



Review

Italian multicentre study for application of a diagnostic algorithm in autoantibody testing for autoimmune rheumatic disease: Conclusive results

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ABSTRACT

Aim: The presence of specific auto-antibodies in serum (i.e., antinuclear antibodies or ANA, anti-extractable nuclear antigens or anti-ENA, and anti-double stranded DNA or anti-dsDNA) is one of the major criteria in the diagnostics of Autoimmune Rheumatic Disease. As such, the request for these tests has grown exponentially in laboratory practice. The aim of this study is to describe the implementation of a joint laboratory-clinics guideline for reducing clinically inappropriate requests for autoantibody testing in a broad geographic area (Parma, Modena, Piacenza, Reggio-Emilia) for the diagnosis of Autoimmune Rheumatic Disease.

Methods: This study, supported by a Regional grant for innovative research projects started in January 2008, is an observational research aimed at comparing the number of ANA, anti-dsDNA and anti-ENA testing as well as the percentage of positive test results before and after implementation of the diagnostic algorithm in hospitalized patients. A multidisciplinary team consisting of clinical immunologist and laboratory scientists was established, with the aim of collecting and analysing diagnostic criteria, clinical needs, laboratory report formats, analytical procedures, as well as the number of tests performed. The laboratory results and the clinical protocol were both validated by data emerging from the clinical follow-up studies.

Results: A joint guideline for auto-antibody testing, placing ANA test at the first level, has been developed and implemented since January 2009. The results for the period January–June 2009 (12,738 tests) were compared with those of the same period in 2008 (13,067 tests). A significant reduction in the number of anti-dsDNA (–26%) and anti-ENA (–15%) was observed. The percentage of second-level tests positivity after implementation of the diagnostic protocol had also consistently increased for both ENA (13% vs 17%) and dsDNA (9% vs 11%).

Discussion: The development and implementation of algorithms for the diagnostics of Autoimmune Rheumatic Disease in hospitalized patients was associated with a reduction in the number of second-level tests, but also with an increased diagnostic specificity. This outcome attests that close collaboration and audit between clinicians, laboratory specialists and healthcare services is effective to develop efficient diagnostic algorithms for both hospitalized patients and outpatients.

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1. Introduction

The field of autoimmunity diseases has been the focus of several clinical and experimental studies on the physiological and pathological mechanisms of immunity over the past few years. Autoimmune diseases are heterogeneous pathologies according to symptoms, organic lesions and prognosis. For all these reasons, these disorders are challenging to manage and need a multidisciplinary approach, both diagnostic and therapeutic [1,2].

The vast majority of autoimmune diseases share a similar pathway, that is reactions against self cells, tissues or organs. A disease-specific antibody set in patient sera is also frequently detected, so that the laboratory plays a critical role to help interpreting a clinical presentation that can often be misleading [3–8].

The recent advances in diagnostic technologies for autoimmune diseases have remarkably impacted on the diagnostic approach to these pathologies [9–12]. Since the presence of anti-nuclear antibodies in the serum, i.e. anti-nuclear antibodies (ANA), anti-extractable nuclear antigens (ENA) and anti-double stranded DNA (dsDNA), is one of the leading diagnostic criteria for Autoimmune Rheumatic Disease [13,14], the test request for these assays has expectedly grown exponentially, due to increasing knowledge of the pathogenetic and diagnostic value of autoantibodies in autoimmune diseases and – less advisably – for the inappropriate use of laboratory resources. This latter problem has not been precisely estimated as yet, and is mainly due to several factors, including also inadequate collaboration or audit between physicians and laboratory professionals [1,15], availability of different techniques and methodologies for assessing biomarkers and the lack of homogeneous terminology and diagnostic algorithms when performing autoantibodies testing.

The containment of the number of clinically inappropriate test requests and the establishment of a reasonable balance between available economic resources and increasing needs is a leading target of the healthcare services worldwide [16]. As such, the most recent project of “governance” issued by the Italian Ministry of Health, has as a specific goal, i.e. “providing citizens with health care services of the highest possible quality, taking into account the available financial support”.

In order to improve the appropriateness of the test requests in auto-antibody testing, it is essential to define and implement reliable and broadly agreed diagnostic algorithms, which should be developed using the available guidelines of the current scientific literature [17–20] and should be shared by all physicians with expertise in the field of clinical Immunology.

The most appropriate strategy for requesting Autoimmune Rheumatic Disease laboratory testing encompasses selective criteria and begins therefore from a clinical suspicion, which is then followed by a logical succession of analyses performed with sensitive tests at an early stage and specific tests in the confirmatory step [20–23]. These assays include ANA as well as testing for antibodies to specific nuclear antigens such as dsDNA or ENA (i.e. SS-A,SS-B, Sm, RNP, Scl70, Jo1). In particular, the diagnostic sensitivity, specificity and overall analytical performances of ANA, anti-ENA and anti-dsDNA tests are critical to identify the most suitable diagnostic algorithm.

A diagnostic protocol for the appropriate use of antinuclear autoantibodies, developed on the basis of the scientific guidelines and shared with the physicians working in clinical immunology, has been implemented in the Academic Hospital of Parma since January 2007,

and has generated a substantial reduction in clinically inappropriate test requests [22].

According to these results, we have further proposed the standardization of a similar algorithm to be used in a multi-centre study supported by a regional grant for innovative research projects, which was started in 2008. The results of this Italian multi-centre study are described here.

2. Materials and methods

2.1. Study methodology

This study is an observational research, aimed to compare the data collected, including both the number of ANA, anti-ENA and anti-dsDNA tests and the percentage of positive results before and after the application of the diagnostic algorithm (first semester 2008 versus first semester 2009).

2.2. Diagnostic algorithm

The ANA test has a very high diagnostic sensitivity for several systemic rheumatic diseases. Indirect immunofluorescence on Hep-2 cells is the recommended and most widely used technique for ANA detection due to its high sensitivity, and because it is easy to perform and relatively inexpensive [24,25].

The antibodies to specific nuclear antigens (anti-dsDNA and anti-ENA) can be detected by several assays which globally display high specificity, but they are less sensitive and more expensive than ANA tests. Due to its diagnostic performances, the ANA test has been proposed by several authors [20–22,26] as first level test in the laboratory diagnosis of autoimmune rheumatic diseases, whereas tests for antibodies to specific nuclear antigens (i.e., second-level tests) should only be performed when ANA screening is positive, or if the patient has clear signs and symptoms suggesting a systemic rheumatic disease. The Flow-chart for the diagnostic protocol is shown in Fig. 1.

The protocol specifically develops as follow:

- ANA test is the first level test performed by an indirect immunofluorescence assay on HEp-2 cells, with a “screening” dilution of 1:80.
- When ANA testing is negative, or is positive for dilutions<1:160, no additional tests for antibodies to specific nuclear antigens (anti-dsDNA and anti-ENA) are performed. These tests, even when not requested, are instead performed when ANA test is positive with any fluorescence pattern for dilutions≥1:160. These tests are performed even when ANA test is negative, and when a specific clinical request has been forwarded to the laboratory (specific clinical suspicion on the request form or computerised note on the request file).

2.2.1. Patients

The eligible patients were those hospitalized in 2009 for Autoimmune Rheumatic Disease diagnosis in the wide geographic area comprehended in this study, which is known as “North Western wide area in the Emilia Romagna district” (AVEN) and includes the administrative districts of the provinces of Parma, Reggio-Emilia, Modena and Piacenza.

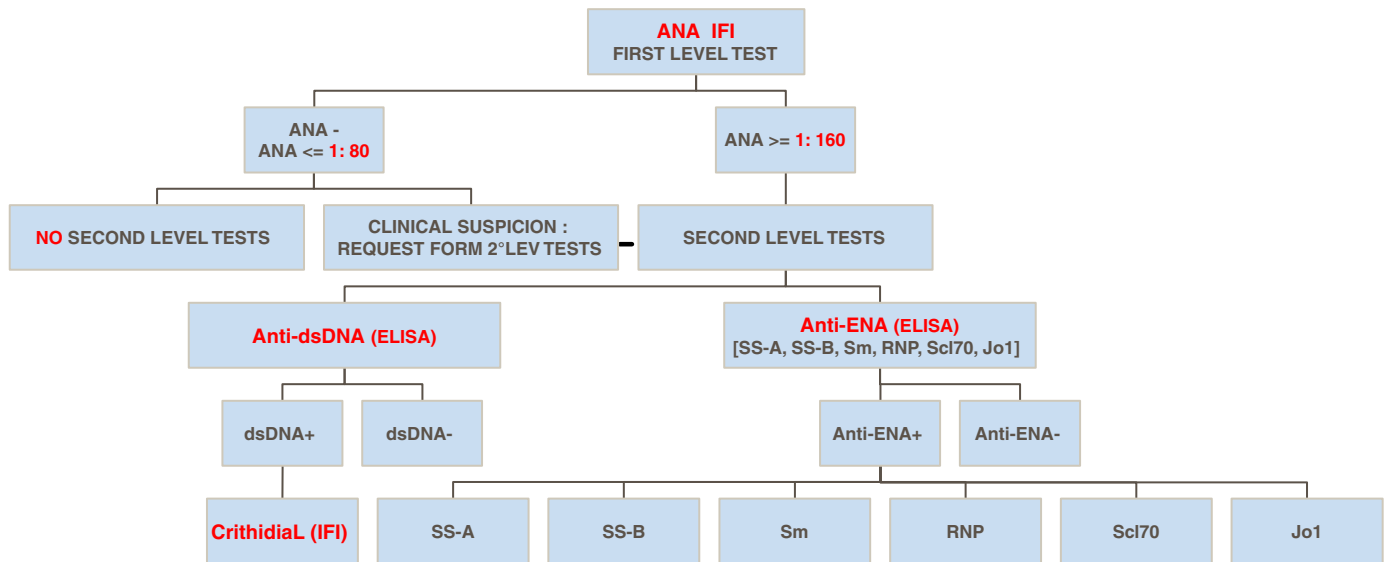


Fig. 1. Flowchart of diagnostic algorithm for autoantibody testing in autoimmune rheumatic disease.

2.2.2. Clinical follow-up

An evaluation of the clinical outcome has been carried out by follow-up of the patients admitted to specialised wards in the first semester of 2009 and who have been classified further as being affected or not by Autoimmune Rheumatic Disease. Both clinical and analytical data have been recorded on specifically designed forms and further analyzed.

2.2.3. Methods

All the different laboratories involved in this project performed ANA testing by indirect immunofluorescence assay on Hep-2 cells. Results of ANA test include a interpretative comment describing whether the test result is negative or positive at the cut-off dilution. In the presence of a positive test result, the report includes a description of the fluorescence pattern and the end-point titre or dilution at which a definable pattern of fluorescence is observed.

The anti-dsDNA test is performed with an immunoenzymatic assay. Positive or uncertain results are confirmed by immunofluorescence assay using a substrate of *Crithidia luciliae*. The anti-ENA assay is performed for screening positive results with an Immunoenzymatic method. The detection of autoantibodies against single antigens (SS-A, SS-B, Sm, RNP, Scl70, Jo1) is performed in the various hospitals in the area by using different confirmation tests (Immunoblotting tests or ENA antigens-ELISA).

3. Results

The multi-centre study for standardisation of a diagnostic algorithm for Autoimmune Rheumatic Disease presented in this article was started in January 2008 and some preliminary results were already gathered. First, a study group of clinical Immunology physicians and Laboratory professionals was established. This working group collected and evaluated several data from each of the different hospitals involved in the project, including diagnostic criteria, formats of request for laboratory tests as well as laboratory reports, analytical procedures and overall number of autoimmunity tests performed. Then, a common guideline for autoantibody testing in Autoimmune Rheumatic Disease, which considers the different clinical needs of the various hospitals, has been developed after discussion of diagnostic problems.

Compliance with the clinical protocol and evaluation of clinical efficacy was assessed at the end of the study (i.e., in 2009), by analysis of both the decrease in number of tests and the percentage of

positivity in second level tests in the different hospitals after introduction of the diagnostic algorithm.

The total volume of activity for the ANA, ENA and dsDNA tests throughout the survey area for both hospitalised patients and outpatients was 13,067 in the first Semester of 2008 and 12,738 in the first Semester of 2009, respectively. As regards the economic/management efficiency expressed in terms of number of tests performed, a significant reduction of anti-dsDNA (–26%) and anti-ENA (–15%) tests was observed when comparing the production statistics of the first Semester of 2008 with those of 2009, whereas the ANA determination increased by 10%, following the trend in increasing requests recorded for autoimmunity tests (Fig. 2).

The analysis of results obtained in each centre (Figs. 3 and 4) shows that the expected reduction in ENA and ds-DNA tests was not accomplished in the Hospital of Reggio Emilia, where the statistics shows a consolidated trend towards an increase. It is however noteworthy that the algorithm for second level tests has been implemented and “proposed” to all the wards in this facility, but was not “officially” applied to the management of laboratory analyses as in all the other hospitals.

As regards the diagnostic specificity of the algorithm, the percentages of positivity observed for the second level tests increased after application of the diagnostic algorithm, in particular for the ENA test (i.e., from 13% to 17%). This better performance has been attributed to

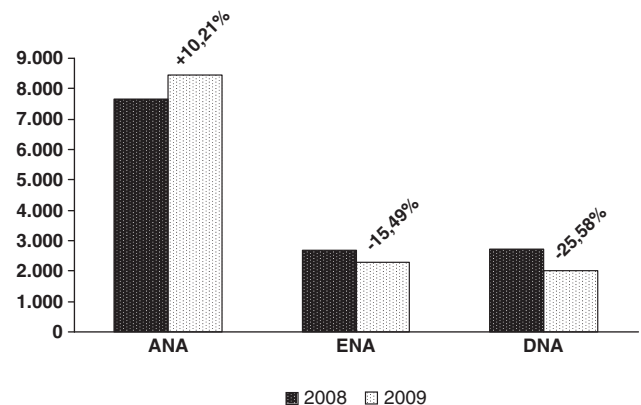


Fig. 2. Total requests ANA, ENA, dsDNA hospitalised patients; all centres 1° term 2008 as against 1° term 2009.

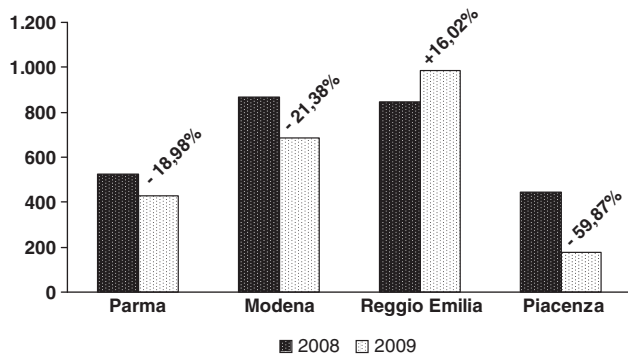


Fig. 3. ENA requests for hospitalised patients; all centres 1° term 2008 as against 1° term 2009.

the fact that this test can be more easily standardized in the various centres as compared with the anti-dsDNA tests, which positivity modestly increased from 9% to 11% (Figs. 5 and 6). As expected, the percentages of positivity was not significantly different for the ANA test, since it was used as a “first line” test.

As regards the effect of the algorithm on the clinical outcome, we have evaluated the effect of the algorithm on the final diagnosis in 54 patients enrolled at Rheumatological Departments of Hospitals involved in the project (43 females and 11 males; average age 60 years old, age range: 28–89 years). Among these patients, 19 had non autoimmune diseases, 34 inflammation of the connective tissue (6 sjögren's syndrome, 7 mixed connective tissue disease, 10 systemic sclerosis, 4 LES, 2 dermatomyositis, 5 rheumatoid arthritis), whereas a single patient suffering from vasculitis was excluded from the following statistical analysis. In these cases, the results of ANA testing have been further stratified among the patients affected by connective tissue disease and the patients with non auto-immune disorders.

The use of a cut-off for ANA testing with a titre $\geq 1:160$ for performing the second level tests allowed to identify 85% (29 out of 34) of the patients affected by Autoimmune Rheumatic Disease. The 5 patients affected by the ANA connective tissue disease, who were negative with the immunofluorescence test, were characterized as follows: two cases rheumatoid arthritis, one of mixed connective tissue disease, one of dermatomyositis, one of systemic sclerosis (Table 1).

After evaluation of second level test results, we observed that in the patients affected by the Mixed connective tissue disease (for whom the clinicians had suggested further tests even if the presence of negative ANA testing), ENA was positive for Jo1, SSA, SSB. As already shown by previous data of scientific literature, the positivity for these antigens might occasionally display a limited cytoplasmic pattern with immunofluorescence analysis. However, the observation of clear cytoplasmic fluorescence might persuade to perform the second level tests, even in presence of a negative ANA testing.

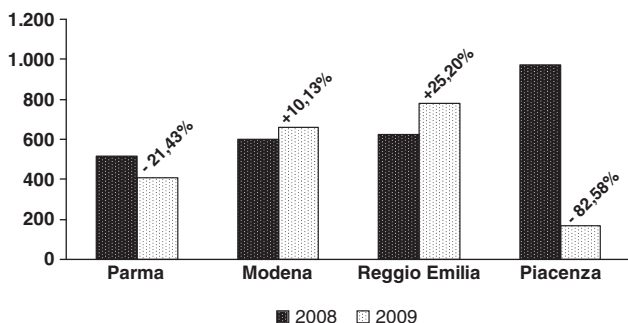


Fig. 4. dsDNA requests for hospitalised patients; all centres 1° term 2008 as against 1° term 2009.

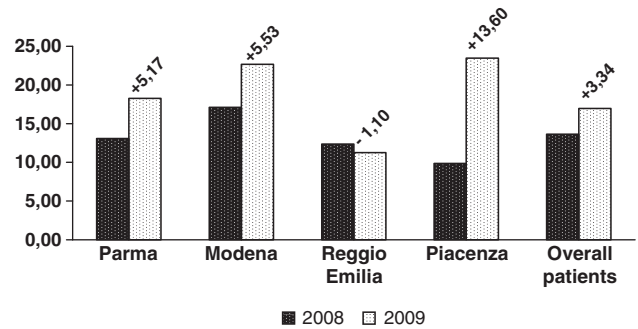


Fig. 5. Positivity percentages ENA test in hospitalised patients; 1° term 2008 as against 1° term 2009.

The patients with positive ANA testing, who were not affected by inflammation of the connective tissue, included one case of hepatitis C, one of hepatitis B, two with cancer and one with psoriatic arthritis. The results of both ENA and anti dsDNA testing were negative in all such patients.

In conclusion, the ANA test was a reliable screening test for performing second line testing in 85% of connective tissue disease including cases with AR and in the 93% of the cases without AR. The second level tests performed by the Laboratory following a physician request or according to results of ANA testing were inappropriate in only 26% of non auto-immune patients (5 out of 19).

4. Discussion

The diagnosis and management of systemic rheumatic diseases is mainly based on clinical parameters. Nevertheless, the autoantibodies assessment has become an integral part of the differential diagnosis. The results of serologic tests for autoantibodies, including tests for ANA, ENA and dsDNA, may hence be a cornerstone for the diagnostics of systemic rheumatic diseases, for example for ruling out misleading pathologies during differential and early diagnosis [1]. Conversely, the inappropriate use of immunological tests can produce misdiagnosis, inappropriate therapy, and waste of healthcare resources. This is a key aspect in the creation of a new approach for the diagnosis of autoimmune diseases, which should encompass the translation from experimental data emerged from several ongoing studies to Evidence Based Laboratory Medicine, and the definition and application of a diagnostic algorithm for test request and for the interpretation of laboratory data may improve the usefulness of test results throughout the challenging patient management. [1,21,22,26–28].

In our experience, a diagnostic protocol including both screening and confirmatory tests should allow selective and multi-step test performance in the diagnosis of Autoimmune Rheumatic Disease, thereby improving the appropriate use of tests for specific autoantibodies.

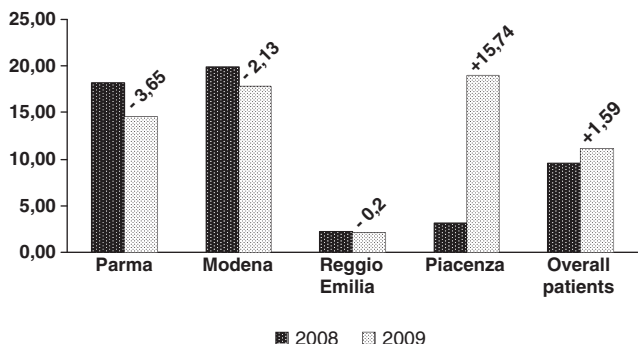


Fig. 6. Positivity percentages dsDNA test in hospitalised patients; 1° term 2008 as against 1° term 2009.

Table 1

Diagnostic algorithm and clinical diagnosis: misclassified patients.

Patients affected by connective tissue disease with ANA < 1:160 (n = 5)
2 Rheumatoid arthritis
1 Mixed connective tissue disease
1 Dermatomyositis
1 Systemic sclerosis
Patients not affected by connective tissue disease with ANA ≥ 1:160 (n = 5)
1 Hepatitis C
1 Hepatitis B
2 Cancer
1 Psoriatic arthritis

Moreover, the diagnostic specificity of the algorithm was also validated by the information emerged from the clinical follow-up.

5. Conclusions

The close collaboration and audit between clinicians and laboratory professionals might allow the standardisation and broad implementation of diagnostic algorithms for a more efficient use of immunological tests in the diagnostic evaluation, prognostic assessment, and monitoring of patients with systemic rheumatic diseases [1].

In our study the evaluation of local and specific clinical needs has increased the feasibility and application of a diagnostic protocol over a broader area. The percentage of second level test positivity has increased both for ENA and dsDNA, after implementation of the diagnostic protocol. The introduction of this diagnostic algorithm has also allowed a significant decrease in the number of second level tests. The large diffusion of this algorithm is an important aspect and it should be considered a strength of this study, as well as the restricted implementation on a single facility represents a drawback of similar studies of appropriateness of laboratory testing after implementation of diagnostic protocols.

Efficiency and efficacy are strongly linked. We expect that the clinical protocol for autoantibody testing which is presented in this article may play an effective role for improving both patient outcome (i.e. efficacy) and efficiency over a broad geographical area.

Take-home messages

- The close collaboration between clinicians, laboratory specialists and healthcare services is effective in developing efficient diagnostic algorithms for the Autoimmune Rheumatic Disease.
- As regards the appropriateness of the test requests in auto-antibodies testing, efficiency and efficacy are strongly linked.

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Is SF-36 a reliable outcome of neuropsychiatric events in patients affected with systemic lupus erythematosus?

Neuropsychiatric events in patients affected with systemic lupus erythematosus (SLE) represent a poor prognostic factor and until now none of the known approaches for the outcome assessment of these patients, such as neuroimaging alone or in combination with clinical variable have been validated.

The Systemic Lupus International Collaborating Clinics (SLICC) proposes the short form SF-36 as reliable outcome measure for the study of neuropsychiatric events in SLE patients (*Ann Rheum Dis* 2011; 50:961–67). The Authors consider a large cohort of 1,400 SLE patients and identified 274 patients that had one or more neuropsychiatric events. The SF-36, particularly the mental component summary, changed in the appropriate direction in association with both clinical improvement and worsening.

In conclusion SF-36 should be considered as a reliable outcome of neuropsychiatric events in SLE patients.