Translational Autoimmunology

Examples from Diagnostic Rheumatology

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With the advancement of new assay principles and new technology platforms it is utterly important to keep in mind that results derived from such technologies must be at least as useful to clinicians taking care of patients as that attained by the use of older methods. Positive and negative predictive values of autoantibodies for the diagnosis of chronic immunoinflammatory rheumatic diseases (often called connective tissue diseases – CTD) mostly have been calculated on the basis of classical autoimmune serology testing using double immunodiffusion, indirect immunofluorescence (IF), enzyme-linked immuno-assay (ELISA), immunoblotting, and radioprecipitation, and thus, data from new technologies cannot be directly extrapolated from the older data without rigorous testing of the new methods. In general, results from new technology testing can only be soundly based on testing sera from a prototype patient group compared against mixed populations of CTD patients, that may cause differential diagnostic problems towards prototype disease in the daily clinical work.

Zusammenfassung

Der diagnostische Wert von Laboruntersuchungen für Autoantikörper steht und fällt mit der Validierung der einzeln zur Anwendung gelangenden Methode. Wir verwenden positive und negative Patientenseren als Referenzproben, um die Produktionskonstanz der einzelnen Methode zu gewährleisten. Dabei ist es von Vorteil, jedenfalls bei der ersten diagnostischen Untersuchung, wenn 2 oder 3 verschiedene Testansätze verwendet werden, um das Vorliegen desselben Autoantikörpers zu dokumentieren, also z.B. ELISA und Immunfluoreszenz. Wenn das diagnostische Labor dem Kliniker die Testverordnung in Form von Algorithmen nahe legt, entsteht für den Patienten ein sinnvolles und für therapeutische Zwecke nützliches Resultatemuster.

Laboratory aspects of autoimmune serodiagnostics

Both the work load and the test repertoire are increasing, so many laboratories feel that the demand for testing is uncontrollable and not firmly based on a pre-test diagnosis of a CTD where a positive finding can be used in a meaningful way to help set diagnosis, estimate prognosis and judge how to follow-up and treat the patient. The great importance of basing orders for autoantibody tests on a few characteristic clinical manifestations instead of one has been illustrated in recent publications [1].

In general, laboratories want to replace old labour-intensive and subjective methods by low cost, commoditized, easy-to-handle techniques, which may be very handy from the laboratory aspect, but can only be of clinical value if the differential diagnostic potential (nosographic sensitivity and diagnostic specificity) has been thoroughly validated locally, since ethnic and genetic as well as extrinsic factors (milieu, infectious pressure, etc.) have an influence on autoantibody production [2].

The role of patients, clinicians, and laboratory experts

Ideally, sera should be collected from patients who are willing to donate their blood for purposes of validation and quality assurance of autoantibody testing, preferentially taken from early diagnostic cases, where laboratory results have maximum impact on diagnostic considerations. Experienced clinicians need to collect clinical data just at the time of serum sampling, while adhering to strict definition of each criterion registered. An important decision to be made by collaborative efforts is the handling of borderline results ("grey area positives"): Should only true positive results be reported as positive after testing by two independent methods? Should the results be reported as positive with a caveat note about the uncertain applicability of it? Or should a new cut-off value for positivity be selected based on optimal differentiation between the prototype disease and its mimics?

Practical laboratory issues

It is worth noting that most autoantibodies are very stably produced, and only a few need to be quantified for reasons of diagnostics and relapse prediction [3, 4]. Thus anti-dsDNA antibodies revealed by radio-precipitation have been shown to predict onset and relapses of systemic lupus erythematosus (SLE), and proteinase 3 (PR3)-anti-neutrophil cytoplasm antibodies (ANCA) similarly shown to predict relapses in ANCA-associated vasculitides.

In routine serology it is practical to start testing using a sensitive assay, e.g. IF technique on high quality cell substrates (HEp-2 cells, buffy coat cells), and then focus on a specific antibody by testing on a pure antigen substrate like native or recombinant autoantigen. Tests used to diagnose or to estimate prognosis of a disease thus need to have a high differential diagnostic potential while the nosographic sensitivity is unimportant [2]. Whether frequent or rare a specific autoantibody commonly indicates an association to a clinically important subgroup of the disease with certain manifestations and a related prognosis [2, 5].

To keep clinicians optimally informed about test ordering, clinical and laboratory directors should agree on setting up a test algorithm, that alleviates rational ordering even by less experienced medical personnel [2]. In Denmark such an algorithm (figure 1) has

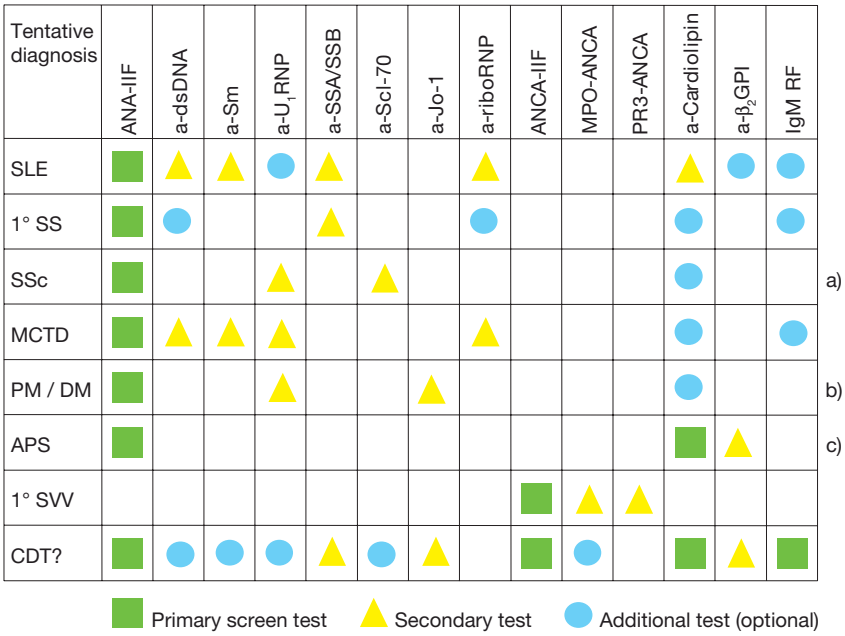

Figure 1. Example of a test ordering algorithm used in Denmark, based on tentative diagnoses (vertical column) and type of tests (horizontal column). a) ideally should include scleroderma panel testing for anti-RNA polymerase I and III, anti-U3RNP, and anti-To/Th RNP antibodies. b) ideally should include myositis panel testing for anti-aminoacyl-tRNA synthetase, anti-SRP, anti-Mi2, anti-PM/Scl, and anti-Ku antibodies. c) should include lupus anti-coagulant.
Abbreviations: **SLE systemic lupus erythematosus, 1'SS primary Sjögren's syndrome, SSc systemic sclerosis, MCTD mixed connective tissue disease, PM/DM poly/dermatomyositis, APS anti-phospholipid syndrome, 1'SVV primary small vessel vasculitis (e.g. Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and oligosymptomatic forms of these).**

been used for about ten years, resulting in more satisfied clinicians as well as technical staff of the laboratory due to a clear fall in unnecessary testing and a better understanding of how to proceed when a screening test comes out positive. This clearly limits costs both for the laboratory and the clinics. The main conceptual aim of this approach has been to leave the doctor who is actually seeing the patient in the decisive role instead of the laboratory operating in the blind by testing for a large battery of antibodies. If due attention is paid to the IF staining pattern found on screening for ANA or ANCA using HEp-2 cells and human buffy coat cells, respectively, as substrates, a much narrower specific test battery is needed [1, 2].

Another approach is to set up the order form in such a way that a customer can tick a box according to a tentative diagnosis, after which the laboratory will test for a previously agreed battery of autoantibodies, while the expert has the freedom to tick just one or two of the disease-related specific antibody (antibodies). The director of a clinic can also construct algorithms for the use of positive and negative test results in relationship to important diagnoses as part of a clinical quality assurance programme.

Economic aspects of laboratory diagnostics

To function optimally in daily clinical use, test results must be delivered early and must have an impact on decision-making, strategy and follow-up planning to be important for the final outcome of disease. Today laboratories mostly consider short-term costs for the laboratory itself, however, what really impacts health economics are the number of visits to clinics, use of costly diagnostic procedures, e.g. imaging techniques, length and cost of stay in hospital, readmission rate, working days lost, and productive years gained.

Résumé

La valeur diagnostique des examens de laboratoire visant la présence d'autoanticorps dépend de la validation des différentes méthodes appliquées. Ainsi, il est recommandé de mettre en évidence les autoanticorps d'une certaine spécificité par des approches différentes, par exemple par l'ELISA et par l'immunofluorescence. Nous utilisons d'emblée des sérums de patients et de contrôle de donneurs bien portants à chaque issu pour ainsi garantir la reproductibilité de la méthode choisie. Un algorithme est rendu aux cliniciens qui pourront ainsi améliorer leur choix des examens demandés au laboratoire tout en pouvant attendre une constellation de différents résultats servant de base à leurs décisions thérapeutiques.

Conclusions

Cutting edge autoimmune diagnostics cannot be exercised without close collaboration between clinical and laboratory experts. More than one method must be available to confirm or refute questionable results. Quality management should be obligatory both in clinics and laboratories, including information about optimal test ordering and result interpretation. All of these issues have to be tailored to local wishes, needs, policy and economics.

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