The Use & Interpretation of
Investigations in Rheumatic Disease

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The Use & Interpretation of Investigations in Rheumatic Disease

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Introduction

- Auto immune rheumatic disease presents in many ways
- Can be problematic to diagnose
- Immunologic laboratory tests serve critical role in care of patients with rheumatic disease
  - Diagnosis
  - Disease activity
  - End organ involvement
  - Prognosis
  - Response to treatment
• Scope for misuse is large
  • Misdiagnosis
  • Inappropriate therapy
  • Unnecessary health care expense

• Increasing emphasis on primary care medicine leads to greater primary care ordering & analysis of such tests

• Aim to focus on utility & interpretation of most frequently used tests
Prior to requesting test physician needs:-
  • Clear indication for test
  • Anticipated response to outcome
  • To consider sensitivity/specificity
  • Positive & negative predictive values

If test will not alter:-
  • diagnosis
  • prognosis
  • therapy

then it may be unnecessary
Sensitivity & Specificity

- Sensitivity -
  - proportion of patients with a disease who have a positive test result
  - Indicates accuracy of test in detecting the disease
  - When test has high sensitivity - negative test rules out the diagnosis (low false - negative)
  - High sensitivity tests are useful for screening
Specificity -

- Proportion of patients **without** the disease who have a negative test result
- Indicates accuracy of test in identifying those without the disease
- When test has high specificity - positive test rules in diagnosis (low false +ve)
- High specificity test is useful for confirmation but not screening
Auto Antibodies

- Modern era rheumatology began in 1940’s with discovery of RF and LE cell
- Auto-antibodies are immunoglobulins which can be produced by most normal individuals to quantitatively detectable levels in immune dysfunction
- Most are not specific for a clinical syndrome
- May be detected in people with markedly different clinical features and normal individuals
• Auto-antibodies may not be detected despite clinical findings consistent with certain conditions

• Research into auto-antibodies has yielded information on pathogenic mechanisms

• Use in clinical practice is an adjunct to diagnosis and management but not a precise guide
Rheumatoid Factor

- RF present in 1% of normal population
- RA occurs in 0.5-1% population
- At least as many individuals who have RF do not have RA as have the disease
- RF found in 70-90% of adults with RA
- High titres tend to correlate with severe articular disease and extra articular manifestations
- False positives occur in many diseases
  - Sarcoid
  - Infective endocarditis
  - Leprosy
  - TB
  - Pulmonary fibrosis
  - Liver disease
  - Syphilis
  - Frequently present in Sjogrens syndrome & marcoglobulinaemia
- Positive RF occurs with increasing age
• Absence of RF in presence of progressive clinical & radiological disease is seen in 10-30% patients (seronegative)

• Patients who become seropositive often do **not** have RF at presentation
  - 33% in first 3 months - 60% in first 6 months

• Not useful for measuring disease activity
• Sensitivity & specificity of RF high in rheumatology clinics but falls in the community & general hospital setting

• RF alone will not exclude or confirm RA

• CRP/ESR may help to identify the presence of inflammatory arthritis & guide use of test
Patients with positive test 80%

Prevalence of +ve test in normals 2/100

Disease prevalence 1/100

Likelihood of disease if +ve test 1/2

Likelihood of disease if +ve test and joint pain 1/1.5
Anti Nuclear Antibodies (ANA)

- ANA directed against variety of nuclear antigens have been detected in serum of patients with many rheumatic and non-rheumatic diseases and patients with no definable clinical syndrome
• ANA is very sensitive for SLE
• >95% of patients with SLE have positive result
• Specificity of test is quite low = 57%
• Low prevalence of SLE in general population (40-50/100,000) means most people with positive ANA do not have SLE (positive predictive value 11%)
• Negative test will help to rule out SLE
• Predictive value of ANA will depend on estimated likelihood of lupus before testing
• Using ANA to screen for SLE if pretest likelihood of lupus is low is undesirable
• ANA’s found in most systemic rheumatic disease
• A positive test is reported both as a particular staining and as a titre
• Four commonly recognised patterns
  • Homogenous
  • Speckled
  • Diffuse
  • Anti-centromere
• Emphasis on staining pattern has diminished with recognition of disease overlap and more specific auto antibody tests

• Low titre values (<1:160) often have minimal clinical significance
## ANA in Rheumatic disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Percentage</th>
<th>Disease Specific Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>&gt;95%</td>
<td>Anti-Sm/Anti ds DNA</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>60-90%</td>
<td>Anti centromere/anti SCL70</td>
</tr>
<tr>
<td>Sjogren’s syndrome</td>
<td>75%</td>
<td>Anti Ro/ Anti La</td>
</tr>
<tr>
<td>MCTD</td>
<td>95-99%</td>
<td>Anti RNP</td>
</tr>
<tr>
<td>Polymyositis /dermatomyositis</td>
<td>25%</td>
<td>Anti Jo - I</td>
</tr>
<tr>
<td>RA</td>
<td>15-35%</td>
<td>RF</td>
</tr>
</tbody>
</table>
Many non rheumatic conditions are associated with ANA

- Infection
- GI disease
- Pulmonary disease
- Endocrine disorders
- Haematologic disorders
- Malignancy
- ESRF
- Post transplant
• ANA has been demonstrated in sera of healthy population ranging between 3-30%

• More common in elderly females, pregnancy, family history of rheumatic disease, drug induced
• In SLE ANA does not reflect disease activity and is not useful for monitoring patients.

• Other variables such as ESR/complement/ds DNA should be utilised with clinical features.
• ANA testing should be ordered in two circumstances

  • 1. To support diagnosis of CTD in patient presenting with multi system disorder suggesting of SLE, Sjogrens, SS

        If test positive then confirm with more specific antibodies

  • 2. To rule out SLE in patient with 1 - 2 features of SLE and no obvious alternative diagnosis

• An inappropriately requested test can be cause confusion for physician and patient if positive
Patients with positive test: 99%

Prevalence of +ve test in normals: 1/100

Disease prevalence: 1/2000

Likelihood of disease if +ve test: 1/20

Likelihood of disease if +ve test and joint pain: 1/5
Anti ds DNA Antibodies

- Less sensitive but more specific for SLE than ANA
- Rarely found in normal individuals
- Many versions of test exist

Fluorescent assay for a DNA-containing organelle in the parasite crithidia lucilliae is the ‘gold standard’

- Evident in 50-80% of untreated patients with SLE
- Other than some cases of Sjogren’s/SS are diagnostic for SLE
• Anti ds DNA antibodies tend to correlate with renal disease and disease activity (with complement levels)

• Useful to establish diagnosis of SLE and monitor lupus nephritis
Scleroderma Antibodies

- Anti centromere antibodies are directed to restricted regions of chromosomes
- Found in 80-90% of patients with CREST variant of scleroderma
  - Calcinosi
  - Raynauds
  - Oesophageal dysfunction
  - Sclerodactyly
  - Telangectasia
• Usually reported positive/negative

• Can be detected in 25% patients with idiopathic Raynauds

• Presence may suggest that patient with Raynauds could develop CREST
• Anti SCL-70 found in 40% of patients with progressive systemic sclerosis
• Directed against topoisomerase I (post transcriptional ribosomal enzyme)
• Detected by immunodiffusion/ELISA and reported as positive/negative
• Prevalence varies in different studies and may depend on racial background
• Often associated with more widespread skin disease and internal organ involvement than anti centromere
• ACA & anti SCL-70 are helpful in confirming diagnosis of scleroderma

• Provide some prognostic information

• Scleroderma variants are diagnosed on clinical features

• Once diagnosis is made these tests do not need repeating

• Some patients may have neither antibody
Extractable Nuclear Antibodies

- May be identified in patients with CTD’s
- Directed against small nuclear ribonucleo proteins involved in RNA processing
- Reported as positive/negative
- Useful in diagnosis but not for monitoring disease activity
- 4 main antibodies
  - Anti Sm
  - Anti U1 RNP
  - Anti Ro (SS-A)
  - Anti La (SS-B)
Anti-Sm Antibodies

- Very specific for SLE
- Lack sensitivity occurring in about 30% patients with SLE
- Seldom present in other conditions
- Higher prevalence in young black females with SLE
- Not to confuse with Anti-smooth muscle antibodies present in autoimmune liver disease
Anti U1 RNP Antibodies

- Not sensitive or specific for SLE
- Present in 40% of patients with SLE
- Described in mixed connective tissue disease
- Patients have features of several CTD’s characteristically
  - Arthritis
  - Raynauds
  - Hand swelling
  - Myositis
  - oesophageal hypomotility

Many can go on to develop RA, scleroderma or other conditions
Anti SS-A/Ro & Anti SS-B/La Antibodies

- Frequently present together
- Found in patients with SLE/Sjogrens and other autoimmune disease
- Anti Ro found in 25% of patients with SLE
  - correlates with prominent skin disease
  - congenital heart block
- Anti-La usually associated with Anti-Ro
  - Less frequent in SLE (10%) and Sjogrens
- Anti-Ro can be useful in screening pregnant women with SLE but absence will not exclude possibility of CHB
**Myositis Specific Antibodies**

- Newly described
- Occur in 50% of patients with idiopathic inflammatory myopathy
- Allow better classification in terms of clinical features/prognosis
- Currently anti Jo-1 is available
- Directed against tRNA histidyl synthetase
- Occurs in 30% IIM
• Associated with 'Anti Synthetase' syndrome
  - Pulmonary fibrosis
  - Fever
  - Raynauds
  - Dry cracked hands
• Others Anti Mi2 / Anti SRP / Anti MAS
• Jo1 not appropriate for screening but useful in newly diagnosed or suspected IIM
ANA Negative

If the ANA is negative, but disease is suspected, test for specific autoantibodies

Positive

- SS-A
- Jo-1, PL-7, PL-12
- aCL, LA
  - SS
  - PM/DM
  - APS

Negative

Clinical Diagnosis

ANA Positive

Homogeneous

- ma DNA
- DNP
- Histone
  - DIL
- SLE

Specified

- PCNA
- Sm
- SS-A/Re
- U1 RNP
- SS-B/La
- High litter U1 RNP
- SS
- GCTD

Nucleolar

Nucleolar Specified Hemag
- Scl-70

Centromere

Confirm by pattern only

Scleroderma
Anti Neutrophil Cytoplasmic Antibodies (ANCA)

- Directed against several neutrophil cytoplasmic components
- Detected in serum by indirect immunofluorescence using ethanol fixed human polymorphs
- 2 main staining patterns
  - (cytoplasmic) c-ANCA
  - (perinuclear) p-ANCA
- Confirmatory testing uses solid phase ELISA assays for defined target antigens
**C-ANCA**

- Correlates with antibodies directed against proteinase 3
- Most frequently observed in Wegners Granulomatosis
- Sensitivity for active WG approx 90%
- Can be found in other systemic vasculitides although p-ANCA more common
- Only moderately sensitive in moderate or limited disease
- Anti PR3 ANCA may have role in disease pathogenesis in WG
- In stable WG, a rise in c-ANCA may herald a clinical exacerbation
**P-ANCA**

- In most cases induced by antibodies against myeloperoxidase (MPO)
- Less specific than c-ANCA
- Anti MPO antibodies occur in
  - Necrotising GN (65%)
  - Microscopic Polyangitis (45%)
  - Churg Strauss Syndrome (60%)
  - Wegners (10%)
- Can also occur in other conditions but target antigen rarely MPO
  - RA
  - IBD
  - Malignancy
  - Infection
**ESR & CRP**

- *C-reactive protein (CRP)* is acute phase protein
- Synthesised in hepatocytes by action of stimulating cytokines (IL6, IL1, INFx, TNFx)
- *Erythrocyte sedimentation rate (ESR)* in part reflects intensity of acute phase response especially fibrinogen and globulins
- Also largely determined by concentration of immunoglobulins which are **not** acute phase reactants
• These proteins have 1/2 lives of days-weeks so rate of change of ESR is slower than CRP
• ESR also influenced by number of red cells
• ESR exhibits diurnal variation
• Reproducability of ESR measurements is poor compared to CRP
• CRP generally reflects disease activity more closely eg. Crohn’s, RA, vasculitis, bacterial infections
• CRP not affected by pregnancy or age
• May be misleading in SLE and capillary leak syndrome

• Typical values

  10-40mg/l mild inflammation & some viral infections

  40-200mg/l acute inflammation & bacterial infections

  >300mg/l extensive trauma & severe bacterial infections
Value of ESR

- Monitoring SLE
- Cancer referrals
- Disease monitoring / treatment protocols

It is rarely necessary to measure both ESR and CRP
**HLA-B27**

Patients with positive test: 90%

Prevalence of +ve test in normals: 6/100

Disease prevalence: 1/300

Likelihood of disease if +ve test: 1/18

Likelihood of disease if +ve test and joint pain: 1/4.8
CONCLUSIONS

• Selection of appropriate auto antibody tests in patients with suspected rheumatologic disorders should be guided by clinical impression

• Before request, be sure of indication & anticipated response to result

• Begin with sensitive tests and if positive use more specific tests to help confirm diagnosis
• Most auto antibody tests need not be serially repeated
  - exceptions cANCA & ds DNA

• CRP is usually preferable to ESR for detecting acute phase response
  - exceptions SLE
    Cancer referrals

• Computer based interventions may help to guide requesting