Autoimmunity Reviews 13 (2014) 292–298

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Automated antinuclear immunofluorescence antibody screening: A comparative study of six computer-aided diagnostic systems

Nicola Bizzaro [a,*], Antonio Antico [b], Stefan Platzgummer [c], Elio Tonutti [d], Danila Bassetti [e], Fiorenza Pesente [a], Renato Tozzoli [f], Marilina Tampoia [g], Danilo Villalta [h],
Study Group on Autoimmune Diseases of the Italian Society of Laboratory Medicine, Italy

[a] Laboratorio di Patologia Clinica, Ospedale San Antonio, Tolmezzo, Italy
[b] Laboratorio Analisi, Ospedale Civile, Santorso, Italy
[c] Laboratorio Centrale, Ospedale Civile, Merano, Italy
[d] Immunopatologia e Allergologia, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy
[e] Microbiologia e Virologia, Ospedale S. Chiara, Trento, Italy
[f] Laboratorio di Patologia Clinica, Dipartimento di Medicina di Laboratorio, Azienda Ospedaliera S. Maria degli Angeli, Pordenone, Italy
[g] Laboratorio di Patologia Clinica, Azienda Ospedaliero-Universitaria, Policlinico, Bari, Italy
[h] Allergologia e Immunologia Clinica, Azienda Ospedaliera S. Maria degli Angeli, Pordenone, Italy

ARTICLE INFO

Article history:
Received 12 October 2013
Accepted 29 October 2013
Available online 9 November 2013

Keywords:
Anti-nuclear antibodies
Immunofluorescence
Automation
Autoimmune rheumatic diseases
Computer-aided systems

ABSTRACT

Background: Indirect immunofluorescence (IIF) plays an important role in immunological assays for detecting and measuring autoantibodies. However, the method is burdened by some unfavorable features: the need for expert morphologists, the subjectivity of interpretation, and a low degree of standardization and automation. Following the recent statement by the American College of Rheumatology that the IIF technique should be considered as the standard screening method for the detection of anti-nuclear antibodies (ANA), the biomedical industry has developed technological solutions which might significantly improve automation of the procedure, not only in the preparation of substrates and slides, but also in microscope reading.
Methods: We collected 104 ANA-positive sera from patients with a confirmed clinical diagnosis of autoimmune disease and 40 ANA-negative sera from healthy blood donors. One aliquot of each serum, without information about pattern and titer, was sent to six laboratories of our group, where the sera were tested with the IIF manual method provided by each of the six manufacturers of automatic systems. Assignment of result (pos/neg), of pattern and titer was made by consensus at a meeting attended by all members of the research team. Result was assigned if consensus for pos/neg was reached by at least four of six certifiers, while for the pattern and for the titer, the value observed with higher frequency (mode) was adopted. Seventeen ANA-positive sera and six ANA-negative sera were excluded. Therefore, the study with the following automatic instrumentation was conducted on 92 ANA-positive sera and on 34 ANA-negative sera: Aklides, EUROPattern, G-Sight (I-Sight-IFA), Helios, Image Navigator, and Nova View. Analytical imprecision was measured in five aliquots of the same serum, randomly added to the sample series.
Results: Overall sensitivity of the six automated systems was 96.7% and overall specificity was 89.2%. Most false negatives were recorded for cytoplasmic patterns, whereas among nuclear patterns those with a low level of fluorescence (i.e., multiple nuclear dots, midbody, nuclear rim) were sometimes missed.
The intensity values of the light signal of various instruments showed a good correlation with the titer obtained by manual reading (Spearman's rho between 0.672 and 0.839; $P < 0.0001$ for all the systems). Imprecision ranged from 1.99% to 25.2% and, for all the systems, it was lower than that obtained by the manual IIF test (39.1%). The accuracy of pattern recognition, which is for now restricted to the most typical patterns (homogeneous, speckled, nucleolar, centromere, multiple nuclear dots and cytoplasmic) was limited, ranging from 52% to 79%.
Conclusions: This study, which is the first to compare the diagnostic accuracy of six systems for automated ANA-IIF reading on the same series of sera, showed that all systems are able to perform very well the task for which they were created. Indeed, cumulative automatic discrimination between positive and negative samples had 95% accuracy. All the manufacturers are actively continuing the development of new and more

* Corresponding author at: Laboratory of Clinical Pathology, San Antonio Hospital, via Morgagni, 18, 33028 Tolmezzo (UD), Italy. Tel.: +39 0433 488261; fax: +39 0433 488697.
E-mail address: nicola.bizzaro@ass3.sanita.fvg.it (N. Bizzaro).

http://dx.doi.org/10.1016/j.autrev.2013.10.015

sophisticated software for a better definition in automatic recognition of patterns and light signal conversion in end-point titer. In the future, this may avert the need for serum dilution for titration, which will be a great advantage in economic terms and time-saving.

Contents

1. Introduction

The detection and measurement of autoantibodies against nuclear and cytoplasmic antigens (the so-called anti-nuclear antibodies — ANA) play a consolidate role for the diagnosis of systemic autoimmune rheumatic diseases (SARDs), such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, idiopathic inflammatory myopathies and systemic vasculitides. Indirect immunofluorescence (IIF) on human epidermoid laryngeal carcinoma cells (HEp-2 cells) is the most established method for ANA screening with the two-step diagnostic strategy for SARDs [1–4]. The high sensitivity of ANA assessment by IIF, able to allow detection of more than 50 antibodies, makes this method an invaluable tool for the initial step of current diagnostic procedures for the detection of systemic autoantibodies [5–7].

However, the IIF method is burdened by some unfavorable features: the need for expert morphologists, the subjectivity of interpretation and a low degree of standardization and automation [4,5,8]. As a consequence, IIF is considered labor-intensive and prone to render bias.

During the last 15 years, the progressive increase of ANA test requests and volume of assays performed in clinical laboratories produced alternative solutions to the ANA-IIF test based on manual or automated monoplex and multiplex immunometric assays (enzymatic immunoassays — EIA; chemiluminescent immunoassays — CLIA; line-immunoassays — LIA), but literature reports demonstrated that these procedures do not provide the same analytical accuracy [5,9].

The need for standardization of ANA testing continues to be a challenge, because its analytical variability continues to be high, without substantial improvement over time [10–12]. Recently, the biomedical industry has proposed technological solutions which might significantly improve the automation of the IIF procedure, not only in the preparation of substrates and slides, but also in microscope reading. This innovation is based on the principle of digitalization of fluorescent images, as an example of computer-assisted diagnosis, and on the classification of patterns using standardized approach (automated positive/negative screening and pattern interpretation) [13–17].

These systems are based on the use of automated microscopes, robotized slide trays, high sensitivity video cameras, and software dedicated to digital image acquisition and analysis. Currently, several commercial systems are available and have been evaluated in preliminary experimental studies on single devices [18–27] with the purpose of assessing the reliability of automated IIF analysis as a standardized alternative for the conventional manual visual approach. Therefore, at present there are no studies comparing the different commercial technological platforms for automated ANA-IIF.

This study was undertaken to verify the level of accuracy of new automatic systems for the reading of ANA samples, specifically in discriminating between ANA-positive and ANA-negative samples. As a second objective, we analyzed the accuracy of these systems in pattern recognition, and checked whether there is correlation between levels of the analytic signal provided by the instruments and the titer obtained with manual IIF.

2. Materials and methods

2.1. Patients and sera

We collected 104 ANA-positive sera and 40 ANA-negative sera. The preliminary selection of ANA-positive sera was made in eight laboratories of the Study Group on Autoimmune Diseases of the Italian Society of Laboratory Medicine (SIMeL) based on five main criteria: a) the source of sera (sera should be obtained from patients with a confirmed clinical diagnosis according to internationally accepted criteria); b) the type of pattern (in order to have a representative number of samples for each of the 15–20 most frequent or clinically more relevant patterns, in accordance with the international nomenclature [28]; c) the presence of a single pattern (i.e. sera with mixed pattern were excluded); d) range