INTERPRETATION OF IMMUNOLOGICAL TESTS

DR MEGHNA GAVALI
ASSISTANT PROFESSOR,
NIMS,HYDERABAD.

Structure

- Introduction

- ANA
  - When and how to order
  - interpretation
  - Pitfalls
  - Utility-diagn,prognosis ,exclusion of disease

- APL Antibodies

Introduction

- Be familiar with the limits and consequences of the test
- Sensitivity
- Specificity
- PPV
- NPV

- Majority of the tests are not sensitive enough to detect all cases
  - - e.g. a test with 70% sensitivity will not detect 30% of cases

- Similarly, the specificity of the tests is well below 100%;
  - e.g. a 90% specific test will result in 10% false-positive cases.

Introduction

- Although autoantibody testing is a useful adjunct to the diagnosis of **autoimmune connective tissue disease, the tests have limited specificity**

- As a result, the tests will have a low *positive predictive value if they are used* indiscriminately

  that is to say,

- *if the tests are performed on patients who have little or no real clinical evidence of relevant disease, most of the positive results will be found in patients without disease*

Anti-nuclear Antibodies (ANA)

ANA

- Family of autoantibodies to nuclear antigens
- -nuclear,nucleolar or perinuclear
- -cellular components- nucleic acid,chromatin,histone,nuclear and ribonuclear proteins.
- Methods: Indirect Immunoflouresence assay, ELISA

When to order ANA

- ***"No test for ANA and for specific autoantibodies to nuclear antigens should be performed without a clinical evaluation that leads to a presumptive diagnosis"***
- Screening tests for diagnosis of CTD

Kavanaugh et al. *Guidelines for clinical use of the ANA test and tests for specific autoantibodies to nuclear antigens*. Arch Pathol Lab Med. 2000;124:71-81.

Methods

- Gold standard screening test for ANA is by **IIF using Hep 2 substrate.**
- Many antibody tests, including ANA screening in some laboratories, are performed via enzyme-linked immunosorbent assay (**ELISA**) because this method affords higher throughput testing, but often - lower specificity.
- Optimal clinical interpretation of ANA tests requires knowledge of the technique(s) used in each specific case.

ANA ELISA negative IIF positive

- antibodies to
- nucleolar components,
- nuclear matrix,
- proliferating cell nuclear antigen,
- nuclear envelope and
- nuclear pore complexes,
- coiled bodies,
- promyelocytic leukemia domains,
- SS-A/Ro 52,
- centromeres and other mitotic spindle apparatus antigens,
- variety of cytoplasmic organelles and structures (e.g. mitochondria, Golgi apparatus, endosomes, a signal recognition particle or ribo-somes)

The use and abuse of commercial kits used to detect autoantibodies

Methods -ELISA

- In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti7 La/SS-B, etc., should be standardized according to national (e.g, CDC) and/or international (e.g., WHO, IUIS) standards

IIF : how to report

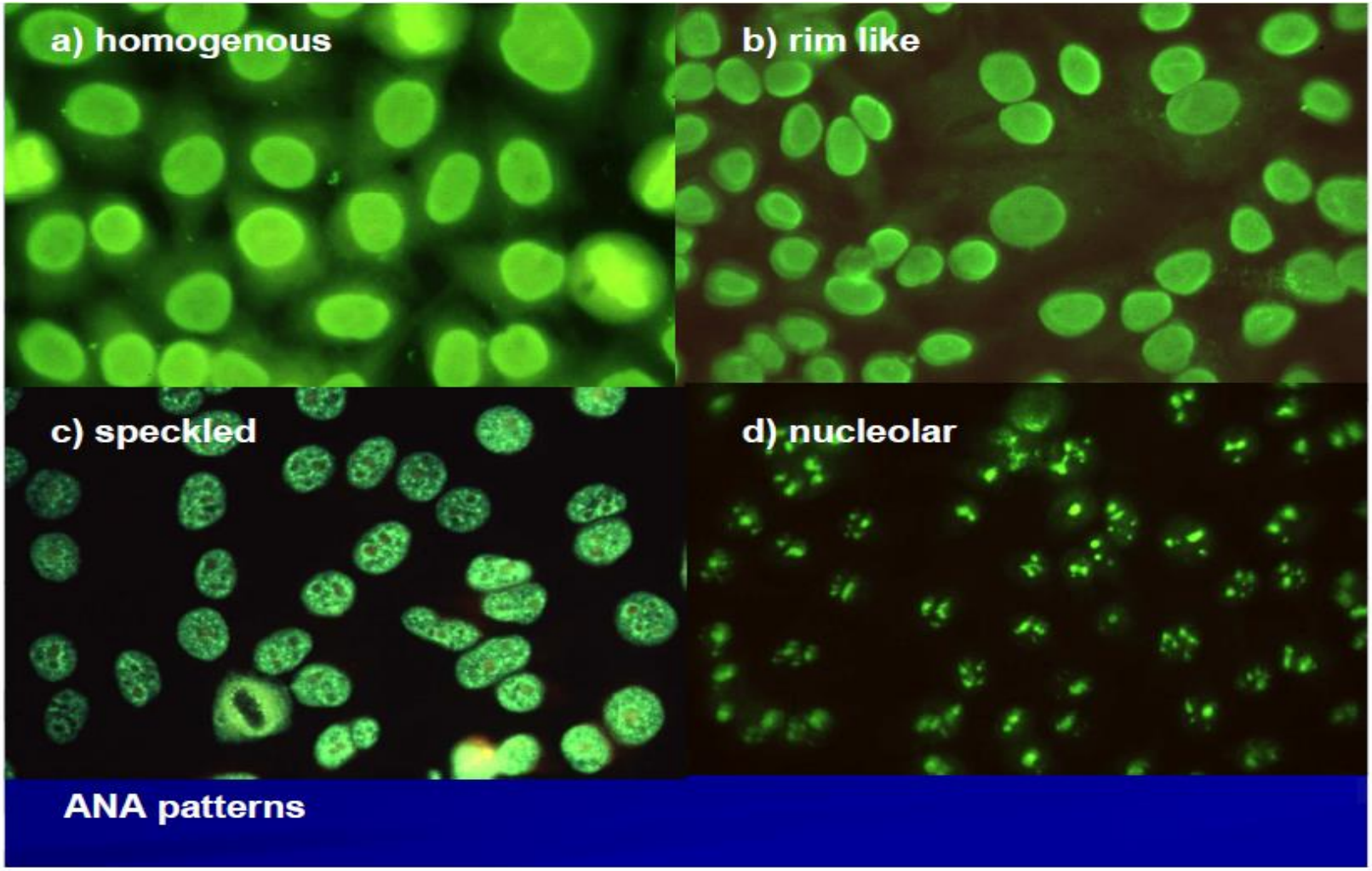
- Positive/negative
- Pattern of positivity - speckled/homogenous/nucleolar
- End titer –

  last dilution at which the IIF is positive

Pattern

- Homogeneous
- Speckled
- Centromere
- Nucleolar
- cytoplasmic

Patterns



Titer

- Titer is simply the highest dilution of the serum which still gives a positive result on IF.
  - Therefore, a titre of 1:640 represents a much stronger autoantibody than a titre of 1:80.

- Is there a titre at which an antibody automatically becomes clinically significant? No, but : an antibody present at 1:640 is *more likely to be significant than an antibody* present at 1:80.

- However- meaningful interpretation is only possible in the context of the clinical findings.

Titer

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

* *Frequency increases with female sex and increasing age*

32% of normal sera were positive at 1/40, it was recommended that a cutoff point of 1/160 is more appropriate

Range of antinuclear antibodies in 'healthy' individuals. Arthritis Rheum 1997, 40:1601-1611.

ANA IIF –interpretation

Condition	Patients with ANAs (%)
Diseases for Which ANA Testing Is Helpful for Diagnosis	
Systemic lupus erythematosus	99-100
Systemic sclerosis	97
Polymyositis/Dermatomyositis	40-80
Sjögren's syndrome	48-96
Diseases In Which ANA Is Required for Diagnosis	
Drug-induced lupus	100
Mixed connective tissue disease	100
Autoimmune hepatitis	100
Diseases In Which ANA May Be Useful for Prognosis	
Juvenile idiopathic arthritis	20-50
Antiphospholipid antibody syndrome	40-50
Raynaud's phenomenon	20-60
Some Diseases for Which ANA Typically Is Not Useful	
Discoid lupus erythematosus	5-25
Fibromyalgia	15-25
Rheumatoid arthritis	30-50
Relatives of patients with autoimmune disease	5-25
Multiple sclerosis	25
Idiopathic thrombocytopenic purpura	10-30
Thyroid disease	30-50
Patients with silicone breast implants	15-25
Infectious disease	Varies widely
Malignancies	Varies widely
Healthy ("Normal") Individuals	
≥1:40	20-30
≥1:80	10-12
≥1:160	5
≥1:320	3

Systemic lupus erythematosus

Sensitivity: 95-100%

Acceptable specificity and positive predictive value

ONLY IF

there is a reasonable *pre test* clinical suspicion of SLE

→should not be used for screening for SLE

Kavanaugh A et al., 2000

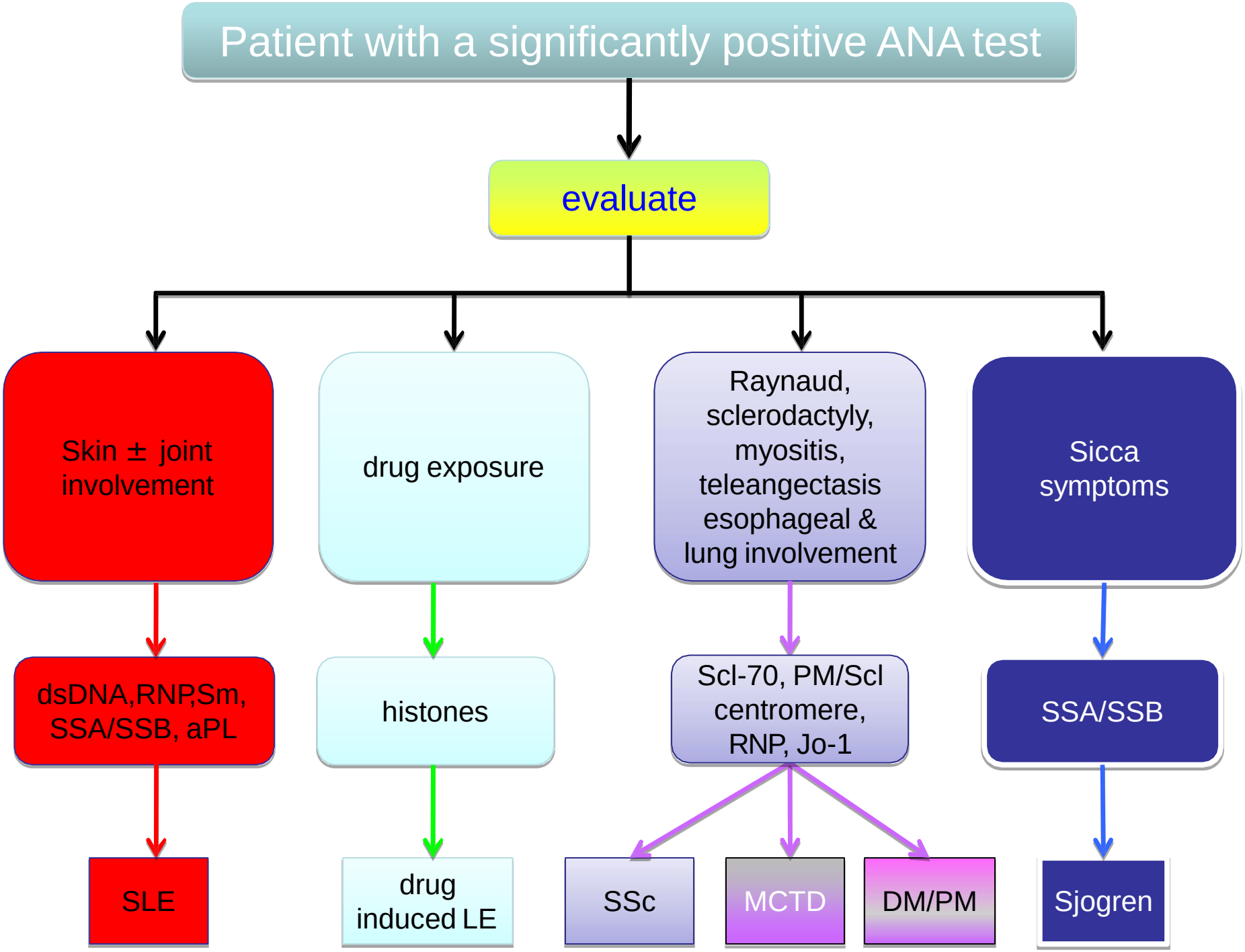
ANA Positive – What next?

Diagnosis	Test
SLE	dsDNA, Sm, Ro/SSA, La/SSB, aCL, anti-beta2-gpI, LLAC
Sjogren	Ro/SSA, La/SSB, RF
PM/DM	Jo1 *(anti-synthetase syndrome)*
Systemic sclerosis	Centromere (CREST), Scl70 (diffuse)
Drug induced-SLE	Histones

So…

what to do?

Always consider history, physical and simple lab tests


Patient with a significantly positive ANA test
evaluate
Skin ± joint involvement
drug exposure
Raynaud, sclerodactyly, myositis, teleangectasis esophageal & lung involvement
Sicca symptoms
dsDNA,RNP,Sm, SSA/SSB, aPL
histones
Scl-70, PM/Scl centromere, RNP, Jo-1
SSA/SSB
SLE
drug induced LE
SSc
MCTD
DM/PM
Sjogren

Auto - antibodies in infertility

- In primary or unexplained infertility higher prevalence of antibodies is seen.
- These maybe anti thyroid, antinuclear or Antiphospholipid antibodies
- Correct interpretation of above as causative agents for infertility requires
  - Studies with standardised antibody testing
  - Studies with standardised definitions of infertility
  - Registries of outcomes of treatments of autoantibody positive infertile women

Reference: Clinical Reviews in Allergy & Immunology. 2017. Female Infertility and Serum Auto-antibodies: a Systematic Review

Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage

Morteza Molazadeh[1] M.Sc., Hadi Karimzadeh[2] M.D., Mohammad R Aziz[3] M.D.

1. *Department of Immunodiagnosis, Erythron Clinical Lab, Isfahan, Iran.*
2. *Department of Rheumatology, Al-Zahra Hospital, Isfahan, Iran.*
3. *Department of Immunology, Fertility and Infertility Center, Isfahan, Iran.*

Corresponding Author:
Morteza Molazadeh, No 232, Parnian Building, Taavaon 2, Ghadir Blvd., Sepahanshahr, Isfahan, Iran.
Email: Morteza_Molazadeh@yahoo .com
Tel: (+98) 3117922491

Received: 16 September 2013
Accepted: 15 December 2013

Abstract

Background: Antinuclear antibodies (ANAs) in women with recurrent miscarriage have been reported. The presence of moderate to high titers of these antibodies represents an autoimmune condition that can endanger the health of the fetus in pregnant women.

Objective: In this study, we evaluated the prevalence of ANAs in Iranian women with a history of two or more unexplained abortion.

Materials and Methods: 560 women with unexplained recurrent miscarriage and 560 healthy controls accounted for this study over a period of 13 months. ANAs were detected by indirect immunofluorescence technique.

Results: ANAs were detected in 74 of 560 (13.21%) patient with recurrent miscarriage, and in only 5 of 560 (0.9%) controls ($p<0.001$). ANA positivity was generally found with low-positive results (1.40-1.80) in about 38% of positive cases, whereas moderate titres (1.160-1.320) and high titres (>1.640) were seen in about 46% and 16% of cases respectively. Finally evaluating of microscopic ANA patterns revealed that about half of positive cases had antibodies against DNA- histone complex, associated with systemic lupus erythematosus disease.

Conclusion: Antinuclear antibodies are not uncommon in women with unexplained recurrent miscarriage, suggesting the possible role of an autoimmune disorder on abortion, at least in a subgroup of patients.

Key words: Recurrent miscarriage, Antinuclear antibodies, Indirect immunofluorescence.

APS work up

- Anti phospholipid antibodies – anti cardiolipin (acl)and beta 2 glycoprotein I
- Lupus anticoagulant (LAC)- Functional assay

- ELISA
- Low positive –less than 40 GPL/MPL units
- Moderate positive – 40-80 GPL/MPL units
- High positive –more than 80 GPL/MPL units
- Repeat after 12 weeks

Take home messages

- No test for ANA should be performed without a clinical evaluation that leads to a presumptive diagnosis (SLE, SSc, Sjögren, PM/DM, JIA, Raynaud, drug-induced SLE, autoimmune hepatic disease, MCTD)

- ANA testing have an extremely low specificity and PPV in the general population.

- ANA and ENA are different tests (ANA more sensitive, anti-ENA more specific)

Take home messages (2)

- Many diseases may cause ANA positivity; many healthy individuals have a positive ANA test.

Ref

- Am J Med. 2013 Apr;126(4):342-8. doi: 10.1016/j.amjmed.2012.09.014. Epub 2013 Feb 8.***The clinical utility of a positive antinuclear antibody test result.***

- Kavanaugh et al. ***Guidelines for clinical use of the ANA test and tests for specific autoantibodies to nuclear antigens.*** Arch Pathol Lab Med. 2000;124:71-81

- **Kelly s textbook of rheumatology**

- Clinical Reviews in Allergy & Immunology. 2017. Female Infertility and Serum Auto-antibodies: a Systematic Review