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Analytical and clinical comparison of two fully automated immunoassay systems for the detection of autoantibodies to extractable nuclear antigens

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ABSTRACT

Background: Detection of antinuclear antibodies (ANA) by indirect immunofluorescence assay (IIFA) is increasingly substituted by fully automated solid phase immunoassays. This study evaluated the performance of an automated chemiluminescence immunoassay (CIA) and fluorescence enzyme immunoassay (FEIA) and compared their performance to that of IIFA.
Methods: The study included an unselected prospective study population suspected of systemic autoimmune rheumatic disease. ANA were measured by IIFA, while in parallel sera were tested by CIA QUANTA Flash CTD Screen Plus on the BIO-FLASH® and FEIA EliA CTD Screen on the Phadia® 250 system. As validation, retrospective cohorts of patients with ANA-associated rheumatic disease (AARD) and healthy controls were tested.
Results: Prospectively, sensitivity of IIFA, CIA and FEIA was 90%, 99% and 92%, respectively. Specificity was 76%, 76% and 84%, respectively. Total percent agreements between the three methods were 75.2% (IIFA vs. CIA), 79.2% (IIFA vs. FEIA) and 85.4% (FEIA vs. CIA). The AUC values were 0.95 for CIA and 0.93 for FEIA and did not significantly differ. Retrospectively in individual AARD cohorts, similar results were obtained comparing both CTD screens.
Conclusions: Both FEIA and CIA CTD screen significantly outperformed IIFA, with a higher specificity for FEIA and higher sensitivity for CIA. Based on ROC analysis, major contributor to the difference between the two solid phase immunoassays was the cut-off.

1. Introduction

Antinuclear antibodies (ANA) are serological hallmarks in the diagnosis of systemic autoimmune rheumatic disease (SARD). Testing for ANA is especially helpful in systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and mixed connective tissue disease (MCTD) and to a certain extent in primary Sjögren's syndrome (SjS) and polymyositis/dermatomyositis (PM/DM) [1]. Hence, these SARDs are defined as ANA-associated rheumatic disease (AARD). ANA testing is less helpful for diagnosing other SARD like rheumatoid arthritis (RA) or juvenile idiopathic arthritis (JIA, except to stratify the risk for uveitis). Consequently, ANA can also be found in patients with non-rheumatic diseases, such as thyroid disease, infectious disease, autoimmune liver diseases, vasculitis, inflammatory bowel disease, malignancy or even in apparently healthy individuals, particularly elderly people [2,3].

The indirect immunofluorescence assay (IIFA) for the detection of ANA was firstly described in 1958 and is still considered the reference method for ANA screening [4]. Nowadays IIFA is performed using HEp-2 cells, a cell line established in 1952 by Moore and colleagues [5], or variants of this cell line (e.g. HEp-2000). These cells form a substrate presenting > 100 autoantibody targets leading to a high sensitivity for particular AARD like SLE and SSc [1]. Hence, ANA as performed by IIFA, is historically included as a classification criterion of SLE [6,7]. On the other hand, IIFA sensitivity is somewhat lower for other AARD like SjS and PM/DM [2,3]. Historically, mainly clinical immunologists and rheumatologists order ANA tests, nowadays a broad spectrum of clinicians are doing so, thereby changing pre- and post-test probability possibly necessitating a more specific ANA test. IIFA is a relatively subjective and labor intensive assay which is difficult to standardize. With an increased demand for ANA testing, IIFA is therefore

Abbreviations: ACR, American College of Rheumatology; ANA, antinuclear antibodies; AARD, ANA-associated rheumatic disease; CIA, chemiluminescent immunoassay; CTD, connective tissue disease; ENA, extractable nuclear antigens; FEIA, fluorescence enzyme immunoassay; IIFA, indirect immunofluorescence assay; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disease; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic disease; SLE, systemic lupus erythematosus; SjS, Sjögren's syndrome; SSc, systemic sclerosis

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increasingly replaced by new assays based on ELISA and automated high throughput multiplex assays, raising concerns on diagnostic accuracy and sensitivity of these new platforms. In 2010, the American College of Rheumatology (ACR) therefore stated that IIFA should remain the gold standard for ANA testing [8]. Later, in 2014, an international workgroup of experts representing 15 European countries developed a set of recommendations for the appropriate assessment and interpretation of ANA determined by different methods. It stated that IIFA should be the reference method for ANA screening [9] but alternative assays might be used under the condition that if clinical suspicion is strong and CTD screen is negative, IIFA should be performed.

During the last decade, several ANA screening assays have been developed on fully automated closed systems such as Phadia®, (Thermo Fisher Scientific, Freiburg, Germany) and BIO-FLASH® (Inova Diagnostics, San Diego, USA) system. The QUANTA Flash CTD Screen Plus (Inova Diagnostics) is a fully automated chemiluminescent immunoassay (CIA) on the BIO-FLASH® system for the qualitative detection of the major extractable nuclear antigens (ENA). The assay detects antibodies against dsDNA, Ro52 (TRIM21), Ro60 (SS-A), SS-B (La), small nuclear ribonucleoproteins (Sm), U1-ribonucleoprotein (U1-RNP), Jo-1, Scl-70, CENP-A and -B, Mi-2, RNA Pol III, PM-Scl, PCNA, ribosomal-P, Ku, and Th/To. Also EliA CTD Screen (Thermo Fisher Scientific) is a fully automated but fluorescence enzyme immunoassay (FEIA) on the Phadia® 250 system which includes dsDNA, Ro52, Ro60, SS-B, Sm, U1-RNP (RNP-70, A, C), Jo-1, Scl-70, CENP-B, Mi-2, RNA Pol III, PM-Scl, PCNA, ribosomal-P and fibrillarin. This study evaluated the analytical and clinical performance of these two automated immunoassays and compared their performance to that of traditional IIFA.

2. Materials and methods

2.1. Patients

The study included an unselected prospective study population suspected of SARD and submitted for routine ANA testing to the Erasmus MC over the course of two months. Afterwards, the medical records of the subjects were evaluated for SARD. Patients categorized as AARD fulfilled the classification criteria for the respective diseases, whereas patients that did not satisfy the classification criteria, were categorized as non-AARD. Also SLE patients in remission (AARD in remission) were categorized as non-AARD. A SLEDAI score of 0 was used as criterion for remission. In addition, a second study population of 120 patients diagnosed with AARD were retrospectively included, consisting of patients diagnosed with SLE (n = 40), SSc (n = 23), SjS (n = 34) or PM/DM (n = 23). Samples were obtained from patients as part of routine screening for autoantibodies in the clinical laboratory. There was informed consent for this study. The control group included apparently healthy blood donors (n = 98).

2.2. Antinuclear antibodies by IIFA and automated immunoassays

All sera prospectively included, were tested for ANA by IIFA using NOVA Lite HEp-2 cells (Inova Diagnostics). The assay was performed according to the manufacturer's instructions, using a screening serum dilution of 1:80. In parallel, antibodies to nuclear target antigens were detected by fluorescence enzyme immunoassay (FEIA) on the Phadia® 250 system using EliA™ CTD Screen and by chemiluminescent immunoassay (CIA) on the BIO-FLASH® system using QUANTA Flash CTD Screen Plus. All patients diagnosed with AARD were subsequently tested in individual QUANTA Flash assays (dsDNA, ENA7, Centromere, Scl-70, Jo-1, Ro52, Ro60, SS-B, Sm and RNP) and EliA assays (dsDNA, Symphony, CENP-B, Scl-70, Jo-1, Ro52, Ro60, La, SmD and U1-RNP).

In the EliA CTD Screen, wells are coated with following antigens: dsDNA, SSA/Ro52, SSA/Ro60, SSB/La, U1-RNP (RNP-70, A, C), SmD peptide, CENP-B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA, and Mi-2 [10]. EliA Symphony contains SSA/Ro52, SSA/Ro60, SSB/La, U1-RNP (RNP-70, A, C), SmD, CENP-B, Jo-1 and Scl-70 [11]. All antigens are human recombinant, except dsDNA and SmD, which are native purified in EliA Symphony and SmD peptide which is synthetic in EliA CTD Screen and individual EliA SmD assay. The QUANTA Flash CTD Screen Plus assay contains recombinant Scl-70, Jo-1, SSA/Ro52, SSA/Ro60, SS-B/La, CENP-A and -B, RNA Pol III, Mi-2, Ku, Th/To, PCNA, native Sm and RNP, synthetic PM-/Scl and Rib-P and synthetic dsDNA [12]. QUANTA Flash ENA 7 contains recombinant Scl-70, Jo-1, SSA/Ro52, SSA/Ro60, SS-B/La, native Sm and RNP [13].

2.3. Statistics

Agreement between the tests was calculated using Cohen's kappa agreement test. k-Values of 0.41–0.60 indicate moderate agreement, k-values of 0.61–0.80 substantial and 0.81–1.00 an almost perfect agreement [14]. McNemar's chi-squared test for paired proportions was used to compare sensitivity and specificity, p-values < 0.05 were considered significant. To compare test accuracy, receiver operating characteristic (ROC) analysis was performed and differences between areas under curves (AUC) were analyzed [15]. Data analysis was performed using MedCalc® (MedCalc Software, Ostend, Belgium) and Graph Pad Prism®, release 7.0.2. 2016 (Graph Pad Software, San Diego, USA).

3. Results

3.1. Prospective results

Prospectively, a total of 322 patients suspected of SARD and submitted for routine ANA testing over the course of two months were included. Seventy-two patients (22%) were diagnosed with AARD (Table 1), of which 14 patients (19%) were investigated and diagnosed with AARD for the first time. Of these 72 AARD patients, 44 were diagnosed with SLE, 16 with SjS, 4 with SSc, 4 with MCTD and 4 with PM/DM.

The group of patients without (active) AARD (n = 250; non-AARD) contained 9 SLE patients in remission (AARD in remission), as well as 8 patients with a (suspected) clinical diagnosis of SLE (n = 6), MCTD (n = 1) or SjS (n = 1) that did not satisfy the classification criteria. RA (n = 12) and JIA (n = 10) were also categorized as non-AARD. The group “other” includes 210 diseased patients, yet without AARD.

ANA as performed by IIFA was compared to the results obtained by EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA) and qualitative agreement was calculated (Table 2). Moderate to good qualitative agreements were obtained between the three methods, with total percent agreements varying between 75.2% (IIFA vs. CIA) and 85.4% (FEIA vs. CIA). The correlation according to *kappa* among IIFA and both CTD screens was moderate, while the correlation among both CTD screens (FEIA vs. CIA) was substantial. Using ROC curve analysis for the discrimination between AARD patients and non-AARD diseased controls (Fig. 1A), the area under the curve (AUC) values were 0.93 (95% CI 0.89–0.96) for FEIA and 0.95 (95% CI 0.92–0.97) for CIA. ROC curves and AUC values were also calculated for SLE patients (n = 44; Fig. 1B) and SjS patients (n = 16; Fig. 1C) compared to disease controls. There were no significant differences in the AUC values of FEIA vs. CIA in AARD, nor in SLE or SjS. No ROC analysis was performed for the other AARD groups due to low sample numbers.

ANA as performed by IIFA had a sensitivity of 90% for diagnosing AARD and a specificity of 76%. CIA also had a specificity of 76%, while sensitivity was 99%. Sensitivity of FEIA was 92% and specificity was 84% (Table 3). Statistical analysis showed that CIA had a significantly higher sensitivity (p = 0.0412) compared to IIFA, while the difference for FEIA was not significant (p ~ 1.000 for FEIA vs. IIFA, p = 0.0736 for FEIA vs. CIA). Conversely, FEIA had a significant higher specificity compared to IIFA (p = 0.0158) and to CIA (p = 0.0340), while specificity of CIA compared to IIFA was similar and not statistically different.

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