Use of Algorithms for High Quality Diagnostics and Handling of Patients with Autoimmune Rheumatic Diseases

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- First priority: use resources to detect systemic rheumatic diseases as early as possible so follow-up can start and therapy be applied when clinically most beneficial
- Better clinical outcome is the goal!

The autoantibody content of serum can be regarded as a “fingerprint” of the disease process including the particular genes that are activated in a patient with an inflammatory rheumatic disease (IRD)

This assumption is supported by family studies, genetic studies and autoantibody profile studies in patients with certain typical clinical manifestations (clinical phenotypes/sub-syndromes)

Major topics of this lecture
- Do we actually use serology right today?
- Where can we make progress?
- Can we improve the clinical usefulness?
- Can IRD be recognized earlier?
- Will new technologies help diagnostics?
- What are the real issues in health economics: money spent at time of diagnosis or cost related to long-term outcome?
Medical challenges I

- Chronic inflammatory diseases have numerous overlapping features
- Autoantibodies are produced by both healthy and sick people
- Many autoantibodies occur in several IRD, only few are disease-specific
- Clinicians are no more running clinical laboratories themselves

Medical challenges II

- High throughput, sensitive techniques are flooding the market, and post-marketing studies have not been done in a clinically relevant fashion
- Experts often speak non-sense language to non-experts!
- Economy today is considered the highest deity to be obeyed!
- Medical education in long term cost estimation is lacking

Autoimmune IRD diagnostics

- Inevitably has to be a merge between clinical experience and laboratory eminence
- Clinicians need to have some insight into laboratory methods and strategy for testing
- Laboratory scientists should master technical as well as clinical advice regarding every laboratory test method used and be able to discuss any individual test result of a patient with the clinic
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Diagnostics/prognostics of IRD

- Clinical history
- Manifestations
- Objective findings
- Radiological signs
- Specialist evaluations
- Histopathology
- Immunopathology
- Laboratory tests to look for signs of inflammation
- Immunoglobulin levels
- Complement activation
- Autoantibodies

Optimal user-defined needs

- The diagnostic potential of a test (e.g., expressed as nosographic sensitivity, diagnostic specificity) must be validated locally before use of a test for routine, employing sera from local prototype patients and a large mixed population of differential diagnostic patients (here called critical controls).
- Performance in early disease diagnostics should be proven in-house on local patients that are followed up until final diagnosis can be made and the initial results can be judged against the final diagnosis.

Tests used for diagnosis of a disease

- Need to show a high diagnostic specificity towards differential diagnostic populations i.e., patients with diseases that mimic the disease searched for (critical disease controls); somewhat different for each specialty!
- Nosographic sensitivity is not very important as positivity usually indicates presence of a defined disease sub-syndrome with certain manifestations and prognosis.
- More focus on disease-specific autoantibodies!
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Laboratory aspects 2006

- Modern quantitative test systems should replace older methods
- "Objective" methods are preferable to those based on "subjective" judgment
- Low overall variation is important (reproducibility, quality assurance)
- High throughput techniques are practical for large daily routine testing when available
- Low cost per patient sample is preferred
- Commoditized assays are preferred (technology already running in the laboratory)

Laboratory medicine 2006

- Work-load is increasing
- Test repertoire is increasing
- Demand seems uncontrollable
- Unnecessary testing is common
- The role of autoantibody results for diagnosis, prognosis, and clinical planning is often unknown to both performers and users!

Some parameters that can be used to indicate clinical value of test results

- Nosographic sensitivity: Percentage of positive results among patients with the disease
- Diagnostic specificity: Percentage of differential diagnostic patients that show negative results
- Positive predictive value: Percentage of [true positives] / [true + false positives]
- Negative predictive value: Percentage of [true negatives] / [true + false negatives]

These parameters are mostly not cited in laboratory reports today.
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Some parameters that can be used to indicate clinical value of test results

- **Positive likelihood ratio:**
  
  \[
  \frac{\text{True positive rate}}{\text{false positive rate}} = \frac{\text{sensitivity}}{1 - \text{specificity}}
  \]

- **Negative likelihood ratio:**
  
  \[
  \frac{\text{True negative rate}}{\text{false negative rate}} = \frac{1 - \text{sensitivity}}{\text{specificity}}
  \]

  These parameters are not often cited in laboratory reports today

Sensitive assays are useful for:

- **Screening** large numbers of sera for the likely presence of an antibody, and when positive – you can focus on the specific autoantibody by use of an antigen-specific assay

- **Following** decrease and increase of an antibody level which needs monitoring to look for fluctuations that may indicate remission induction / imminent exacerbation or ongoing disease activity

  But sensitive assays may result in clinically irrelevant positive results and should not be used without criticism!

Anti-dsDNA antibodies – using two assays

Two assays should be used in borderline positive cases!
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Screening by indirect immunofluorescence (IIF)

Broad screening potential

Fluorescence

Fluorochrome conjugate

ANA

HEp-2 cell

Advantage:
Positive results are seen both when reaction takes place with well known and less well known autoantigens, all of which are potentially helpful for diagnostics

My postulate

• We will never get a better autoantigen array or multiparameter test platform than the well-preserved HEp-2 cell substrate containing a practical mixture of resting and dividing cells!

• We just need to teach people how to read staining patterns in the same internationally accepted way in laboratories worldwide, most practically through use of the internet!

Cellular regions: HEp-2 cells

Use of IIF staining patterns for diagnosis?

Note! autoantibodies target cellular organelles and structures

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Modern testing platforms: are they better?

- Multiantigen micro-arrays
- Addressable laser bead immuno-assays
- Luminescence ELISA systems
- Microfluidics systems

Autoantigen arrays

18 different autoantigens coated on solid phase

Reactivity with one antigen is shown by colour development on one spot; location of that antigen reactivity known by data system

Addressable laser bead immuno-assay

Beads ➔ Unbound autoab.

Coated antigen ➔ Autoab. binding ➔ Bead colour shows antigen source ➔ Fluorescent conj. ➔ Bound autoab. ➔ Unbound autoab. ➔ Fluorescence shows autoantibody binding
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Reading the reactions

Precision fluids align the microspheres in a single file, and passes them through the lasers one at a time
Advantage: quantitative read-out

Limitations of solid phase assays
(ELISAs, bead assays, antigen arrays etc.)

- Molecular conformation is a “sine qua non” as regards optimal autoantibody binding
- Among clinically important autoantigens only few can be obtained in such native form (at present around 10)
- Several autoantigens thus need to be manufactured as recombinant molecules to be obtained in sufficient amounts, but these replacement molecules often lack the conformation of the native autoantigen
- Many autoantigens do not bind well to the solid supports used today, important epitopes are hidden as they attach, or they loose the necessary conformation for reactivity

Diagnosis vs. differential diagnosis
(critical controls)

Not really needed!
Prototype
Mimic

Healthy individual
Differential diagnostic patients

Note that critical disease controls may differ from one to another subspecialty in medicine!!
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Criteria-based clinical diagnostics

Criteria = rigorously defined items

Clinical criterion 1
Clinical criterion 2
Clinical criterion 3
Absent
Present
Not yet found

* Note that serologic results have special value in early diagnostics

Criteria-based clinical diagnostics

Ideal clinical and laboratory collaboration involves:

- Collection of sera from well-characterized patients
- Collection of clinical data in early cases with recording of symptoms found at the time of serum sampling
- Adherence to strict definitions of each clinical and paraclinical manifestation registered

- Follow-up at time final diagnosis has been reached
- Agreement on the report and use of borderline results
- Agreement on the use of local test algorithms
- Note! use of new methods needs to be agreed by both parties!
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Making diagnostics appropriate

Clinical tentative diagnosis/symptoms: clinician

Lab. info about test ordering

Lab. info about interpretation (cut-off, ranges, levels/ strength)

Lab. info about prognostics

Lab. info about follow-up tests

All of these should be agreed between clinicians and the laboratory scientists

Make diagnosticians aware of the most frequent early clinical and laboratory signs of IRD

- Produce clinical algorithms for less experienced doctors to help them recognize potentially chronic inflammatory rheumatic diseases as early as possible
- Teach doctors to order and use appropriate laboratory screening in early cases of disease
- Teach rheumatologists to recognize early laboratory features of these diseases (single autoantibodies and autoantibody profiles) for setting a correct diagnosis and estimate a likely prognosis

Algorithm for family practice

just an example

<table>
<thead>
<tr>
<th>Disease suspected:</th>
<th>Common early signs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Rash, arthralgia, fatigue, etc.</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Fatigue, arthralgia, dryness</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Raynaud’s phen, arthralgia</td>
</tr>
<tr>
<td>Mixed connect. tissue disease</td>
<td>Raynaud’s phen, arthralgia</td>
</tr>
<tr>
<td>Juvenile chronic arthritis</td>
<td>Arthritis, fever, stiff joints</td>
</tr>
<tr>
<td>Adult rheumatoid arthritis</td>
<td>Arthritis, stiffness in small joints</td>
</tr>
<tr>
<td>Autoimmune thrombosis/pregnancy loss</td>
<td>Deep venous or arterial thrombosis, fetal loss</td>
</tr>
</tbody>
</table>

In any event, refer the patient to a specialist in internal medicine or rheumatology if clinical suspicion is present!!
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Principle of autoimmune test guide

a) Ideally should include SSc panel testing for anti-RNA polymerase I and III, anti-U3RNP and anti-3/7 RNP antibodies
b) Ideally should include PM/DM panel testing for anti-aminocyl-tRNA synthetase, anti-SRP, anti-M-2, anti-Pm/Scl, and anti-Ku antibodies
c) Should also include lupus anticoagulant

An algorithm to aid in the ordering of autoantibody tests based on a tentative diagnosis

Algorithm for clinical utility

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Nosographic sensitivity</th>
<th>Diagnostic specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE: Anti-dsDNA</td>
<td>50 – 60%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>10%</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>Anti-ribonucleoprotein A</td>
<td>3%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Anti-ribonucleoprotein B</td>
<td>10%</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>Anti-U1RNP</td>
<td>30%</td>
<td>low</td>
</tr>
<tr>
<td>Anti-Ro</td>
<td>30 - 40%</td>
<td>low</td>
</tr>
<tr>
<td>Anti-La</td>
<td>15 - 20%</td>
<td>low</td>
</tr>
<tr>
<td>ANA</td>
<td>&gt; 98%</td>
<td>extremely low</td>
</tr>
</tbody>
</table>

Inflammatory rheumatic diseases in which autoantibody specificity is associated with different diagnostic sub-syndromes

- Systemic lupus erythematosus
- Primary Sjögren’s syndrome
- Polymyositis/dermatomyositis
- Juvenile chronic arthritis
- ANCA-associated vasculitides
- Scleroderma
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Autoantibody-associated lupus sub-syndromes

- IgG anti-dsDNA, anti-C1q
- IgM RF, anti-SSB
- Anti-SSA/SSB
- Anti-phospholipid antibody/lupus inhibitor
- Anti-U1RNP antibody
- Anti-ribosomal RNP antibody
- Anti-histone, MPO-ANCA, anti-cardiolipin profile

Did you know that around 140 different autoantibodies have been described in SLE?

ANA are found in glomerular lesions of SLE patients

Lupus immune complex nephritis

Extracts from glomeruli contain several lupus-associated ANA and their cognate antigens (most prominently anti-dsDNA/DNA)

Production of antinuclear antibodies (ANA) before onset of SLE mean 3.3 years before, up to 9.4 years

- In 130 patients who later on developed SLE at least 1 SLE autoantibody tested for was positive in 88%
- ANA at 1/120 dilution were found in 78%
- Anti-dsDNA antibodies in 59%
- Anti-SSA/Ro-60 antibodies in 47%
- Anti-SSB/La antibodies in 34%
- Anti-Sm antibodies in 32%
- Anti-U1RNP in 26%
- Anti-phospholipid antibodies in 18%

Arbuckle MR et al., N Engl J Med

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Production of lupus-related ANA before onset of clinical SLE

- Typical SLE-associated ANA found up to 9.4 years before onset of clinical disease

Production of antiphospholipid antibodies (APA) before onset of SLE

- 18% of persons who later developed SLE were found to produce IgM and/or IgG anti-cardiolipin antibody (mean 3.0 years, range: 7.6 years – 1 month) before onset
- APA heralded worse disease: More criteria (6.1 vs. 4.9), more common development of nephritis, clotting events, thrombocytopenia, and CNS involvement
- APA preceded clotting events by 3.1 years

Genes and autoantibody production in SLE

- 1506 individuals from 229 multiplex SLE pedigrees were studied for autoantibody profile and for their linkage to genes by genome-wide linkage analyses:
  - Anti-SSB production was linked to chromosome 3q21,
  - Anti-Sm/RNP was linked to chromosome 3q27,
  - Anti-SSA/SBB was linked to chromosome 4q34-q35
  - Anti-phospholipid was linked to IgM to chromosomes 13q14

Conclusion: autoantibody production is a genetically complex trait

Ramos PS et al., Genes Immuno 2006
Even infrequent ANA are important!
examples of scleroderma sub-syndromes

- Limited scleroderma
- Diffuse scleroderma
- Limited scleroderma
- Overlap scleroderma

Survival rates, scleroderma

Anti-centromere associations:
limited scleroderma

- Raynaud’s phenomena
- Digital skin involvement
- Arthritis/arthralgias
- Biliary cirrhosis
- Decreased CO diffusion capacity
- Mostly Caucasian patients
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Anti-Th/To RNP associations:
limited scleroderma

- Raynaud’s phenomena
- Puffy fingers
- Intestinal involvement
- Pulmonary hypertension
- Often associated with hypothyroidism

Anti-Scl-70 associations:
diffuse scleroderma

- Fibrozing alveolitis
- Restrictive lung disease
- Digital ischaemia/ulcers
- HLA-DR5 association

Anti-RNA polymerase 1 associations:
diffuse scleroderma

- Tendon and joint involvement
- Kidney involvement
- Malignant arterial hypertension
- Acute onset
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### Anti-U3 RNP associations:
**diffuse scleroderma**
- Pulmonary hypertension
- Cerebral haemorrhage
- Peripheral neuropathy
- Associated with HLA DR4
- Black patients

### Anti-U1RNP associations:
**overlap scleroderma**
- Arthritis/arthralgias
- Sicca complex symptoms
- Pulmonary hypertension
- Peripheral neuropathy
- Polymyositis

### Anti-PM/Scl associations:
**overlap scleroderma**
- Polymyositis
- Heliotropic lesions
- Gottron's lesions
- Arthritis/arthralgias
- Associated with HLA-DR3

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Evidence-based laboratory medicine

- Long term economic outcome
  - Number of visits to clinics
  - Length of stay/cost of stay
  - Readmission rate
  - Working days lost
  - Productive years gained

SF-36 based evaluation by the patient should be satisfactory!

Ultimate importance: clinical outcome

Patient → Decision → Final outcome

Test → Intervention

As early as possible

Future efforts should be devoted to research that can indicate which tests and autoantibodies have a strong impact on long-term clinical outcome!!

Perspectives: health economics

Consequences to economy of the society → Society → Region → Hospital → Laboratory → Test

Decisions are commonly made by laboratory directors only

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Conclusions I

- Cutting edge diagnostics cannot be exercised without a close collaboration between clinicians and laboratory experts, but also patients and the kit industry have very important roles.
- More than one method must be available to confirm/refute questionable results.
- At present the use of "subjective" methods like HEp-2 cell interpretation is still necessary.

Conclusions II

- Quality management should be obligatory both for clinics and laboratories.
- To secure optimal appropriateness of testing and post-test use of results regular consultations between clinicians and laboratories are required, and the use of algorithms is advised.

Conclusions III

- However, solutions should be optimized according to local wishes and strategies, needs, possibilities, economy etc.
- To ensure early diagnostics of IRD family doctors need to be involved, and interaction between rheumatologists/internists and practitioners should be encouraged to help patients get optimal work-up and rational therapy and better clinical outcome.
Some references

- Cervera R et al., Medicine 1993; 72: 113-24
- Kuwana M et al., Arthritis Rheum 1994; 35: 75-83
- Ramos PS et al., Gene Immun 2006; 7: 417-32