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Expert Opinion

Is immunofluorescence about to disappear in clinical laboratories?

Running title: autoantibody testing: the role of immunofluorescence

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Abstract

In recent years an emerging question was raised. Is indirect immunofluorescence for autoantibody testing going to decline or even to disappear? Evolution of autoantibody testing has emerged. methods such as double immunodiffusion and counter-immunoelectrophoresis have been progressively abandoned in favor of much more reliable and reproducible methods. An important step towards the possible replacement of IIF has been made with the introduction of fully automated methods. But are those new technologies appropriate. In this article, we discuss the pros and cons of the evolving issues and we raise some more questions which need to be addressed.

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I. INTRODUCTION

One of the questions we have been asking ourselves for some years now is whether the indirect immunofluorescence method (IIF) to search for antibodies has had its day and it is time to replace it with other techniques that are more accurate, faster and more automated. There has always been an evolution in the immunological diagnosis of rheumatic diseases: methods such as double immunodiffusion and counter-immunoelectrophoresis have been progressively abandoned in favor of much more reliable and reproducible methods. Radioimmunoassay and immunoenzymatic methods (ELISA) are also recording a progressive and constant decline and will probably be completely replaced within a few years. Only IIF still resists more than 60 years after its introduction in laboratories. No other technique can boast such a long duration in the history of laboratory medicine. Twenty years ago, we had already focused attention on alternative methods to detect antibodies against cellular antigens (ANA - antinuclear antibodies) [1]. The study came to the conclusion that the time was not yet ripe and that the ELISA methods, innovative at the time, did not guarantee the same diagnostic performance as IIF on HEp-2 cells. Eight years later, in 2011, Marvin Fritzler asked himself the same question in an editorial on Arthritis & Rheumatism entitled "The Antinuclear Antibody Test: Last or Lasting Gasp?" [2], concluding that within a few years IIF would be replaced by better performing methods. Today, twelve years later, we are still looking for answers but in this period of time significant

changes and technological innovations have taken place that pose the same question again with more data and more evidence available. Why then should we perhaps shelve this method? The main reasons, known to all those who deal with these diagnostics, are that IIF is a laborious technique, still not standardized, semi-quantitative, lacking in specificity and, above all, dependent in the interpretative phase on the experience of the operator [3]. Moreover, its sensitivity, although high in general, does not allow in some cases to identify some antibodies such as anti-Ro60 which are an important classification criterion of Sjögren's syndrome, anti-ribosomal P in systemic lupus erythematosus, anti-Ro52 in neonatal lupus or anti-synthetase in autoimmune myositis.

An important step towards the possible replacement of IIF has been made with the introduction of fully automated fluoroimmunoenzymatic (FEIA) and chemiluminescent (CLIA) methods. In screening for systemic autoimmune rheumatic diseases these methods are slightly less sensitive but more specific than the IIF HEp-2 method [4, 5]. The reason for the lower sensitivity of the new solid-phase methods is above all linked to the still incomplete panel of antigens compared to that present in a HEp-2 cell. When a method characterized by a much greater number of antigens was used, the sensitivity was in fact comparable to that of the IIF HEp-2, also confirming a clearly higher specificity [6]. These data highlight how new multiparametric systems with superior diagnostic efficiency are starting to act as a concrete alternative to the IIF HEp-2.

Notwithstanding this evidence, a not indifferent role in the opposition to change, it is played by the vast number of studies on the IIF method, its consolidated use over time and above all the fact that clinicians struggle to accept changes on diagnostic aspects with which they grew up and which they have incorporated into many classification criteria of autoimmune diseases. Resistance to change also comes from the world of the laboratory. The recognition of the morphological patterns is still considered very rewarding and professionally qualifying. So much so that in the last survey carried out among Italian laboratories in 2019, as many as 98.2% of them declared that they still use the IIF method [7]. Conversely, in US laboratories it is used by only 55%, signaling a strong propensity to move towards automated solid-phase methods [8]. The choice is dictated by practical reasons: greater speed of execution and reporting, indispensable in particular where the Laboratory services have been strongly consolidated, and elimination of any interpretative aspect (need for training and possible source of legal dispute).

From a clinical point of view, an often-underestimated aspect is whether it is preferable to use more sensitive or more specific methods. Although by definition screening tests should favor diagnostic sensitivity, in a context in which tests are now required by almost all specialists and general practitioners with a very low pre-test probability and in which the target diseases, with the due exceptions, are chronic pathologies with very slow onset and development, false positives have a much greater impact than false negatives. It is now established that in situations of low pre-test probability, immunometric methods perform better than IIF.

However, it should be noted that when we speak of IIF we must consider that we are not referring only to ANA, but also to all the other antibodies that are still being searched for in immunofluorescence, such as the anti-dsDNA antibodies in lupus, the anti-endomysial in celiac disease, anti-gastric parietal cell in autoimmune gastritis, anti-mitochondrial in primary biliary cholangitis, anti-smooth muscle and anti-liver kidney microsomal in autoimmune hepatitis, anti-pancreatic islet in type 1 diabetes, anti-skin in bullous autoimmune dermatitis and anti-adrenal in Addison's disease. Giving up IIF in the diagnosis of rheumatic diseases, which accounts for more than 90% of IIF tests performed in the autoimmunology laboratory, would therefore also involve other diagnostics with an impact that no one has yet taken into consideration at the moment. It therefore appears evident that before discontinuing the IIF method for ANA, screening methods must be made available for all the antibodies that can be found when using the IIF on HEp-2 cells and, more broadly, also for all antibodies that are now being searched for with this analytical method.

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II. CONCLUSION

Finally, as in many other sectors of Laboratory Medicine, the choices will be strongly conditioned by organizational aspects. If readers of IIF slides will fail, the solution will be to eliminate the problem at its source. It therefore seems unlikely that IIF can continue to be considered as the reference method for a long time while new technologies are already available that are much more suitable for the current context of autoimmune diagnostics. These questions, currently still unresolved, will keep us busy in the coming years to ensure that autoimmunology laboratories provide ever more accurate and clinically useful results, adopting the most suitable and most effective methods.

CONFLICT OF INTEREST

The Author declares no conflict of interest.

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