Autoimmune (AI) Disorders

- Affect up to 50 million people in the U.S.
- 80–100 types, dozens more suspected
- #2 cause of chronic illness

- Women are more likely to be affected than men
- Symptoms overlap and are nonspecific
- Patients with one AI or AI disorder in their family are at higher risk

- Rheumatoid arthritis
- Scleroderma
- Systemic lupus erythematosus
- Sjögren's syndrome
- Systemic sclerosis
- Mixed connective tissue disease
- Polymyositis
- Dermatomyositis

American Autoimmune Related Diseases Association (AARDA). Available at: https://www.aarda.org/news-information/statistics.

What You Do Matters!

- Diagnosis is often delayed (average 5 years)
  - Delay in presentation to PCPs
  - Non-specific presentations
  - Non-availability of one single diagnostic test
  - Multiple tests combined with clinical findings are required to make a diagnosis
- However, prompt diagnosis and treatment of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and other autoimmune diseases will lead to improved long-term prognosis

Kumar K et al. *Rheumatology (Oxford)*. 2007;46:1438-40. Raza K. *Rheumatology (Oxford)*. 2010;49:406-10.

Conditions Associated with a Positive Antinuclear Antibody (ANA) Test

Disease	Sensitivity %	Specificity %
Systemic lupus erythematosus	93 – 95	57
Sjögren's syndrome	48	52
Systemic sclerosis	85	54
Juvenile idiopathic arthritis	57	39
Juvenile idiopathic arthritis with uveitis	80	53
Rheumatoid arthritis	41 – 86	56
Polymyositis/dermatomyositis	61	63
Drug-induced lupus*	NA	NA
Mixed connective tissue disease*	NA	NA

*For both drug-induced lupus and mixed connective tissue disease, the diagnostic criteria require a positive ANA, and therefore specificity and sensitivity cannot be determined.

Scholz J et al. *Clin Chem Lab Med*. 2015;53:1991-2002. Colglazier CL, Sutej PG. *South Med J*. 2005;98:185-91.

ANA IFA vs ANA ELISA: Which Lab Test?

ANA IFA

- Still considered the gold standard by ACR
- Higher titers are generally associated with greater likelihood of AI disease, but do not reflect disease activity
- When positive, results reported as a titer with a particular type of immunofluorescence pattern
- Different patterns are associated with a variety of autoimmune disorders
- Automated tiered testing possible when positive results obtained

ANA ELISA

- More economical
- Allows for large volume of testing
- Less labor-intensive
- Tests for only several biomarkers at a time
- Reports a number for positivity
- Reliability and accuracy system-dependent
- Results in comparison with IFA variable
- Tiered testing possible when positive results obtained

ELISA=enzyme-linked immunosorbent assay; IFA=immunofluorescence.

Meroni PL, Schur PH. *Ann Rheum Dis*. 2010;69:1420-2. Mahler M et al. *Immunopharmacol Immunotoxicol*. 2016;38:14-20.

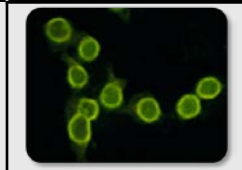
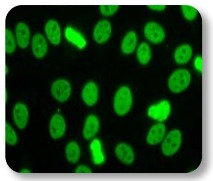
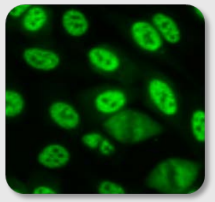
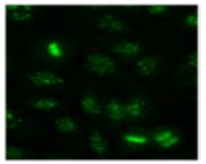
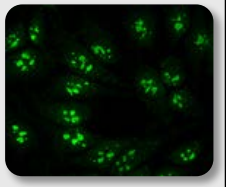
ACR Position Statement: Methodologies of Testing for Antinuclear Antibodies

- ACR supports the ANA IFA test using Human Epithelial type 2 (HEp-2) substrate as the gold standard.
- Laboratories should specify the methods utilized for detecting ANAs.
- Laboratories using alternative multiplex platforms or other assays for detecting ANAs must provide requested data that the assay has the same or improved sensitivity compared to ANA IFA.
- In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, etc, should be standardized according to national (eg, CDC) and/or international (eg, WHO, IUIS) standards.

American College of Rheumatology (ACR). https://www.rheumatology.org/Practice-Quality/Administrative.../Position-Statements

ANA by Immunofluorescence Antibody: Reporting Titer and Pattern

- ANA titers (generally):
  - <1:40 negative
  - 1:40–1:80 low antibody level
  - >1:80 elevated antibody level
- Any titer above 1:40 along with pattern interpretations is reported
- Patterns aid in differential diagnosis

Pattern	Picture	Antibody	Disease State(s)
Rimmed/ peripheral		Anti-DNA	SLE
Homogenous		Anti-DNA Anti-histone	RA & SLE Misc. Disorders
Speckled		Anti-Sm & RNP Anti-Ro & La Anti-Jo-1 & Mi-2 Anti-Scl-70	SLE & SSc PM/DM
Centromere		Anti-centromere	SSc & Sjögren's
Nucleolar		Anti-nucleolar	SLE & SSc

DM=dermatomyositis; lcSSc=limited cutaneous of systemic sclerosis; PM=polymyositis; RA=rheumatoid arthritis; RNP=ribonucleoprotein; SSc=systemic sclerosis; SLE=systemic lupus erythematosus.

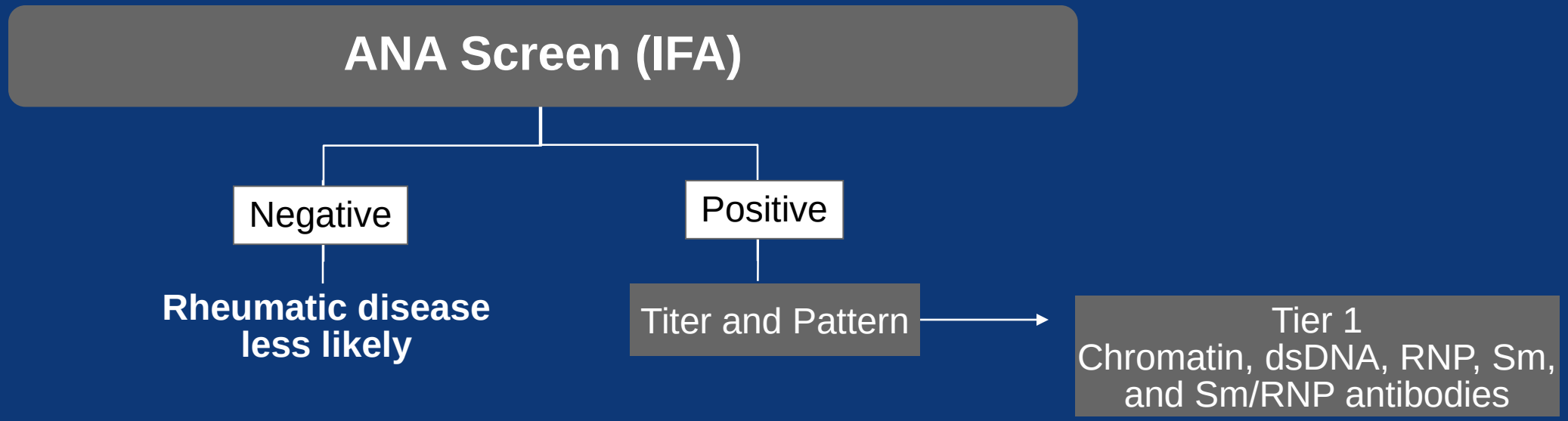
ANA IFA: Testing Subserologies when ANA is Positive

A tiered approach to testing may be used to identify autoimmune (AI) disorders when ANA is **positive**

After Positive ANA	Antibodies tested	Potential Diagnosis when Positive
Tier 1	Chromatin, dsDNA, RNP, Sm, and Sm/RNP	SLE, MCTD
Tier 2	Jo-1, Scl-70, SS-A, and SS-B	Sjögren's, SSc, Polymyositis/Anti-synthetase syndrome
Tier 3	Centromere B and ribosomal P	Limited-cutaneous SSc, Neurologic SLE

MCTD=mixed connective tissue disease.

ANA IFA: Subserologies (cont.)



ANA IFA: Subserologies (cont.)

Tier 1
Chromatin, dsDNA, RNP, Sm, and Sm/RNP antibodies

Negative

Tier 2
Jo-1, Scl-70, SS-A, and SS-B antibodies

Positive

Antibody Test	Systemic Lupus Erythematosus	Mixed Connective Tissue Disease
dsDNA	+ (high specificity)	–
Chromatin	+ (high specificity)	–
Sm	+ (high specificity)	–
Sm/RNP	+	+ (high titer)
RNP	+	+ (high titer)

ANA IFA: Subserologies (cont.)

Tier 2
Jo-1, Scl-70, SS-A, and SS-B antibodies

Negative

Tier 3
Centromere B and ribosomal P antibodies

Positive

Antibody Test	Sjögren's Syndrome	Systemic Scleroderma	Polymyositis
SS-A	+	–	–
SS-B	+	–	–
Scl-70	–	+	–
Jo-1	–	–	+

ANA IFA: Subserologies (cont.)

Tier 3
Centromere B and ribosomal P antibodies

Negative

No evidence of rheumatic disease shown by analytes tested

Positive

Antibody Test	Limited cutaneous SSc	Neurologic SLE
Centromere	+	–
Ribosomal P	–	+

ACR *Choosing Wisely* Recommendation When Considering ANA Testing

American College of Rheumatology

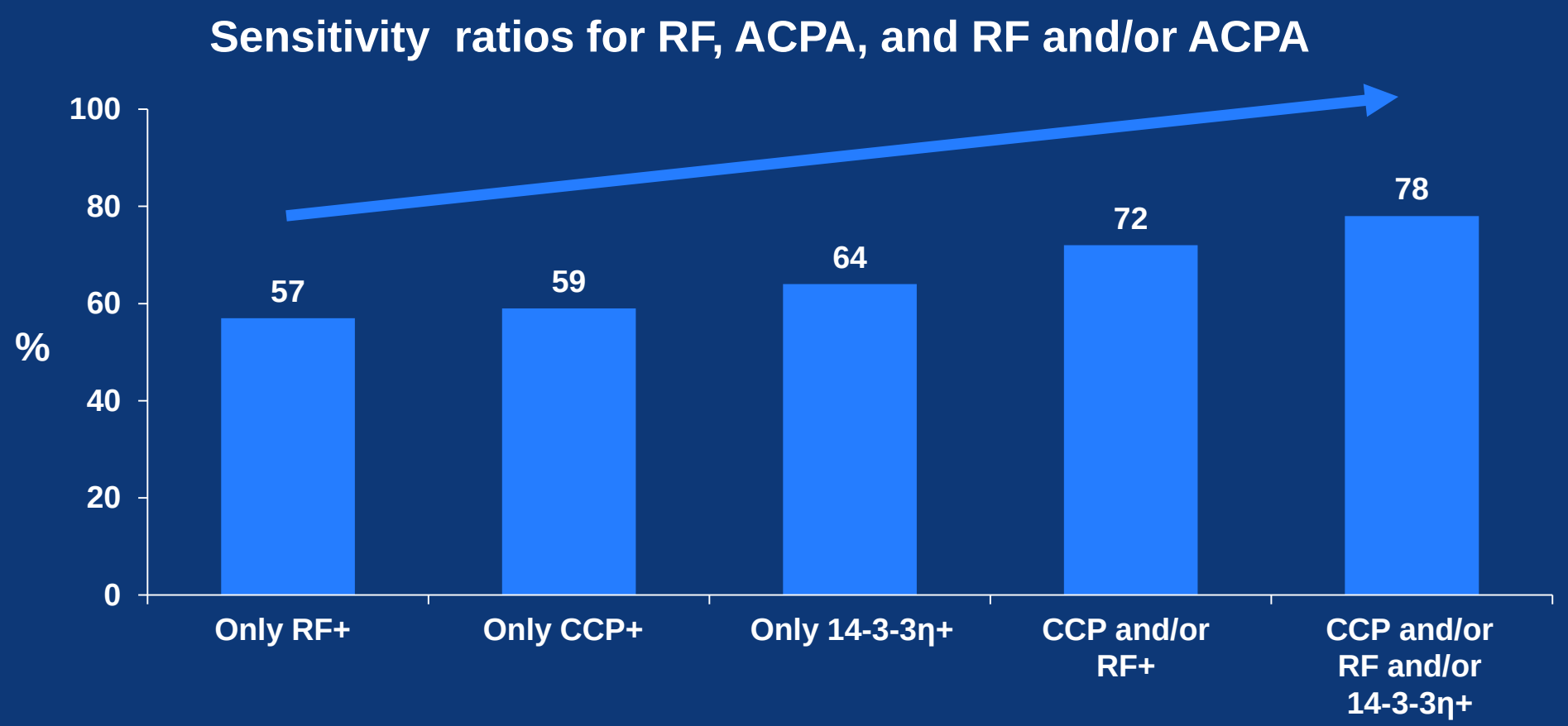
Don’t test ANA sub-serologies without a positive ANA and clinical suspicion of immune-mediated disease

- Tests for anti-nuclear antibody (ANA) sub-serologies are usually negative if the ANA is negative
- Broad testing of autoantibodies should be avoided; instead the choice of autoantibodies should be guided by the specific disease under consideration
  - Exceptions include anti-Jo1, which can be positive in some forms of myositis, or occasionally anti-SSA, in the setting of lupus or Sjögren’s syndrome

ACR. Available at: http://www.choosingwisely.org/societies/american-college-of-rheumatology

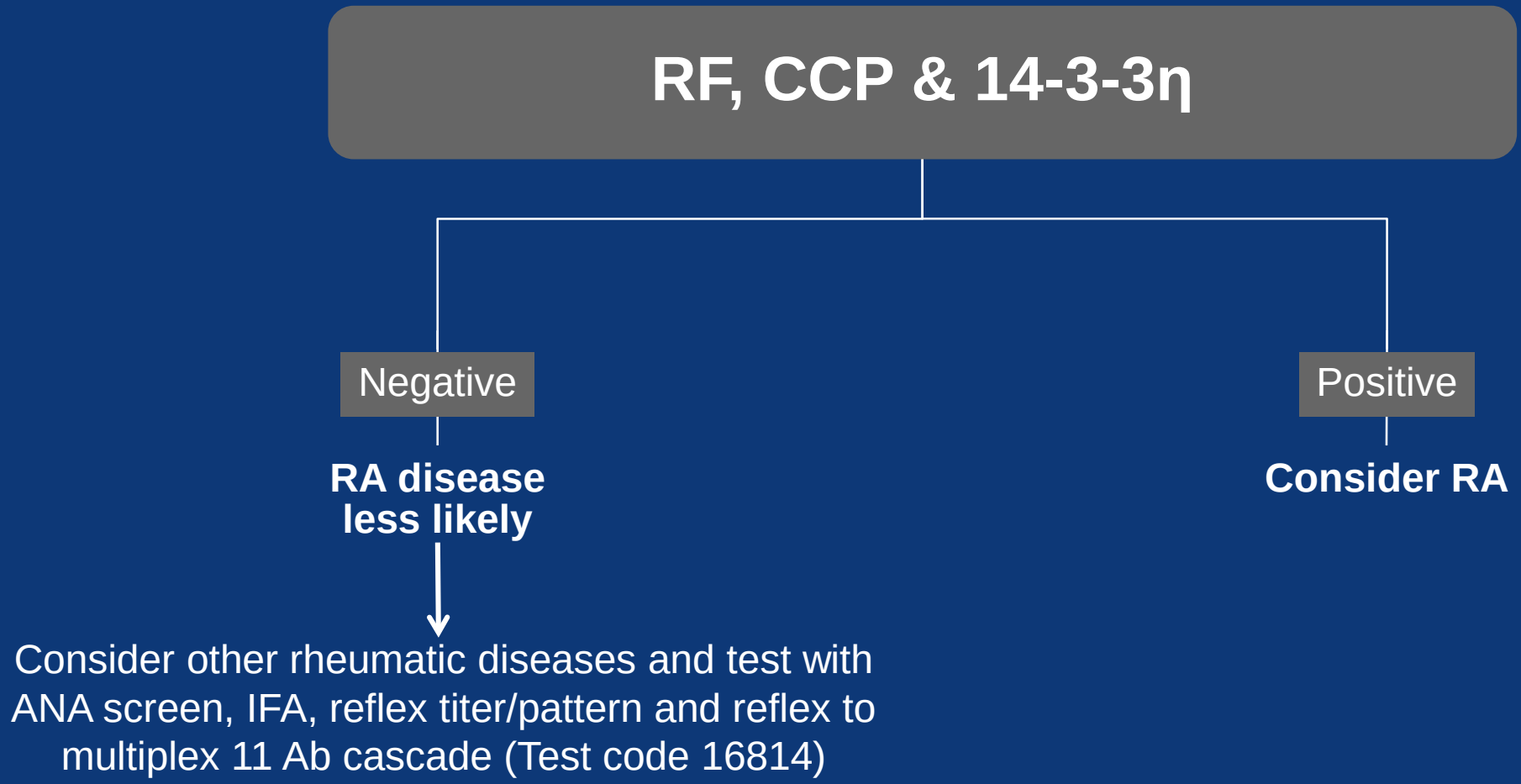
Potential for Combination Serological Assessment To Improve Diagnostic Utility in Early RA Detection

Early RA (n=99) vs healthy controls (n=189)



ACPA=anti-citrullinated protein antibody; CCP=cyclic citrullinated peptide; RF=rheumatoid factor. 14-3-3η = joint-derived proinflammatory mediator found in the synovial fluid and serum of patients with arthritis. Maksymowych WP et al. *J Rheumatol.* 2014;41:2104-13.

New Panel for Suspected Rheumatoid Arthritis



14-3-3η Is also a Marker for Joint Damage…

- A positive 14-3-3η test and higher titers at baseline indicate high joint damage progression risk 5 years out
- **Persistent negative 14-3-3η values show better outcomes**
  - A higher percentage of patients achieved SDAI remission based on a persistently negative test for 14-3-3η

van Schaardenburg et al. American College of Rheumatology meeting. 2013;Abstract L13.

Testing for Sjögren’s Syndrome

Diagnostic

- SS-A (Ro)
  - The main anti-body used to aid in diagnosis
- SS-B (La)
  - The significance of a positive SS-B in the setting of a negative SS-A is debated
- Rheumatoid factor and ANA
  - Each may be positive in about ½ of Sjögren's patients
  - Having both +ANA ≥1:320 and a +RF may be used to aid in diagnosis

Additional testing

- Lip biopsy
- Ocular staining for integrity of tear film
- Schirmer test

Testing for Systemic Lupus Erythematosus (SLE)

Diagnostic

- An ANA is reported to be "**positive**"
  - Titer = 1:320
  - Pattern = Homogeneous or speckled
- Specific antibodies-SS-A/SS-B; Smith (Sm); anti-DNA (dsDNA); anti-chromatin
- Antiphospholipid antibody positivity
- Complement levels (C3/C4/CH50)
- Complete blood count with differential & platelets
- Creatinine & urinalysis
- Erythrocyte sedimentation rate/C-reactive protein (ESR/CRP)

Disease Activity

- Anti-native DNA antibody (dsDNA)
- C3/C4
- CBC w/ diff & platelets
- Creatinine
- ESR/CRP
- Urinalysis, urine protein/creatinine ratio

ANA: Once this is positive, there is no need to repeat

Continue to treat or refer to Rheumatologist

Drugs Implicated in the Development of Drug-induced SLE

Definite	Probable	Possible	Case Reports
Hydralazine Procainamide Isoniazid Methyldopa Quinidine Minocycline Chlorpromazine	Sulfasalazine Antithyroid Anticonvulsants Ethosuximide, Phenytoin Primidone, Valproate Zonisamide, Carbamazepine Statins Lovastatin, Simvastatin Fluvastatin, Pravastatin Atorvastatin Terbinafine Penicillamine Fluorouracil agents Hydrochlorothiazide	Antibiotics Ciprofloxacin Penicillin Tetracycline Nitrofurantoin Cefepime Cefuroxime NSAIDS Ibuprofen Diclofenac Miscellaneous Lithium Interferons Gold salts	Infliximab Etanercept Interleukin-2 Zafirlukast Clobazam Tocainide Lisinopril Bupropion

Cessation of offending therapy offers the best outcome

Garza A. *Pharmacy Times*. Available at: http://www.pharmacytimes.com/publications/issue/2016/january2016/drug-induced-autoimmune-diseases?p=1.
Vasoo S. *Lupus*. 2006;15:757-61. Araújo-Fernández S et al. *Lupus*. 2014; 23:545-53.

Systemic Sclerosis – Diffuse Cutaneous

- Skin tightness proximal to elbows or knees often with truncal involvement
- “Salt and pepper” pigment changes
- Scl-70 positive
- Pulmonary fibrosis
  - Secondary pulmonary arterial hypertension
- Renal/hypertensive crisis
- Raynaud’s phenomenon
- GERD

Testing for Systemic Sclerosis

- Serologic
- ANA positive
  - Titer=1:320
  - Pattern=Speckled, nucleolar, or centromere
- Anti-centromere antibody
  - Limited cutaneous systemic sclerosis (formerly CREST)
  - Associated with higher risk of pulmonary hypertension
- Anti-nucleolar antibody
  - Diffuse cutaneous systemic sclerosis (formerly PSS)
- Anti-Scl-70 (Topoisomerase I)
  - Suggests high risk of pulmonary involvement with fibrosis
- RNA polymerase 3
  - Associated with higher risk of developing diffuse cutaneous disease, renal crisis, and a temporal relationship to malignancy

Continue to treat or refer to Rheumatologist or refer to Scleroderma Center

Rheumatic Diseases to Consider when ANA Test is Negative

- Autoimmune thyroid disease
- Sjögren's syndrome
- Rheumatoid Arthritis
- Ankylosing spondylitis
- Inflammatory bowel disease
- Psoriatic arthritis
- Multiple sclerosis
- Myasthenia gravis
- Autoimmune neuropathies and vasculitis
- Celiac disease and bullous disease
- Gout / pseudogout
- Idiopathic myositis / polymyositis (not associated with MCTD)

When to Refer to a Rheumatologist: Treatment Help…

- Rheumatic manifestations of other diseases
  - Genetic, paraneoplastic, infections, neuropathic, and hematologic disorders
- Other inflammatory/musculoskeletal conditions
  - Complex regional pain syndrome
  - Serum sickness
  - Inflammatory eye disease
  - Vasculitis
  - Osteoarthritis
  - Metabolic bone disease
  - Fibromyalgia
  - Sarcoidosis