Making the Most of the Immunology Laboratory in the Rheumatic Diseases

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Overview

- Autoantibodies and Disease
- Associations with specific syndromes
  - SLE and neonatal lupus
  - Other connective tissue diseases
  - Rheumatoid arthritis
  - Myositis
  - Vasculitis and ANCA-associated syndromes
- Case studies
Clinical Problem Solving is Often an Puzzle with Missing Pieces
Two Maxims

▪ Acknowledge the limitations of disease definitions, recognizing that there are often exceptions (especially in autoimmune rheumatic diseases)
  ▪ There are many situations that will fall outside of the defined borders of known diseases or not fit cleanly into established definitions

▪ Diagnostic tests are applied to add to the clinical observation and to test the clinical hypothesis, not replace them
  ▪ There are seldom absolutely perfect tests or diagnostic criteria (is RF a test for rheumatoid arthritis? How specific a test is it?)
“Gathering the pieces of the puzzle” follows these general principles:

- Use clinical information: history, physical examination – still the key to the diagnosis
- Laboratory testing can be intimidating: realize the strengths and limitations of tests
- Pattern recognition: using experience to know what the picture will likely be – you almost never get “all the pieces”
### Tool #1: Knowing the Range of Measured Autoantibodies

#### 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

#### Ana Profiles in ANA-Positive Rheumatic Disease

<table>
<thead>
<tr>
<th>Antibody Specificity</th>
<th>Active SLE</th>
<th>MCTD</th>
<th>PSS</th>
<th>CREST</th>
<th>Primary Sjogren’s</th>
<th>RA</th>
<th>Drug-Induced SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>70-90%</td>
<td>60-90%</td>
<td>&gt;70%</td>
<td>40-50%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>60%</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Rare</td>
<td>Rare</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>30%</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>30%</td>
<td>&gt;95% (high titer)</td>
<td>Common (low titer)</td>
<td>Negative</td>
<td>Rare (low titer)</td>
<td>Rare</td>
<td>10-20% (low titer)</td>
</tr>
<tr>
<td>Anti-Centromere</td>
<td>Rare</td>
<td>Rare</td>
<td>10-15%</td>
<td>60-90%</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Ro (SS-A)</td>
<td>30%</td>
<td>Rare</td>
<td>Rare</td>
<td>Negative</td>
<td>70%</td>
<td>10-15%</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-La (SS-B)</td>
<td>15%</td>
<td>Rare</td>
<td>Rare</td>
<td>Negative</td>
<td>60%</td>
<td>Rare</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Nucleolar</td>
<td>Occasional</td>
<td>Negative</td>
<td>Common</td>
<td>Negative</td>
<td>Occasional</td>
<td>Rare</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Scl-70</td>
<td>Rare</td>
<td>Negative</td>
<td>10-20%</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Histone</td>
<td>24-95%</td>
<td>Occasional</td>
<td>Occasional</td>
<td>Occasional</td>
<td>Occasional</td>
<td>20%</td>
<td>Procainamide: 67-100% Sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hydralazine: 50-100% Sensitivity</td>
</tr>
</tbody>
</table>

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.
Tool #2: Knowing the Range of Suspicious Clinical Symptoms

- Use the mnemonic “RASH PAIN O MD” which is derived from 1982 SLE classification criteria for SLE
  - **R** – renal
  - **A** – arthritis
  - **S** – serositis
  - **H** – hematologic
  - **P** – photosensitivity
  - **A** – ANA positive
  - **I** – immunologic
  - **N** – neurologic
  - **O MD** – oral ulcers, malar rash, discoid rash

- Other typical signs and symptoms
  - Raynaud’s, dysphagia, GERD, skin thickening
  - Edema
  - Sinusitis, neuropathy, inflammatory ocular
  - Low complement protein levels
### Tool #3:
**Certain Clinical Symptoms or Signs May Raise or Lower the Clinical Suspicion for Autoimmune Diseases**

<table>
<thead>
<tr>
<th></th>
<th><strong>Higher Suspicion (specific)</strong></th>
<th><strong>Lower Suspicion (non-specific)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal</strong></td>
<td>Progressive azotemia, heavy proteinuria, inflammatory casts</td>
<td>Stable renal function, trace/1+ proteinuria, edema, flank pain, recurrent UTIs</td>
</tr>
<tr>
<td><strong>Arthritis</strong></td>
<td>Specific swollen joints or tendons on exam</td>
<td>Joint pain without swelling, whole hand puffy</td>
</tr>
<tr>
<td><strong>Serositis</strong></td>
<td>Documented pericarditis, pleuritis</td>
<td>Nonspecific chest pain</td>
</tr>
<tr>
<td><strong>Photosensitivity</strong></td>
<td>Objective photosensitive rash</td>
<td>Fatigue after sun exposure</td>
</tr>
<tr>
<td><strong>Raynaud’s</strong></td>
<td>At least bluish discoloration on cold exposure, tri-color changes</td>
<td>Pain with cold exposure, no color change</td>
</tr>
<tr>
<td><strong>Rash</strong></td>
<td>Objective inflammatory rash</td>
<td>Transient rashes</td>
</tr>
<tr>
<td><strong>Sicca</strong></td>
<td>Sipping water constantly, dental issues, corneal abrasion</td>
<td>Non-severe dry eyes, dry mouth</td>
</tr>
<tr>
<td><strong>Alopecia</strong></td>
<td>Patchy, moth eaten hair loss</td>
<td>Generalized hair loss, receding hairline, androgenic pattern</td>
</tr>
</tbody>
</table>
Tool #4: Knowing that there are certain correlations between antibodies and clinical features

- Clinical correlation with certain antibodies exist:
  - Skin, joint, mucocutaneous – anti-SSA, SSB
  - Nephritis – anti-dsDNA, anti-chromatin
  - Scleroderma-like features – anti-centromere, anti-Scl70, anti-RNP
  - Raynaud’s – anti-centromere, anti-RNP
  - Thrombosis, pregnancy loss – antiphospholipid, lupus anticoagulant
  - Interstitial lung disease – RF, anti-Jo-1
  - Neonatal lupus – anti-SSA, -SSB

- Low complement levels (C3, C4) usually signify that immune-complexes are present and are driving an inflammatory process
Systemic Lupus

- Lupus subsets:
  - Milder disease, mostly skin, joint involvement: SSA, SSB (+)
  - Severe disease, visceral involvement: anti-dsDNA, -RNP, C3 low, C4 low
  - Clinically quiescent-serologically active lupus
  - Risk of having lupus with (-)ANA: 0.14 percent. If anything, these may be anti-SSA/B positive

- Extractable Nuclear Antigen (ENA) panel
  - Anti-SSA, -SSB
  - Anti-Smith (not Smooth Muscle!)
  - Anti-RNP

- Serologic markers of disease activity
  - ANA is not reliable – up to 1:160 is borderline; Mayo Lab: 1.1 - 2.9 Units (Weakly Positive)
  - dsDNA antibody and C3, C4 probably best, but changes often occur before clinical manifestations are apparent
The Autoantibody-Disease Correlation

- Many immune-mediated diseases are accompanied by the production of autoantibodies
- Many measured autoantibodies are directly pathogenic, for example
  - Anti-SS-A, -SS-B antibodies (neonatal lupus, congenital heart block)
  - Anti-CCP antibodies (rheumatoid arthritis)
  - Anti-dsDNA antibodies (lupus nephritis)
  - Antiphospholipid antibodies (Hughes’ syndrome)
  - ANCA in vasculitis
- Many measured autoantibodies are not directly pathogenic, and are merely immune epiphenomena
  - ANA (many autoimmune diseases, e.g. lupus, Hashimoto’s)
  - Anti-Smith (SLE)
  - Anti-centromere (limited scleroderma, aka CREST syndrome)
  - Anti-Scl70 (diffuse scleroderma)
The Autoantibody-Disease Correlation

- Scleroderma
  - Centromere Ab: 30% of limited scleroderma
  - Scl70 antibody: 30% of diffuse scleroderma

- Mixed connective tissue disease (MCTD): anti-RNP positive, anti-Smith negative

- Overlap syndromes: mainly a clinical diagnosis, with no standard antibody pattern
  - lupus-like, but “borrows” manifestations from other CTDs (remember that these diseases are in a spectrum, so overlap is common). A mix of autoantibodies are possible

- Undifferentiated connective tissue disease: just does not fit “inside the lines”
Rheumatoid Arthritis

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

- Negative serology for lupus and other “connective tissue diseases”

- 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%
    - Inflammatory back pain
    - Peripheral oligoarthritis (often large joint)
    - Inflammatory skin, ocular (uveitis) or bowel disease
    - Psoriasis
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
Myositis

- Remember that myositis (polymyositis, dermatomyositis) can be a manifestation of SLE and other systemic autoimmune diseases
- Remember drug-related myopathies: hydroxychloroquine, colchicine
- Remember inclusion body myositis in an older male with distal=proximal weakness
- Remember metabolic myopathies with non-inflammatory features
Note: this is very esoteric and not always clinically necessary in primary care
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
<table>
<thead>
<tr>
<th>DISEASE CATEGORY</th>
<th>C-ANCA</th>
<th>P-ANCA</th>
<th>ANTI-MPO</th>
<th>ANTI-PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEGENER’S GRANULOMATOSIS</td>
<td>3-4+</td>
<td>1+</td>
<td>1+</td>
<td>3-4+</td>
</tr>
<tr>
<td>Active - generalized</td>
<td>2-3+</td>
<td>Occasionally</td>
<td>&lt;1+</td>
<td>2-3+</td>
</tr>
<tr>
<td>Active - limited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic Necrotizing and Crescentic Glomerulonephritis without Immune deposits (Pauci-Immune)</td>
<td>Rare</td>
<td>4+</td>
<td>3-4+</td>
<td>Rare</td>
</tr>
<tr>
<td>Microscopic Polyarteritis</td>
<td>1+</td>
<td>2-3+</td>
<td>2-3+</td>
<td>1+</td>
</tr>
<tr>
<td>Churg-Strauss Syndrome</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>Classic Polyarteritis Nodosa</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
</tr>
<tr>
<td>Polyangitis Overlap Syndrome</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>INFLAMMATORY BOWEL DISEASE</td>
<td>Absent</td>
<td>2-4+</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Ulcerative Colitis</td>
<td>Absent</td>
<td>1+</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Crohn’s Disease</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

GRADING SYSTEM: 1+(15-25%); 2+(26-50%); 3+(51-75%); 4+(76-100%)
Case 1

A 30-year-old man sees you with a complaint of multiple joint pain for the last 6 months. He has a history of chronic hepatitis C infection, without cirrhosis or hepatic synthetic dysfunction. He is otherwise healthy.

He describes pain involving the small joints of the hands, wrists, knees, and sometimes ankles. Lately, his feet have also been symptomatic. He complains of being stiff in the morning, for one hour before finding relief, and has noted some swelling especially in the hands and wrists.

He has tried anti-inflammatory medications, with some relief. However, because of his liver condition, he is reluctant to take it.

His clinical examination reveals minor synovial hypertrophy in the MCP and PIP joints. The joints are nontender. There is no overlying erythema. Range of motion is preserved, and there are no deformities. His general medical examination is normal. There are no signs of chronic liver disease. His abdomen is benign. Skin examination reveals no rash or nodules.
You suspect rheumatoid arthritis. What antibody tests will confirm this?

A. Rheumatoid factor (RF) alone will confirm the diagnosis of rheumatoid arthritis

B. HCV antibody confirms rheumatoid arthritis in the presence of hepatitis C infection

C. CCP antibody will be necessary to confirm rheumatoid arthritis in this man

D. There are no useful antibodies in this situation
Case 2

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 the 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 comprehensively (“all the way”)
Case 3

A 37-year-old Caucasian female presents with multiple symptoms and a suspicion of systemic autoimmunity and connective tissue disease. Her local doctors have told her that she has lupus.

For the past year she has been feeling extremely fatigued, and having joint pain in a generalized distribution. She states that she has a rash on her cheeks which is intermittent, and has had several ulcers in her mouth. She does not recall specific joints being swollen, but states that both her hands are diffusely puffy at times. She feels very stiff in the morning, and muscle ache endures for the whole day. She denies Raynaud’s phenomenon, serositis, edema, neuropathy, bruising, and has not had a history of pregnancy loss or vascular thrombosis. Physical exam reveals no synovitis but tender points of fibromyalgia. Mucous membranes are clear, and saliva production appears normal.

Review of outside lab results shows that she has a positive ANA in a titer of 1:80, homogeneous staining pattern. Her doctor also drew an anti-dsDNA and C3 and C4 levels, all of which were negative or normal.

You perform a complete autoimmune panel.

What would be your diagnosis if her anti-SSA and SSB antibodies were positive?

What would be your diagnosis if her ANA came back at 1.2 and everything else was negative?
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.