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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 ($p \sim 1.000$ 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.

Table 1
Overview of positive rates in each disease group for IIFA (ANA), EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA) among AARD patients and disease controls.

Prospective disease cohort	n = 322	ANA		FEIA CTD		CIA CTD	
		n Pos	(%Pos)	n Pos	(%Pos)	n Pos	(%Pos)
Systemic lupus erythematosus (SLE)	44	41	(93%)	43	(98%)	44	(100%)
Sjogren's syndrome (SjS)	16	12	(75%)	13	(81%)	15	(94%)
Systemic sclerosis (SSc)	4	4	(100%)	2	(50%)	4	(100%)
Mixed connective tissue disease (MCTD)	4	4	(100%)	4	(100%)	4	(100%)
Polymyositis/dermatomyositis (PM/DM)	4	4	(100%)	4	(100%)	4	(100%)
Total AARD	72	65	(90%)	66	(92%)	71	(99%)
AARD in remission	9	9	(100%)	2	(22%)	2	(22%)
AARD unproven	9	7	(78%)	6	(67%)	6	(67%)
Rheumatoid arthritis (RA)	12	4	(33%)	5	(42%)	5	(42%)
Juvenile idiopathic arthritis (JIA)	10	0	(0%)	2	(20%)	4	(40%)
Other	210	40	(19%)	25	(12%)	43	(20%)
Total non-AARD	250	60	(24%)	40	(16%)	60	(24%)

Table 2
Qualitative agreement between methods.

Methods	% PPA	% NPA	% TPA	*Kappa* (95% CI)
FEIA vs. ANA	71.0	83.8	79.2	0.54 (0.45–0.64)
CIA vs. ANA	68.8	79.4	75.2	0.48 (0.38–0.58)
FEIA vs. CIA	80.0	88.5	85.4	0.69 (0.61–0.77)

PPA positive percent agreement, *NPA* negative percent agreement, *TPA* total percent agreement.

Table 3
Performance characteristics for IIFA (ANA), EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA).

Prospective disease cohort	ANA	FEIA CTD	CIA CTD
Specificity	76%	84%	76%
Sensitivity	90%	92%	99%
Area under the curve (AUC)	[a]	0.93 (0.89 to 0.96)	0.95 (0.92 to 0.97)
LR +	3.76	5.73	4.11
LR −	0.13	0.10	0.02

[a] AUC for IIFA (ANA) was not calculated since it has binary results.

Additionally, positive and negative likelihood ratios (LR) were calculated (Table 3).

All sera from patients diagnosed with AARD (n = 72) were subsequently tested in individual QUANTA Flash assays and EliA assays for the single ENA (Fig. 2). A total of 59/72 (81.9%) AARD-patient sera were positive by QUANTA Flash ENA7 CIA. When ENA7 results were combined with dsDNA CIA, a total of 65/72 (90.3%) AARD-patient sera were positive. The prevalence of autoantibodies to individual antigens tested by QUANTA Flash CIA was: RNP (26.4%), Sm (23.6%), Scl-70 (1.4%), Jo-1 (5.6%), Ro60 (59.7%), Ro52 (37.5%), SS-B (29.2%), dsDNA (44.4%) and CENP-B (5.6%). For EliA Symphony FEIA a total of 58/72 (80.6%) AARD-patient sera were positive. When EliA Symphony results were combined with dsDNA FEIA, a total of 64/72 (88.9%) AARD-patient sera were positive. Prevalence of autoantibodies to individual antigens tested by EliA FEIA was: RNP (30.6%), Sm (15.3%), Scl-70 (0%), Jo-1 (5.6%), Ro60 (55.6%), Ro52 (36.1%), SS-B (27.8%), dsDNA (44.4%) and CENP-B (5.6%).

When analyzing the individual ENA tested in these AARD patients, there were no substantial differences, except for Sm (Fig. 2). Four SjS patients and one patient without AARD (polyarthritis) tested Sm-positive by CIA but negative by FEIA. Consequently, Sm-specificity for SLE was 94% and 100%, relatively.

3.2. Retrospective results

Retrospectively, both CTD screens (FEIA vs. CIA) were compared in a cohort of 120 patients with AARD. Good qualitative agreements were obtained between both CTD screens with a total percent agreement of 88.5% with a substantial correlation according to *kappa* (0.77; 95%CI 0.69–0.86). This AARD cohort consisted of 40 patients with SLE, 34 patients with SjS, 23 patients with SSc and 23 patients with PM/DM. In addition, 98 healthy blood donors were tested as controls (Table 4). The results of both CTD screens are depicted in Fig. 3. Sensitivity and specificity for AARD were 88% and 84% for CIA and 83% and 92% for FEIA, respectively. Although there was a trend towards higher sensitivity for CIA and higher specificity for FEIA, these differences were not statistically significant. Sensitivity of CIA and FEIA in the individual disease groups (SLE, SjS, SSc, and PM/DM) are summarized in Table 4 and were not significantly different either. ROC curve analysis was performed and showed an AUC value of 0.91 (95% CI, 0.86–0.95) for CIA and 0.95 (95% CI, 0.92–0.97) for FEIA. AUC values in the

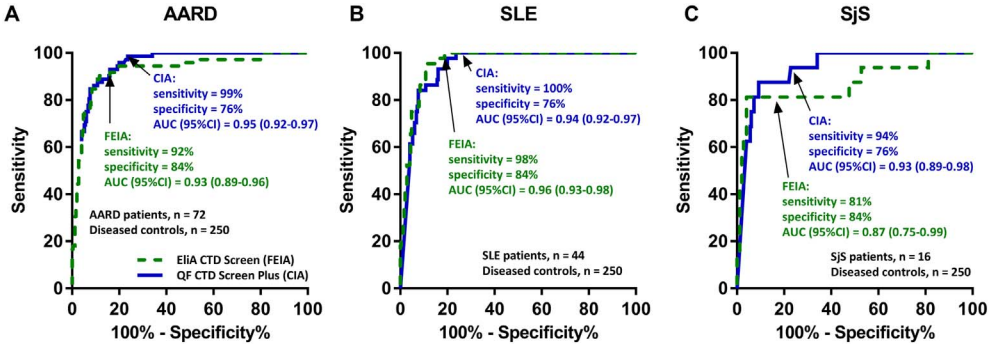

Fig. 1. Receiver operating characteristics (ROC) analysis comparing EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA) among (A) AARD (n = 72), (B) SLE (n = 44) and (C) SjS (n = 16) patients and diseased controls (n = 250).

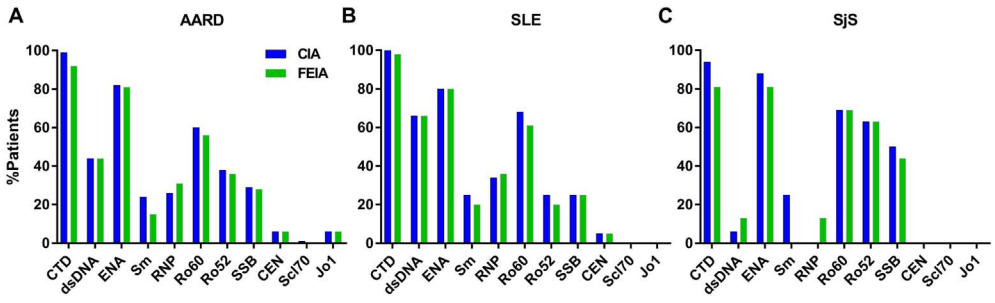

Fig. 2. Prevalence of anti-ENA antibodies among (A) AARD (n = 72), (B) SLE (n = 44) and (C) SjS (n = 16) patients measured by CIA QUANTA Flash and FEIA EliA assays.

Table 4
Overview of positive rates in retrospective disease group for EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA).

Retrospective disease cohort	n = 120	FEIA CTD		CIA CTD	
		n Pos	(%Pos)	n Pos	(%Pos)
Systemic lupus erythematosus (SLE)	40	36	(90%)	38	(95%)
Sjogren's syndrome (SjS)	34	33	(97%)	32	(94%)
Systemic sclerosis (SSc)	23	17	(74%)	17	(74%)
Polymyositis/dermatomyositis (PM/DM)	23	14	(61%)	18	(78%)
AARD	120	100	(83%)	105	(88%)
Healthy donors	98	8	(8%)	16	(16%)
Specificity		92%		84%	
Sensitivity		83%		88%	
LR +		10.21		5.36	
LR −		0.09		0.15	

individual disease groups are depicted in Fig. 4. There were no significant differences in AUC values between both CTD screens in AARD, nor in the individual disease groups.

4. Discussion

Testing for ANA is important in the diagnosis of SARD [1]. With the introduction of fluorescence enzyme immunoassays (FEIA) and more recent chemiluminescent immunoassays (CIA) developed on fully automated closed systems, high throughput ANA screening has become available as an attractive alternative to traditional ANA screening using IIFA. This study evaluated the analytical and clinical performance of two automated immunoassays (FEIA vs. CIA) and compared their performance to that of traditional IIFA. For this purpose, an unselected prospective study population suspected of SARD was included. ANA were measured by IIFA and in parallel samples were tested by CIA and FEIA CTD screen. Secondly, parallel measurements on both CTD screens were performed in retrospective cohorts of AARD patients and healthy controls.

When comparing IIFA to CIA and FEIA CTD results, good qualitative agreement was found with total agreement of 75.2% (IIFA vs. CIA), 79.2% (IIFA vs. FEIA) and 85.4% (FEIA vs. CIA). When analyzing the difference in clinical performance between the three methods in this prospective study, it is important to note that up to 72/322 patients were diagnosed with AARD (22%, Table 1). Since the Erasmus MC is a tertiary care center (university hospital) ANA prevalence and pretest probability for AARD might expected to be higher compared to primary care [16,17]. Secondly, > 80% of these AARD patients were diagnosed with SLE or SjS, so these analyses are predominantly influenced by these patients. It is known that IIFA sensitivity for SLE is high, but relatively low for SjS and PM/DM [2]. On the contrary, solid phase assays are superior to IIFA in diagnosing SjS and PM/DM, but perform less well in SLE. For this reason, ACR stated in 2010 that IIFA should remain the gold standard [8]. A case report was hereby cited in which the diagnosis of SLE was significantly delayed because of a false negative non-IIFA method [18]. Interestingly, in the present prospective study sensitivity of all three methods for AARD was high ranging from 90% for IIFA, 92% for FEIA up to 99% for CIA. Sensitivity of FEIA for AARD was not significantly different compared to IIFA, but CIA sensitivity did differ significantly (99% vs. 90%, p = 0.0412). When analyzing individual disease groups, CIA sensitivity for SLE was even 100% compared to 93% for IIFA. IIFA was negative in 3 SLE patients, which were negative for anti-dsDNA, but two of these positive for Ro60. Four SjS patients were IIFA-negative of which one was positive for Ro52. It is known from literature that solid phase assays detect antibodies to extractable nuclear antigens that might be missed by IIFA, especially Ro60, Ro52 and anti-SSB/La [19–21]. CIA was negative in 1/16 SjS

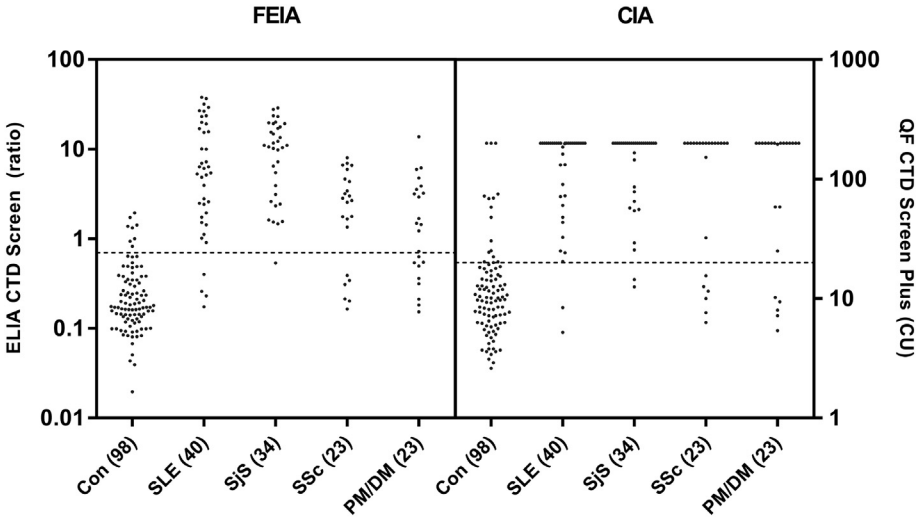

Fig. 3. Results of EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA) among individual groups of AARD patients (n = 120) and healthy controls (n = 98).

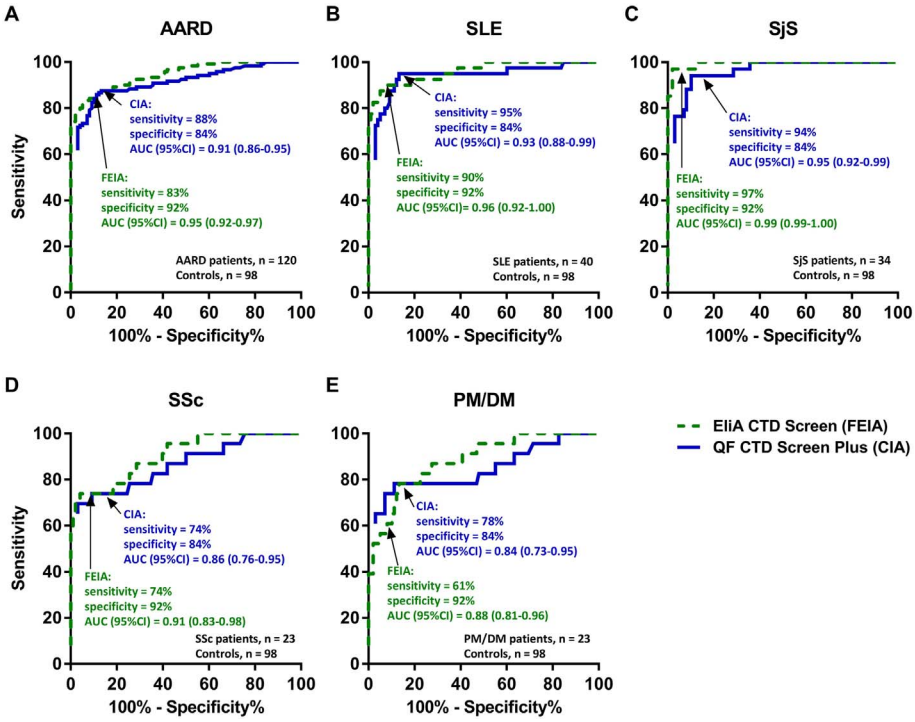

Fig. 4. Receiver operating characteristics (ROC) analysis comparing EliA CTD Screen (FEIA) and QUANTA Flash CTD Screen Plus (CIA) among (A) AARD (n = 120), (B) SLE (n = 40), (C) SjS (n = 34), (D) SSc (n = 23), (E) PM/DM (n = 23) patients and healthy controls (n = 98).

patients and FEIA in 3/16. These latter 3 patients were shown to be seronegative for SS-A/SS-B as measured for individual ENA by CIA and FEIA. To what antigen CIA CTD Screen Plus reacted in the 2 remaining seronegative SjS patients and the so far seronegative SLE patient has to be further analyzed.

FEIA CTD was negative in 2/4 SSc patients prospectively included, which were all 4 positive by IIFA and CIA CTD. This might be explained by difference in antigen composition of both CTD screens. In contrast to HEp-2(000) cells presenting more than hundred antigens, both CTD screens only allow the measurement of antibodies to a limited number of ENA. While FEIA EliA CTD Screen includes CENP-B and fibrillarin, CIA QUANTA Flash CTD Screen Plus instead contains both CENP-A and -B, Th/To and Ku. With the inclusion of Th/To, CIA QUANTA Flash aims for another subgroup of SSc patients [22,23] compared for fibrillarin included in EliA FEIA [24]. In the prospective study, this difference in antigen composition partially explained the discordance between FEIA and CLIA for two SSc patients that were negative by FEIA, but positive by CIA CTD. One patient indeed was positive for Th/To (Rpp25 and Rpp38 subunits) as assessed by CIA, while the other patient was positive for Scl-70 as assessed by CIA, while EliA single antigen assay was negative.

When further analyzing the individual ENA tested in these AARD patients, there were no substantial differences, except for Sm which is known to be highly specific for SLE (Fig. 3). Four SjS patients and one patient without AARD (polyarthritis) tested Sm-positive by CIA (94% Sm-specificity for SLE), but negative by FEIA (100% Sm-specificity for SLE), indicating that CIA Sm-specificity for SLE is lower. Whereas Sm antigen in CIA QUANTA Flash is native purified from calf thymus, FEIA EliA includes a SmD peptide, which is probably more specific than native purified Sm [25].

Another difference in antigen composition between both CTD screens is the Ku antigen in QUANTA Flash CTD Screen Plus [26], which is absent in EliA CTD Screen. While prospectively, no differences between CIA and FEIA were observed (all four PM/DM patients were Jo-1 positive), retrospectively FEIA was negative in 4 PM/DM patients of which 3 turned out to be Ku-positive by immunoblot (EUROLINE Myositis profile 3, EuroImmun, Lübeck, Germany).

Interestingly, 9 patients with an SLE clinically in remission (categorized as non-AARD) were positive by IIFA, while 7 of these patients tested negative in CIA and FEIA CTD. No earlier diagnostic blood samples from these 7 patients were available, therefore we could not confirm whether CTD screen sensitivities might be influenced by disease state and moment of blood drawing i.e. at diagnosis, at flares of active disease or during remission.

Specificity of IIFA and both CTD screens were analyzed using diseased patients, yet without AARD. In addition, specificity was analyzed retrospectively using a control group of healthy blood donors (Fig. 4). FEIA demonstrated a high specificity in both healthy and diseased control groups. In the diseased control group FEIA specificity was significantly higher compared to IIFA ($p = 0.0158$) as well as CIA ($p = 0.0340$). Also Op de Beéck et al. [10] evaluated the diagnostic performance of FEIA compared to IIFA and demonstrated high specificity in diseased patients as well (97% compared to 92% in our study). Bentow et al. [12] recently evaluated the clinical performance of CIA QUANTA Flash CTD Screen Plus and showed 96% specificity with a total of 6/146 (4%) positivity by CIA in a healthy Biobank population (ProMedDx, Norton, MA, USA). Nevertheless, in the present study 16/98 (16%) healthy blood bank volunteers (Sanquin, Amsterdam, The Netherlands) tested positive resulting in a lower specificity of 84%. Possibly age, gender or local population might explain this disagreement.

In the prospective study, patients with RA (42% CTD-positive), JIA (20–40% CTD positive) and suspected but unproven AARD (67% CTD-positive) were categorized as non-AARD, which is obviously also influencing specificity in the diseased non-AARD control group. When specificity was solely analyzed in the non-AARD category “other” (n = 210, Table 1), specificity of IIFA, CIA and FEIA for AARD was 84%, 83% and 90%, respectively. This might be of importance when comparing specificities with other studies published.

Although there were differences in specificity and sensitivity between both CTD screens, AUC values were similar in both the prospective as retrospective study. Based on ROC analysis (Figs. 1 and 4), the major contributor to the observed difference between the two solid phase immunoassays was the selected cut-off.

In short, we found a significantly higher specificity for FEIA CTD and higher sensitivity for CIA CTD compared to IIFA with several SLE

and SjS patients negative by IIFA, testing positive by CTD screen. Recently, Bossuyt [27] evaluated the added value of FEIA EliA CTD Screen to IIFA and conclude that combining both IIFA with solid-phase assays increases the diagnostic accuracy. For SLE and SjS, the highest diagnostic accuracy was achieved by combining both tests and for SSc screening with IIFA and performing FEIA on IIFA-positive samples was the best option. More recently, also Robier et al. demonstrated that FEIA EliA CTD Screen represents an appropriate diagnostic tool for ANA screening and also recommended sequential or parallel screening in case of strong clinical suspicion for SARD [28]. Our data support these recommendations and show that both CTD screens might be attractive alternatives or additives to traditional ANA screening.

Some laboratories perform ENA screen together with anti-dsDNA assay as alternative for traditional ANA screening. When combining QUANTA Flash ENA7 with dsDNA CIA or EliA Symphony with dsDNA FEIA, we could demonstrate a sensitivity of 90% and 89% for AARD, respectively. Sensitivity of IIFA, CIA QUANTA Flash CTD Screen Plus and FEIA EliA CTD Screen for AARD was 90%, 92% and 99%, respectively. These data show that sensitivity of both CTD screens for AARD is higher compared to IIFA or ENA screen combined with a dsDNA assay, implying an added value of both CTD screens.

A limitation of our study is that we did not evaluate sensitivity of IIFA and both CTD screens for less common disease-specific autoantibodies. For EliA CTD Screen it was shown that sensitivity for anti-fibrillarin and anti-RNA polymerase III was low, 68% and 67%, respectively [29]. Such a particular study for QUANTA Flash CTD has not been performed thus far. Although prevalence of these antibodies is low and patients are not often monospecific for these autoantibodies, CTD screens might be false-negative in such cases. Therefore, although both CTD screens are good alternative methods to IIFA, one should keep in mind that false negative and false positive ratio of both CTD screens may be different compared to traditional IIFA.

5. Conclusions

In this prospective study, both FEIA EliA CTD Screen and CIA QUANTA Flash CTD Screen Plus outperform IIFA. FEIA is relatively more specific, while CIA has a higher sensitivity. Based on ROC analysis, major contributor to the difference between the two solid phase immunoassays was the cut-off. Both FEIA and CIA are reliable CTD screening tests with high specificity and sensitivity and therefore both CTD screens might be attractive alternatives for traditional ANA screening. Still, in case of a strong clinical suspicion of AARD and a negative CTD screen, additional IIFA should be performed.

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Conflict of interest

Thermo Fisher Scientific and Inova Diagnostics were involved in making the study design, but had no part in collection, analysis and interpretation of data or in the decision where submit the report for publication. Before submission, Thermo Fisher Scientific and Inova Diagnostics were allowed to review and comment on the written report, however without any further obligations to the authors.

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