

PAPER

European attempts to set guidelines for improving diagnostics of autoimmune rheumatic disorders

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The rational way to set a diagnosis and estimate a prognosis in rheumatology is to start by setting a tentative diagnosis and then follow a fixed scheme for laboratory testing, eg, by using an agreed algorithm. The use of order algorithms can be extended to post-test algorithms that will assist clinicians in approaching the right diagnosis and prognosis. New methods used in autoimmune serology do not deliver results that can be directly compared to those of older methods, and thus the new methods need to be thoroughly tested with sera from differential diagnostically relevant disease controls to set a clinically meaningful cut-off for positivity. Borderline positive results need to be treated with special care to avoid misuse. Early diagnosis is of great importance, and serological results can be very useful if used the right way. European efforts to secure rational diagnostic work-up in autoimmune rheumatic disease have led to a better dialogue between clinicians and laboratory scientists in several countries. *Lupus* (2006) 15, 391–396.

Key words: autoantibodies; clinical use; early diagnosis; guidelines; methods; reporting

Introduction

Several chronic disorders within the spectrum of rheumatic diseases are classified as autoimmune rheumatic diseases (ARD) based on existence of rheumatic complaints and manifestations and the finding of one or more characteristic autoantibodies in serum. No matter whether such antibodies are assumed to have a pathogenetic impact on the disease or just reflect a particular disease process they have become important tools for diagnostic classification and estimation of prognosis.^{1,2} The diseases categorized most frequently as ARD are listed in Table 1. Some of these conditions are diagnosed by clinical as well as serological criteria, and in other conditions clinical classification criteria are used to set a diagnosis but detection of an autoantibody in that setting is an important support for the diagnosis (Table 1).

At the time when disease criteria were agreed upon decades ago the laboratory test procedures were quite different from those used in most serologic routine settings today, but the criteria still are used as if the technology by which they are detected has little influence on diagnosis. This is far from being true as exemplified in more recent scientific literature.^{3,4} Therefore, it seems more and more important to set up guidelines for autoimmune serology testing to attain optimal support for clinicians in setting a correct diagnosis and estimating a likely prognosis.⁵

Setting up such guidelines inevitably involves intensive collaboration and discussion between experienced clinicians and laboratory scientists, and they must rely on the support of patients who are willing to deliver blood samples for this particular aspect of quality assurance.⁵

When experience with different interactive strategies for diagnosing autoimmune diseases has been tested out by different groups the next step is to determine which strategy will be the optimal in a hospital or a larger health district. The optimal strategy in a small hospital may be very different from that chosen for a

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Table 1 ARD diagnostics using autoantibodies as criteria or diagnostic support

Disease criteria	SLE	ANA, anti-DNA, anti-Sm, anti-ribo P, anti-CL
	MCTD	Anti-n-RNP
	1. SjS	Anti-Ro/La
	APS	Anti-CL, LAC
Diagnostic support	SSc	Anti-centromere, anti-Scl-70, anti-U3RNP, and so on
	PM/DM	Anti-tRNA synthetases, anti-nRNP, and so on
	RA	Anti-CCP, RF
	SVV	NSA, MPO-ANCA, PR3-ANCA, anti-GBM

SLE: systemic lupus erythematosus, MCTD: mixed connective tissue disease, 1. SjS: primary Sjögren's syndrome, APS: antiphospholipid syndrome, SSc: scleroderma, PM/DM: poly-/dermatomyositis, RA: rheumatoid arthritis, SVV: necrotizing small vessel vasculitis, ANA: antinuclear antibodies, DNA: deoxyribonucleic acid, Sm: Smith antigen, ribo P: ribosomal RNP P proteins, CL: cardiolipin, nRNP: U1 nuclear ribonucleoprotein, LAC: lupus anticoagulant, Scl-70: scleroderma 70 antigen, U3RNP: U3 nucleolar ribonucleoprotein, tRNA: transfer ribonucleic acid, SRP: signal recognition particle, CCP: cyclic citrullinated peptide, RF: rheumatoid factors, NSA: neutrophil-specific antibodies, MPO: myeloperoxidase, PR3: proteinase 3, GBM: glomerular basement membrane.

big hospital or from the strategy agreed upon for a larger area in the health system. Nevertheless certain general principles are important to govern the quality of ARD diagnostics, and these principles are discussed in this communication.

It should be emphasized that positive serologic findings at an early phase of a disease have a greater impact on diagnostic considerations, since clinical symptoms in that phase are often faint and less characteristic than later in disease development. Unfortunately, clinical disease classification criteria often demand presence of manifestations that are known to develop late in the disease and thus are of less value for recognizing early disease, typically exemplified by the current ACR criteria for rheumatoid arthritis⁶ that need to be modified to recognize early disease.⁷

Some of the questions addressed in this article are:

- How can we use results derived from use of new technologies?
- Are such results as valuable for diagnostics as those of classical techniques?
- Do we use serological results rationally in clinical work?
- Where can we make progress in clinical diagnostics?
- Do we need more than one method to ascertain a positive result?
- Who decides which methods are optimal for clinical work?

- Will new technologies help solve our problems in diagnostics?
- Can we limit costs for serodiagnostics by use of algorithms for test ordering?
- Can long-term costs for the patient be limited by early diagnosis and intervention?

This communication does not try to answer all these questions but is meant to be a catalogue of issues to be discussed between active partners in the health system.

What is the common situation today?

In a small hospital setting the autoimmune serology service may be located in the department itself close to the patient, and results can easily be discussed in relation to difficult clinical cases. Expertise then comes both from the clinician and the laboratory scientist.

That situation is not very common today. In most diagnostic centres the autoimmune serology laboratory is located distant from the clinician and the patient, and the laboratory acts more like a clinical biochemistry laboratory delivering results that have to be interpreted and used by the clinician without supportive comments (Figure 1). The ideal situation for interactive diagnostics is illustrated in Figure 2. Here the responsibility for setting a correct diagnosis is equally shared between the clinician and the laboratory

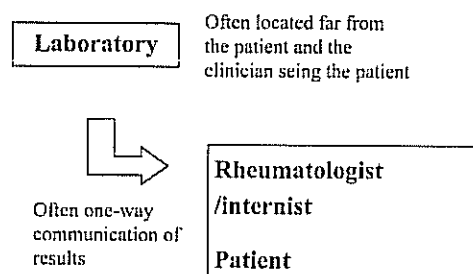


Figure 1 What are the relations between the diagnostic laboratory, the clinician and the patient today?

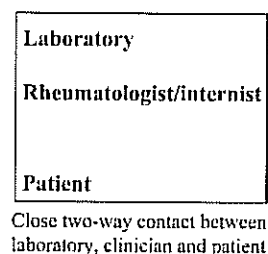


Figure 2 What would be the ideal for clinical diagnostics?

specialist, who collaborate on all levels to ensure high quality diagnostics and the optimal use of test results to the benefit of the patient. The physical distance between the clinic and the laboratory is unimportant if positive results come with some comments for the potential clinical use of the result.

Who decides on the methods used for autoantibody determination?

Today autoimmune serology has become an essential part of the tasks performed by large clinical biochemistry or pathology laboratories. The methods chosen to detect an autoantibody are rarely chosen from the clinical perspective but rather from the aspects of the performing laboratory. This commonly means that the preferred technologies are chosen from a daily work condition aspect such as:

- 1) Assays that can run on automated systems which are already available in the laboratory.
- 2) Avoidance of steps that are labour-intensive to keep cost for testing low.
- 3) High throughput technical platforms, constructed to meet an uncontrollable testing demand.
- 4) Methods that involve subjective interpretation eg, indirect immunofluorescence (IIF) interpretation by fluorescence microscopy should be avoided.

A common excuse for eliminating IIF from routine autoantibody testing has been that 'few persons can accurately classify the images seen' and 'the receiving doctor does not know how to use such results.' Arguments like that are not used in many other areas of medicine based on morphologic recognition by persons eg, histopathology and sophisticated image interpretation and still they are used. In addition, detailed studies have shown that skills in reading and interpreting HEp-2 cell staining patterns are advanced in many persons, both experienced and unexperienced in the field.⁸ Whether the receiver can use a result in a meaningful way or not totally depends on the laboratory that just needs to deliver IIF based results together with useful comments on the potential use of the results.

The main impetus for choosing a method for detecting an autoantibody should always be the wish to deliver results of importance for optimal clinical decision-making and thus benefit the patient. Decisions about technologies for autoantibody demonstration and use have to be taken on the grounds of solid literature knowledge and with long-term disease outcome as an ultimate goal for the rational choice. Decisions about methodologies, thus, ought to be based on a

mutual agreement on what will be optimal for the patient.

Positive results may be clinically misleading

Positive results can be expected to appear in about 5% of individuals who are healthy and in a higher percentage in inflammatory rheumatic conditions if such positive results have not been ruled out or at least limited by the way results are filtered in the laboratory before being sent to the clinic.⁵ Positive results that are clearly clinically 'out-of-context' may be misused by less experienced doctors as support for wrong diagnosis. The more autoantibodies are tested for in an individual patient the greater the chance to get irrelevant positive results. Cut-off limits for positivity should be set after testing a broad spectrum of sera from both local healthy controls and local patients with inflammatory diseases, after which a strategy for delivering results has to be discussed between the involved parties.

To minimize unwanted use of borderline positive results clinicians and laboratory scientists have to agree on how to proceed. They may choose to set a higher positive cut-off value based on their own data, or they may tell the receiving doctor that a borderline positive result cannot be used as a diagnostic criterion just like a definite positive result, or they may select to do additional testing using another test procedure to confirm or refute the borderline result seen at first. Unfortunately, most laboratories just send out 'grey area' positive results without any comment.

It is important to realize that the industry producing kits for autoimmune serodiagnostics normally has very limited access to sera from relevant disease groups. They therefore have to base a positive cut-off value on findings in serum collections from healthy blood donors, selecting a value that is clearly above the mean of the values obtained in healthy persons (eg, the mean value $+2-3 \times SD$). This is a serious limitation to laboratories and clinics wanting to use such kits for testing abnormalities that are differently expressed in a variety of somewhat similar inflammatory conditions. Thus a kit can only become useful after testing a sufficient number of differential diagnostically relevant sera among a local patient clientele after obtaining their consent to use sera for that purpose. Even in the event that data from broad testing of different ARD has been undertaken, it may still be necessary to test an assay using sera from local patients. If the testing by a company has been done eg, in the United States it must be realized that genetic backgrounds, ethnic differences and environmental pressures are considerably different from that in Europe, resulting in great variation as to the frequency, the antigenic specificity and

the associated manifestations of an autoantibody seen in the two populations.

How do we secure appropriateness of laboratory testing?

Some laboratories demand that orders for laboratory testing are furnished with clinical information about the patient in order to secure appropriate testing, but actually such information is often insufficient and may be potentially misleading.⁹ A positive screening result may be used to guide further testing based on the primary screening results. A better principle is to base testing on continually updated information from the doctor following the patient through the diagnostic process and then he is the one who directs further testing.⁵ In this situation it is advantageous to keep sera in a serum repository for some months to allow later rational testing without having to take new blood samples.

In many laboratory centres blood specimens are received without clinical information and testing is based solely on the request for tests, ordered by any doctor with or without expertise, and too often based on an eagerness not to forget any test that might come out positive. Positive results are often delivered without comments on the potential clinical utility though the level of expertise of the receiving doctor is unknown.

Algorithms for ordering laboratory tests

The demand for laboratory testing largely ought to depend on local recommendations and the strategy laid out for rational testing. Such a strategy needs to be discussed between expert users and test performers to reach an agreement on use of clinically optimized testing procedures, agreement on which results are needed quickly and which are not, discussion on whether a rational user-friendly test order algorithm should be constructed to ensure that the ordering procedure follows agreed rules and so on.

An algorithm may be based on an initial screening step using an appropriately broad and sensitive test system eg, screening for antinuclear antibodies (ANA) using HEp-2 cells² or screening for neutrophil-specific antibodies (NSA) using a human leukocyte smear as substrate¹ followed by a planned search for the specificity of the autoantibody in positive cases. Such algorithms have been used for many years in the Nordic countries. The current Danish version is shown in Figure 3. In Denmark the clinician involved in the diagnostic work-up who continuously adjusts the most likely diagnosis according to the most recent clinical and para-clinical findings has been the one to select

which tests to perform after a positive (or negative) screen test has been done.² Alternatively, the same guide can be used in the laboratory to autonomously do stepwise testing for important antibodies after a positive screen test has been found, starting with the first priority tests and only going on with the second priority tests after an agreed strategy for testing or consultation with the clinician in charge of the patient.⁹

A different approach to testing strategy is to construct the order form in such a way that the tentative diagnosis given by the clinician will guide the whole test procedure, starting with a screening test and continuing with rationally selected specific tests.² This is an advantage to the less experienced doctor (junior doctor or family practitioner). The experienced doctor can tick any one or several boxes that will satisfy his needs.

Simple guidelines for autoantibody screening can also be adapted for use by family practitioners, so they are helped to suspect or recognize chronic inflammatory rheumatic diseases at an early stage. In many ARD the simple detection of a strong ANA can strengthen the suspicion that a patient suffers from an ARD, and form a sound basis for handing the patient over to a specialist. Other examples are testing for rheumatoid factors and anti-CCP in a patient with polyarthritis, or testing for anti-cardiolipin antibody in a patient with recurrent venous or arterial thrombosis and/or repeated pregnancy loss, or testing for ANCA in a patient with unexplained recurrent upper airway inflammation. In any case, the guideline must stress that early hand-over to a selected specialist in cases suspected of an ARD is mandatory to provide the patient with a definite diagnosis and avoid organ damage and extension of disease using appropriate follow-up and carefully chosen therapy.

European efforts to set guidelines for ARD diagnostics

During the last five years a steering group consisting of the authors of this communication has been working to harmonize different approaches to ARD diagnostics and attempt to improve the communication between clinical rheumatologists and laboratory immunologists responsible for autoimmune serology. Another aim has been to develop better tools for doctors with different levels of expertise to set diagnosis, estimate prognosis and plan follow-up in such a way that treatment is implemented at the most appropriate time of disease development. The group has been named European Autoimmunity Standardization Initiative (EASI), although it is evident that this name does not truly reflect the activities of the group.

During the meetings of the EASI steering group we have especially discussed the benefits of having similar

	ANA	DNA	Sm	nRNP	Ro/La	Sci70	Jo1	RiboP	NSA	PR3	MPO	CL	b2-GP1	RF	CCP
SLE	1	2	2	3	2			2				2	3	3	
SjS	1	3			2			3				3		2	
SSc x)	1			2	3	2						3			
MCTD	1	3	3	2	3			3				3		2	
PM/DM y)	1			2			2					3			
RA					3									1	1
APS z)	1											1	2		
SVV									1	2	2				
DI-LE	1*								1	2	2	1	2		

1 = primary screen test, 2 = second priority test 3 = third priority test, * especially anti-histone.

Primary screen tests can conveniently be done through the family practitioner, secondary and tertiary tests through the relevant clinical specialist.

Figure 3 Algorithm used for test ordering in Denmark. An example of how an algorithm for test ordering has been set up for use in Denmark. The rheumatic disorders are listed in the left column, and the tests that will be relevant in the upper panel, here using a commonly used abbreviation for each test (see below). The digit 1 denotes the test(s) most appropriate for the first screen test, the digit 2 denotes second priority tests which usually depend on the results of the primary screening results. The digit 3 is used for tests of third priority that may be relevant in some cases. The x) has been placed to make the experts aware of the fact that certain ANA, primarily those directed to nucleolar antigens, cannot be detected with routine tests, but demands use of special testing eg, by radio-immunoprecipitation. The y) denotes that many autoantibodies found in patients with polymyositis/dermatomyositis cannot be detected without use of special testing eg, by radio-immunoprecipitation. The z) should help the ordering doctor remember that lupus anticoagulant should also be ordered in a case suspect of anti-phospholipid syndrome.

SLE; systemic lupus erythematosus, SjS; Sjögren's syndrome, SSc; scleroderma, MCTD; mixed connective tissue disease, PM/DM; poly- and dermatomyositis, RA; rheumatoid arthritis, APS; anti-phospholipid syndrome, SVV; primary small vessel vasculitis, DI-LE; drug-induced lupus erythematosus, ANA; antinuclear antibodies, DNA; anti-double-stranded DNA, Sm; anti-Smith, nRNP; anti-U1RNP, Ro/La; anti-Ro/anti-La, Sci70; anti-Sci70, Jo1; anti-Jo1, Ribo P; anti-ribosomal P protein, NSA; neutrophil-specific autoantibodies, PR3; proteinase 3-ANCA, MPO; myeloperoxidase-ANCA, CL; anti-cardiolipin, b2-GP1; anti-β₂-glycoprotein 1, RF; rheumatoid factors, CCP; anti-citrullinated protein antibodies.

guidelines for appropriate test ordering across Europe and Israel using algorithms, but also agreeing how to handle practical problems arising with borderline positive results (commonly seen with solid phase assays). We also wanted to help people involved in diagnostics to evaluate the effect of switching from classical to new technologies, especially when it comes to the use of results for differential diagnostics. The fast advancement of new technologies in the autoimmunity field is a great challenge both to the industry, the clinical laboratories, and the clinicians, and our endeavour is to help evaluating the kit market products by setting up critical post-marketing evaluations before a new test is approved for routine use.^{5,10} Another goal was to pinpoint when an autoantibody can be reported as positive only after being run by two different methods eg, anti-dsDNA to ensure its value as a criterion for systemic lupus erythematosus.^{3,4} The former use of the Farr RIA test for anti-dsDNA antibodies have largely been replaced by ELISA techniques with too low recommended cut-off values, although the Farr technique gave the most clinically valuable results. We are now in

the process of creating national EASI teams headed by an expert in rheumatology and an expert in clinical immunology who will take suggestions from the steering group as topics for discussion at the national level. In some instances it is foreseen that some amendments to eg, the algorithm for test ordering (Figure 3) have to be done, but the important thing is to start a dialogue that can lead to a greater harmonization of diagnostic habits and strategies to decrease costs and avoid irrational testing that can lead to wrong diagnosis. The national teams will be organized in a multinational EASI Forum that is planned to meet annually in conjunction with an international meeting trying to summarize the advancement of the EASI efforts into national and European guidelines. Every second year it is planned to have a special session on the issues and results derived from the EASI collaborative agreements for everyone interested in these matters. The EASI network is displayed in Figure 4.

At the present time national teams consisting of clinical and laboratory experts are working in Italy, Spain, France, Germany, the Netherlands, and the

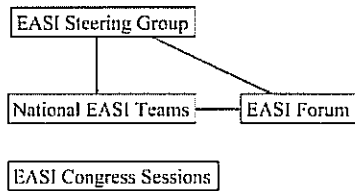


Figure 4 The EASI network.

Nordic Countries, while we are waiting to start EASI activities in the United Kingdom and several other countries that have expressed an interest in joining these activities. We do hope that these efforts will lead to an open dialogue between involved parties all over Europe, and hopefully in the rest of the World.

The EASI Steering Committee is now exploring how the EASI collaborative network can get directly involved with EULAR.

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