WISELY ORDERING RHEUMATOLOGIC ANTIBODIES

Benjamin Wang, M.D., FRCPC
Division of Rheumatology
Mayo Clinic
Jacksonville, FL

Baptist Health South Florida
Boca Raton Regional Hospital
Annual Internal Medicine Conference
March 22-24, 2024

1

DISCLOSURE OF RELEVANT FINANCIAL RELATIONSHIP(S) WITH INELIGIBLE COMPANIES

Benjamin Wang, M.D., faculty for this educational activity, has no relevant financial relationships with ineligible companies to disclose, and has indicated that the presentation or discussion will not include off-label or unapproved product usage.

2

“PROBLEMS WORTHY OF ATTACK PROVE THEIR WORTH BY FIGHTING BACK.”

— PIET HEIN

3

LEARNING OBJECTIVE

- To recognize broad categories of autoimmune antibodies used in diagnosing rheumatic diseases
- To learn the correlation of certain autoantibodies with specific clinical manifestations
- To utilize rheumatology immunologic testing to confirm the diagnosis of autoimmune syndromes

4

OVERVIEW

- Autoantibodies and Disease: clinical strategy
- Associations with specific syndromes
  - SLE and neonatal lupus
  - Other systemic autoimmune diseases
  - Rheumatoid arthritis
  - Myositis
  - Vasculitis and ANCA-associated syndromes
- Case study

5

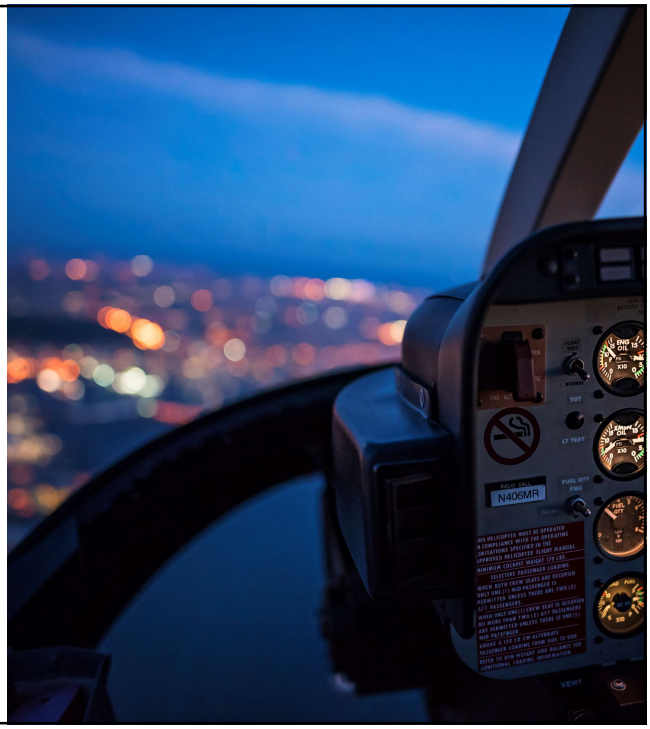
CLINICAL PROBLEM SOLVING IS A PUZZLE WITH MISSING PIECES DERIVED FROM MULTIPLE SOURCES

- **Clinical evaluation**
  - Symptoms
  - Signs
- **Laboratory testing**
  - Organ function
  - Inflammation
  - Immunology
- **Diagnostic testing**
  - Imaging
  - Functional testing
  - Histologic examination
- **Pattern recognition**

6

STANDARD OPERATING PRINCIPLES

- **Be clinically based**
  Confirm diagnoses based on what you see and hear
- **Be hypothesis-driven**
  Avoid “fishing expeditions” which can mislead
- **Be aware that disease classification has limitations**
  Many cases will not fit cleanly into established definitions
- **Diagnostic tests add to clinical observations, not replace them**
  No test is absolute or perfect (is RF a test for rheumatoid arthritis? How specific a test is it?)



7

ANTIBODIES IN THE SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASE (SARD) GROUP

ANA PROFILES IN ANA-POSITIVE RHEUMATIC DISEASE

ANTIBODY SPECIFICITY	ACTIVE SLE	MCTD	PSS	CREST	PRIMARY SJOGREN'S	RA	DRUG-INDUCED SLE
ANA	>95%	>95%	70-90%	60-90%	>70%	40-50%	100%
Anti-dsDNA	60%	Negative	Negative	Negative	Rare	Rare	Negative
Anti-Sm	30%	Negative	Negative	Negative	Negative	Negative	Negative
Anti-RNP	30%	>95% (high titer)	Common (low titer)	Negative	Rare (low titer)	Rare	10-20% (low titer)
Anti-Centromere	Rare	Rare	10-15%	60-90%	Negative	Negative	Negative
Anti-Ro (SS-A)	30%	Rare	Rare	Negative	70%	10-15%	Negative
Anti-La (SS-B)	15%	Rare	Rare	Negative	60%	Rare	Negative
Anti-Nucleolar	Occasional	Negative	Common	Negative	Occasional	Rare	Negative
Anti-Scl-70	Rare	Negative	10-20%	Negative	Negative	Negative	Negative
Anti-Histone	24-95%	Occasional	Occasional	Occasional	Occasional	20%	Procainamide: 67-100% Sensitivity Hydralazine: 50-100% Sensitivity

Abbreviations: SLE – systemic lupus erythematosus; MCTD – mixed connective tissue disease; PSS – progressive systemic sclerosis (here meaning diffuse scleroderma); CREST – calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly; telangiectasia (here meaning limited scleroderma); RA – rheumatoid arthritis

Morris, R. RDL Laboratories, Inc. Los Angeles, CA

8

CLINICAL SYMPTOMS OR SIGNS MAY RAISE CLINICAL SUSPICION FOR AUTOIMMUNE DISEASES

	Higher Suspicion (specific)	Lower Suspicion (non-specific)
Arthritis	Specific swollen joints or tendons on exam	Joint pain without swelling or prolonged AM stiffness (<30')
Rash	Objective inflammatory rash	Transient rashes
Sicca	Sipping water constantly, dental issues, corneal abrasion	Non-severe dry eyes, dry mouth
Raynaud's	At least bluish discoloration on cold exposure, tri-color changes	Pain with cold exposure, no color change
Photosensitivity	Objective photosensitive rash	Fatigue after sun exposure
Alopecia	Patchy, moth eaten hair loss	Generalized hair loss, receding hairline, androgenic pattern
Renal	Decreased function, heavy proteinuria, inflammatory casts, edema	Stable renal function, trace/1+ proteinuria, edema, flank pain, recurrent UTIs
Serositis	Documented pericarditis, pleuritis	Nonspecific chest pain

9

GENERAL CORRELATIONS BETWEEN CLINICAL FEATURES AND SPECIFIC ANTIBODIES

CLINICAL MANIFESTATION	SEROLOGIC TEST
Skin, joint, mucous membrane	anti-SSA, SSB
Nephritis	anti-dsDNA, anti-chromatin, anti-Smith, low C3 / C4
Sclerodactyly (skin thickening)	anti-centromere, anti-Scl70, anti-RNP, anti-RNA polymerase III
Raynaud's	anti-centromere, anti-Scl70, anti-RNP, anti-RNA polymerase III
Thrombosis, pregnancy loss	antiphospholipid, anti-β_2 glycoprotein I, lupus anticoagulant
Cytopenias	dsDNA, Smith,
Interstitial lung disease	RF, ANA, anti-Jo-1, myositis-related antibodies
Neonatal lupus	anti-SSA, anti-SSB

10

ORDERING ANTIBODY TESTS ACCORDING TO THE CLINICAL PICTURE

	HIGHER SUSPICION (SPECIFIC)	LOWER SUSPICION (NON-SPECIFIC)	APPROPRIATE ANTIBODIES
Arthritis	Specific swollen joints or tendons on exam	Joint pain without swelling or prolonged AM stiffness (<30')	RF, CCP ANA, SSA, SSB, dsDNA
Rash	Objective inflammatory rash	Transient rashes	ANA, SSA, SSB
Sicca	Sipping water constantly, dental issues, corneal abrasion	Non-severe dry eyes, dry mouth	ANA, SSA, SSB Mitochondrial
Raynaud's or skin thickening or esophageal	At least bluish discoloration on cold exposure, tri-color changes; distal skin thickened	Pain with cold exposure, no color change	ANA, RNP, Scl-70, centromere, RNA pol III Myositis-related Abs
Photosensitivity	Objective photosensitive rash	Fatigue after sun exposure	SSA, SSB
Alopecia	Patchy, moth eaten hair loss	Generalized hair loss, receding hairline, androgenic pattern	ANA, SSA, SSB dsDNA, centromere, Scl-70
Renal	Decreased function, heavy proteinuria, inflammatory casts	Stable renal function, trace/1+ proteinuria, edema, flank pain, recurrent UTIs	ANA, dsDNA, Smith C3, C4
Serositis	Documented pericarditis, pleuritis	Nonspecific chest pain	ANA, SSA, SSB, dsDNA

11

SYSTEMIC LUPUS – SOME FINE POINTS

- **Lupus tends to segregate into subsets:**
  - Milder disease, mostly skin, joint involvement: SSA, SSB (+)
  - Severe disease, visceral involvement: Strong ANA, anti-dsDNA, -RNP, C3 low, C4 low
  - Clinically quiescent-serologically active lupus
  - Risk of having lupus with a negative ANA: almost zero (0.14 percent). If anything, these may be anti-SSA/B positive (test if clinically suspect)
- **What is the Extractable Nuclear Antigen (ENA) panel?**
  - Anti-SSA, -SSB
  - Anti-Smith, RNP
  - Anti-Scl70, Jo-1
- **Which antibodies are correlated with disease activity?**
  - ANA is not reliable – up to 1:160 is borderline; Mayo Lab ELISA: 1.1 - 2.9 Units (Weakly Positive)
  - dsDNA antibody and C3, C4 probably best, but changes often occur before clinical manifestations are apparent

12

MORE AUTOANTIBODY-DISEASE CORRELATION

- **Scleroderma**
  - Centromere Ab: 30% of limited scleroderma
  - Scl70 antibody: 30% of diffuse scleroderma
- **Mixed connective tissue disease (MCTD)**

  anti-RNP positive, PUFFY HANDS, Raynaud's phenomenon
- **Overlap syndromes**
  - two or more concomitant autoimmune diseases; mainly a clinical diagnosis, no standard antibody pattern
  - RA-SLE ("Rhupus") (note: Sjogren's syndrome may be positive for ANA, RF, SSA, SSB, and CCP
- **Undifferentiated connective tissue disease**
  - "Incomplete Lupus," "Lupus that doesn't check all the boxes"

13

RHEUMATOID ARTHRITIS

- Rheumatoid factor
- Anti-cyclic citrullinated peptide antibody (CCP)

14

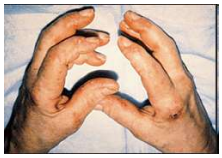
SPONDYLOARTHRITIS

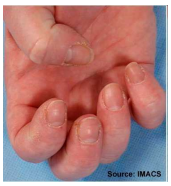
- Negative serology for lupus and other SARDs
- HLA-B27 is the only diagnostic lab marker
  - This allele occurs in 8% of general U.S. population
  - In a patient with typical symptoms, its presence increases the likelihood of true disease to over 95%
- Key Clinical Features
  - Inflammatory back pain
  - Peripheral oligoarthritis (often large joint)
  - Inflammatory skin, ocular (uveitis) or bowel disease
  - Psoriasis

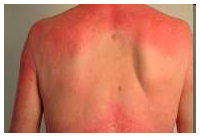
15

MYOSITIS

- Inflammatory, autoimmune myositis such as polymyositis and dermatomyositis may have associated autoantibodies that have prognostic value:
  - Anti-Jo-1: interstitial lung disease, Raynaud's phenomenon, arthritis, and mechanic's hands
  - Anti-Mi2: acute onset of classic DM with erythroderma and the shawl sign
  - Anti-SRP: severe myopathy and aggressive disease







16

Myositis-Specific Abs, Myositis-Associated Abs and Their Clinical Associations

Antibody	Myositis-Specific Abs	Myositis-Associated Abs	PM	DM	Anti-Synthetase Syndrome	Overlap Syndrome	Myositis (Juvenile)	ILD	Arthritis
Mi-2*	4-14%	Not Applicable	Rare	Common	Not Applicable	Common	10%	Rare	Occas
SRP*	4%	Not Applicable	Common	Occas	Not Applicable	Not Described	Uncommon	Common	Common
Jo-1*	20%	Not Applicable	Common	Occas	Marker	Common	Reported	Common	Common
PL-7	1-4%	Not Applicable	Occas	Common	Marker	Common	Reported	Common	Common
PL-12	1-4%	Not Applicable	Occas	Common	Marker	Common	Reported	Common	Common
OJ	1-4%	Not Applicable	Occas	Common	Marker	Common	Reported	Common	Common
EJ	1-4%	Not Applicable	Occas	Common	Marker	Common	Reported	Common	Common
PM/Scl	Not Applicable	8%	Uncommon	Uncommon	Not Applicable	Common	Occas	Rare	Common
Ku	Not Applicable	% Unknown	Not Described	Not Described	Not Applicable	Common	Occas	Not Described	% Unknown
U2 snRNP	Not Applicable	% Unknown	Not Described	Not Described	Not Applicable	Common	Not Described	Not Described	% Unknown

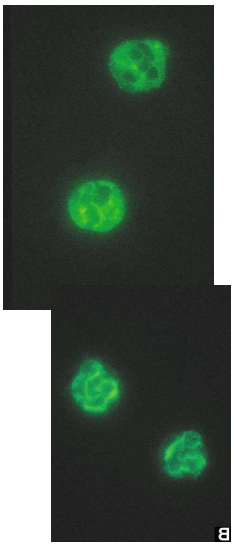
Note: this is very esoteric and not always clinically necessary in primary care

Morris, R. RDL Laboratories, Inc. Los Angeles, CA

17

VASCULITIS AND ANCA-ASSOCIATED SYNDROMES

- c-ANCA and p-ANCA are positive in a limited number of systemic vasculitides
- They are associated with certain finer specific antigens
  - c-ANCA – proteinase 3 (PR3)
  - P-ANCA – myeloperoxidase (MPO), lactoferrin, and other non-MPO antigens
- When c-ANCA and p-ANCA are positive but PR3 and MPO are negative, this may point to the presence of and “atypical ANCA,” which are often seen in inflammatory bowel disease



18

Frequency of C-ANCA, P-ANCA, MPO & PR-3 Abs in Vasculitis

DISEASE CATEGORY	C-ANCA	P-ANCA	ANTI-MPO	ANTI-PR3
WEGENER'S GRANULOMATOSIS Active - generalized Active - limited	 3-4+ 2-3+	 1+ Occasionally	 1+ <1+	 3-4+ 2-3+
Idiopathic Necrotizing and Crescentic Glomerulonephritis without Immune deposits (Pauci-Immune)	Rare	4+	3-4+	Rare
Microscopic Polyarteritis	1+	2-3+	2-3+	1+
Churg-Strauss Syndrome	1+	2+	2+	1+
Classic Polyarteritis Nodosa Polyangitis Overlap Syndrome	Rare 1+	Rare 1+	Rare 1+	Rare Rare
INFLAMMATORY BOWEL DISEASE Ulcerative Colitis Crohn's Disease	 Absent Absent	 2-4+ 1+	 Absent Absent	 Absent Absent
GRADING SYSTEM: 1+(15-25%); 2+(26-50%); 3+(51-75%); 4+(76-100%)				

Morris, R. RDL Laboratories, Inc. Los Angeles, CA

19

CLINICAL CASE

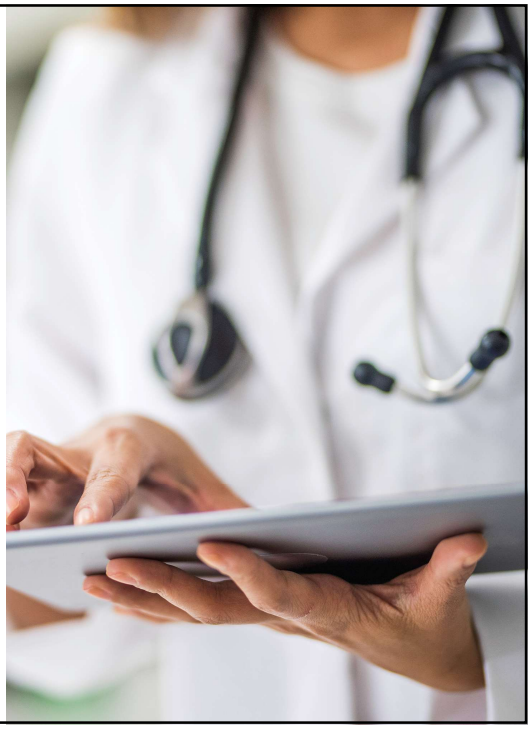
A 68-year-old woman presents with severe dryness of the eyes and mouth. A review of medications does not disclose any strongly implicated drugs that may be causing this.

She also complains of joint pain with swelling involving her hands and wrists.

Physical examination is remarkable for a mild left foot drop and pinprick sensory deficit along the left L5 dermatome. Her mucous membranes are severely dry, and several dental caries are seen. She has a faint bilateral conjunctival injection. There is mild synovitis of the right wrist and 2 MCP joints of each of the right and left hand. There is no palpable lymphadenopathy.

Considering that she may have a systemic autoimmune disorder, what would your diagnostic approach be?

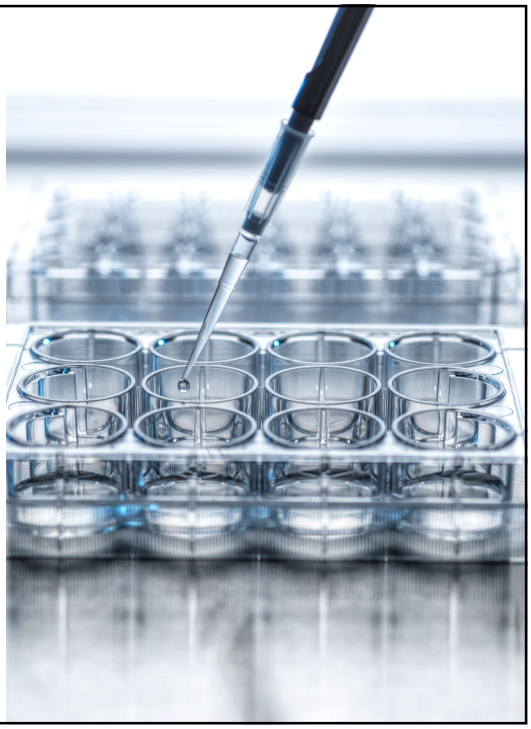
A. Consider the possibility of systemic lupus: obtain ANA, dsDNA, ENA panel (SSA, SSB, RNP, Smith)
B. Consider the possibility of primary Sjogren's Syndrome: SSA, SSB, ANA, RF
C. This may be RA with secondary Sjogren's: RF, CCP
D. All of the above may apply – test all of the above antibodies



20

CONCLUSION

- The immunology lab can be powerful to define diseases but is often a source of confusion
- The lab must be applied with knowledge of the clinical scenario, understanding what the clinical likelihood of disease is; the lab tests are used to confirm or refute the clinical suspicion
- Knowing the range of antibody tests, the symptoms and signs of autoimmune diseases, and the associations between the two will aid pattern recognition to arrive at the diagnosis of these syndromes



21

QUESTIONS & ANSWERS

22