Autoimmunity Reviews 9 (2009) 17–22

Contents lists available at ScienceDirect

Autoimmunity Reviews

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

Challenges of automated screening and differentiation of non-organ specific autoantibodies on HEp-2 cells

Rico Hiemann [a], Thomas Büttner [b], Thorsten Krieger [c], Dirk Roggenbuck [b], Ulrich Sack [d], Karsten Conrad [e,*]

[a] Department of Biology, Chemistry and Process Technology, Lausitz University of Applied Sciences, Senftenberg, Germany
[b] GA Generic Assays GmbH, Dahlewitz, Germany
[c] Institute of Immunology, University Hospital Eppendorf, Hamburg, Germany
[d] Institute of Clinical Immunology and Transfusion Medicine, University of Leipzig, Germany
[e] Institute of Immunology, Technical University Dresden, Germany

ARTICLE INFO

Article history:
Received 15 February 2009
Accepted 17 February 2009
Available online 24 February 2009

Keywords:
Autoantibodies
HEp-2
Immunofluorescence
Autoimmune diseases
Automated screening
Standardisation

ABSTRACT

Analysis of autoantibodies (AAB) by indirect immunofluorescence (IIF) remains the hallmark of diagnosing autoimmune diseases despite the introduction of multiplex techniques. Non-organ specific AAB are screened in routine diagnostics by IIF on HEp-2 cells. However, IIF results vary due to objective (e.g., cell fixation) and subjective factors (e.g., expert knowledge). Therefore, inter- and intralaboratory variance is relatively high. Standardisation of AAB testing by IIF remains a critical issue in and between routine laboratories and may be improved by automated interpretation systems. An overview of existing interpretation techniques will be given taking into account own data of the first fully automated reading system AKLIDES. The novel system provides fully automated reading of IIF images and software algorithms for the mathematical description of IIF AAB patterns. It can be used for screening and preclassification of non-organ specific AAB in routine diagnostics regarding systemic autoimmune and autoimmune liver diseases. Furthermore, this system paves the way for economic data processing of cell-based IIF assays and can contribute to the reduction of interlaboratory variance of AAB testing. More sophisticated pattern recognition algorithms and novel calibration systems will improve standardised quantifications of IIF image interpretation.

Contents

Abbreviations: AAB, Autoantibodies; ACA, anti-centromere antibody; AMA, anti-mitochondrial antibody; ANA, Antinuclear antibodies; DAPI, 4',6-diamidino-2-phenylindol; dsDNA, Double stranded DNA; ENA, extractable nuclear antigens; GFU, Green fluorescence units; IIF, Indirect immunofluorescence; MCTD, Mixed connective tissue disease; NuMA, nuclear mitotic apparatus; PDH, pyruvate dehydrogenase; SLE, Systemic lupus erythematosus.

* Corresponding author. Institute of Immunology, Medical Faculty "Carl Gustav Carus", Fetscherstraße 74, 01307 Dresden, Germany. Tel.: +49 351 458 6540; fax: +49 351 458 6308.
E-mail address: karsten.conrad@tu-dresden.de (K. Conrad).

1568-9972/$ – see front matter.
doi:10.1016/j.autrev.2009.02.033

1. Introduction

The serological hallmark of autoimmune diseases is the occurrence of disease specific autoantibodies (AAB). In the multi-stage diagnostic approach to many of the autoimmune diseases, the screening for non-organ specific AAB by indirect immunofluorescence (IIF) on HEp-2 cells is well established. AAB, especially antinuclear antibodies (ANA), are essential diagnostic markers for identifying a number of chronic inflammatory rheumatic and autoimmune liver diseases [1–5]. Currently, different strategies for screening and identification of non-organ specific AAB are used [6–8]. The most common approach is the combination of screening by IIF on HEp-2 cells and specific AAB analysis by immunoassays using highly purified or recombinant autoantigens. This strategy allows a cost-effective and high-quality serological diagnosis of a variety of autoimmune diseases due to: (i) high sensitive detection of most clinically relevant non-organ specific AAB, (ii) optimal combination of immunoassays for further evaluation of specific AAB taking into account IIF pattern and suspected diagnosis, (iii) detection of diagnostically relevant AAB without further need of specific immunoassays (e.g., anti-centromere antibodies), and (iv) assessment of AAB only detectable by this method since the autoantigenic targets have not been identified or commercial assays are not available yet.

Given the pivotal role of HEp-2 screening, a consistent reproducibility and high quality are required for the detection of non-organ specific AAB [7,9,10]. However, the main drawback of HEp-2 cell assays is still the visual and subjective evaluation. Results are significantly influenced by qualification and individual experience causing high intra- and interlaboratory variance. To overcome this issue, fully automated IIF interpretation systems with pattern-recognition software have been introduced recently [11]. Furthermore, automation is required for high-throughput diagnostics.

In this review, the focus will be the current state and the challenges encountered regarding automation of first-line screening and differentiation of non-organ specific autoantibodies on HEp-2 cells. Own data will be summarised and perspectives will be discussed in terms of the main problems and challenges of automated image processing by the novel techniques.

2. Technical and mathematical challenges of automated IIF interpretation systems

Automated interpretation of routine cell-based IIF tests has not been in the main focus of experts yet. Current concepts of automated interpretation of HEp-2 cell assays with subcellular pattern detection are summarised in Table 1. The main disadvantage of most of these systems is the overestimation of the concluding steps of image interpretation like image extraction and, in majority of publications, classification [12–16].

In our opinion, especially self-learning classificators [14] are inappropriate for routine laboratories since local erroneous self-learning cannot improve interlaboratory variance. Frequently, high quality images are subjectively preselected and may lead to indeterminable human bias. Remarkably, automated classification has been shown to obtain higher classification accuracy than subjective classification by experts, provided features for pattern description had been correctly selected. The classifier was able to discriminate between two anti-golgi protein patterns (giantin and gpp130) with 77–78% accuracy that initially were thought to be indistinguishable [12].

Outcome and success of automated reading depends essentially on the first processing steps. Thus, increased efforts are required especially for high-quality image acquisition and quality control of image taking [16]. In this regard, the most critical steps for automation usually underestimated, are (i) automated focusing, (ii) adjustment of image intensity, (iii) quality control of sharpness and brightness, (iv) artefact detection and exclusion, (v) real-time data processing including pattern evaluation, and (vi) automated system calibration regarding light source and fluorescence fading.

A standardised interpretation of HEp-2 cell assay by fully automated processing and evaluation may allow reproducible

Table 1
Current concepts of automated interpretation of HEp-2 cell assays with subcellular pattern detection.

	A	B	C	D	E	Remarks
Commercial application						
HEp-2 cell analyzer (AID Advanced Imaging Devices GmbH, Strassberg, Germany)	+					Positive/negative signal evaluation
HEp-2 PAD© (ImageInterpret Ltd., Leipzig, Germany) [14]			+	+	+	
AKLIDES© (Medipan GmbH, Dahlewitz, Germany) [11,16]	+	+	+	+	+	Fully automated
Research application						
Rigon et al. [18]; Soda and Iannello [15,19]			+	+	+	
Hu and Murphy [12]				+	+	Data for pattern classification accuracy only, no data for HEp-2 routine
Glory and Murphy [13]				+	+	Data for pattern classification accuracy only, no data for HEp-2 routine

A: automated image acquisition, B: quality control of images, C: image segmentation for object detection, D: feature extraction for object description, E: classification of detected objects.