Diagnostic performance of particle-based multi-analyte technology compared to indirect immunofluorescence in screening for anti-nuclear antibodies in patients with autoimmune rheumatic diseases

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Abstract

Objective

In autoimmune rheumatic disease (ARD), ANA testing is crucial for orienting clinical diagnosis and further diagnostic workups. We evaluated the performance of a fully automated system using particle-based multi-analyte technology (PMAT) and compared it to indirect immunofluorescence (IIF) on HEp-2 cells.

Methods

Serum samples from 1241 subjects were collected in 13 Italian rheumatology centres. The ARD group (782 samples) included 164 patients affected by systemic lupus erythematosus, 277 by Sjögren's syndrome, 132 by systemic sclerosis, 106 by idiopathic inflammatory myopathy, and 103 by undifferentiated connective tissue disease. The control group comprised 120 healthy donors, 221 patients affected by other autoimmune/inflammatory disorders, and 118 patients affected by acute or chronic infections.

Results

In the overall ARD population, HEp-2 IIF showed higher sensitivity when compared to Aptiva/PMAT (92.8 vs. 82.6%) except in the case of idiopathic inflammatory myopathy (58.5% vs. 82.1%). Conversely, Aptiva/PMAT showed higher specificity (77.9% vs. 54.0%) and a higher likelihood ratio for positive results (3.81 vs. 2.08). Double-positive samples provided an LR for positive results higher than one method alone (6.31).

Conclusion

This is the first study comparing Aptiva/PMAT against HEp-2 IIF in ANA detection. While the diagnostic sensitivity of this novel method is lower than that of HEp-2 IIF, its high specificity is a valuable tool in the diagnosis of patients affected by ARD and improves their stratification into specific disease subsets. The combined use of HEp-2 IIF and Aptiva/PMAT assays increases diagnostic accuracy and significantly enhances the potential to accurately classify patients affected by ARDs.

Key words

anti-nuclear antibodies, ANA, autoimmune rheumatic diseases, particle-based multi-analyte technology.

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Introduction

Autoantibody testing plays a pivotal role in diagnosing suspected autoimmune rheumatic disease (ARD). In routine clinical practice, anti-nuclear antibodies (ANA) are currently the entry test; they support physicians in the early diagnostic phase when signs and symptoms may overlap, guiding further diagnostic processes. Moreover, ANA sub serology (*e.g.* ANA-specific antibodies) is performed to stratify patients into subsets with different clinical features, treatment responses, and disease outcomes (1, 2). Indirect immunofluorescence (IIF) on human epidermoid laryngeal carcinoma (HEp-2) cells is considered the "gold standard" technique for ANA screening (3), and current criteria for classifying different ARDs were developed based on this method. In recent years, automated computer-aided (CAD) systems were introduced for HEp-2 IIF reading. Although it does not enable reliable classification in terms of fluorescence signal and pattern, digital microscopy partially reduces the extensive workload, improves the learning curve in non-expert operators and facilitates the exchange among specialists via high-quality image archives (4-7). However, the disadvantages of this technique include a lack of standardisation and high false positivity rates. To overcome these limitations, new solid-phase assays (SPAs), also known as connective tissue disease (CTD) screen assays, have been developed to screen for the 15-16 most common ANA using fluor enzymatic (FEIA) or chemiluminescence (CLIA) immunoassays. SPAs are faster, fully automated, reproducible methods of ANA screening. This characteristic makes them suitable for application in laboratories with less expertise in ANA HEp-2 pattern recognition. Recent studies have compared newly developed SPAs to IIF in order to evaluate their performance and establish their positioning in ARD autoantibody testing. Compared to HEp-2 IIF, SPAs have higher diagnostic accuracy overall but slightly lower sensitivity (8-12). For this reason, some authors recommend the use of SPAs in combination with traditional ANA screening using HEp-2 IIF (13, 14), while others have evaluated the option of fully replacing the IIF method (15, 16). In this study, we compared a novel fully automated system using particle-based multi-analyte technology (PMAT), which presents a wider number (29) of antigens than FEIA and CLIA CTD screen assays, to the HEp2-IIF method for ANA screening.

Materials and methods

Patients

Serum samples from 782 ARD subjects were collected at 13 different Italian rheumatology centres. All patients were diagnosed according to internationally accepted classification criteria (701 females/81 males, males; mean age: 41.3 years; range: 16–88 years). In total, 164 patients were diagnosed with systemic lupus erythematosus (SLE), 277 with Sjögren's syndrome (SjS), 132 with systemic sclerosis (52 with diffuse cutaneous SSc and 80 with limited cutaneous SSc), 106 with idiopathic inflammatory myopathy (IIM), and 103 with undifferentiated connective tissue disease (UCTD).

The control group included 459 patients (288 females/171 males; mean age: 44.8 years; range: 5–88 years) distributed as follows:

120 healthy donors in which ARD or other pathologies were excluded; 221 patients suffering from other autoimmune/inflammatory disorders classified according to single diseases: autoimmune gastritis (AIG, n=30), autoimmune thyroid disease (AITD, n=25), coeliac disease (CD, n=25), osteoarthritis (OA, n=3), polymyalgia rheumatica (PMR, n=23), fibromyalgia (FMG, n=17), rheumatoid arthritis (RA, n=15), ankylosing spondylitis (ASp, n=36), primary biliary cholangitis (PBC, n=30), and psoriatic arthritis (PsA, n=17); 118 patients affected by acute or chronic infections: cytomegalovirus (CMV, n=22), Epstein-Barr virus (EBV, n=21), hepatitis B virus (HBV, n=25), hepatitis C virus (HCV, n=27), and syphilis (Syp, n=23).

Methods

Sera were tested using the Aptiva instrument (Inova Diagnostics, San Diego, CA, USA). Aptiva is a novel digital system that uses particle-based

Table I. Main characteristics of IIF and Aptiva/PMAT for ANA detection.

	Indirect immunofluorescence (IIF)	Particle-based multi-analyte technology (PMAT)
Substrate	Human laryngeal carcinoma cells (HEp-2 cells)	Mixture of suspended micro particles individually coated with a different antigen
Antigens	Potentially all nuclear and cytoplasmic antigens	29 nuclear and cytoplasmic antigens (16 RUO)
Identification of antibody specificity	No (except for anti-centromere antibodies)	Yes
Results	Semiquantitative (titre)	Quantitative (arbitrary units)
Interpretation	Subjective interpretation of fluorescence intensity	LED technology, high resolution charged coupled device (CCD) sensor

multi-analyte technology (PMAT) to simultaneously measure multiple autoantibodies in one step. This technology is based on the use of a mixture of suspended microparticles individually coated with different antigens. Colour coding allows for the identification of single antigens during the process. After incubation with diluted sera, particles are washed and incubated with a human IgG conjugated with phycoerythrin. Finally, after a washing cycle, the particles are aligned in a monolayer and analysed using digital imaging double-LED technology. A red LED is first used to identify the analyte, while a subsequent green LED enables the measurement of the fluorescence intensity. The reaction data are captured digitally by a sophisticated high-resolution charge-coupled device (CCD) sensor. The acquired image is stored in the analyser database for the calculation and distribution of quantitative results. To verify the instrument's functionality, the system uses quality control samples that contain antibodies specific to each analyte tested (17). Samples were centralised at Pordenone Laboratory and tested using PMAT with the following antigenic panels: CTD IgG Essential™ (dsDNA, DFS70, U_1RNP, Sm, Ro60, Ro52, La, Scl70, Jo1, CENP-B, and Ribo-P), CTD IgG Comprehensive™ (RNA pol III, Th/To, Ku, BICD2, and PM/Scl) (Research use only), and Autoimmune Myopathy IgG™ (Mi2, HMGCR, NXP2, MDA5, PL-7, PL-12, EJ, SRP, TIF, SAE and OJ) (Research use only). The Aptiva instrument uses ready-to-use cartridges containing all the specific reagents for the analytical reaction, including a mixture of the microspheres coated with the different antigens defined in each profile (17).

ANA were detected via indirect immunofluorescence using an automated HEp-2 IIF method (NOVA Lite, Inova Diagnostics). Sera were diluted to 1:80 with phosphate-buffered saline (PBS), and positive samples were titrated to a maximum dilution of 1:1280. Incubations and washing of the slides were performed using the QuantaLyser 3000 (Inova Diagnostics) automated processor according to the manufacturer's instructions. Slides were read by two expert pathologists without knowledge of the clinical diagnosis or the results from the Aptiva/PMAT analyser (blind experiment) using a Zeiss LED fluorescence microscope with an eyepiece set at 10x and a lens set at 40x, for a total magnification of 400x. According to the International Consensus on ANA Patterns (ICAP), nuclear, cytoplasmic, and mitotic patterns were considered (18).
A summary description of the main characteristics of the two methods for ANA detection is provided in Table I.

Statistical analysis

The optimal cut-off for Aptiva/PMAT was selected according to a receiver operating characteristic (ROC) curve analysis and set at 5 arbitrary units (AU)/ml for antibodies included in CTD Essential and 1 AU/ml for both the Comprehensive and Autoimmune Myositis panels. The diagnostic sensitivity and specificity and the likelihood ratio for positive or negative results (LR+ or LR-), were calculated both for Aptiva/PMAT and HEp-2 IIF cumulatively on all ARD patients and for each single ARD. The diagnostic accuracy of HEp-2 IIF and Aptiva/PMAT was quantified as the area under the ROC curve (AUC). Statistical analysis was performed using MedCalc software v. 15.2.1 (Ostend, Belgium). A p-value <0.05 was considered statistically significant.

Ethics

This study was approved by the Ethics Committee of the Umbria Region, Italy (authorisation no. 3574/19). All participants provided informed consent, and their records and information were anonymised and de-identified prior to analysis, according to the Declaration of Helsinki and Italian legislation (authorisation of the privacy guarantor no. 9, 12/12/2013).

Results

The diagnostic accuracy of the two assays for ANA screening (HEp-2 IIF and Aptiva/PMAT) was evaluated in 782 diagnostic samples from ARD patients and 459 control samples. Among the ARD patients, 612 samples were positive and 22 samples were negative for both HEp-2 IIF and Aptiva/PMAT. Conflicting results were found for 148 samples; 114 were positive only with HEp-2 IIF, and 34 were positive only with Aptiva/PMAT. In the control group, 57 were positive and 203 were negative for both HEp-2 IIF and Aptiva/PMAT, while 154 were positive only with HEp-2 IIF and 45 were positive only with Aptiva/PMAT.
Comprehensive results from the ARD and control groups according to antibody levels are shown for both Aptiva/PMAT (AU/ml) and HEp-2 IIF (titre dilution) in Figure 1. The yellow quarter (upper right) indicates samples that both methods identified as positive.
Overall, sensitivity was 82.6% for Aptiva/PMAT *vs*. 92.8% for HEp-2 IIF, and specificity was 77.8% *vs*. 54.0%, respectively. The LR+ was 1.97 for

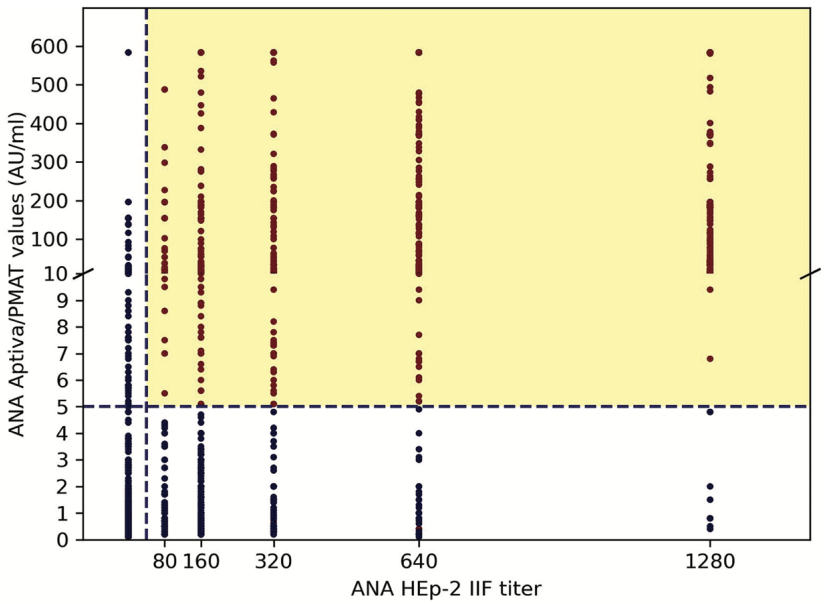

Fig. 1. Plot analysis comparing Aptiva/PMAT (values) and HEp-2 IIF (titre) results (dashed lines show cut-offs). Since cut-off levels of CTD IgG Essential™ (5 arbitrary units (AU)/ml) and of CTD IgG Comprehensive™ / Autoimmune Myopathy IgG™ (1 AU/ml) are different, to enable comparison of results, all test values of CTD IgG Comprehensive™ and Autoimmune Myopathy IgG™ have been multiplied 5x.

Table II. Sensitivity (SE), specificity (SP) and likelihood ratio for positive (LR+) and negative results (LR-) for HEp-2 IIF and for Aptiva/PMAT in ARD patients.

		SE % (95%CI)	SP% (95%CI)	LR+	LR-
AARDs (n=782)	HEp-2 IIF	92.8 (90.8 – 94.5)	54.0 (48.1 – 57.5)	1.97	0.14
	Aptiva/PMAT	82.6 (79.8 – 85.2)	77.8 (73.7 – 81.7)	3.81	0.22
	Double POS	78.0 (74.9 – 80.9)	87.6 (84.2 – 90.5)	6.31	0.25
SLE (n=164)	HEp-2 IIF	92.0 (86.7 – 95,7)	59.0 (54.2 - 63.7)	2.24	0.14
	Aptiva/PMAT	90.2 (84.5 – 94.3)	77.9 (73.7 – 81.7)	4.08	0.13
	Double POS	89.0 (83.1 – 93.3)	87.6 (84.1 – 90.5)	7.15	0.13
SjS (n=277)	HEp-2 IIF	87.4 (82.9 – 91.0)	59.0 (54.2 – 63-7)	2.13	0.21
	Aptiva/PMAT	80.9 (75.7 – 85.3)	77.9 (73.7 – 81.7)	3.66	0.25
	Double POS	78.7 (73.4 – 83.4)	87.6 (84.1 – 90.5)	6.33	0.24
SSc (n=132)	HEp-2 IIF	97.0 (92.4 – 99.1)	59.0 (54.2 – 63.7)	2.36	0.05
	Aptiva/PMAT	90.9 (84.7 – 95.2)	77.9 (73.7 – 81.7)	4.11	0.12
	Double POS	89.4 (82.8 – 94.1)	87.6 (84.1 – 90.5)	7.18	0.12
IIM (n=106)	HEp-2 IIF	58.5 (48.5 – 69.0)	59.0 (54.2 – 63.7)	1.43	0.70
	Aptiva/PMAT	82.1 (73.4 – 88.8)	77.9 (73.7 – 81.7)	3.71	0.23
	Double POS	57.5 (47.6 – 67.1)	87.6 (84.1 – 90.5)	4.84	0.44
UCTD (n.=03)	HEp-2 IIF	81.4 (72.4 – 88.4)	59.0 (54.2 – 63.7)	1.98	0.32
	Aptiva/PMAT	65.7 (55.6 – 74.8)	77.9 (73.7 – 81.7)	2.96	0.38
	Double POS	59.8 (49.6 – 69.4)	87.6 (84.2 – 90.5)	4.81	0.46

HEp-2 IIF and 3.81 for Aptiva, and the LR- was 0.14 for HEp-2 IIF and 0.22 for Aptiva/PMAT (Table I).

HEp-2 IIF failed to find any antibodies in 34 ARD samples (20 patients with IIM, six with SjS, two with SLE, two with SSc and four with UCTD). The missed antibodies were Ro60 (in seven samples), Ro52 (13 samples), Jo1 (10 samples), anti-synthetase (7 samples) and other in three samples. At the same time, Aptiva/PMAT was positive in the control groups for Ro60 (12 samples), Ro52 (two samples) anti-dsDNA (seven samples), anti-Scl70 (six samples), and other antibodies in 18 cases. The distribution of the diagnoses of these subjects was uniform for the different inflammatory or infectious diseases.

When differentiating between single ARDs, HEp-2 IIF reached the maximum sensitivity in SSc (97%), and the lowest in IIM (58.5%). For Aptiva/PMAT, the maximum sensitivity was observed in SSc (90.9%) and the lowest sensitivity was observed in UCTD (65.7%). In double-positive samples (HEp-2 IIF and Aptiva/PMAT), specificity was 87.6%, with a consistently increasing LR+ value in each individual disease compared to the LR+ of each assay. Sensitivity, specificity, and LR values for ARDs overall and for each disease are reported in Table II.

Regarding IIM, 20 (18.9%), 19 (17.9%), and 67 (63.2%) samples were positive only with Aptiva/PMAT, only with HEp-2 IIF, and with both methods, respectively. All 20 samples with isolated positivity on Aptiva/PMAT were also positive for myositis-specific antibodies (MSA).

In the control group, HEp-2 IIF was positive in 211/459 (46.0%) patients compared to 102 (22.2%) patients analysed using Aptiva/PMAT. Positivity was distributed in three subgroups as follows: a) in healthy donors, HEp-2 IIF and Aptiva/PMAT were positive in 29/120 patients (24.2%) and 17/120 patients (14.2%), respectively; b) in the "other inflammatory disease group", 120/221 patients (54.3%) and 62/221 patients (28.0%) were identified as positive via HEp-2 IIF and Aptiva/PMAT, respectively (Table IIIa). Double positivity was found in 43 samples (19.5%).

In controls affected by acute or chronic infections, positivity was found in 58/120 (48.3%) patients with HEp-2 IIF and in 20/120 (16.7%) with Aptiva/PMAT. Data for healthy donors and patients with each disease are provided in Table IIIb.

The accuracy of the two classifiers for ARD overall and for each disease was expressed using ROC AUC models (Fig. 2). For ARD patients, the AUC indicates a significant difference between the two assays (p=0.003), with the AUC being slightly higher for Aptiva/PMAT (0.879 Aptiva/PMAT *vs*. 0.841 HEp-2 IIF). However, when analysing data on each disease, the trait was confirmed only for IIM (0.671 for HEp-2 IIF vs. 0.875 for Aptiva/PMAT, p=0.001) and for SLE (0.891 for HEp-2 IIF *vs*. 0.931 for Aptiva/PMAT, p=0.048). No significant difference in AUC values was ob-

Table IIIa. Number of positive samples detected by HEp-2 IIF and Aptiva/PMAT stand alone, and double positive samples for each single disease included in the control group named 'other autoimmune/inflammatory disorders'.

Disease	n.	Only Aptiva/PMAT positive	Only HEp-2 IIF positive	Double positive	Double negative
AIG	30	2 (6.7%)	13 (43.3%)	6 (20%)	9 (30%)
AITD	25	3 (12%)	10 (40%)	5 (20%)	7 (28%)
CD	25	3 (12%)	6 (24%)	6 (24%)	10 (40%)
FM	17	8 (47%)	2 (11.8%)	2 (11.8%)	5 (29.4%)
OA	3	0	1 (33.3%)	0	2 (66.7%)
PBC	30	4 (13.3%)	4 (13.3%)	6 (20%)	16 (53.4%)
PMR	23	3 (13%)	7 (30.4%)	2 (8.7%)	11 (47.9%)
PsoA	17	2 (11.8%)	5 (29.4%)	3 (17.6%)	7 (41.2%)
RA	15	1 (6.7%)	9 (60%)	3 (20%)	2 (13.3%)
AS	36	1 (2.8%)	19 (52.7%)	10 (27.8%)	6 (16.7%)
Total	221	27 (12.2%)	76 (34.4%)	43 (19.5%)	75 (33.9%)

AIG: autoimmune gastritis; AITD: autoimmune thyroid disease; CD: coeliac disease; FM: fibromyalgia; OA: osteoarthritis; PBC: primary biliary cholangitis; PMR: polymyalgia rheumatica; PsA; psoriatic arthritis; RA: rheumatoid arthritis; AS: ankylosing spondylitis.

Table IIIb. Number of positive samples detected by HEp-2 IIF and Aptiva/PMAT stand alone, and double positive samples for each single disease included in the control group named 'acute/chronic infections'.

Disease	n.	Only Aptiva/PMAT positive	Only HEp-2 IIF positive	Double positive	Double negative
HBV	25	4 (16%)	11	0	10
CMV	22	2 (9.1%)	11	4	5
EBV	21	4 (19%)	7 (33.3%)	0	10 (47.7%)
HCV	27	2 (7.4%)	9 (33.3%)	2 (7.4%)	14 (51.9%)
SYP	23	1 (4.3%)	11 (47.8%)	2 (8.7%)	9 (39.2%)
Total	118	13 (11.1%)	49 (41.5%)	8 (6.8%)	48 (40.6%)
HD	120	11 (9.1%)	23 (19.2%)	6 (5%)	80 (66.7%)

CMV: cytomegalovirus; EBV: Epstein Barr virus; HBV: hepatitis B virus; HCV: hepatitis C virus; SYP: syphilis; HD: healthy donors.

served in SSc (p=0.314), SjS (p=0.340), or UCTD (p=0.988).

Discussion

Anti-nuclear antibodies are hallmarks in ARD screening, and their accurate detection and proper reporting are strongly recommended in rheumatological guidelines (19). Recently, new SPAs in addition to HEp-2 IIF have been developed for ANA screening, namely the FEIA and CLIA "CTD screen" assays. Current data in the literature report that, globally, HEp-2 IIF shows higher sensitivity and lower specificity when compared with the new CTD screen assays (9, 11, 12). This finding was confirmed in the present study, where HEp-2 IIF showed a higher sensitivity than Aptiva/PMAT (92.8 *vs*. 82.6%), while specificity was much higher for the latter (77.8%) compared to HEp-2 IIF (54%). This feature was consistent across all ARDs except for IIM, for which the sensitivity of HEp-2 IIF was far lower than that of Aptiva/PMAT (58.5% *vs*. 82.1%). The low sensitivity of HEp-2 IIF in IIM is consistent with data reported in the literature and underlines the relevance of testing for a wide panel of MSA when IIM is clinically suspected (20). Notably, some anti-aminoacyl-tRNA synthetase autoantibodies generate a cytoplasmic pattern on

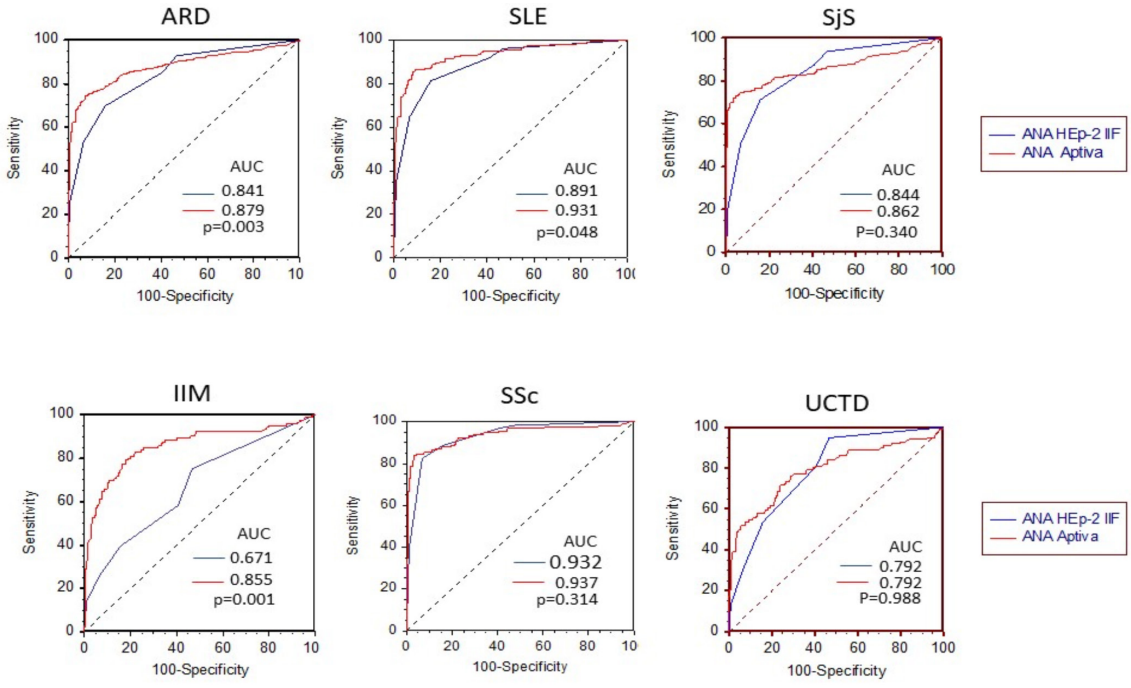

Fig. 2. Receiver operating characteristics (ROC) curves of HEp-2 IIF and Aptiva/PMAT methods for ARD patients overall, SLE, SjS, IIM, SSc, UCTD.

HEp-2 cells (*i.e.* AC-20 in case of Jo1 and AC-19 in case of PL-7 or PL-12 autoantibodies) which, especially at low titres, may not be detected. Indeed, in nine cases in the present cohort, anti-Jo1 antibodies were only detected with Aptiva/PMAT and not with HEp-2 IIF. Anti-M2, anti-SAE, anti-NPX-2, and anti-TIF1 produce a nuclear fine speckled pattern on HEp-2 cell, frequently with negative nucleoli, which is difficult to differentiate from the speckled pattern produced by other autoantibodies. In addition, in MDA5 positive patients, HEp-2 IIF is often negative. As a consequence, HEp-2 IIF may be negative in about 20% of IIM patients (21, 22).

MSA detection plays an important role in clinical practice, not only in diagnosing IIM but also in stratifying patients into specific disease subsets (1, 23-25). This expanding role demands reliable and routinely applicable MSA assays. Currently, the laboratory work flow in IIM is based on screening with HEp-2 IIF followed by line- and dot-immunoassays as confirmatory methods, as the latter are currently the only type of assay that provides an adequate panel of antigens (10-12) for detecting MSA. Limitations in using these methods include prolonged reporting times, increased costs, and the need for highly trained staff. In addition, immunoblot methods lack specificity (21, 26). Given the excellent results obtained by the Aptiva/PMAT assay in this study, it is conceivable that the Autoimmune Myopathy panel can be used for the detection of MSA (with the additional advantage of being able to provide quantitative data on antibody levels) as an alternative to the line- and dot-immunoassays, which are currently the most used methods for this type of investigation.

When comparing Aptiva/PMAT with other SPA (*e.g.* the CTD screen assays) for ANA screening, Aptiva/PMAT presents higher diagnostic accuracy in IIM patients. In this disease, Claessens *et al.* (11) found sensitivities of 52% for FEIA and 56% for CLIA, which are lower than that observed in this study using Aptiva/PMAT (82.1%). Furthermore, Aptiva/PMAT also showed better accuracy than line- and dot-immunoassays in detecting difficult autoantibodies, such as TIF1 (20, 23). For other antigens such as OJ, for which available commercial blot-based assays present shortcomings probably due to the presence of conformational epitopes (24), Aptiva/PMAT is a potential novel diagnostic method. Taken together, there is evidence that the wider antigenic panel for MSA provided by Aptiva/PMAT represents a strength that could positively impact clinical and laboratory diagnostics for timely and accurate IIM diagnosis.

SSc is another disease that deserves consideration. As observed for IIM, in the landscape of SPAs for nuclear antigens, Aptiva/PMAT uses a wider panel of SSc-specific antigens. In the present study, Aptiva/PMAT demonstrated lower sensitivity than HEp-2 IIF (90% *vs.* 97%), but much higher specificity (77% *vs.* 59%) and significantly higher LR+ (4.11 Aptiva/PMAT *vs.* 2.36 HEp-2 IIF). When compared to to the CTD screen assays, Aptiva/PMAT has higher sensitivity (90.9%) than FEIA (80.5%) or CLIA (85.9%) (11). The antigens introduced in the "Comprehensive" profile of Aptiva/PMAT are clinically relevant and can support clinicians in the diagnostic phase and patient stratification, according to the risk of severe disease and major organ impairment.

Limitations and potential of HEp-2 IIF and Aptiva/PMAT

HEp-2 IIF is a consolidated, widespread, and low-cost technique (27). Its high sensitivity is advantageous in revealing a wide spectrum of autoantibodies. Whilst high sensitivity is a strength of screening methods, the low diagnostic specificity of HEp-2 IIF can potentially lead to overdiagnosis if not appropriately managed. As such, there are ongoing discussions on the importance of setting the screening titre for ANA HEp-2 IIF at an acceptable specificity value. There is a strong consensus in the literature that a titre of 1:80 is the best compromise between diagnostic sensitivity and specificity (28-31). In the present study, the titre of 1:80 showed high sensitivity (92.8%), but the best compromise between sensitivity and specificity was obtained using the cut-off titre of 1:320, as shown via ROC curve analysis. However, although the use of this titre allows for an increase in specificity (84.1%), sensitivity falls to 69.9%, a value hardly suitable for a screening test.

Though less sensitive, Aptiva/PMAT showed higher specificity, supporting the correct identification of true positive samples. In addition, the potential of Aptiva/PMAT to cluster ARD patients according to their autoantibody levels was previously demonstrated in a wide population. Clustering according to autoantibody levels allows for the identification of ARD subjects with similar clinical features but different prognoses (23, 32). Although these preliminary results need to be confirmed by further studies to ensure that a level of standardisation/reproducibility at least equal to that of the IIF method is achieved, it is important to underline that this multiparametric technology has shown a level of diagnostic accuracy similar to that of the most consolidated fluor enzymatic and chemiluminescence immunoassays.

Combining HEp-2 IIF and AptivaPMAT

The analysis of double-positive samples (determined by both HEp-2 IIF and Aptiva/PMAT) yielded a valuable result. As illustrated in Table I, ARD samples with double positivity have a higher LR+ than those scored positive via an individual method. These data are consistent across all the diseases studied. Thus, as demonstrated for HEp-2 IIF and the CTD screen assays, the combined use of the two assays significantly enhances the potential to accurately classify patients affected by the disease (13).

To the same extent, in non-ARD subjects, the combined use of the two methods identifies double-negative samples and increases specificity. In clinical pratice, this approach could help decrease access to outpatient services and potentially reduce costs related to follow-up or reflex tests.

Conclusions

Demand for ANA testing by a variety of specialists, no longer rheumatologists or immunologists alone, is increasing. This impacts pre-test probability, which must be considered when looking at the future of ARD screening (33). The development of SPAs has improved ANA test-

ing, increasing diagnostic accuracy. Aptiva/PMAT, with its highly comprehensive antigen panels, could be a valuable tool in the diagnosis of patients affected by ARD, improving their stratification into specific disease subsets. However, at present, HEp-2 IIF should not be considered outdated, as the combined use of HEp-2 IIF and Aptiva/PMAT could find its application especially in the early diagnostic phase. These data are even more valuable in the perspective of the development of possible future scores for combined positive results in the classification of ARDs.

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