REVISTA BRASILEIRA DE REUMATOLOGIA

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SOCIEDADE BRASILEIRA DE REUMATOLOGIA

Original article

Usefulness of anti-dsDNA antibody screening with chemiluminescence followed by confirmation by indirect immunofluorescence

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ARTICLE INFO

Article history:
Received 28 May 2012
Accepted 23 April 2013

Keywords:
Anti-dsDNA
Autoimmune diseases
Systemic lupus erythematosus
Chemiluminescence
Indirect immunofluorescence

ABSTRACT

Objective: The purpose of this study was to evaluate the performance of a chemiluminescent immunoassay (CLIA) to detect anti-dsDNA antibodies, using the indirect immunofluorescence test (IIF) on Crithidia luciliae as a reference.
Methods: The automation system demonstrated 81% efficiency, 100% sensitivity and 82% specificity according to the intrinsic validation process performed using 179 consecutive samples from 169 patients in the beginning of 2011. These patients were subsequently divided into 3 groups according to the co-reactivity of anti-dsDNA results using the 2 methods (reactive, non-reactive and discrepant results).
Results: Upon data analysis, 77% (129/169) of the tests were requested by rheumatologists, and 57% (97/169) of the samples were from lupus patients. Both the reactive and non-reactive results of the CLIA were well defined and standardised, and automation reduced the manual labor required by 70% in a safe and high-quality manner. Furthermore, the high prevalence of patients with lupus and nephritis among the CLIA false-positive results corroborates the hypothesis that the actual index of CLIA false positivity is lower than that initially found in this study.

Study conducted at the Unit of Rheumatology and Clinical Pathology of Hospital Geral de Fortaleza, Fortaleza, CE, Brazil.

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0482-5004/$ - see front matter.

Utilidade da triagem dos anticorpos anti-dsDNA por quimioluminescência, seguida de confirmação por imunofluorescência indireta

Palavras-chave:
Anti-dsDNA
Doenças autoimunes
Lúpus eritematoso sistêmico
Quimioluminescência
Imunofluorescência indireta

RESUMO

Objetivo: Avaliar o desempenho de um imunoensaio quimioluminescente (CLIA) para os anticorpos anti-dsDNA, utilizando como referência o teste de imunofluorescência indireta (IFI) sobre *Crithidia luciliae*.

Métodos: O sistema de automação foi previamente aprovado com 81% de eficiência, 100% de sensibilidade e 82% de especificidade, por processo de validação intrínseca em 179 amostras consecutivas de 169 pacientes no início de 2011. A seguir, esses pacientes foram subdivididos em três grupos de acordo com os resultados da pesquisa dos anticorpos anti-dsDNA nas duas metodologias (reagentes, não reagentes e resultados discrepantes).

Resultados: Na análise dos dados: 1) 77% (129/169) dos exames haviam sido solicitados por médicos reumatologistas; 2) 57% (97/169) das amostras eram de pacientes lúpicos; 3) Os resultados de CLIA, reagentes e não reagentes, estavam bem definidos e padronizados; 4) A automação reduziu em 70% as passagens pela técnica manual com segurança e qualidade; 5) A alta prevalência de pacientes lúpicos e com nefrite entre os resultados de CLIA falso-positivos corrobora a hipótese de que o índice real de falsa positividade do CLIA seja menor que o encontrado inicialmente neste estudo.

Introduction

The study of anti-double-stranded DNA (anti-dsDNA) autoantibodies is useful for the diagnosis and management of systemic lupus erythematosus (SLE),[1] especially in patients with lupus nephritis.[2] Automated assays have been introduced as a more rapid alternative for anti-dsDNA antibody screening in the major laboratories.[3] Although radioimmunoassay tests are recognised as a more specific method, such tests are less commonly used because they require the use of radioactive material.[4] Automated assays process large volumes of clinical samples quickly and at lower cost than traditional methods.[5,6]

The implementation of serological testing in a clinical pathology laboratory requires an intrinsic validation process to evaluate test performance by comparison to a reference method, according to sensitivity, specificity and efficiency parameters. This validation process evaluates features of the new test rather than those of the population to which it is being applied, which enables the collection of consistent results that are independent of disease prevalence.[7] These validation methods may be approved for replacing techniques (change of reactive supplier), improving quality (an addition to the technique in use) and/or reducing laboratory operating costs. This type of analysis does not require approval of an ethics committee because the origin of the biological sample should not be disclosed.

From 2002 to 2006, the prevalence of positive antinuclear antibody (ANA)-Hep-2 test results at the Hospital Geral de Fortaleza (HGF) was studied. Among the 6,000 samples analysed, negative results were obtained in 84% of cases,[8] which justified the performance of autoimmune screening tests using an automated method to reduce the test time and chance of human error resulting from the interface with the equipment.

The objective of this study was to analyse the performance of a chemiluminescent immunoassay (CLIA) for the detection of anti-dsDNA antibodies, using the indirect immunofluorescence assay (IFA) on *Crithidia luciliae* as a reference. Upon approval of an internal protocol of the Clinical Pathology Laboratory of HGF for the intrinsic validation of an automation system for screening ANA and anti-dsDNA antibodies, this project was developed to analyse medical records of the clinical samples studied. This study was approved by the Research Ethics Committee of HGF under protocol number 060705/11; all authors signed the trustee statement and declared no conflicts of interest.

Materials and methods

Sample

From February to March 2011, serum samples sent to the HGF laboratory for the detection of anti-dsDNA antibodies were examined in the immunofluorescence unit and subsequently forwarded to the automation unit. The tests were carried out independently by the two technical teams. The IFA results were released in a timely manner without detriment to the patients. The intrinsic validation of the automation system was approved with 81% efficiency (results in agreement with IFA), 100% sensitivity and 82% specificity. Demographic (age and gender), epidemiological and clinical (reason for the request, diagnoses, duration of symptoms and laboratory results) data for patients (n = 169) included in this study were obtained from the laboratory database and medical records after approval from the ethics committee.

Anti-dsDNA antibody tests were requested in subsequent consultations for patients with discrepant results between the two methods during the CLIA validation period and were monitored for one year (March/11 to March/12), without involvement of the study authors.

Laboratory analysis

Chemiluminescence assay (CLIA): LIAISON_dsDNA (DiaSorin, Saluggia, Italy) is a CLIA that uses magnetic particles coated with a synthetic dsDNA oligonucleotide, which ensures the absence of contamination with histones and other nuclear proteins. A monoclonal antibody labelled with an isoluminol derivative is used as a conjugated antibody to detect IgG anti-dsDNA antibodies.[5] All test procedures were performed automatically in a primary sample using the LIAISON® system. The reactivity pattern was defined by the manufacturer as non-reactive (< 20 IU/mL), in the grey zone (20-25 IU/mL) or reactive (> 25 IU/mL).

Indirect immunofluorescence assay (IFA): These tests were performed using the commercially available method (Euroimmun, Lubeck, Germany) according to the manufacturer's technical recommendations. The sera were 1/10 diluted in phosphate-buffered saline solution and incubated on glass slides with the antigen substrate (*Crithidia luciliae*), where the anti-dsDNA antibodies present bind to the kinetoplast and are revealed by a specific fluorescein isothiocyanate-labelled anti-gamma-globulin. Internal positive and negative controls were conducted in each test routine. Cell staining was examined using a fluorescence microscope (model Nikon YS2H) under 400x magnification. Sera with positive results in the 1/10 screening were expressed in semiquantitative titres.

Statistical analysis

The data were collected in a Microsoft Excel® spreadsheet. Sensitivity, specificity and efficiency tests were carried out for validation of the serological test using the analysis of anti-dsDNA antibodies by IFA as a reference test.

Results

The intrinsic evaluation was performed with 179 serum samples, which were analysed using both techniques. The CLIA was positive in 41 (23%) serum samples, negative in 132 (74%) and indeterminate (grey area) in six samples (3%). The six indeterminate sera samples were grouped with the positive samples for comparing sensitivity, specificity and efficiency of the method compared to IFA. The comparison between the two methods revealed that 15 samples (8.4%) were positive in both techniques, 132 (73.7%) were double negative, 32 (17.9%) were false positive in CLIA, and none were false negative in CLIA, revealing a sensitivity of 100%, specificity of 82% and an efficiency of 81% for CLIA. After this analysis, the laboratory implemented screening of anti-dsDNA antibodies by automation, in which positive results were re-evaluated by IFA for confirmation. In this new screening process, the manual phase was reduced by 74% (132/179) of the previous total test-bench effort, limiting the need for manual testing in each of the 4 tests previously performed using the IFA method.

The intrinsic evaluation (179 serum samples) comparing the CLIA and IFA methods involved 169 patients with eight duplicate sera and one triplicate sample. The CLIA results of multiple samples were negative in seven patients and discrepant in one patient (32 and 13.8 IU/mL), with double serum samples positive in the patient with three anti-dsDNA antibody test requests over a 2-month period (154.6, 46 and 37.5 IU/mL). All these sera samples were negative using the IFA method. An analysis of these results will be presented later.

The epidemiological and demographic characteristics of this patient sample are shown in Table 1. Patients were classified according to the diagnoses in their medical records. One third of the sample (55 patients) comprised patients under diagnostic investigation due to clinical suspicion of SLE, where the test requests were made due to the presence

Table 1 – Epidemiological characteristics of the sample (n = 169).

Clinical sample characteristics	n (%)
Gender	
Male	16 (9)
Female	153 (91)
Age range (years)	(n = 166)[a]
Children (< 11)	3 (2%)
Adolescents (12 to 19)	20 (12%)
Adults	
20-29	54 (33%)
30-39	43 (26%)
40-49	28 (17%)
50-59	12 (7%)
60-69	6 (4%)
Clinic requesting test	
Rheumatology	123 (73%)
Medical clinic	19 (11%)
Nephrology	7 (4%)
Gynaecology/obstetrics	7 (4%)
Paediatric rheumatology	6 (4%)
Other clinic[b]	7 (4%)
Patient diagnosis	
SLE	92(54%)
SLE overlap syndrome	5 (3%)
Investigation of autoimmune disease[c]	55 (33%)
Other autoimmune diseases	17 (10%)
Primary APLS	4
Rheumatoid arthritis	3
Vasculitis	2
Devic's disease	2
Mixed connective tissue disease	1
Sjögren's syndrome	1
Ankylosing spondylitis	1
Linear systemic sclerosis	1
Autoimmune thyroiditis	1
Multiple sclerosis	1

SLE, systemic lupus erythematosus; APLS, antiphospholipid antibody syndrome.
[a]Three patients did not have records and their ages were not mentioned in the laboratory records.
[b]Emergency, endocrinology, neurology and ICU.
[c]The patients without medical records (n = 3) were included in this group.

of several signs or symptoms (e.g., arthralgia, arthritis, kidney failure, haemolytic anaemia, purpura, Raynaud's disease and paresthesias) related to or present in SLE.

Patients were divided into three groups as defined in Table 2, according to the results obtained in the anti-dsDNA antibody testing using the two methodologies. The sera from Group I belonged to 15 lupus patients. Of these, 12 patients had a previous diagnosis of lupus nephritis, one demonstrated serositis, and another had suffered from SLE for seven months, presenting with evidence of positive inflammatory activity, lymphopenia and consumption of the complement components C3 and C4. The last patient, who had juvenile rheumatoid arthritis (JRA) and had been in treatment for two years, tested positive for ANA and anti-dsDNA antibodies in this laboratory revaluation nine months before meeting the criteria for a diagnosis of SLE. The results of the screening for anti-dsDNA antibodies using the CLIA technique in the 15 sera samples from Group I remained in the 240 IU/mL to 32.6 IU/mL range with a mean of 167 IU/mL, median of 198 IU/mL and mode of 240 IU/mL, and the IFA titres ranged from 1/640 to 1/20. Eleven sera samples demonstrated readings greater than 125 IU/mL in the CLIA and IFA titres of 1/320 or 1/640. The values for the four remaining sera samples (63, 39, 36 and 32.6 IU/mL) showed titres of 1/320, 1/20 1/160 and 1/320, respectively.

The relevant information recorded in the medical records of each patient in Group II (clinical condition and laboratory changes, with reasons for requesting the anti-dsDNA tests) is listed in Table 3. These data showed that 87% (26/30) of the cases labelled as false-positive in CLIA had SLE, and 65% (17/26) of these patients had a previous diagnosis of lupus nephritis, with a description of signs and/or symptoms of clinical progression of disease in 50% (13) of cases. In addition, isolated laboratory changes compatible with active disease were present in 23% (6/26) of patients (cases 5, 8, 11, 19, 25 and 29).

The results of the anti-dsDNA antibody testing using CLIA in Group II (Table 4) was in the range of 184-20 IU/mL, with a mean of 59 IU/mL and median 45 IU/mL. Four sera samples (cases 1 to 4) were positive with values 5 times greater than the cut-off point indicated by the manufacturer. Sera classified in the 'grey zone' accounted for 3.5% (6/169) of the sample studied.

The clinical condition of each patient in Group II (Table 4) was paired with the historic presence of autoantibodies and the progression of detection of anti-dsDNA antibodies in sera since disease onset. The ANA results were available and positive in 97% (29/30) of patients. Anti-Sm antibodies were detected in 38% (10/26) of SLE patients in this group. A previous history of reactivity to dsDNA (anti-dsDNA by IFA) occurred in 50% (13/26) of lupus patients; however, this information was not available for two patients from other units (cases 25 and 26), and four patients under diagnostic investigation were undergoing tests for the first time (cases 1, 4, 12 and 21).

Further evaluations of anti-dsDNA antibodies were requested in 77% (23/30) of patients within one year. Among the seven remaining patients, three did not have SLE (cases 1, 12 and 21), three had lupus in clinical and laboratory remission (3, 10 and 18) and case number 5, with clinical remission, had haematuria at the time of intrinsic validation.

Two patients (cases 2 and 17) were studied using multiple sera samples. Case 2, which demonstrated a triple positive evaluation in CLIA (154.6, 46 and 37.5 IU/mL) had been diagnosed with SLE and lupus nephritis for four years. This patient showed a positive result for anti-dsDNA antibodies by enzyme-linked immunosorbent assay (ELISA) (80 U) at the onset of the disease (2007), which was not confirmed by IFA in three tests conducted in the 2009 to 2010 period, although the IFA test became positive after this patient experienced convulsive symptoms for one month. Case 17, which demonstrated discrepant results in the CLIA (32 and 13.8 IU/mL), had been diagnosed with SLE and lupus nephritis 2 months prior, although anti-dsDNA results using both CLIA and IFA for this patient remained negative after six months.

Ten patients became reactive by IFA within one year of their first evaluation (cases 2, 9, 23 and 26 up to 3 months; case 29 after five months; cases 14 and 15 after 9 months and cases 7, 20 and 22 after an interval of 12 months).

The remainder of the sample (Group III) consisted of 58% (56/97) of the total number of patients with SLE, 95% (52/55) of the patients being tested for autoimmune diseases and the majority (94%, 16/17) of patients affected by other autoimmune diseases. There were seven duplicate samples in the intrinsic evaluation of this group that belonged to five patients with SLE, 1 under diagnostic investigation and one with rheumatoid disease. The CLIA results in Group III showed values in the range of 19 to 0.5 IU/mL (Fig. 1), with a mean of 5.5 IU/mL, median of 4 IU/mL and mode of 0.5 IU/mL.

A prior history of reactivity to dsDNA using IFA in all SLE patients who participated in the study (n = 97) was investigated using the medical and/or laboratory records (Table 5), and we found that 48% (47/97) of the samples were reactive, with five patients in Group I (positive in both methodologies) undergoing anti-dsDNA testing for the first time. Clinical activity measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was not available in all records during the test request period, which prevented the study of clinical progression (periods of disease activity or remission) related to the presence of anti-dsDNA antibodies.

Table 2 – Definition of strata for analysis (n = 169).

Group	Definition	Stratum	n	Total
I	Samples positive in both techniques (CLIA and IFA)	SLE	15	15
II	Samples positive in CLIA and negative in IFA (false positives)	SLE	24	30
		SLE overlap syndrome	2	
		Autoimmune thyroiditis	1	
		Investigation of autoimmune diseases	3	
III	Samples negative in both techniques (CLIA and IFA)	SLE	53	124
		SLE overlap syndrome	3	
		Other autoimmune pathologies	16	
		Investigation of autoimmune diseases	52	

CLIA, chemiluminescent immunoassay; IFA, indirect immunofluorescence assay; SLE, systemic lupus erythematosus.

Table 3 – Relevant information for Group II patients (n = 30)

Pat.	G	Age	Reasons for requesting anti-dsDNA test	Time	Current condition	
					Clinical	Laboratory changes
1	F	35	Undefined arthralgia	1 y	under investigation	ANA-reactive
2	F	25	SLE + nephritis	4 y	activity (convulsion)	lymphopenia, ↓ C ', proteinuria
3	F	60	SLE	15 y	remission	no change
4	F	34	Additive polyarthritis	2 y	under investigation	ANA-reactive
5	F	33	SLE + nephritis	11 y	remission	haematuria ++
6	F	21	SLE + nephritis	7 m	activity	↓C', haematuria, proteinuria
7	F	24	Mucocutaneous SLE	2 m	remission	no change
8	F	57	SLE	22 y	remission	lymphopenia
9	F	28	SLE + nephritis	5 y	activity	anaemia, ↓C', proteinuria
10	F	18	SLE + nephritis	2 y	remission[a]	no change
11	F	49	SLE	5 y	remission	CRP+, ↓C3
12	F	49	Autoimmune thyroiditis	3 y	arthralgia	haematuria +
13	F	33	SLE + nephritis	8 y	activity	lymphopenia, ↓ C ', haematuria
14	F	22	SLE	10 m	remission	no change
15	M	13	SLE	2 m	activity	↓C'
16	F	43	SLE + nephritis	5 y	activity	lymphopenia, haematuria, proteinuria
17	F	34	SLE + nephritis	2 m	activity	lymphopenia, ↓C', ESR and CRP, haematuria, proteinuria
18	F	27	SLE + DM/DP	4 y	remission	CRP
19	F	14	Mucocutaneous SLE	5 m	remission	↓C'
20	F	27	SLE + nephritis	6 y	activity	↓C', proteinuria
21	F	49	Undefined kidney failure	1 m	under investigation	proteinuria
22	F	22	SLE + nephritis	4 y	activity[a]	haematuria, proteinuria
23	F	23	SLE + nephritis	7 y	activity[a]	proteinuria
24	F	28	SLE + nephritis	3 y	activity[a]	haematuria, proteinuria
25	F	19	SLE + nephritis	5 y	remission	lymphopenia
26	M	34	SLE + nephritis	3 y	activitya, haemodialysis	↓C', haematuria, proteinuria, creatinine
27	F	46	SLE + nephritis	5 y	activity[a]	leukocyturia
28	F	29	SLE + nephritis	18 m	activity, haemodialysis[b]	↓C3, proteinuria, leukocyturia
29	F	61	SLE + SS	10 y	remission	lymphopenia, ↓C',
30	F	22	SLE + nephritis	3 y	evaluation after pregnancy	no change

Pat, patient; G, gender; F, female; y, year; ANA, antinuclear autoantibody; SLE, Systemic lupus erythematosus; C', complement; m, month; CRP, C-reactive protein; M, male; ESR, erythrocyte sedimentation rate; DM/DP, dermatomyositis/dermatopolymyositis; SS, systemic sclerosis
[a]In the presence of pulse therapy with methylprednisolone and cyclophosphamide.
[b]Use of rituximab in previous year.

Discussion

Currently, the most commonly used techniques for detecting anti-dsDNA antibodies are immunoenzymatic assay and IFA, the latter being more specific and capable of detecting antibodies with moderate and high affinity related to SLE activity.[9] ELISA-based methods, although quantitative, reproducible and automated, exhibit lower precision in terms of clinical performance because they detect low-avidity anti-dsDNA autoantibodies, which generally have little clinical relevance and may be present in other connective tissue diseases, inflammatory or infectious diseases and in normal subjects.[10] However, in recent years, a new generation of ELISAs for the detection of anti-dsDNA antibodies has been introduced into the market, and these new reagents provide greater antigen purification, making them more selective for the detection of intermediate- and high-avidity antibodies.[5] The CLIA method evaluated in this study is included in this group.

The performance of the CLIA-LIAISON assay in this intrinsic evaluation was satisfactory and produced 100% sensitivity, 82% specificity and 81% efficiency when compared to IFA. This same CLIA reagent has been tested by the Italian Society of Laboratory Medicine Study Group on Autoimmune Diseases[5] in an extrinsic evauation[7] with a clinical samples from 52 patients with SLE, 28 patients with other connective tissue diseases, 36 patients with hepatitis C virus (HCV) and 24 patients with other acute viral diseases. These authors reported 84.6% sensitivity, 82.9% specificity and 83.6% efficiency of the method, which is similar to the results obtained in the present study, although the difference in sensitivity may be attributed to the clinical samples examined. This study also analysed the performance of the automated test for the detection of anti-dsDNA antibodies, according to the reality experienced by the local population, where the majority (57%) of patients who undergo this exam have SLE. The Italian study also included patients with HCV who eventually had positive CLIA testing for anti-dsDNA antibodies.[5]

The performance of the CLIA test in identifying a negative reaction was adequate in this study, with measures of central tendency in Group III convergent with values less than three times the maximum negativity suggested by the manufac-

Table 4 – Presence of autoantibodies in the serum of Group II patients (n = 30).

Pat.	Current clinical condition	Previous history of other autoantibodies	Anti-dsDNA history				
			Previous	Current	Later evaluations		
			(IFA)	CLIA (IU/mL)	CLIA (IU/mL)	IFA (titre)	(interval)
1	Investigation	ANA	tnc	183.5	tnc	tnc	-
2	Activity	ANA	NR	154.6	49.0	1:80	1 m
3	Remission	ANA	R	134.1	tnc	tnc	-
4	Investigation	ANA, Cardio G and M	tnc	125.5	tnc	NR	5 m
5	Remission	ANA, Sm, Cardio G and M	R	111.1	tnc	tnc	-
6	Activity	ANA, Sm, RNP	NR	92.5	199.9	NR	10 m
7	Remission	ANA	NR	92.1	54.1	1:80	12 m
8	Remission	ANA	R	85.6	19.0	tnc	5 m
9	Activity	ANA, Ro, La	NR	84.6	189.3	1:320	3 m
10	Remissiona	ANA	R	68.6	tnc	tnc	-
11	Remission	ANA, Sm	R	51.5	96 e 15.2	NR	2 e 6 m
12	Investigation	ANA, Ro	tnc	50.9	tnc	tnc	-
13	Activity	ANA, Sm	NR	49.5	38. 2 e 52	NR	3 e 12 m
14	Remission	ANA	NR	45.2	45.1	1:160	9 m
15	Activity	ANA, Sm, Ro	NR	44.9	26.7	1:80	9 m
16	Activity	ANA, Ro, La	R	36.1	4.75	tnc	12 m
17	Activity	ANA	NR	32.0	12.5	NR	6 m
18	Remission	ANA	R	31.4	tnc	tnc	-
19	Remission	ANA, Sm, Cardio G	tnc	31.1	28.4	NR	3 m
20	Activity	ANA, Sm, RNP	R	30.2	45.7	1:80	12 m
21	Investigation	tnc	tnc	28.7	tnc	tnc	-
22	Activity[a]	ANA	R	26.6	33.6	1:40	12 m
23	Activity[a]	ANA	R	26.5	34.4	1:160	1 m
24	Activity[a]	ANA, Sm, RNP, anti-p	NR	25.4	tnc	NR	2 m
25	Remission	un	un	22.7	22.2	NR	10 m
26	Activity[a]	ANA, Ro, Cardio G	un	22.1	56.3	1:40	2 m
27	Activity[a]	ANA, RNP	R	22.0	45.6	NR	2 m
28	Activity	ANA, Sm, Cardio G	NR	20.9	8.33	tnc	10 m
29	Remission	ANA, Sm, RNP	R	20.6	32.5	1:40	5 m
30	Remission	ANA	R	20.3	18.3	tnc	11 m

Pat, patient; IFA, indirect immunofluorescence assay; CLIA, chemiluminescent immunoassay; ANA, antinuclear autoantibody; tnc, test not conducted; NR, non-reactive; m, month; R, reactive; Cardio G, anticardiolipin G; Cardio M, anticardiolipin M; Sm, anti-Sm; RNP, anti-RNP; Ro, anti-SSA(Ro); La, anti-SSB(La); un, evaluation unknown; anti-p, anti-ribossomal p.
[a]In the presence of pulse therapy with methylprednisolone and cyclophosphamide, anti-ribosomal p.

turer (up to 19 IU/mL). In addition, the low frequency (3.5%) of grey zone results enabled a clear definition of the positivity of the method. The identification of patients who constituted Group III demonstrated the specificity for anti-dsDNA antibodies used in the test; of the 45 CLIA-reactive sera, 91% (41/45) were from SLE patients.

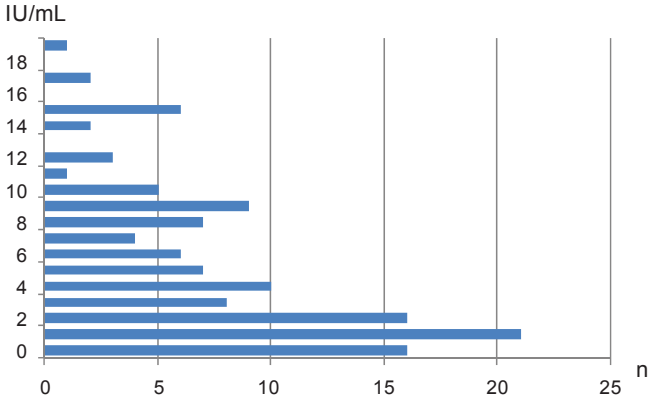

Fig. 1 – Frequency of CLIA results (IU/mL) in Group III (n = 124).

The availability of the clinical samples also facilitated qualitative analysis of the type of patient who receives anti-dsDNA testing in the hospital, and these results revealed a clinical and epidemiological profile similar to that found in the pathology of lupus where this autoantibody is prevalent.[11] The vast majority of patients were female and in the young adult age range (20-39 years), with the Rheumatology Unit accounting for over 70% of the test requests. The low prevalence of children and adolescents may be explained by the hospital's focus on the tertiary care of adults.

Table 5 – Previous history of anti-dsDNA (IFA) in lupus patients (n = 97).

Group	Reactive	Non-reactive	1st time	UN	Total
I	10	0	5	0	15
II	13	10	1	2	26
III	19	32	5	0	56
Total	42	42	11	2	97

un, evaluation unknown.

After the establishment of automated screening for anti-dsDNA antibodies, positive samples and those with results in the grey zone in CLIA were tested by IFA, using *Crithidia luciliae* as a substrate. The implementation of this routine led to the optimisation of time and laboratory personnel,[6] reducing the requirement for manual procedures by more than 70% and also reducing the likelihood of procedural and random errors that could compromise the quality and accuracy of the released tests. The potential for cost reduction with this new detection approach will be analysed in a subsequent study.

International trials recommend the use of automated reagents for the detection of anti-dsDNA antibodies,[3,12-15] although the gold standard method in clinical and laboratory research remains IFA.[12,16] Because the array of laboratory methods for the detection of anti-dsDNA antibodies is continuously increasing, tests traditionally used in routine work are still far from becoming standardised and widely accepted. Moreover, physicians should be aware that the agreement rates between laboratories, the interpretation of results and the diagnostic accuracy are dependent on the analytical variability and the population of patients being studied.[17] In the present study, the technical laboratory conditions and the referral of patients' serum samples were maintained within the normal working routine of the institution.

This study demonstrated that screening of anti-dsDNA autoantibodies using CLIA is a safe (100% sensitivity) and rapid method that could improve the quality of tests available to patients. Among the study findings, it should be noted that most of the CLIA results labelled as false positives belonged to lupus patients with clinical and/or laboratory disease activity, some of whom were confirmed months later as positive by IFA.

Conflicts of interest

The authors declare no conflicts of interest.

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