

Advances in Autoantibody Testing & Clinical Applications

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- Introduction to the Autoantibody Standardization Committee (ASC)
- Progress in diagnostic technologies used for autoantibody detection
- Short overview of advances in the study of ribosomal P autoantibodies

Standardization of autoantibody testing is a MAJOR ISSUE

ASC Sub-Committee of the:

IUIS – International Union of Immunological Societies

WHO – World Health Organization

AF – Arthritis Foundation

CDC – Centers for Disease Control and Prevention

www.AutoAb.org

ASC: Roles and Responsibilities

- Evaluate & make available consensus autoantibody standards through Centers for Disease Control
- Serve as an education, advisory and advocacy body to physicians, patients and industry
- Undertake and publish research studies

ASC: Reference Standards

- ANA #1 FANA homogeneous (anti-dsDNA)
- ANA #2 FANA speckled (anti-SS-B/La)
- ANA #3 FANA speckled (Sm/RNP)
- ANA #4 Anti-U1 RNP
- ANA #5 Anti- Sm
- ANA #6 FANA Nucleolar (anti-fibrillar)
- ANA #7 Anti-SS-A/Ro
- ANA #8 FANA Discrete Speckled (centromere)
- ANA #9 Anti- Scl-70 (DNA topoisomerase I)
- ANA #10 Anti-Jo1 (histidyl-tRNA synthetase)
- ANA #11 Anti-PM/Scl
- ANA #12 Anti-Ribosomal P
- #13 human IgG anti-cardiolipin monoclonal
- #14 human IgM anti-cardiolipin monoclonal
- ANA #15 Anti-RNA Pol III (in progress)
- #16 cANCA (in progress)
- #17 Anti- cyclic citrullinated peptide CCP (in progress)

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A Perspective on the Relative Importance of Diagnostics & Therapeutics

The first line of defense in clinical medicine is prevention of disease.
If prevention fails, or is not possible, a **TIMELY** and **ACCURATE DIAGNOSIS** is the next most important thing the physician can do.



The Patient of 2015

- **Complaints:**
Fever, rash, arthritis, chest pain, dark urine
- **Objective findings:**
fever, 'butterfly' rash, pericardial friction rub
- **Investigations:**
 - Anemia
 - Pericardial effusion on ECHO
 - Proteinuria, hematuria and RBC casts
- **Diagnosis? Will it matter?**



Personalized Arthritis Therapy

With Blockbuster Drugs in Jeopardy Firms Look To Pharmacogenetics

Catherine Shaffer

Risk reports of an increased risk of heart attack with the two 2-tubulinase drugs Xarelto and Cabomtyl have resulted in a sharp setback for arthritis therapeutics. These drugs are being studied for the treatment of osteoarthritis (OA) and rheumatoid arthritis, both debilitating inflammatory diseases.

It seems that the only hope of keeping these drugs available would be to find some way to identify patients who are susceptible to the cardiovascular effects, possibly by means of a genetic test.



The FDA has recently begun to solicit data on a patient's genetic background as a factor in drug response, and some time this year will issue a list of genes to be tested for pharmacogenetics.

One size does not fit all
THERANOSTICS

Which tests do you order or do?



What are the costs of not doing the right tests?

Autoantibody Profile Snapshot

SLE	Scleroderma	Myositis	Sjögren's Syndrome
chromatin dsDNA histone	topoisomerase I (Scl-70)	Jo-1 other tRNA synthetases	SS-A/Ro 60 Ro 52 SS-B/La
Sm U1-RNP	RNA Pol III PM/Scl	Mi-2 PM/Scl	Muscarinic (M3) receptor
Ribo P proteins assemblyosome	CENP-A,-B To/Th	Signal Recognition Particle	Golgins GW Bodies
ASE-1, HsEg5 CLIP-170	Fibrillarin B23	Exosome	NuMA TS-1 RNA
Cardiolipin β-2 glycoprotein I	Myenteric plexus tPA-fibrin	Ro 52	CENP-C ? α-fodrin ??

1 patient = 1 (meaningful) autoantibody.....rare!

Over 120 autoantibodies in SLE*

Separating the wheat from chaff (Prof. Ian Mackay)

- ◆ Which are PREDICTIVE? **
 - Appear before disease: Sm, CENP, cardiolipin, Ro, Ribo P
- ◆ Which are PATHOGENIC?
 - dsDNA — nephritis
 - Nucleosome — nephritis — renal failure — transplant
 - SSA60/La — neonatal lupus syndrome
- ◆ Which are PROTECTIVE?
 - RNP 70, CENP ??

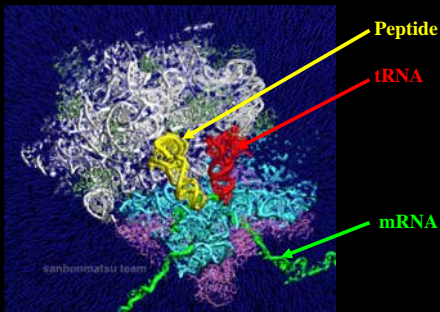
* Shoenfeld, Fritzler, et al. *Sem. Arthritis Rheum.* 34: 501-537, 2004

** Harel & Shoenfeld *Ann. NY Acad Sci.* 1069: 322, 2006.

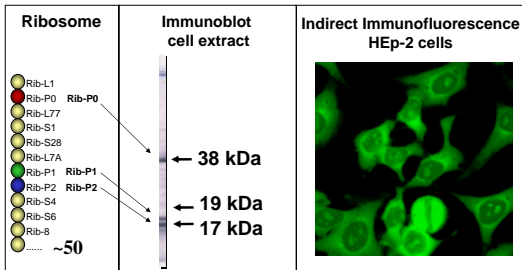
...MORE questions than answers

- ◆ Which are disease specific?
 - Sm D3, C22 Ribo P, dsDNA, topo I
- ◆ Which are "organ" specific?
 - Rib P — psychosis ??
- ◆ Relationship to MHC, SNPs
- ◆ Relationship to cytokines, other biomarkers?
- ◆ Relationship to apoptosis, necrosis, senescence, extracellular exosomes?

Autoantibodies to Ribosomes

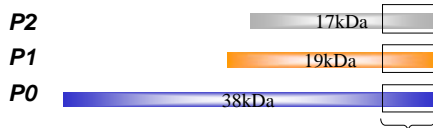


Ribosomal P Autoantigen



Ribosomal P

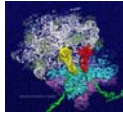
- Immunodominant C-terminal region



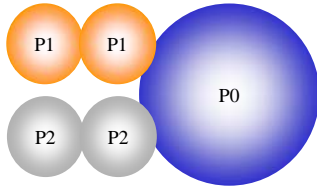
K V E A K K E E * S E E * S D D D M G F G L F D

Mahler M, Kessenbrock K, Raats J, Williams R, Fritzler MJ, Bluthner M. Characterization of the human autoimmune response to the major C-terminal epitope of the ribosomal P proteins. J Mol Med. 2003 Mar;81(3):194-204.

Ribosomal P



Newer assays based on the pentameric complex



Anti-Rib P in SLE

- More than 100 studies - specificity >90%
- Sensitivity 10 – 40% (geographical variable)
- 27.4% anti-Rib-P antibody positive SLE patients are anti-dsDNA negative and anti-Sm negative.
- Some studies have shown that anti-Rib-P correlates with SLE disease activity
- Controversy: anti-Rib-P antibodies are associated with internal organ damage (CNS, kidney, liver) or feature (NPSLE).

Mahler M, Kessenbrock K, Szmyrka M, Takasaki Y, Garcia-De La Torre I, Shoenfeld Y, Hiepe F, Shun-He G, von Muhlen CA, Loch H, Hopff P, Wiik A, Reeves W, Fritzler MJ. International multicenter evaluation of autoantibodies to ribosomal P proteins. Clin Vaccine Immunol. 2006 Jan;13:77-83.

Summary Rib-P Antibodies

Anti-ribosomal P antibodies represent a predictive, highly disease specific and independent variable for the diagnosis and prognosis of systemic lupus erythematosus.

ASC Consensus Recommendation

Anti-Ribosomal P should be added to anti-Sm + anti-dsDNA in the Classification Criteria for SLE



Patient of 2015:

- What is the **DIAGNOSIS**?
- Will it matter??
 - “Rib P-C22, immune complex mediated, Th2 dependant, IL-10 deficient, SNP 4p16-15.2, hRrp41 positive”

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