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Renato Tozzoli, Nicola Bizzaro

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**THE CLINICAL AUTOIMMUNOLOGIST AND THE LABORATORY AUTOIMMUNOLOGIST: THE TWO SIDES OF THE COIN**

Renato Tozzoli <sup>a</sup>, Nicola Bizzaro <sup>b,\*</sup>

<sup>a</sup> Laboratory of Clinical Pathology, Department of Laboratory Medicine, Azienda Ospedaliera S. Maria degli Angeli, Pordenone, Italy

<sup>b</sup> Laboratory of Clinical Pathology, S. Antonio Hospital, Tolmezzo, Italy

\* Corresponding author at:

Laboratory of Clinical Pathology  
Ospedale S. Antonio  
via Morgagni, 18  
33028 Tolmezzo (UD)  
Italy  
phone +39-0433-488261  
fax +39-0433-488697  
e-mail: nicola.bizzaro@ass3.sanita.fvg.it

*Abstract*

The objective of this review is to analyze the causes of the increasing interest of Laboratory Medicine in the definition and characterization of a new sub-discipline, laboratory autoimmunology, concerning the development and the clinical use of tests for measuring circulating autoantibodies and the study of autoimmune serological reactions. The laboratory autoimmunologist's task includes knowledge of the range of technical solutions available to identify the different types of autoantibodies and provide laboratory consult for improving utilization of laboratory results. Along with the figure of the laboratory autoimmunologist, the position of clinical autoimmunologist not only has been proposed but has already found its first concrete applications. This is a specialist with a wide knowledge of those symptoms and diagnostic procedures necessary to identify autoimmune diseases, and who is familiar with the range of therapies available to treat various diseases. Cooperation between the two autoimmunology specialists will lead to a more up-to-date and efficient management of autoimmune patients.

**Key words:** Autoimmunology, autoimmune diseases, autoimmunology laboratories

**1. Introduction**

Autoimmune diseases (AIDs) are human pathologies increasing in frequency worldwide. Medical reports from industrialized countries show that nearly 5-10% of individuals (80% females) suffer from one or more autoimmune diseases [1-3]. This phenomenon is equivalent to an epidemic, and is probably due to the improvement of social and economic conditions of the population and the related reduction of infectious stimuli on the immune system (hygiene hypothesis) [4].

Considering that AIDs cumulatively are the third cause of illness and disability behind heart disease and cancer, in the last two decades many efforts have been made aimed at early diagnosis and effective therapy of AIDs. Recently, some authors emphasized the necessity of creating a new medical specialty, autoimmunology [5-9], and a new specialist, the autoimmunologist.

Although these definitions have been recently updated [5], they are not new in the medical literature: the term autoimmunology first appeared nearly 50 years ago in a paper of E. Kass [10] on systemic AIDs. However, in the following 10 years, only two publications — one on psoriasis [11] and one on cardiovascular diseases [12] — referred to autoimmunology.

As a result, the concept of autoimmunology as a distinct discipline is established principally in the arena of laboratory diagnostics and, since the '90's, numerous studies have been produced by physicians and scientists interested in the laboratory diagnostic aspects of many AIDs. In time, therefore, within the laboratory a specialist evolved as a physician/scientist with multidisciplinary competence in the field of autoimmune diseases. The reasons for this fusion of abilities can be found in the increase in antibody tests developed by the biomedical industry and by the enormous evolution and increased complexity of methods to measure autoantibodies. This has brought about a progressive and diffuse creation of sectors within the laboratory dedicated to diagnostics of autoimmune diseases.

The need for a specialist role—the laboratory autoimmunologist—is more obvious in light of some fundamental changes that have taken place in recent years in the field of laboratory diagnosis of AIDs,

namely, a new view on the importance of autoantibodies, the parallel evolution of technology, and the organizational consolidation of clinical laboratories.

## **2. The evolution of the role (diagnostic, prognostic, predictive) of autoantibodies.**

As of the production of high-affinity specific autoantibodies (AABs) represents a hallmark of autoimmune reactions, the use of immunoassay methods for their detection has gradually entered clinical practice as a tool for the diagnosis of AIDs. One or more laboratory tests constitute criteria for the diagnosis and/or the classification of AIDs. For example, anti-nuclear antibodies (ANA), anti-dsDNA, anti-Sm and anti-phospholipid antibodies mark systemic lupus erythematosus (SLE); rheumatoid factor and anti-citrullinated peptide antibodies (ACPA) are indicative for rheumatoid arthritis (RA); anti-thyroid peroxidase for Hashimoto's thyroiditis; anti-gliadin (AGA) and anti-tissue transglutaminase (tTGAb) for celiac disease (CD). The quantitative measurement of AABs also allows for monitoring of the clinical course of AIDs, especially disease activity and response to treatment, as in the case of anti-dsDNA in SLE and anti-TSH receptor antibodies (TRAb) in Graves' disease [13].

Besides their diagnostic and prognostic role, many specific AABs are early screening indicators, as they can be detected in the sera of asymptomatic subjects who later eventually develop an overt systemic [14, 15] or organ-specific [16-18] AID. These studies are important because they redefined the natural history of AIDs, as a group of human diseases characterized by a long latency period and by the appearance of AABs when clinical manifestations are still absent. Indeed, AABs can precede the clinical symptoms of the disease by years, and can be used for early diagnosis purposes, including screening studies.

## **3. The evolution of immunoassays for autoantibody detection: from 1<sup>st</sup> to 2<sup>nd</sup> and 3<sup>rd</sup> generation**

In the nineties, the discovery of new autoantigens and the progressive refinement of purification techniques increased the accuracy of AABs tests, improving the diagnosis and classification of several important AIDs. Some examples of this development are the detection methods of anti-nucleosome antibodies, ACPA, tTGAb and TRAb (**table 1**). In addition, the availability of recombinant antigen preparations has significantly increased the analytical and diagnostic sensitivity of commercial immunoassays, which are largely superior to the values obtained with 1<sup>st</sup> generation home-made techniques. These results were achieved without reducing the analytical and diagnostic specificity of the tests, thus providing great advantage in clinical practice [19].

Among the research methods for AABs, classifiable in a modern perspective as "monoplex" and "multiplex" [20], indirect immunofluorescence (IIF) and immunometric assays have had, and still have, a relevant role. The IIF method, which can be considered as the first multiplex method, being able to detect more than 60 AABs [21], has been used for a long time in academic or research laboratories, similarly to other first-generation qualitative immunological methods (reviewed in 18) (**table 2**). Since the '80's, this method has had its place in the repertoire of commercial technologies of hospital clinical laboratories. During the same period, following the saga of the radioimmunoassay method, the monoplex quantitative 2<sup>nd</sup>-generation and later on 3<sup>rd</sup>-generation commercial non-isotopic methods were made available for clinical pathology laboratories and they were rapidly implemented with automatic analyzers which were capable of executing different types of tests simultaneously, to detect various analytes such as hormones, humoral biomarkers, cytokines, as well as antibodies.

Since the year 2000, research has produced an additional rapid advancement in diagnostic technologies directed at detection and quantification of AABs. This technological evolution has been brought about by

the refinement of methods for purifying autoantigens and by the introduction of proteomic technologies, which bring the possibility of simultaneous determination of several antibodies in the same reaction environment (multiplexing) (**figure 1**).

#### ***4. The evolution of clinical immunology laboratories: from academic research laboratories to autoimmunology laboratories***

In the last 15 years, as in other subdisciplines of laboratory medicine, the clinical immunology laboratory has advanced toward a phenomenon of technological evolution which had been anticipated during the end of the second millennium [22]. In any case, although the progressive improvement in technology had been foreseen due to the accelerating effort to introduce total laboratory automation in diagnostic immunology [23], it was not predicted that such a large increase in demand for autoantibody tests would occur [24]. The increase in demand and the increasing automation of testing has produced an organizational consolidation, with testing becoming more concentrated in general clinical pathology laboratories rather than in academic research laboratories. Thus, in clinical laboratories in the USA and Europe, specialty sectors dedicated to autoimmunology testing have arisen. These are the new autoimmunology laboratories.

#### ***5. The evolution of immunology laboratory specialists***

As pointed out by M. Plebani [25], the progressive development of specialized sectors in clinical laboratories and the correlated increase in the number of tests and their complexity have given rise to difficulties in therapy prescribing and in data interpretation by general practitioners and physicians receiving laboratory tests outside their own specialty area. These professionals are, in general, not familiar with specifics of autoantibodies and autoimmunity-related tests. In any case, it is now clear that laboratory information is only partially utilized. As in the case of routine tests [26], probably also in the case of autoimmunity-related tests, physicians encounter difficulties in interpreting abnormal test results. For these reasons, there is a need for a laboratory specialist that helps physicians in the prescription and clinical interpretation of laboratory results. This type of specialist in the field of AIDs is necessarily the laboratory autoimmunologist.

However, in immunology laboratories, two types of autoimmunity specialist have developed, each with its own operative logic and expertise. The first type (usually the senior consultant with a clinical background) is primarily dedicated to individual clinical cases with relevance to the internist (rheumatology, endocrinology, etc), closely interconnected with the clinician and keenly interested in case-finding. The second type—the biochemist—is progressively involved in managing large volumes of tests and is strictly dedicated to maximizing efficiency and turn-around time (TAT) of laboratory tests. The recent phenomenon of consolidation of laboratories has brought to the concentration in immunology laboratories the two types of specialist which have, more and more, shared their own expertise, becoming experts in diagnostic technology and management, while retaining interest in case-finding and in interpretation of results. Therefore, a new professional role is defined, one who is able to forge into one the professional characteristics of the clinical pathologist: familiarity with the necessary technologies, the organizational ability to manage the through-flow of samples and information; and the capacity to counsel the clinician on diagnostics and monitoring of therapy for individual patients.

Today, the responsibilities of the laboratory autoimmunologist concern all three phases of laboratory procedure—pre-analytic, analytic, and post-analytic—to optimize the use of autoimmunity tests, through the use also of laboratory management systems.

In the pre-analytical phase the main task is to regulate test requests (appropriateness) [27, 28], which encompasses promoting awareness of correct procedures and guidelines related to autoantibody testing [29, 30], predisposing adequate forms for test request [31, 32], and suggesting diagnostic rules and algorithms based on reflex or reflective tests [33], including first-line tests [32,34,35].

In the analytical phase the laboratory autoimmunologist must be responsible for up-to-date knowledge of technologies and methods, for measuring AABs, for choosing systems to introduce to his/her own lab, for the correct and professional use of manual (i.e morphologic identification of fluorescence pattern on IIF) and automated technologies (i.e semi- and fully-automated immunoassay analyzers), for their standardization [36] and application of procedures to ensure the quality of autoimmune diagnostic tests [37]. It is now clear that the evaluation of the impact of new technologies requires constant updating on the state-of-the-art of laboratory autoimmunology which no longer can be left in non-expert hands.

In the post-analytical phase, the laboratory autoimmunologist must accurately define the diagnostic strength of the tests [32, 38] and provide interpretative comments on reports for improving interpretation and utilization of laboratory results by the clinical autoimmunologist [24,25,39-41]. There are many instances in which the clinical value of an autoimmunity test can be considerably enhanced by an accompanying 'canned' or 'narrative' written comment. They include: a) false (i.e. due to an interference) or true cases of unexpected autoantibody positivity [42]; b) positive finding of AABs with low or unexpected clinical correlation; and c) finding of inconsistent results when two or more methods are used to detect the same antibody [39].

Besides, analytical quality specifications of AAb tests change over time, thus affecting the related comparative values (reference ranges and decision limits). The correct definition and updating of reference values and decision levels, by means of direct or indirect methods based on current data, is an important task of the laboratory autoimmunologist [43].

## ***6. The laboratory autoimmunologist and the clinical autoimmunologist: two cooperating and collaborating specialists***

We maintain that the motivations of laboratory physicians are the same as those which have recently brought the world of internal medicine to propose the establishment of autoimmunology as a new clinical discipline. These motives arise from the constantly increasing number of patients with AIDs, from the recognition that many diseases have an autoimmune etiopathogenesis, often with pathogenic mechanisms in common, and from the availability of therapeutic treatments that are ever more efficacious and targeted.

We note, therefore, that currently there are two branches of this new specialty: clinical autoimmunology and laboratory autoimmunology. According to the proposal of Y. Shoenfeld [5], clinical autoimmunologists and laboratory autoimmunologists must cooperate and collaborate within the autoimmunology department. The first must make use of their knowledge of symptoms and of diagnostic tests necessary to identify AIDs, and should be familiar with the range of therapies available to treat the different types of diseases [44]. The second must contribute to diagnosis through expertise in the management of immunologic laboratory tests and knowledge of the range of technical solutions available to identify the

different types of autoantibodies or autoimmune reactions (source of antigens, type of reagents and immunological reaction in automated analyzers, etc). We believe that the integration of clinical and laboratory competencies is achievable in all hospital settings, whether large hospital complexes, university hospitals, or small local hospitals.

In large hospital settings or university hospitals the organizational model is represented by the Department of Autoimmunology [5] or Autoimmune Diseases Unit [7, 44, 45], where a pool of clinical specialists from diverse disciplines, including laboratory autoimmunologists, will diagnose, treat and follow patients with any AID.

In smaller hospitals, where the departmental model is not possible due to the lack of a sufficient number of specialists for diagnosis and treatment of autoimmune diseases, the integration of capabilities assigns a central role to the laboratory autoimmunologist. In creating an autoimmunology diagnostics unit within the clinical laboratory, the laboratory autoimmunologist must choose the most appropriate methods and plan to execute the most commonly used autoantibody tests. He/she must also be ready to furnish information on the diagnostic strength of these tests through interpretative reports provided to hospitalists and to general practitioners [24,25,39].

Why assign this role to the laboratory autoimmunologist? Because, while diagnosis and therapy of individual autoimmune diseases requires the expertise of individual specialists in the various disciplines of internal medicine, and therefore the evolution of the role of the clinical autoimmunologist is only just beginning, the laboratory diagnosis of AIDs depends on a common analytical approach (use of similar monoplex e/o multiplex methods and technologies for the different types of autoantibody) and a cross-cutting activity of interdisciplinary clinical counseling. In practice, the process of coordinating different disciplines within the laboratory is by now complete, under the guides of standardization of technology.

Moreover, in smaller hospitals, the lab autoimmunologist can be a cornerstone of a layer of specialization for clinical internists who are interested in autoimmune diseases. A more multidisciplinary and cross-cutting role will evolve in time: there will thus be an autoimmunology unit, similar to the model in the large hospitals, even with the constraints of a small hospital.

Whichever be the model adopted in relation to the size of the hospital and local exigencies, the cooperation and collaboration between different autoimmunology specialists will allow the improvement of the laboratory-clinical interface and will allow for better diagnostic and therapeutic actions for patients. Probably it will take a bit of time for these organizational models to become manifest in practice, but we believe that the road has been laid and that the two types of autoimmunologist (clinical and laboratory) need only follow it.

**Conflict of interest:** None to declare

**Take home messages**

- The increase in antibody tests developed by the biomedical industry and the huge evolution and increased complexity of methods to measure autoantibodies, has brought about a progressive and diffuse creation of autoimmune disease sectors within the clinical laboratories and the consequent development of a laboratory specialist with multidisciplinary competence in the field of autoimmune diseases.
- The responsibilities of the laboratory autoimmunologist concern all phases of laboratory procedure (pre-analytic, analytic, and post-analytic) to optimize the use of autoimmunity tests, also through the involvement of laboratory management systems.
- A major task of the laboratory autoimmunologist is to provide counseling on test results, also through interpretative comments on reports, to hospital specialists and to general practitioners.
- The clinical autoimmunologist is a specialist with a wide knowledge of symptoms and diagnostic procedures necessary to identify autoimmune diseases, and who is familiar with the range of therapies available to treat various diseases.
- Clinical autoimmunologists and laboratory autoimmunologists must cooperate within the autoimmunology department. Their collaboration will allow for the improvement of the laboratory-clinical interface and for better diagnostic and therapeutic actions for patients.

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| Autoimmune disease           | 'Old' antigen<br>(1 <sup>st</sup> generation) | 'New' antigen<br>(2 <sup>nd</sup> - and 3 <sup>rd</sup> -generation) | Autoantibodies                       |
|------------------------------|-----------------------------------------------|----------------------------------------------------------------------|--------------------------------------|
| Systemic lupus erythematosus | Intact nucleosome                             | H1-stripped nucleosome                                               | ANuA-IgG                             |
| Rheumatoid arthritis         | CCP1 (1 peptide)                              | CCP2 (10 peptides)<br>MCV, VCP, ECP-1                                | ACPA-IgG                             |
| Celiac disease               | Guinea pig tTG<br>Gliadin                     | Human recombinant tTG<br>Deamidated gliadin peptides                 | tTG-IgA, tTG-IgG<br>DGP-IgA, DGP-IgG |
| Graves' disease              | Porcine TSHR                                  | Human recombinant TSHR<br>M22 MoAb                                   | TRAb-IgG                             |

Table 1. 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> generation conventional immunoassay methods for autoantibody detection. ANuA, anti-nucleosome antibodies; CCP, cyclic citrullinated peptides; MCV, mutated citrullinated vimentin; VCP, viral citrullinated peptide; ECP-1, enolase citrullinated peptide; tTG, tissue transglutaminase; TSHR, TSH receptor; MoAb, monoclonal antibody.

|                                                                                   |             |
|-----------------------------------------------------------------------------------|-------------|
| <b>1<sup>st</sup>-generation monoplex immunoassay methods</b>                     |             |
| Double immunodiffusion                                                            | ID          |
| Complement fixation                                                               | CF          |
| Indirect immunofluorescence                                                       | IIF         |
| Passive agglutination                                                             | PHA, LPA    |
| Radio-immunoprecipitation                                                         | RIPA        |
| Westernblot                                                                       | WB          |
| <b>2<sup>nd</sup>- and 3<sup>rd</sup>-generation monoplex immunoassay methods</b> |             |
| Radioimmunoassay, Immunoradiometric assay                                         | RIA-IRMA    |
| Radio-receptor assay                                                              | RRA         |
| Enzymeimmunoassay                                                                 | ELISA, IEMA |
| Immunoblot                                                                        | IB          |
| Immunodot                                                                         | DB          |
| Chemiluminescent immunoassay                                                      | CLIA-ILMA   |
| Fluoroimmunoassay                                                                 | FIA-IFMA    |
| <b>Multiplex immunoassay methods</b>                                              |             |
| Addressed microbead immunoassay                                                   | MBA         |
| Line immunoassay                                                                  | LIA         |

Table 2. Classification of immunoassay methods for autoantibody detection.

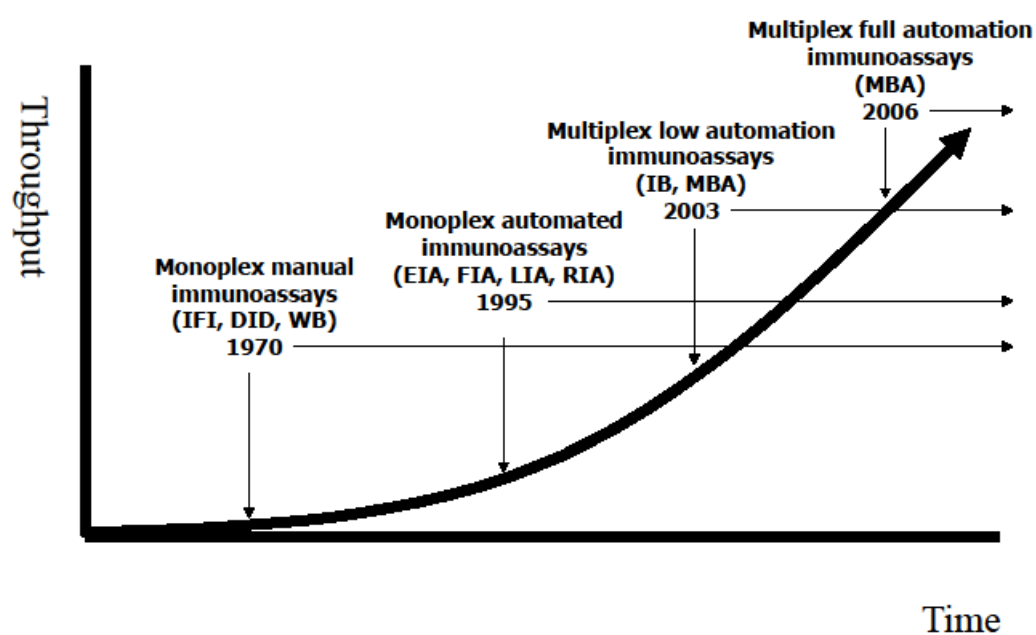


Fig. 1. The evolution of technologies for autoantibody detection: the role of automation. (See table for legends).