Comparison and evaluation of different anti-dsDNA antibody detection methodologies in a cohort of Hungarian patients

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Introduction

Anti-double stranded DNA (dsDNA) antibodies are useful for the diagnosis of systemic lupus erythematosus (SLE). However, these antibodies are very heterogeneous and low avidity anti-dsDNA antibodies may occur in other rheumatic diseases as well. As a consequence of their heterogeneity, detection of anti-dsDNA antibodies partly depends on the type of assay used. If a commercially available enzyme-linked immunosorbent assay (ELISA) is performed for detection and quantification of anti-dsDNA antibodies, generally both low and high avidity antibodies will be detected. In contrast, the immunofluorescence test on *Crithidia luciliae* and the radioimmunoassay by the Farr method will only detect high avidity antibodies. Differences in assay performance may lead to important discrepancies between results, because high avidity dsDNA antibodies are considered highly specific for SLE.

Aim of the study

Since our routine laboratory diagnostic protocol mandates the detection of anti-dsDNA antibodies with ELISA method without any confirmatory testing, the aim of the current study was to compare our results with five different anti-dsDNA antibody assays, including the gold standard Farr test in order to set up an algorithm for better differentiation of high avidity anti-dsDNA antibodies from low avidity ones in our patient population.

Patients and Methods

Patients: From sera of 1446 consecutive patients sent to our laboratory for anti-dsDNA antibody measurements during a 4-month period, all sera that were found positive with our routine ELISA test (Quanta Lite® dsDNA ELISA, INOVA Diagnostics, San Diego, CA, USA – **ELISA1**) were included in the study: 90 samples from SLE, 38 from RA, 28 from other connective tissue disease (CTD) and 34 from patients with other inflammatory diseases (190 in total). Sera of 20 healthy blood donors were used as control.

Methods: All selected sera were tested for anti-dsDNA antibody with two additional commercially available enzime-linked immunoassays (anti-dsDNA ELISA, Orgentec Diagnostica GmbH, Mainz, Germany – **ELISA2** and Quanta Lite® HA dsDNA ELISA, INOVA diagnostics, San Diego, CA, USA – **ELISA3**), with two *Crithidia luciliae* immunofluorescence tests (commercially available Nova Lite dsDNA *Crithidia luciliae* slides, Inova Diagnostics, San Diego, CA, USA – **CLIFT1** and in house test – **CLIFT2**) and with in house Farr method used as the gold standard **(FARR)**. All commercially available kits were used according to the manufacturer's instructions. For ELISA1 the cut-off value was established according to a previously used in house method, while for ELISA2 and ELISA3 the cut-off indicated by the respective manufacturer was used (Table 1.). A sample dilution of 1:10 in PBS was applied for both Crithidia tests, while bound antibodies were detected by FITC – conjugated anti-human rabbit IgG (DAKO, Denmark) for CLIFT1 and FITC-conjugated sheep anti-human total immunoglobulins (Sanquin Reagents, Amsterdam The Netherlands) for CLIFT2, respectively. All ELISA tests and the Farr assay were standardized against the international WHO reference material (Wo/80). An internal reference serum was included in each assay. Statistical analysis was performed using the IBM SPSS Satistics 20.0 and Statistica 11.0 programs.

Table 1. Main characteristics of the six anti-dsDNA antibody assays

Assay	Manufacturer	Technology	Isotype Detection	dsDNA Source	Cut-Off
ELISA1	Inova Diagnostics	ELISA / manual	IgG	Calf thymus	99 WHO U/mL
ELISA2	Orgentec	ELISA / manual	IgG	Recombinant	19 IU/mL
ELISA3	Inova Diagnostics	ELISA / manual	IgG	Calf thymus	30 IU/mL
CLIFT1	Inova Diagnostics slides	IIF / manual	IgG	*Crithidia luciliae*	1:10
CLIFT2	In house	IIF / manual	IgG, IgA, IgM	*Crithidia luciliae*	1:10
FARR	In house	RIA / manual	IgG, IgA, IgM	^{125}I-plasmid dsDNA	5 IU/mL

Abbreviations: ELISA: enzyme-linked immunosorbent assay, CLIFT: Crithidia luciliae fluorescnece test, IIF: indirect immunofluorescence assay, RIA: radioimmunoassay

Results

In the selected group, out of the six methods the FARR, CLIFT2 and CLIFT1 had the highest specificity (93%, 91%, 82%, but lowest sensitivity for SLE (30%, 32.22%, 40%). ELISA2 had a lower specificity (39%), but still high sensitivity (93.33%) (Table 2.). The highest agreement measured by the Cohen's Kappa was seen between CLIFT1 and CLIFT2 (0.558), Crithidia tests and Farr (0.531 and 0.489) and ELISA2 and ELISA3 (0.472), all considered as moderate agreement. There was a fair agreement between ELISA1 and ELISA 3 (0.338), while comparison of ELISA's with FARR or Crithidia's result in slight agreement, with one single exception (ELISA2 vs. CLIFT2) (Table 3.). It should be noted that all Farr positive samples (n=23) were also positive in ELISA's. The levels of anti-dsDNA antibodies detected with ELISA2 and ELISA3 correlated significantly (Spearman rank correlation coefficient Rho: 0.755; $p<0.05$). All other quantitative methods had a lower correlation coefficient (Figure 1.). The percentage of positive results detected with both Crithidia's and FARR assay are considerably lower in other groups than SLE (especially other CTD and Inflammatory Diseases) (Figure 2.).

Table 2. Diagnostic sensitivity and specificity for SLE obtained with five different anti-dsDNA antibody assays in 190 sera of Hungarian patients with systemic autoimmune diseases

Assay	Diagnostic Sensitivity %	Diagnostic Specificity %	PPV %	NPV %
ELISA1	*	*	*	*
ELISA2	93.33	39.00	57.93	86.67
ELISA3	78.89	45.00	56.35	70.31
CLIFT1	32.22	91.00	76.32	59.87
CLIFT2	40	82.00	66.67	60.29
FARR	30	93.00	79.41	59.62

** Not stated (only positive samples)*

Table 3. Correlation of six assays for detection of anti-dsDNA antibodies in 210 sera of Hungarian patients with systemic autoimmune diseases and controls

Kappa*	ELISA1	ELISA2	ELISA3	FARR	CLIFT2
CLIFT1	0.045	0.173	0.172	0.531	0.558
CLIFT2	0.070	0.226	0.167	0.489	
FARR	0.040	0.153	0.195		
ELISA3	0.273	0.472			
ELISA2	0.338				

** Kappa: Cohen's kappa coefficient*

Figure 1. Correlation of the levels of anti-dsDNA antibodies detected with ELISA methods in 210 sera of Hungarian patients with systemic autoimmune diseases and controls

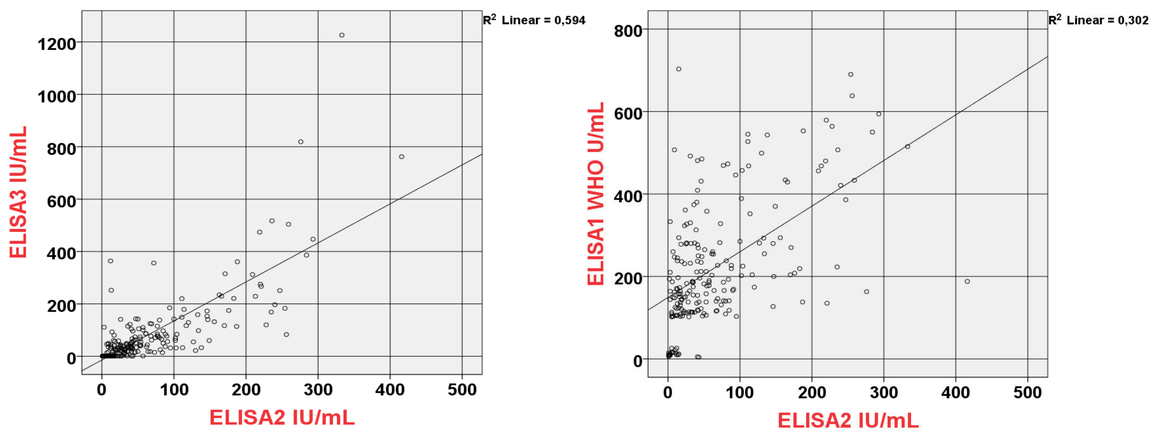
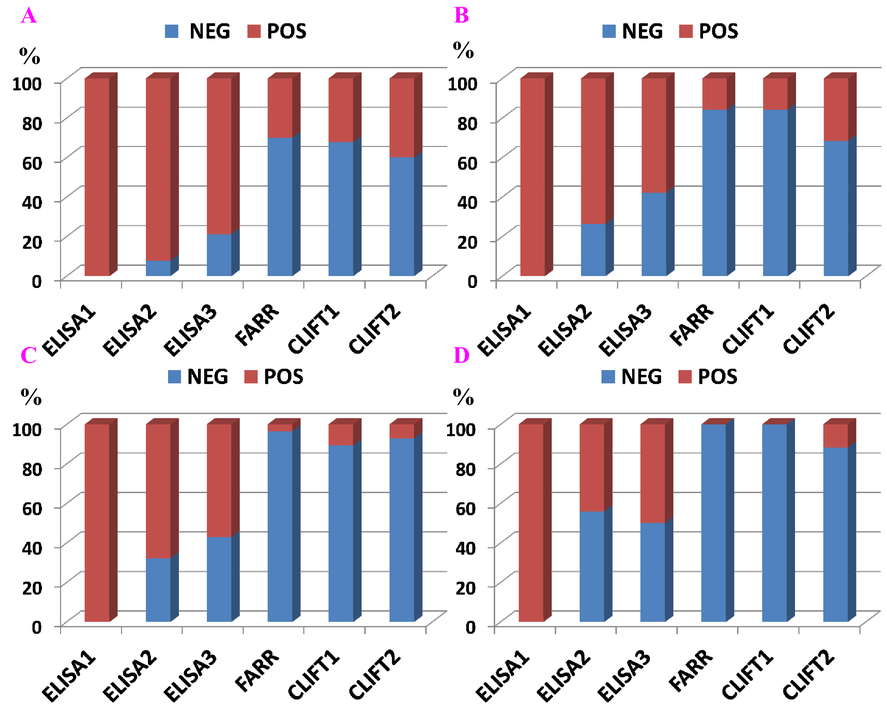


Figure 2. Percentage of negative and positive results with six anti-dsDNA antibody assays in different patient groups. A: SLE, B: RA, C: Other CTD, D: Inflammatory Diseases



Conclusions

Our results clearly show that for the diagnosis of SLE a screening of anti-dsDNA antibody solely with ELISA assay is insufficient. For the results proved to be positive by ELISA, even with a high-avidity one, a second confirmatory step is necessary to differentiate SLE patients form other patient groups.

For this reason, a two-step algorithm is suggested for the detection of anti-dsDNA antibodies: after screening samples with an ELISA test as the primary method (preferably ELISA2), the presently available CLIFT1, which performed as well as the gold standard Farr assay, should be used to confirm positive results obtained in step one.