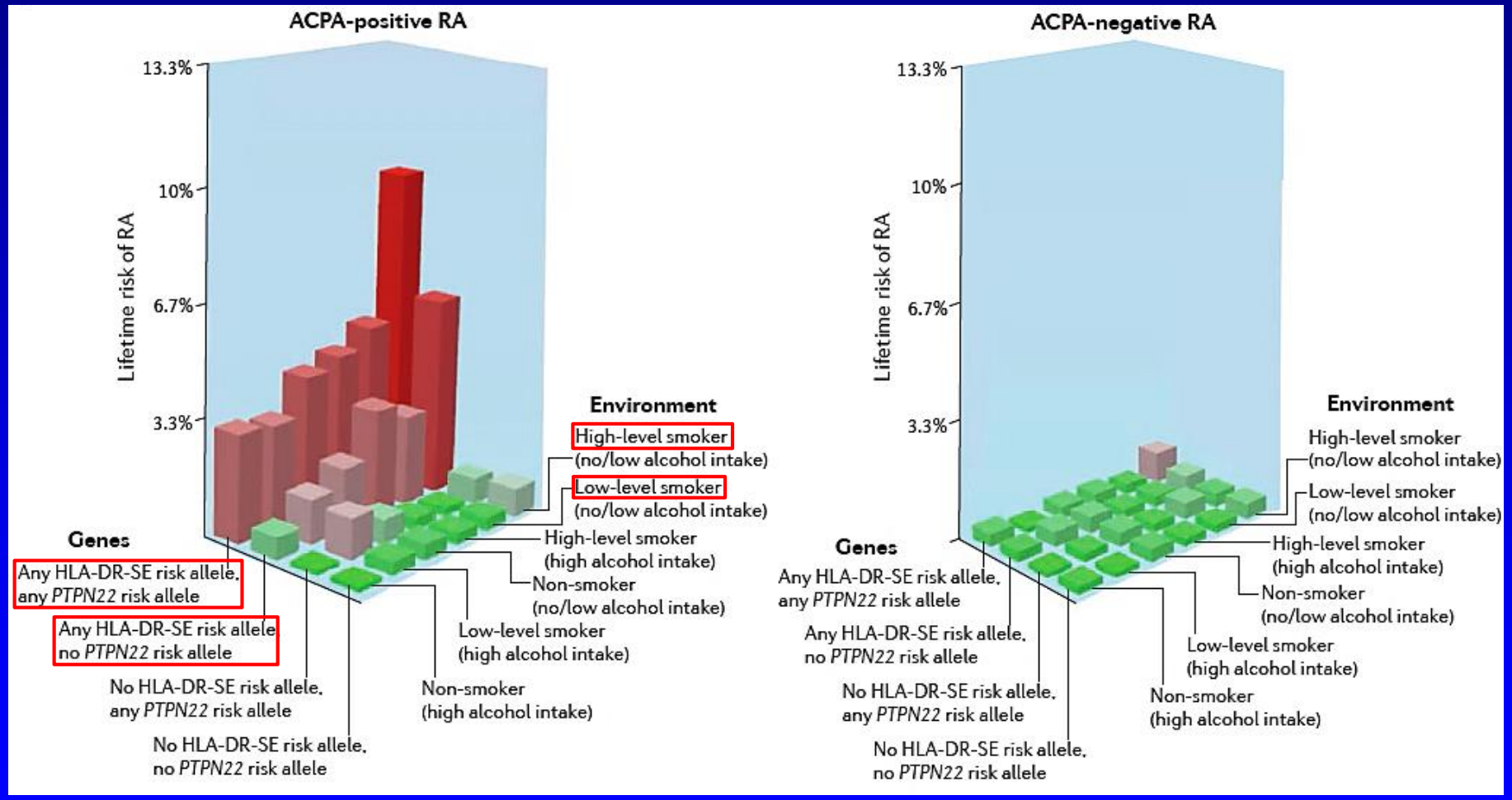
-increased PAD4 and citrullinated proteins identified in inflamed synovium

-cost ~$79-$189



Increased expression of citrullinated proteins in rheumatoid synovium. ACPA targets?
A. RA synovium C. normal synovium (Arthritis Rheum. 2004. 50:380.)

Risk of Developing RA: Genes, Environment and ACPAs



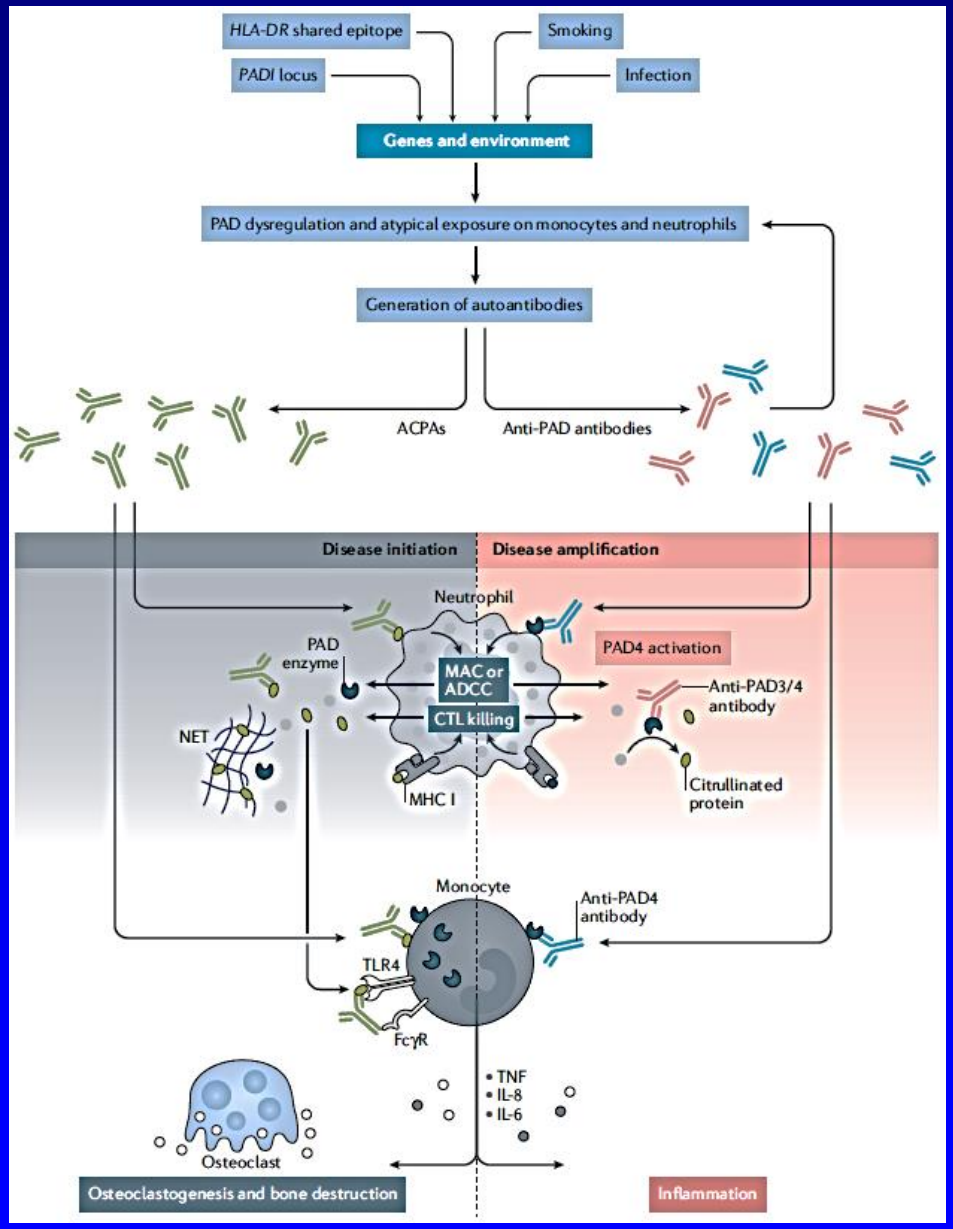
-the class II HLA-DR shared epitope alleles contribute strongly to the RA ACPA pathogenicity

-other risk alleles and environmental factors less strongly contribute to ACPA pathogenicity

-less clear what genetic and environmental factors contribute to ACPA formation

Nature Reviews Immunology. doi:10.1038/nri.2016.124 .Published online 5 Dec 2016.

The Role Of ACPAs in RA: The Two Hit Model



Curran AM, et. al.
Nature Reviews Rheumatology.
2020. 16:301.

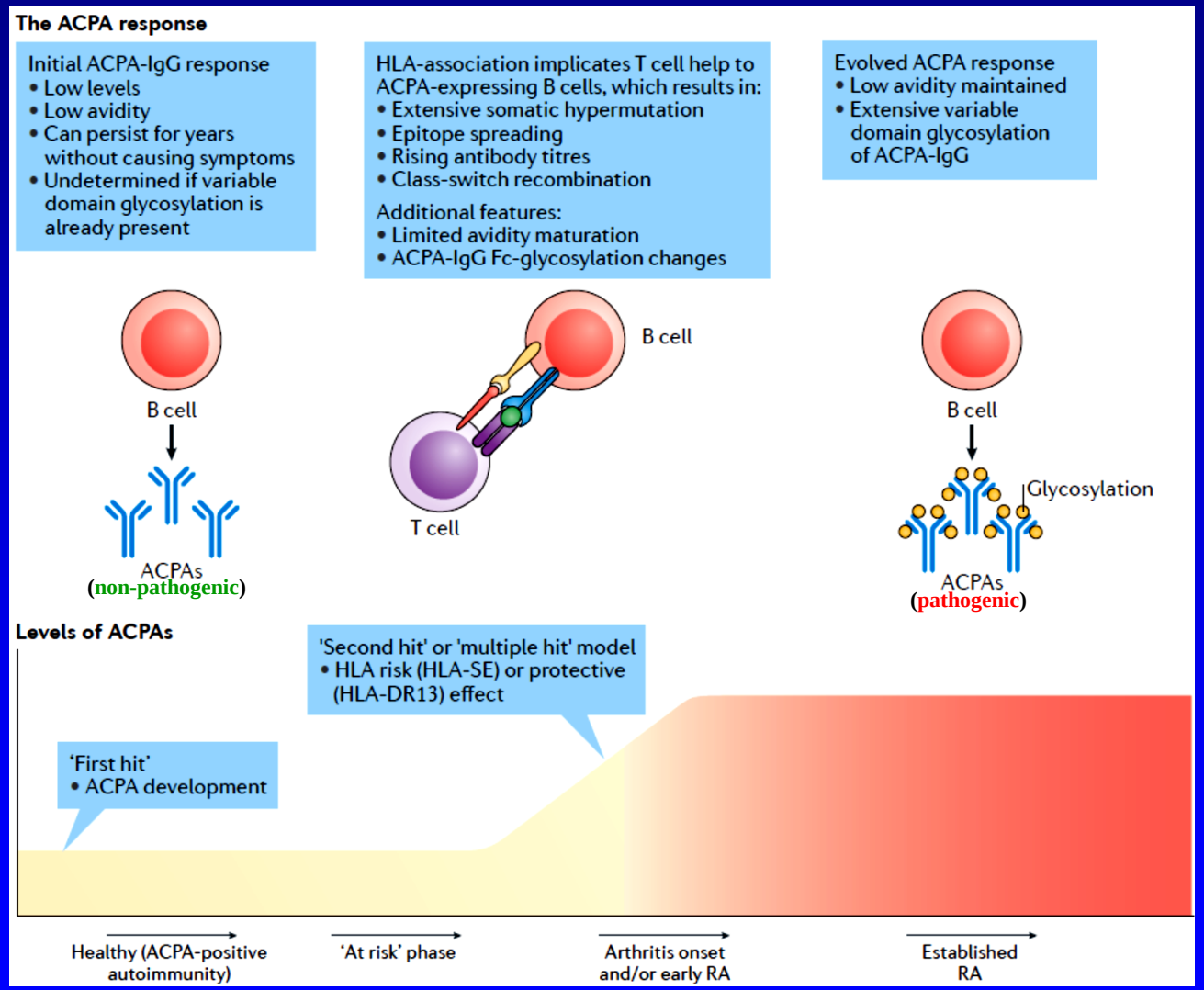
1st Hit:

protein citrullination at mucosal surfaces in setting of inflammation results in ACPA production

2nd Hit:

ACPAs activate macrophages promoting inflammation, activate osteoclasts promoting bony erosions, and target other citrullinated proteins in the joint contributing to tissue damage

The Evolution of ACPA Pathogenicity and the Development of RA



Scherer HU, et. al. Nature Rev. Rheum. 2018. 14:157.

-early ACPAs are not pathogenic

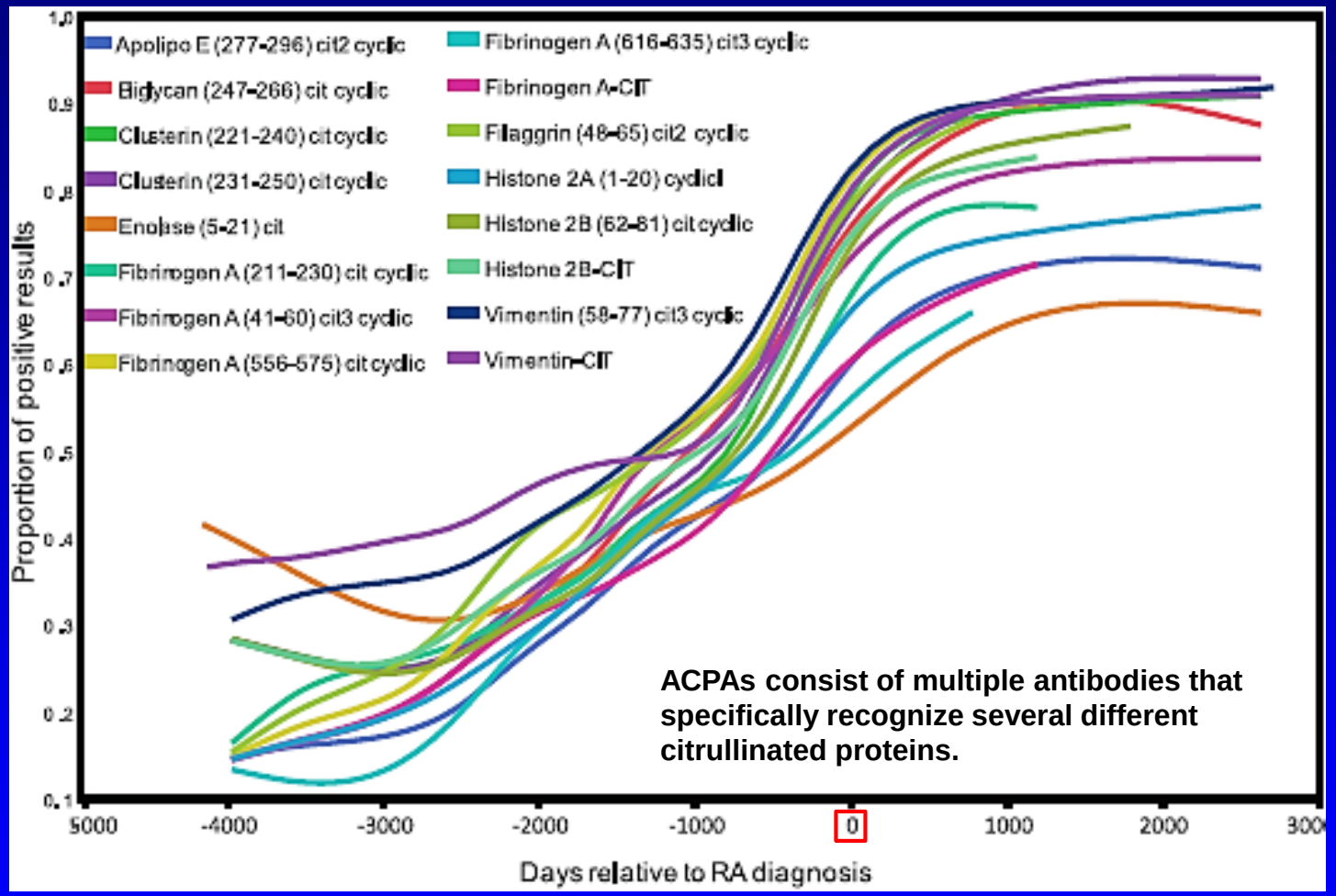
-genetic, environmental, and other immune response factors contribute to changes in the ACPA structure conferring increased pathogenicity

-clinical anti-CCP/ACPA assays only detect their presence and titer

-clinical anti-CCP/ACPA assays <u>do not inform about pathogencity</u>:

Interpretation requires clinical context!

ACPAs and RA Preclinical Autoimmunity

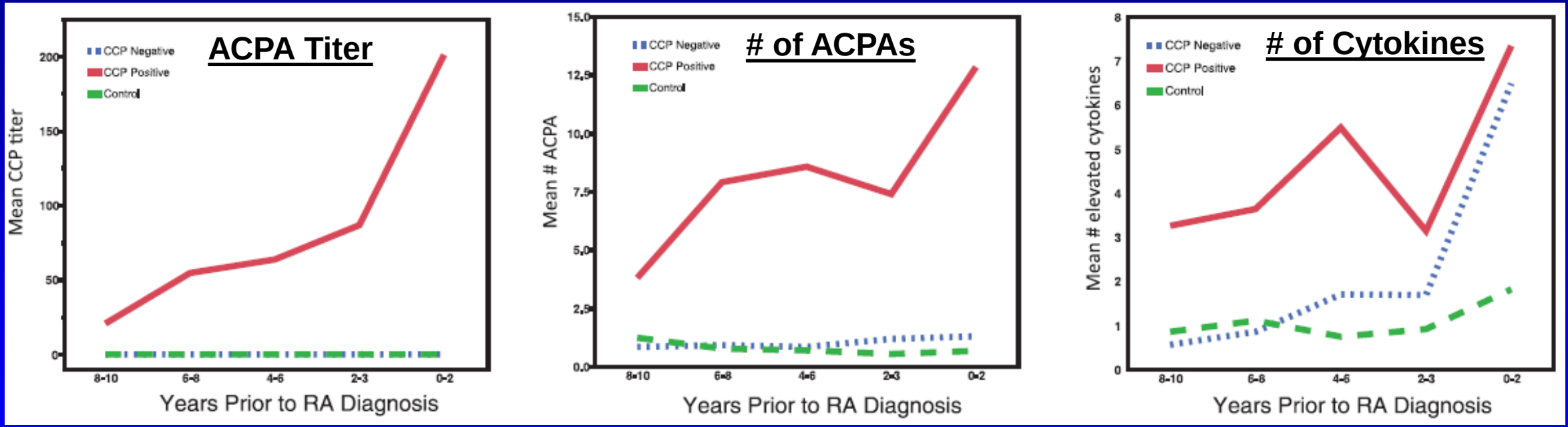


PLoS ONE. 2012. 7(5).

Prior to the development of RA (day 0):

- ACPAs to many different citrullinated proteins are present
- the number and concentration of ACPAs to different citrullinated proteins increases as the time to diagnosis approaches

ACPAs and Cytokines and RA Progression



PLoS ONE. 2012. 7(5).

ACPA Prevalence In Other Rheumatologic Conditions

	Anti-CCP2		
Disease	References	Patients (No)	Positive anti-CCP test No (%)
Systemic lupus erythematosus	17, 18, 21, 23, 24, 30, 33, 34, 43, 45, 48, 49, 50, 52	567	49 (9)
Sjögren's syndrome	17, 19, 23, 30, 33, 45, 46 48, 49, 52, 68, 69	521	27 (5)
Hepatitis C virus	17, 41, 42, 70	219	3 (1)
Wegener's granulomatosis	19, 24, 34, 48, 52	67	1 (1)
Ankylosing spondylitis	18, 21, 23, 24, 34,43, 45, 48, 50, 52	181	5 (3)
Psoriatic arthritis	14, 21, 34, 46,48, 50, 52, 66, 67	424	36 (8)
Polymyalgia rheumatica	47	49	0
Palindromic rheumatism	71	63	28 (44)

modified from Ann Rheum Dis 2006;65:845–851.

Diagnosis of RA: PPV and NPV of the RF and ACPA

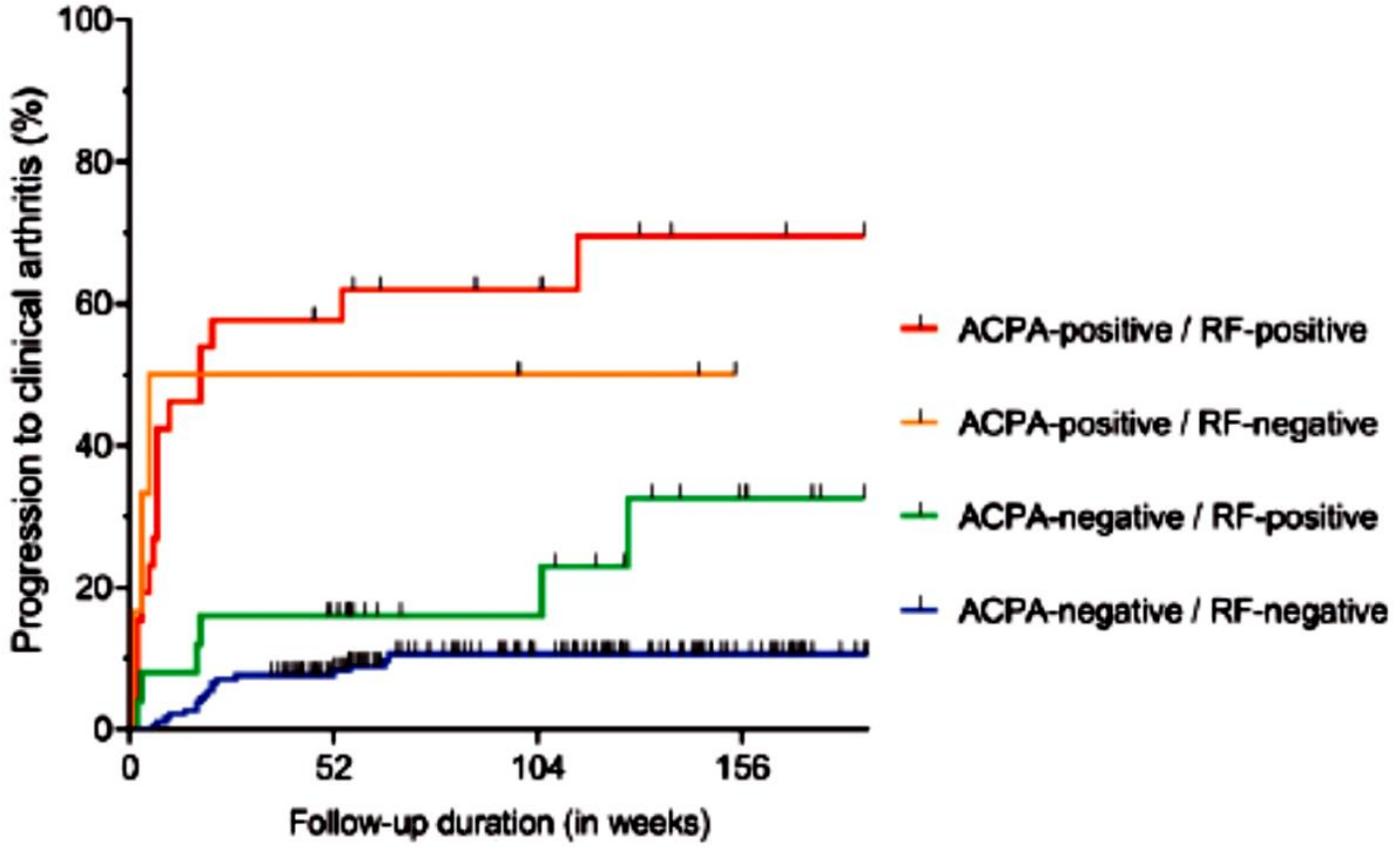
	Sensitivity	Specificity
Anti-CCP1	53 (10) 54 (41–68)	96 (3) 97 (90–99)
Anti-CCP2	68 (15) 68.5 (39–94)	95 (5) 97 (81–100)
RF	60 (18) 65 (25–95)	79 (15) 81 (31–95)

modified from Ann Rheum Dis 2006;65:845–851.

Pre-Test Probability	1%	50%	75%
RF			
PPV	3.3%	77.4%	91.1%
NPV	99.6%	69.8%	43.5%
Anti-CCP2			
PPV	3.0%	75.5%	90.2%
NPV	99.9%	95.8%	88.4%

RF and ACPA Positivity Associated with Progression to Clinical RA in Patients with Arthralgia

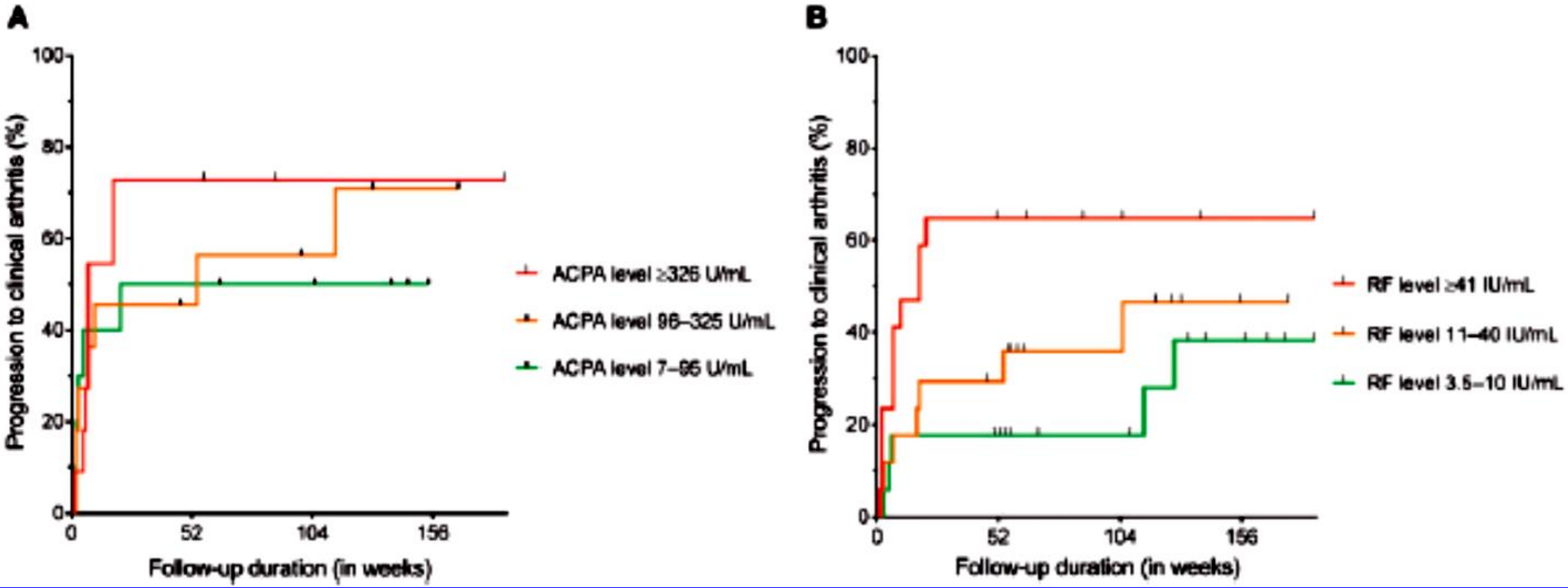
FIG. 2 Kaplan–Meier plots with combinations of ACPA and RF and risks for progression to clinical arthritis



doi:10.1093/rheumatology/kex340doi:10.1093/rheumatology/kex340

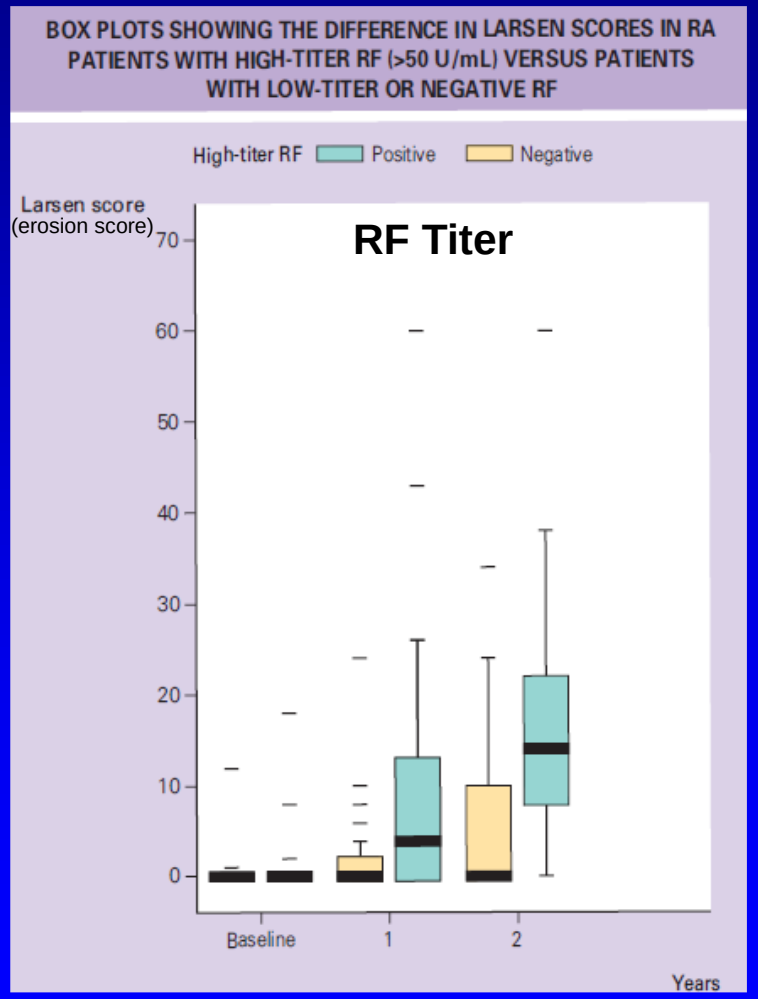
Increased ACPA and RF Levels Associated with Increased Risk for Progression to RA in Patients with Arthralgia

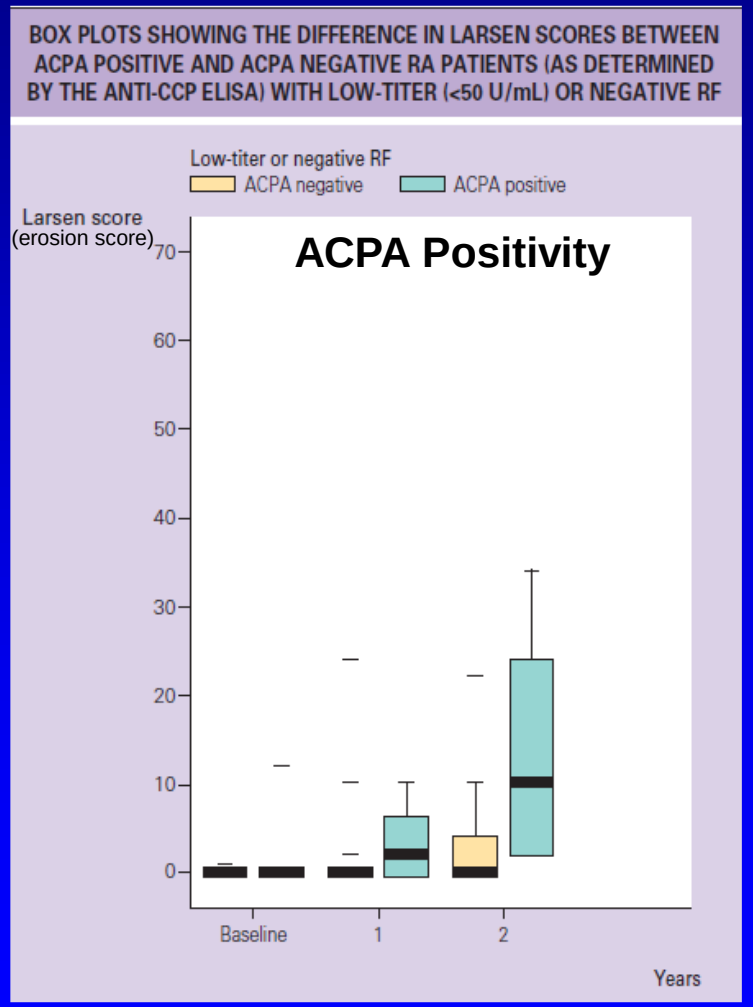
FIG. 3 Kaplan–Meier plots with (A) ACPA levels in ACPA-positive patients and (B) RF levels in RF-positive patients and risks for progression to clinical arthritis



doi:10.1093/rheumatology/kex340doi:10.1093/rheumatology/kex340

High Titer RF and ACPA Positivity Associated with an Increased Risk of Erosive RA





Diagnosing SLE…and Other Things:

The Antinuclear Antibody

2019 EULAR/ACR Classification Criteria for SLE

Entry criterion

Antinuclear antibodies (ANA) at a titer of ≥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 ≥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
Constitutional		***Antiphospholipid antibodies***	
Fever	2	Anti-cardiolipin antibodies OR	
Hematologic		Anti-β2GP1 antibodies OR	
Leukopenia	3	Lupus anticoagulant	2
Thrombocytopenia	4	***Complement proteins***	
Autoimmune hemolysis	4	Low C3 OR low C4	3
Neuropsychiatric		Low C3 AND low C4	4
Delirium	2	***SLE-specific antibodies***	
Psychosis	3	Anti-dsDNA antibody* OR	
Seizure	5	Anti-Smith antibody	6
Mucocutaneous			
Non-scarring alopecia	2		
Oral ulcers	2		
Subacute cutaneous OR discoid lupus	4		
Acute cutaneous lupus	6		
Serosal			
Pleural or pericardial effusion	5		
Acute pericarditis	6		
Musculoskeletal			
Joint involvement	6		
Renal			
Proteinuria >0.5g/24h	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.

-the diagnosis of SLE is clinical and based upon the history, exam findings, and laboratory results

-SLE is not diagnosed from a single clinical finding or single laboratory test

Arthritis Rheumatology. 2019. 71:1400/

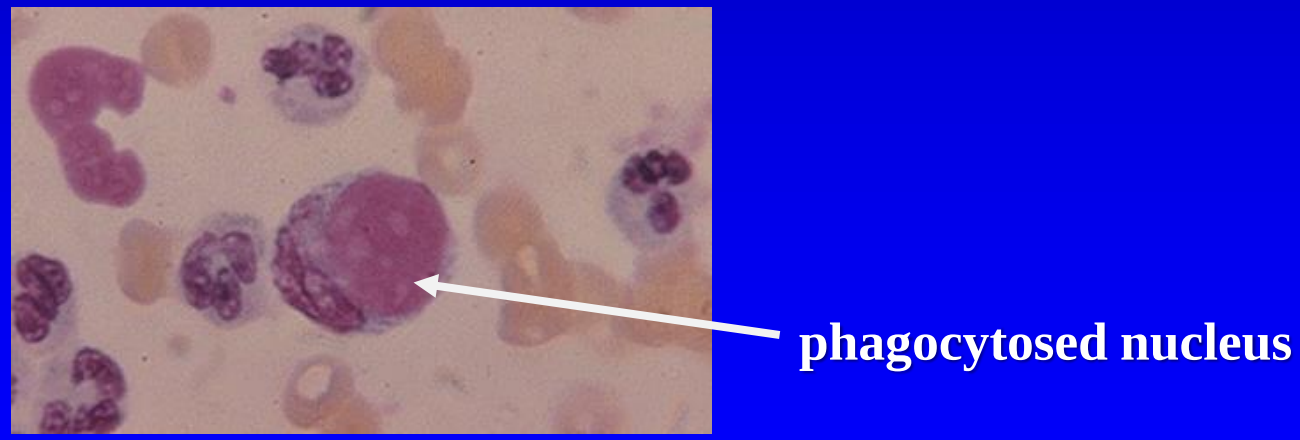
What Are Antinuclear Antibodies (ANA)?

-Hargraves and Morton 1948:

identified the LE cell, a PMN leukocyte with a phagocytosed nucleus found to be increased in lupus patients;
subsequently found to be caused by antinuclear antibody binding to nuclei promoting their phagocytosis by PMNs (opsonization)

-the ANA detects antibodies directed against several different antigens in the nucleus

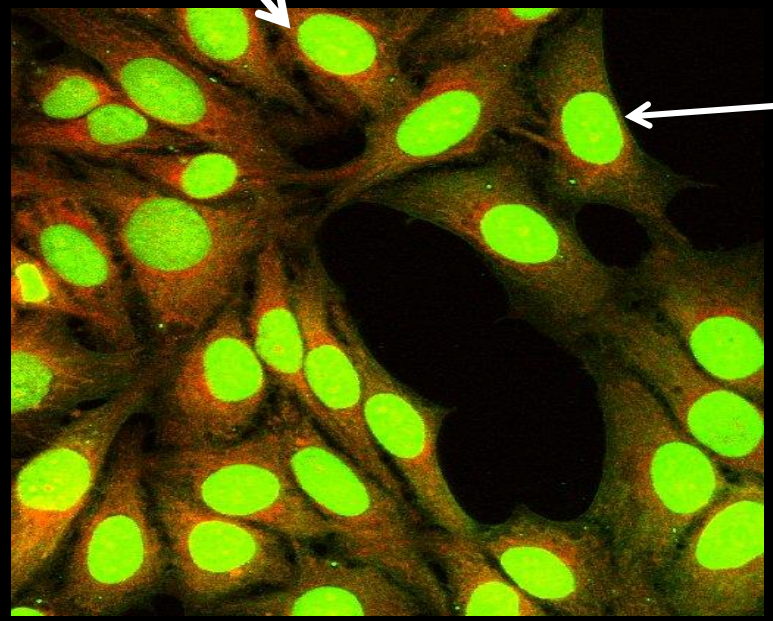
-almost always positive in patients with lupus, but they are not specific for lupus;
positive ANAs can be found in many other rheumatologic and non-rheumatologic conditions



LE Cell

The Gold Standard of ANA Detection: Direct Immunofluorescence Assay (ANA DIFA)

human HEp-2 cells



immunofluorescent nucleus (positive signal)

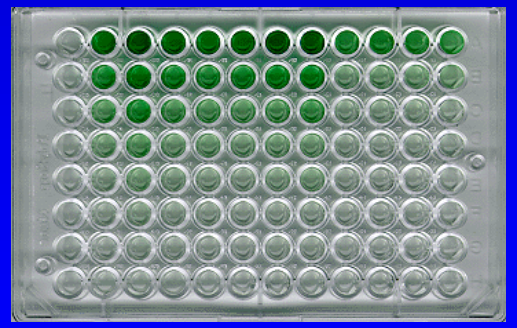
-ANAs bind to the nucleus and are detected by a labelled anti-Ig antibody
-the titer is the highest serial dilution of serum giving a positive signal
-an abnormal result is usually a titer of ≥1:40 (≥1:80 by new criteria)
-there are many different antigens in the nucleus that can detect many different ANAs
- cost ~$29-$159

DIFA ANA vs. ELA ANA

-some clinical labs may perform a screening ANA test by an ELISA assay

-although easier and quicker, it is less specific

-it must be followed up by a confirmatory DIFA ANA

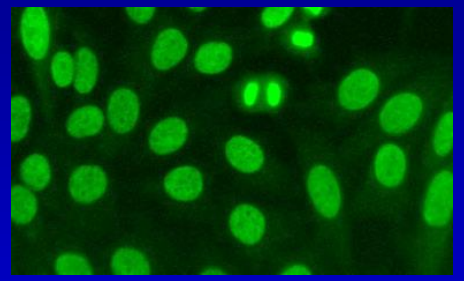


ELISA Assay for detecting and measuring specific ANAs:

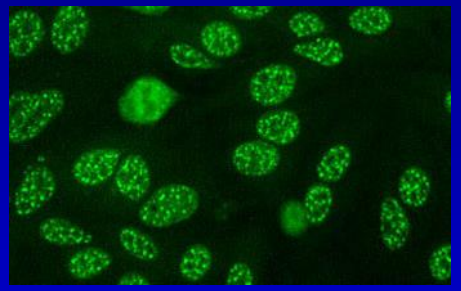
- -screening ANA: each well contains a mixture of nuclear antigens bound to the bottom of the well
- -specific ANA testing: an individual specific nuclear antigen is bound to the well
- -although sensitive, non-specific binding increases the rate of false positive results (esp. for anti-dsDNA ABs

ANA Patterns and Specific Antinuclear Antibodies

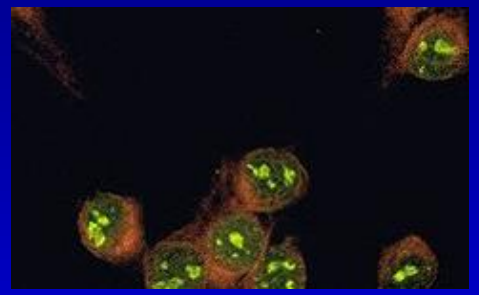
Staining patterns



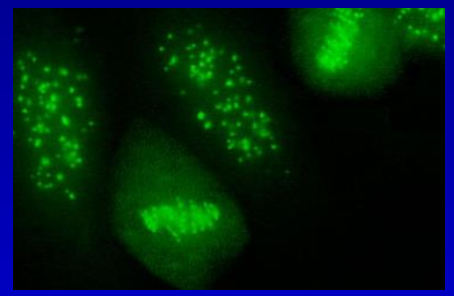
Homogenous



Speckled



Nucleolar

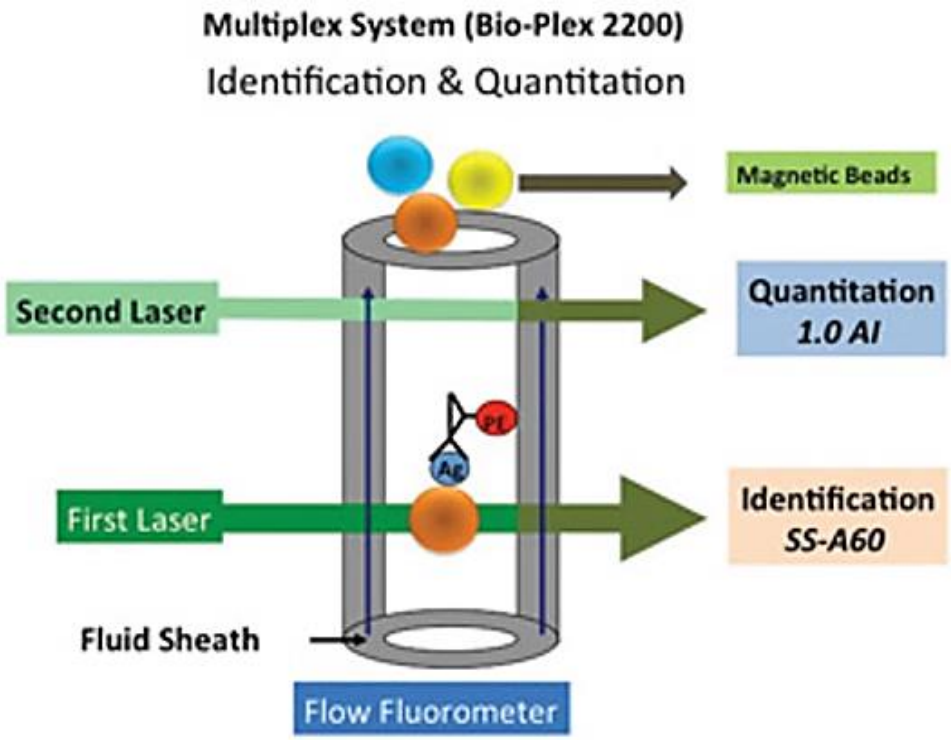


Centromere

-different patterns of staining can be associated with different types of ANAs (e.g. homogenous pattern and anti-DNA antibodies, speckled pattern and anti-ribonucleoprotein antibodies)

-specific antinuclear antibodies are associated with certain SLE clinical features and other rheumatologic diseases

Mulitplex Bead Assay for Detection of ANAs



Problems:

- **-multiplex immunoassays for autoantibodies differ significantly depending on the manufacturer or kits**
- **-do not always show the intended specificity and sensitivity**
- **-lack of standardization of test methodology**
- **-lack of validation against a standard assay for many of these**

ANA DIFA Testing Results Can Vary From Lab to Lab

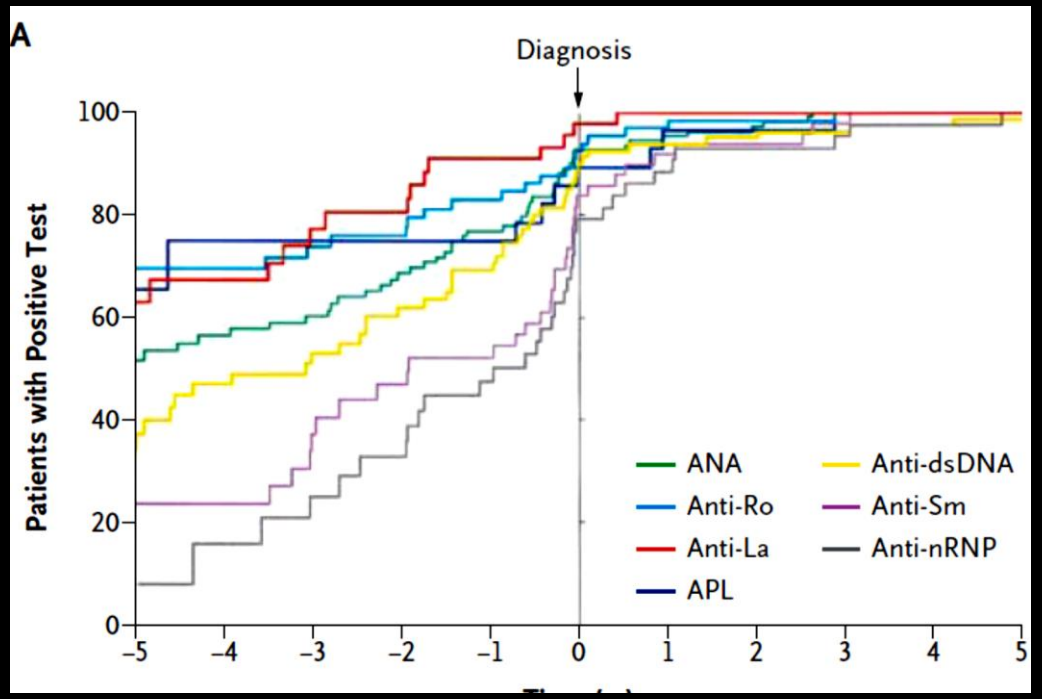
Individual Patient Serum Samples Tested by Two Different Labs

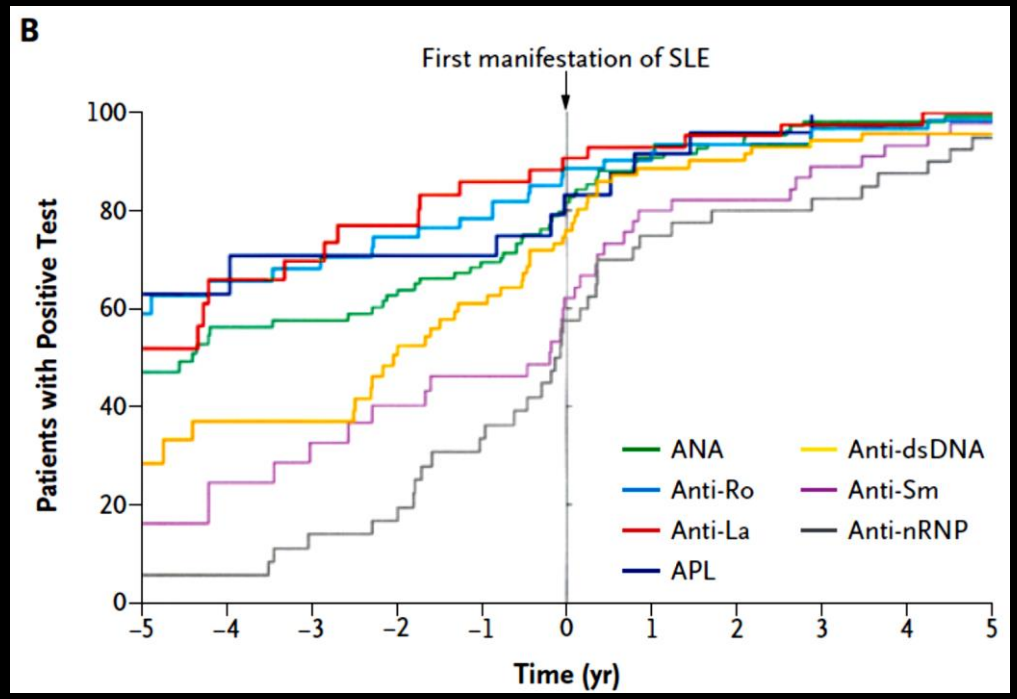
Negative	9	78
≥1:40 and <1:80	12	1
≥1:80 and <1:160	10	3
≥1:160 and <1:320	30	1
≥1:320 and <1:640	14	3
≥1:640 and <1:1280	18	11
≥1:1280 and <1:2560	4	4
≥1:2560	4	0
Total	101	101

ANA results by titer at laboratories 1 and 2. This table demonstrates that in this patient cohort, ANA titers drawn at laboratory 1 were much more likely to be positive

Clin Rheumatol. Antinuclear antibody testing: discordance between commercial laboratories. Abeles AM et. al. 2016. 35:1713.

ANAs Can Develop Years Before SLE Clinical Symptoms And Findings Occur





N Engl J Med 2003;349:1526.

Some Limitations of using the ANA for the early diagnosis of SLE:

- **not everyone with a positive ANA goes on to develop SLE**
- **early symptoms may not be associated with a positive ANA**
- **currently no other available tests to better stratify risk of progressing to SLE**

Rheumatic Disease ANA Associations

Rheumatic Diseases (sensitivity: % with positive ANA)

- -systemic lupus erthematosus (99%)
- -drug-induced lupus (95-100%)

highly sensitive for SLE

- -scleroderma/systemic sclerosis (60-80%)
- -mixed connective tissue disease (100%)
- -polymyositis/dermatomyositis (61%)
- -Sjogren's syndrome (40-70%)
- -rheumatoid arthritis (30-50%)

positive in many other rheumatologic diseases: not very specific for any given rheumatic condition

specificities from 49-92%

Schur. The Rheumatologist. February, 2009.

Non-Rheumatic ANA Associations

Non-Rheumatic Conditions (further contributing to its non-specificity)

- -normal individuals:
  - females>males, increasing age (20% in those >70yrs), healthy relatives of patients with SLE (15-25%), pregnancy
- -liver disease
- -pulmonary disease
- -chronic infections
- -hematologic conditions
- -malignancies
- -other

Healthy ("Normal") Persons	
≥1:40	20-30
≥1:80	10-12
≥1:160	5
≥1:320	3

titers tend to be more mildly elevated with non-rheumatic conditions

Salzman BE et al. Clinics in Family Practice. 7:335-358. 2005.

PPV and NPV of the ANA for SLE

Pre-Test Probability*	0.4%	50%	75%
ANA			
PPV	4.69%	92.50%	97.40%
NPV	>99.99%	97.87%	93.88%

***for an ANA titer of 1:80, sensitivity of 99% and specificity of 92%**

-the high ANA sensitivity for SLE makes a <u>negative result very helpful for ruling out SLE</u>

-the lower ANA specificity for SLE requires a higher pretest probability for it to be helpful in the diagnosis of SLE

Specific Antinuclear Antibodies

Disease	*Anti-dsDNA	Anti-RNP	*Anti-Sm	Anti-SSA	Anti-SSB	Anti centromere	Anti-SCL70	Anti-Jo1
SLE	60%	30%	30%	30%	15%	rare	-	-
RA	-	-	-	Rare	rare	-	-	-
MCTD	-	high titer >95%	-	Rare	rare	rare	-	-
Systemic Sclerosis	-	low titer	-	Rare	rare	10-15%	20-30%	-
Limited Sclerosis	-	-	-	-	-	60-90%	10-15	-
Sjogren's Syndrome	-	rare	-	70%	60%	-	-	-
Polymyositis	-	-	-	-	-	-	-	20-50%

*The anti-dsDNA and anti-Sm antibodies are very specific for SLE (not typically associated with other rheumatologic diseases). That is why they are included in the immunologic criteria in the SLICC and 2019 EULAR/ACR Classification Criteria.

Antinuclear Antibody SLE Associations

Antibody	Frequency (%)	Clinical Associations
dsDNA	60-90	high sensitivity and specificity- helpful diagnostically for SLE; correlates with disease activity (esp. nephritis)
ssDNA	90	low specificity, not helpful diagnostically
Histones	50-70	drug-induced lupus
Ro(SS-A)	20-60	neonatal lupus (with anti-LA), photosensitivity, subacute cutaneous lupus,
La(SS-B)	15-40	neonatal lupus (with anti-Ro)
Sm	10-30	high specificity for SLE (low sensitivity)- helpful diagnostically; no SLE associations
RNP	10-30	no associations with SLE subsets
ribosomal-P	10-15	specific for SLE; ?association with psychiatric disease, particularly depression
neuronal (detected in the CSF)	10% SLE w/o neuropsychiatric 75% SLE w/ neuropsychiatric	? neuropsychiatric lupus
NMDA receptor		? association with neuropsychiatric lupus

The Clinical Utility of a Positive ANA Test Result:

Results of Referrals for Subspecialty Evaluation of a Recently Ordered Positive ANA

The American Journal of Medicine. 2013. 126:342.

Table 2 Antinuclear Antibody-Associated Rheumatic Diseases Diagnosed in Patients, by Antinuclear Antibody Titer

Reported ANA Titer	No. of Patients	No. of Patients with AARD (and Specific Diagnoses)
≥1:40 (and <1:80)	27	0
≥1:80 (and <1:160)	28	0
≥1:160 (and <1:320)	71	1 (SLE)
≥1:320 (and <1:640)	34	1 (SjS)
≥1:640 (and <1:1280)	31	4 (2 SLE, 2 SjS)
≥1:1280 (and <1:2560)	23	8 (2 SLE, 4 SjS, 1 SSc, 1 UCTD)
≥1:2560 (and <1:5120)	6	2 (1 SSc, 1 SjS)
≥1:5120	7	4 (1 MCTD, 1 SSc, 2 SjS)
No titer	5	1 (UCTD)

Total: 232 21 → 9.1%

SLE: 232 5 → 2.2%

Table 4 Common Reasons for Antinuclear Antibody Test (and Ultimate Diagnoses)

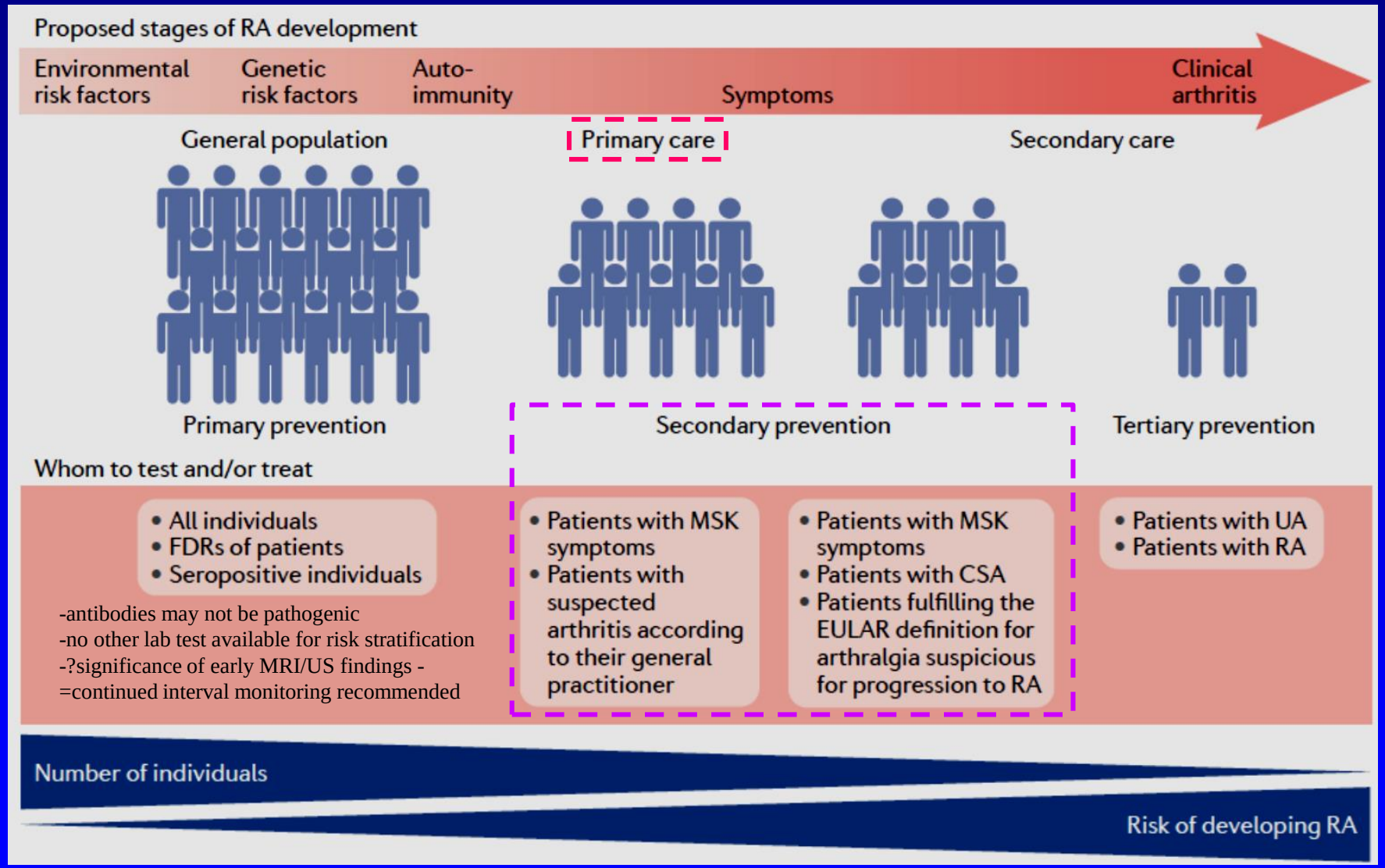
Reason for ANA Test	No. of Patients (% of Cohort)	Ultimate Diagnosis (or Diagnoses)
Widespread pain	54 (23.2%)	Fibromyalgia in 46 (8 with other diagnoses)
Hand pain	21 (9.1%)	Osteoarthritis (7), carpal tunnel syndrome (6) (1 patient with osteoarthritis and carpal tunnel syndrome), rheumatoid arthritis (2), psoriatic arthritis (2), pseudogout (1), trigger finger (1), post-fracture pain (1), mixed connective tissue disease (1), congenital dystonia (1)
Knee pain	9 (3.9%)	Osteoarthritis
Chronic lower back pain	8 (3.4%)	Osteoarthritis, regionalized pain syndrome, ankylosing spondylitis
Chronic headache	5 (2.2%)	Headache syndrome
Unilateral hip pain	4 (1.7%)	Trochanteric bursitis (3), hip osteoarthritis (1)
Unilateral shoulder pain	3 (1.3%)	Mechanical shoulder pain (tendinopathy/bursitis)
Fatigue	3 (1.3%)	Chronic fatigue (no clear cause)
Thinning hair	2 (0.9%)	Age-related hair loss

ANA = antinuclear antibody.

Miscellaneous (n = 1) reasons for an ANA test (and ultimate diagnoses) included chronic rhinorrhea (chronic rhinitis), intermittent toe pain and swelling (podagra/gout), right-sided jaw pain (temporomandibular joint syndrome), groin rash (tinea cruris), unilateral foot pain (immediately after foot surgery), 3 days of generalized arthralgias (immediately after zoledronic acid infusion), loss of motion in the left fourth digit (Dupuytren's contracture), left hip and left shoulder pain (that began after falling from a ladder), and elevated erythrocyte sedimentation rate (active osteomyelitis in a diabetic foot).

-low pretest probability for an autoimmune condition
-ANAs ordered indiscriminately

Autoantibody Testing and Evaluation/Management of Rheumatologic Diseases



Nature Review Rheumatology. Van Steenbergen HW et. al. 2018. 14:32.

Take Home Points

1. most rheumatologic autoimmune/inflammatory diseases are uncommon with disease prevalences of ≤1%

2. the RF and ACPA for diagnosing RA and ANA for diagnosing SLE or other autoimmune syndromes are most helpful in the setting of a moderately raised pretest probability
   - they should not be used as screening tests
   - the history and exam should be the prime determinant of the pretest probability
   - how will positive or negative test results change management?

3. multitest rheumatologic panels should not be ordered (⬆false pos rate) 

4. both the autoantibody and clinical responses can evolve over time: importance of interval follow-up

Medicine is a science of uncertainty and an art of probability.

-Sir William Osler