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Review

Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis

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ABSTRACT

Background: Methods to detect anti-nucleosome antibodies (ANuA) have been available for more than 10 years and the test has demonstrated its good sensitivity and high specificity in diagnosing systemic lupus erythematosus (SLE). Despite these data produced through clinical and laboratory research, the test is little used.

Objective: To verify the diagnostic performance of methods for measuring ANuA and to compare them with those for anti-dsDNA antibodies.

Data sources: A systematic review of English and non-English articles using MEDLINE and EMBASE with the search terms “nucleosome”, “chromatin”, “anti-nucleosome antibodies” and “anti-chromatin antibodies”. Additional studies were identified checking reference lists in the selected articles.

Study selection: We selected studies reporting on anti-nucleosome tests performed by quantitative immunoassays, on patients with SLE as the index disease (sensitivity) and a control group (specificity).

A total of 610 titles were initially identified with the search strategy described. 548 publications were subsequently excluded based on abstract and title. Full-text review was undertaken as the next step on 62 publications providing data on anti-nucleosome testing; 25 articles were then excluded because they did not include either SLE patients or a control group, and 37 articles were selected for the metanalysis. Finally, a sub-metanalysis study was conducted on the 26 articles providing data on both ANuA and anti-dsDNA antibody assays in the same series of patients.

Data extraction: Extraction of data from selected articles was performed by two authors independently, using predefined criteria: the number of patients with SLE as the index case, and the number of healthy or diseased controls; specification of the analytical method used to detect anti-nucleosome and anti-dsDNA antibodies; the cut-off used in the study; and the sensitivity and specificity of the assay. Demographic and clinical data on the population investigated (adults or children; lupus patients with or without nephritis; patients with active or inactive disease) were also recorded and analyzed in a separate evaluation.

Results: The systematic review and metanalysis showed that the overall sensitivity of the ANuA assay is 61% (confidence interval-CI, 60–62) and the specificity 94% (CI, 94–95). The overall positive likelihood ratio is 13.81 (CI, 9.05–21.09) and the negative likelihood ratio 0.38 (CI, 0.33–0.44). The odds ratio for having SLE in ANuA-positive patients is 40.7.

The comparative analysis on anti-dsDNA antibodies conducted on the 26 studies which provided data for both antibodies showed that ANuA have greater diagnostic sensitivity (59.9% vs 52.4%) and a specificity rating only slightly higher (94.9% vs 94.2%). The probability that a subject with positive ANuA have SLE is 41 times greater than a subject with negative ANuA, while for anti-dsDNA the probability is 28 times greater.

These figures are even more impressive in children, in whom ANuA have an odds ratio for the diagnosis of SLE of 146, compared to 51 for anti-dsDNA antibodies.

In selected studies, ANuA ($p < 0.0001$) but not anti-dsDNA antibodies ($p = 0.256$) were significantly associated with disease activity measured by the international score systems. However, neither antibody appears to correlate with kidney involvement.

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Conclusions: Data from the metanalysis have shown that ANuA have equal specificity but higher sensitivity and prognostic value than anti-dsDNA antibodies in the diagnosis of SLE. Despite a certain heterogeneity among the various studies, the use of ANuA appears more efficacious than anti-dsDNA.

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1. Introduction

The nucleosome represents the basic element of chromatin and is the fundamental package unit of double stranded (ds)DNA, which is composed of approximately 200 base pairs of DNA wrapped twice around the (H2A–H2B–H3–H4)₂ histone octamer, with histone H1 bound on the outside, similar to a finger on a knot to tighten the complex (reviewed in [1]). Anti-nucleosome antibodies (ANuA) are a large family of autoantibodies directed against histone epitopes exposed in chromatin, against dsDNA and against conformational epitopes created by the interaction between dsDNA and core histones (reviewed in ref. [2]).

ANuA have been recently shown to be a good diagnostic marker for systemic lupus erythematosus (SLE) and, indeed, they represent the first serological marker described in association with this disease. In fact, it has been demonstrated that the LE phenomenon (described in 1947 by Hargraves [3]) was caused by the opsonization of nuclear material by an ‘LE cell factor’, which was later demonstrated to be an autoantibody directed against nucleosomes.

The presence of autoantibodies able to bind to the intact nucleosome, but not its individual components (dsDNA and histones), was described in the late 1980s. Later, it was demonstrated that these autoantibodies may precede the appearance of other nuclear antibodies in SLE and that the nucleosome is both the driving immunogen and the target of lupus anti-dsDNA, anti-histone and anti-nucleosome specific antibodies.

There is abundant evidence that nucleosome antibodies play an important part in the pathogenesis of SLE and especially in the development of nephritis, a major complication of the disease. The major evidence supporting this role are:

- nucleosome-specific T cells stimulate B cells to produce nucleosome-specific, dsDNA and histone autoantibodies through epitope-spreading. Nucleosome-specific autoantibodies are detected in murine lupus models before the onset of other autoantibody specificities [1];

- in SLE patients and murine lupus the apoptosis is abnormal, chromatin components appear at the surface of apoptotic cells, the removal of apoptotic debris is defective and the release of apoptosis-modified nucleosomes in the circulation is massive, inducing the recognition by the immune system (T and B cells) and the production of autoantibodies;

- nucleosomes play a pivotal role in the development of kidney lesions by mediating binding of autoantibodies to basal membranes [4,5].

The definition of the role of ANuA reactivity in the pathogenesis of SLE and of their nephritogenic potential confirmed the importance of this autoantibody specificity, and the first commercial monoplex immunoenzymatic assay method (ELISA) for the measurement of these autoantibodies appeared on the market in the late 1990s.

Anti-dsDNA antibodies, first detected and measured 55 years ago, are the best studied of all autoantibodies found in SLE and are still ubiquitously used to help diagnose and manage this disease, because this autoantibody correlate with disease activity and may be involved in the pathogenesis of the disease [6]. Although it has been suggested that ANuA are as good as or better than anti-dsDNA antibodies in diagnosing lupus, tests for ANuA are rarely ordered by physicians.

In conducting this systematic review, we therefore aimed at answering three questions: (1) is the ANuA test characterized by an adequate level of diagnostic accuracy for diagnosing SLE? (2) In case of ANuA positivity, does the test allow us to exclude the presence of other connective tissue diseases? (3) Is the ANuA test comparable, for use in diagnostics, to the test for anti-dsDNA antibodies?

2. Methods

A systematic review and metanalysis were performed according to the framework outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to increase standardization and quality in reporting [7]. A protocol with predefined inclusion

and exclusion criteria was prepared before starting the study selection.

2.1. Data sources and study selection

We reviewed the most relevant papers published from 1990 to 2011, using the electronic database MEDLINE and EMBASE with the search terms: “nucleosome”, “chromatin”, “anti-nucleosome antibodies” and “anti-chromatin antibodies”. There was no language restriction. The last search was run on November 20th, 2011.

From the initial search we identified 603 potentially relevant papers. Additional 7 publications were identified checking reference lists in the selected articles. We subsequently excluded 548 papers as not appropriate for the study design as ascertained by title and abstract, mainly because they were not dealing with test assays. Reviews and case reports were also excluded (Fig. 1).

The remaining 62 papers were evaluated by two independent reviewers (NB and DV) and 25 were excluded after full-text review because no data were provided on both the sensitivity and the specificity of the assays. Articles were included if they reported data from an original study on the diagnostic accuracy of tests for ANuA detection. Inclusion criteria consisted of: a) description of patients with SLE as the index pathology; and a control group, either healthy individuals or patients with diseases other than SLE. Articles reporting data only on sensitivity (e.g., only SLE patients studied) or specificity (e.g., only patients other than SLE) were not included in the metanalysis; b) diagnosis made using accepted international criteria for each disease considered

in the study. Studies where the diagnosis was based on the presence of a positive antibody test were excluded; c) appropriate description of the diagnostic methods used to detect anti-nucleosome antibodies; d) specification of the cut-off used to discriminate between positive and negative results. Both studies using the manufacturer's cutoff or the cutoff established by ROC curve analysis were deemed acceptable; e) studies performed by the same group of authors and including the same series of patients or controls were considered only once; the publication providing the most relevant information with respect to diagnostic accuracy of the tests was reviewed. However, studies performed on the same population for comparison of two or more analytical methods were maintained.

For this reason, the number of included studies exceeds the number of selected articles.

Finally, 44 studies from 37 articles providing data on ANuA were considered eligible for inclusion in the data analysis. All papers were assessed for methodological quality and statistical data on diagnostic accuracy of the tests using available guidelines for conducting diagnostic systematic reviews [8].

Special care was dedicated to the assessment of risk of bias related to the choice of the cutoff used to discriminate between positive and negative findings.

The diagnostic accuracy of H1-stripped nucleosomes versus intact nucleosomes as the coating antigen in the ANuA assays was also compared.

As a subgroup analysis, studies reporting on ANuA and on anti-dsDNA antibodies in the same set of patients were selected for

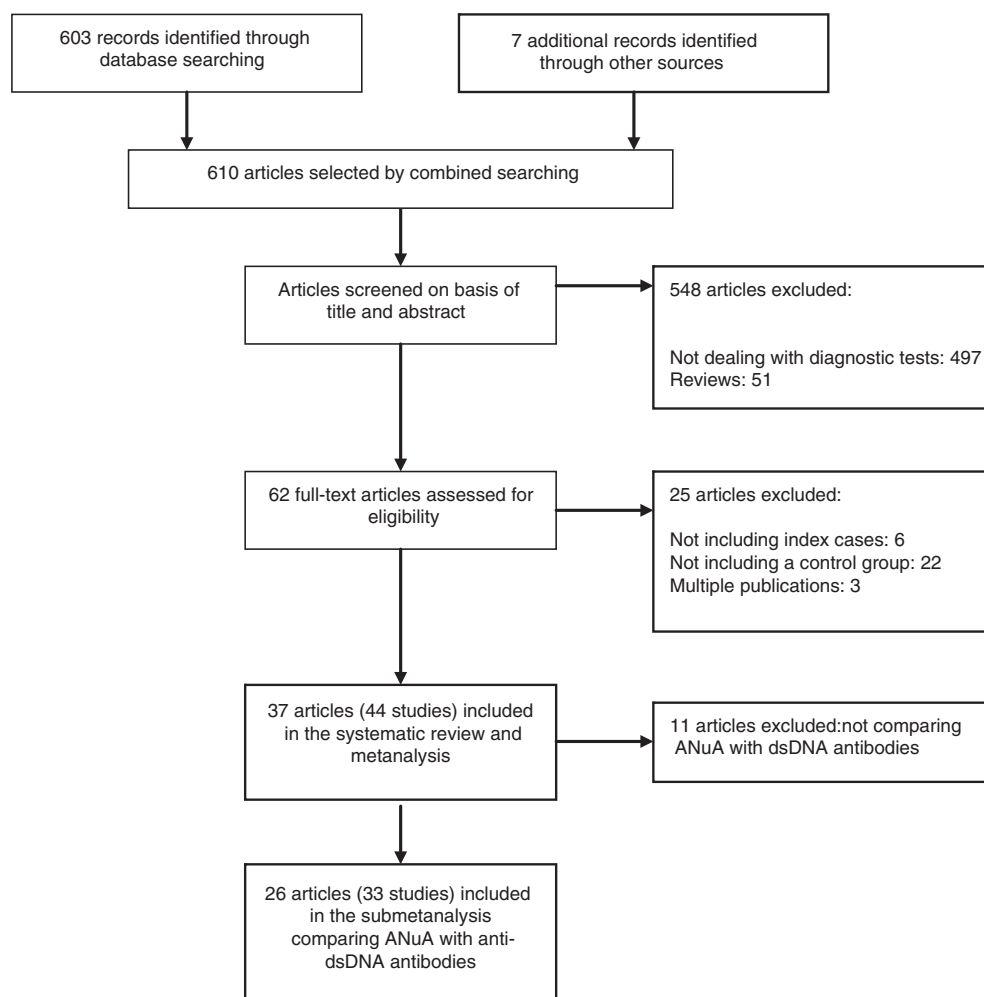


Fig. 1. Flow diagram of study selection for the systematic review.

comparison between the two tests. 26 articles met this requisite, providing data on 33 different studies on ANuA and anti-dsDNA antibody detection.

In addition, the different methods to detect anti-dsDNA antibodies were subgrouped to verify whether the summary effects varied in relation to specific characteristics of the analytical method used. ANuA and anti-dsDNA antibody assays were also compared in studies including children with SLE.

Finally, a post-hoc analysis was conducted including studies reporting on ANuA in SLE patients with kidney involvement and studies that correlated ANuA concentrations with internationally validated disease activity scores (SLEDAI, ECLAM, SLAM or BILAG).

2.2. Data extraction

Data retrieved from the articles included criteria for patient recruitment; the number of patients with SLE as the index case, and the number of healthy or diseased controls; specification of the analytical method used to detect anti-nucleosome antibodies (either in-house or commercial and, in this latter case, subdivided by the manufacturer); characteristics of the nucleosomal antigen (intact molecule or H1-stripped); the cut-off used in the study; sensitivity and specificity of the assay, and the number of true positive, false negative, true negative and false positive results, demographic and clinical data on the population investigated (adults or children; lupus patients with or without nephritis; patients with active or inactive disease).

The same data were extracted also for anti-dsDNA antibody assays from those studies that performed both tests (ANuA and anti-dsDNA) in the same patient series.

Diseased controls were further subclassified for specific disease and prevalence, and specificity of anti-nucleosome antibodies was also calculated for each pathology group.

One author (NB) extracted data from included studies and plotted them in tables; two other authors (DV and DG) independently checked the data and confirmed accuracy of selection.

2.3. Statistical analysis

Data were analyzed using Meta-DiSc (version 1.4) [9].

The accuracy of each study was measured as sensitivity, specificity, positive and negative likelihood ratio (LR+ and LR−) and diagnostic odds ratio (DOR). Sensitivity can be defined as the proportion of positives among people with disease, and specificity as the proportion of negatives among people without disease. The likelihood ratios express how much more frequent the respective result is among subjects with disease than among subjects without disease; LR+ was calculated by dividing the pooled sensitivity by 1-specificity; LR− was calculated by dividing 1-sensitivity by specificity. The DOR expresses how much greater the odds of having the disease are for the people with a positive test result than for the people with a negative test result. It is a single measure of diagnostic test performance that combines both likelihood ratios by dividing LR+ by LR−.

The overall DOR was calculated by combining each study's diagnostic odds ratio, using a random-effects model. A correction factor of one-half was added to each cell to avoid calculation problems by having a value of zero in the 2×2 table [10].

Heterogeneity for threshold effect (i.e. differences in sensitivity and specificity occurring because of different cut-offs used in different studies) was explored by representation of accuracy estimates from each study in a summary receiver operating characteristic (sROC) space, and by the computation of Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity.

The sROC curve analysis was based on a regression analysis of logit transformation of the data, which plots the difference (D) between the logit of the true-positive (TPR) and the logit of the false-positive (FPR) rates ($D = \text{logit TPR} - \text{logit FPR}$) on the y axis and the sum ($S =$

$\text{logit TPR} + \text{logit FPR}$) on the x axis. The y axis (D) is equivalent to the log diagnostic odds ratio, and the x axis (S) is a measure of how the test characteristics vary with the test threshold. A regression eq. ($D = \alpha + \beta S$) derived from the sROC curve analysis can be used to assess the heterogeneity among study results. If the β coefficient is near zero and not statistically significant, then evidence of significant heterogeneity is not present. The symmetric sROC curve was created using the overall DOR of all the studies. The asymmetric sROC curve was created using the (unweighted) method as described by Moses et al. [11].

Cochran's Q statistic [12] and Inconsistency Index [13] were used to test for the presence of heterogeneity between the selected studies, other than threshold effect. Data were pooled in a random effects model using DerSimonian Laird methods [14]. Forest plots and summary estimates with their 95% confidence interval (CI) were made by constructing sROC regression curves, using each study's paired sensitivity and specificity. The area under the curve (AUC) was also calculated.

Potential reasons for heterogeneity (such as variations in analytical methods, H1-stripped methods, age of studied populations) were explored using subgroup and metaregression analyses using diagnostic odds ratios not only as a global measure of accuracy, but as a suitable method to compare the overall diagnostic accuracy of different tests, according with Littenberg and Moses linear model [11]. The model compares DOR in subgroups to verify significant differences. The outputs of meta-regression are ratios of DOR. If a particular study level covariate is significantly associated with diagnostic accuracy, then its coefficient will have a low p value, and the ratio of DOR will give a measure of magnitude of the association. A relative DOR <1.0 indicates that studies with a particular characteristic (e.g. those that considered only children subjects) have a lower DOR than studies without this characteristic.

3. Results

3.1. Diagnostic accuracy of the ANuA assays

The 44 included studies involved 4239 patients with SLE and 6667 controls [15–51]. In 43/44 (97.7%) studies the analytic method consisted of a solid phase immunoenzymatic test (ELISA), except in one case where an immunofluorimetric test (ALBIA – Addressed Laser Beads Immuno Assay) was used. Of the ELISA methods used, 36 were manufactured by 11 different commercial companies and 7 were manufactured in-house.

A pooled analysis of data from the studies showed that ANuA were positive in 2586 out of 4239 patients with SLE and in 361/6667 of the controls. Therefore, the overall sensitivity was 61% (CI, 60–62) and the specificity was 94% (CI, 94–95) (Fig. 2). Overall positive LR for having SLE in ANuA-positive patients was 13.81 (CI, 9.05–21.09) and the negative LR was 0.38 (CI, 0.33–0.44). The DOR was 40.7, with a CI of 26.2–63.3 (Fig. 3).

The AUC for symmetric sROC curve was 0.873, while it was 0.862 for the asymmetric sROC curve, indicating that a very moderate threshold effect was present and that the modest heterogeneity was due to the low number of studies, while most studies gave results that were quite homogeneous among themselves (Fig. 4).

Finally, the specificity of the ANuA was calculated separately for each group of pathology and in healthy controls. The specificity was 91.5% in the 4108 patients affected by diseases other than SLE and was 99.5% in 2559 healthy subjects. The pathologies with the highest prevalence of ANuA were mixed connective tissue disease (MCTD) 25.3% and systemic sclerosis (SSc) 14.9% (Table 1).

3.2. Nucleosome core particles (H1-stripped) versus intact nucleosome assays

31 studies used assays with H1-stripped nucleosomes and 10 used intact nucleosomes (in the remaining 3 studies, the characteristics of

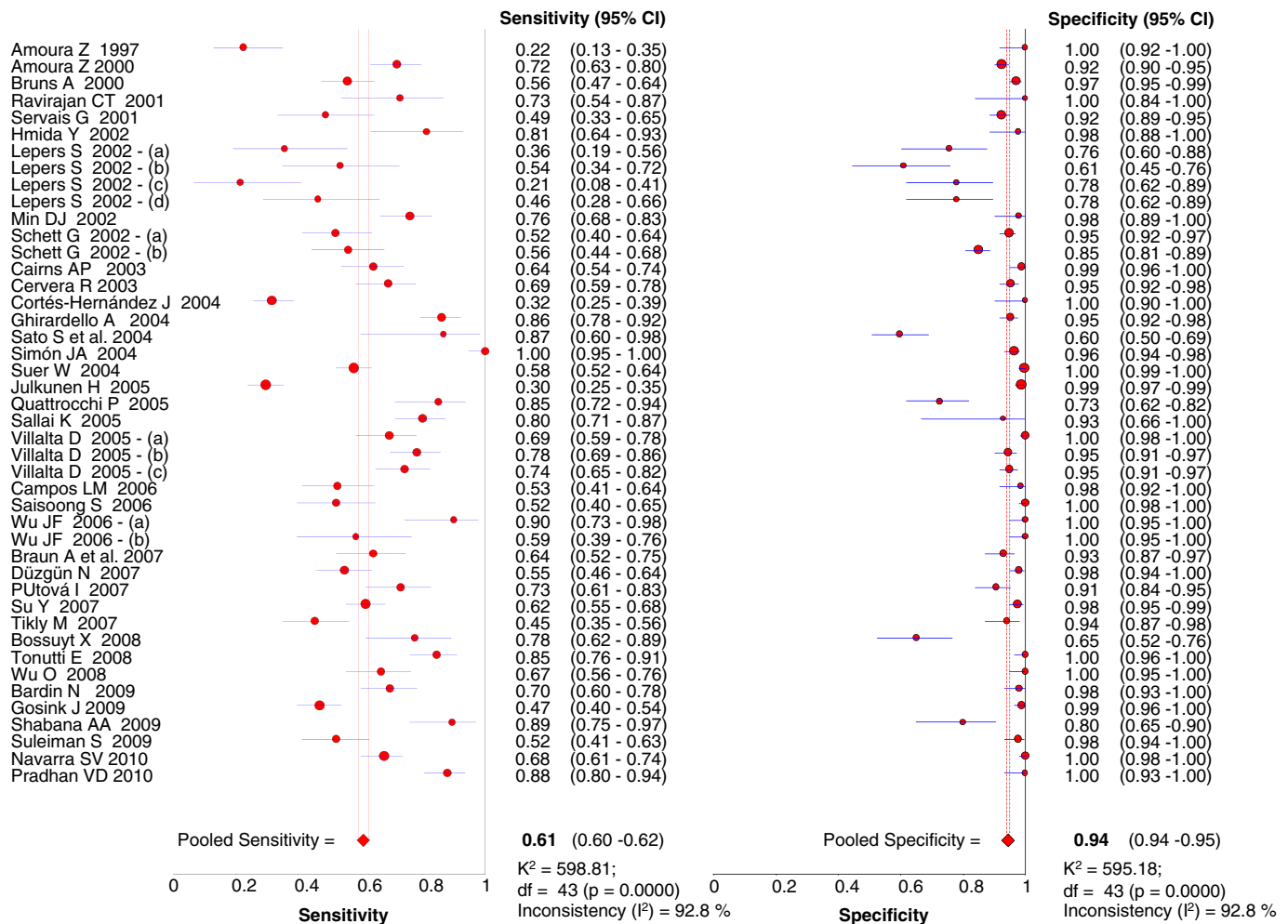


Fig. 2. Forest plots representing the estimated pooled sensitivity and specificity of 44 studies on anti-nucleosome antibody assays. Mean sensitivity is 0.61 (95%CI 0.60–0.62) and mean specificity is 0.94 (95%CI 0.94–0.95).

the antigen were not specified). The comparative analysis resulted in close sensitivity values (61.4% vs 59.5%) for both groups, but higher specificity (95.7% vs 87.5%), LR+ (14.7 vs 5.1) and DOR (42.8 vs 14.5) for the tests using H1-stripped nucleosomes compared to those using the intact nucleosomal antigen (Table 2).

3.3. Comparison of ANuA with anti-dsDNA antibodies

33 studies presented data both for ANuA and for anti-dsDNA, such that it was possible to compare them within the same population of subjects, constituted in total by 3191 patients with SLE and 5529 controls [26–51]. The results showed greater diagnostic sensitivity for ANuA (59.9%) than for anti-dsDNA (52.4%), with a comparable specificity (94.9% vs 94.2%, respectively) (Table 3). While positive and negative LR were similar, the DOR was higher for ANuA (41.0) than for anti-dsDNA (27.8).

The symmetric sROC curves showed an AUC of 0.862 for ANuA and 0.719 for anti-dsDNA. The AUC of the asymmetric sROC curve was 0.848 for ANuA and 0.695 for anti-dsDNA (Fig. 5).

If one analyzes the data of the anti-dsDNA antibodies subgrouped per analytical method, mean sensitivity and mean specificity are: 55.8% and 92.5% respectively, for ELISA methods (20 studies); 33.6% and 97.2% for the IIF assay on Crithidia luciliae (5 studies); and 60.2% and 96.7% for the Farr technique (7 studies).

3.4. ANuA and anti-dsDNA antibodies in children

In the subgroup analysis taking into account the 3 studies performed on pediatric patients, and including 475 patients with SLE and 710 controls [49–51], anti-dsDNA antibody testing was slightly more sensitive than ANuA (66.5% vs 64.1%), while specificity slightly favored ANuA (98.8% vs 97.1%). However, the positive LR and the DOR were much better for ANuA than for anti-dsDNA: 43 vs 17 and 146 vs 51, respectively (Table 4).

3.5. Correlation of ANuA and anti-dsDNA with disease activity of SLE

In 19 studies out of 22 (86.4%) where such analyses were performed, the authors reported the presence of a correlation between ANuA and disease activity, and the global data was statistically significant (chi-square 20.49; $p < 0.0001$). Vice versa, although 6 studies out of 9 (66.7%) had shown a correlation between anti-dsDNA and disease activity, the overall correlation was not significant (overall chi-square 0.894; $p = 0.344$) (Table 5).

3.6. Correlation of ANuA and anti-dsDNA with nephropathy

A correlation between ANuA and nephropathy was demonstrated in 13 of the 22 studies examined (59.1%), but in total these did not

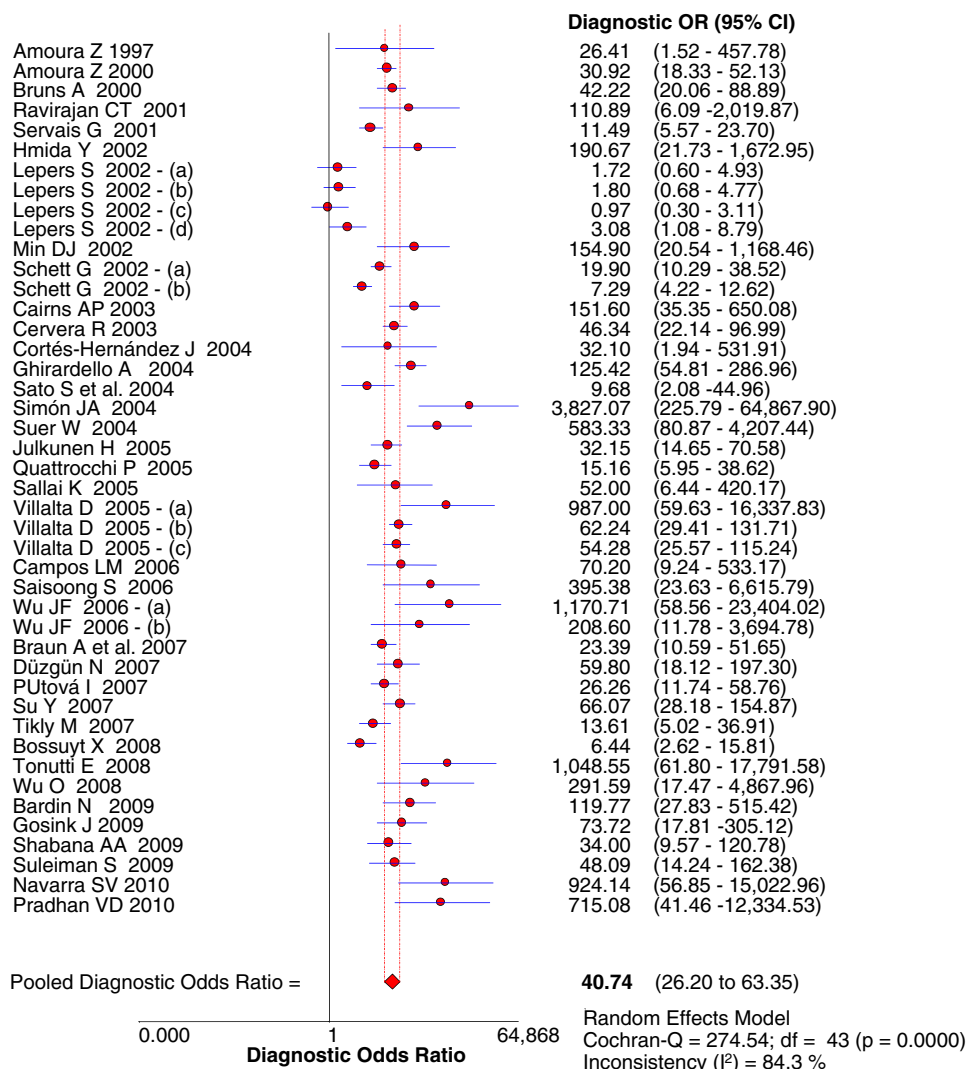


Fig. 3. Forest plot representing the pooled diagnostic odds ratio of 44 studies on anti-nucleosome antibody assays. The p value = 0.0000 indicates that no significant difference was present in the diagnostic accuracy of the selected studies.

reach statistical significance (chi-square 0.820; $p = 0.365$). Analogous results were also obtained for anti-dsDNA in that 4 of 6 studies (66.7%) showed a correlation with nephropathy, although again they did not reach a statistically significant overall value (chi-square 0.336; $p = 0.562$) (Table 5).

3.7. Cost of testing

Finally, as none of the study included costs of testing as an outcome, it was not possible to evaluate which of the two tests be more advantageous economically.

4. Discussion

Although dozens of clinical studies undertaken with several analytical methods by diverse researchers have demonstrated that the test for identifying ANuA can be useful in SLE diagnosis [52], and accurate commercial methods having been available for more than 10 years, these tests are not used except sporadically. The reasons may be several; the most important is probably the fact that anti-dsDNA were introduced many years before and are included among the classification criteria for SLE; but another important reason must be that the diagnostic performance of ANuA is not well known. To clarify this point, we have conducted a systematic review

and a metaanalysis of all the studies published in the last 15 years on the diagnostic accuracy of the analytical methods to search for ANuA.

The data gathered from the studies available in the literature have allowed us to answer the questions we posed at the beginning of the study, the first of which was “Does the ANuA test provide an adequate level of diagnostic accuracy for diagnosing SLE?”

4.1. Sensitivity of ANuA assays

The data on diagnostic accuracy tell us that the mean sensitivity of the ANuA test is 61% without significant differences in relation to the commercial or in-house method used to measure it. The very narrow confidence interval (0.59–0.62) may lead to the conclusion that this sensitivity value represents the natural limit of prevalence of this antibody in subjects with SLE and that the differences found in the various studies are due to the selection of patients.

Only 4 studies found sensibility data very much lower than the mean. Amoura et al. [15] studied 58 SLE patients and 44 controls, and found a sensitivity of ANuA of 22.4% and a specificity of 100%. For their study, however, they have used an in-house method and adopted a very restrictive cutoff, the mean optical density (OD) + 3 standard deviations (SD) obtained in 93 healthy subjects, which privileges specificity than sensitivity.

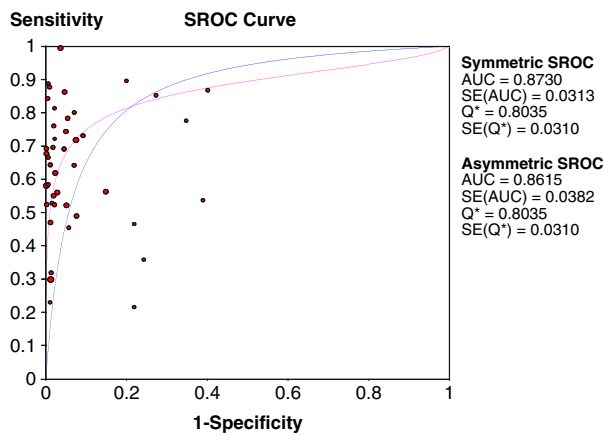


Fig. 4. Summary ROC curves from 44 studies on anti-nucleosome antibody assays. The blue line depicts the symmetric (conventional) curve, while the red line represents the asymmetric curve. The symmetric sROC curve was created using the overall diagnostic odds ratio by combining each study's diagnostic odds ratio, using a random-effects model. The asymmetric sROC curve was created using the (unweighted) method. The slight difference between the symmetric and asymmetric sROC curves indicates that a weak threshold effect is present.

As for the study of Cortes et al. [30], the same is true as of the Amoura study. These authors also adopted the cutoff of mean OD + 3SD in 50 healthy subjects, finding a sensitivity of 31.7% and a specificity of 100%. As the authors of this study did not perform a ROC curve analysis, it is not possible to know at what value the sensitivity would have rise in relation to inferior specificity values.

In the study conducted by Julkunen et al. [34] the sensitivity of the ANuA test in SLE is 30% and the specificity 98.7%. In this study they tested serum samples from routinely followed SLE patients using a commercial ELISA method, adopting the cutoff value proposed by the manufacturer. Because in any case in this series of patients even the sensitivity of the anti-dsDNA antibodies measured with an ELISA method manufactured by the same company is 29%, one can maintain that the low sensitivity values are due to the characteristics of the selected population.

Lastly, Lepers et al. [35] studied 28 patients with SLE using 4 ELISA commercial methods at the manufacturer's cutoff. They found sensitivity values ranging from 21.4% to 53.6%. These notable differences in the results obtained by same authors with 4 different methods are very probably to be imputed to the analytical methods used, some of which have very low sensitivity.

4.2. Specificity of ANuA assays

Specificity results fell between 61% and 100%, with a mean value of 95%. It is interesting to note that 11/44 studies showed 100% specificity. If, in some cases [19,20,40–42] this may be due to the

characteristics of the control group which was made up exclusively of healthy subjects, in the majority of other studies the control group was composed of diseased subjects affected by pathologies which were quite heterogenous within the group. For example, Cortes [30] used only patients with renal pathologies and Amoura et al. [15] used only subjects infected with the human immunodeficiency virus (HIV).

On the other hand, some studies [26,35] using as controls only patients with the same autoimmune diseases as those being studied by the majority of other studies, obtained lower specificity results (from 60% to 70%). These results, between their heterogeneity and sometime contradictions, lead us to hold that, if without doubt the criteria for control group selection influence the specificity results of individual studies, when these same are evaluated cumulatively the mean specificity value is sufficiently balanced, as shown by the very narrow confidence interval (93%–94%).

The sROC curves showed that only a modest bias related to the choice of cutoff is present, as demonstrated by the favorable superimposition of the symmetrical and asymmetrical curves. Globally these data, which enable us to answer the first question we had posed, tell us that the ANuA test has high diagnostic specificity and that the sensitivity value of 60% is probably the highest obtainable in patients with SLE. They also demonstrate that a patient with positive ANuA has a probability of being affected by SLE which is 40.7 times greater than a patient in whom the ANuA are negative.

The second question we posed was: “In case of positivity of ANuA, does the specificity of the test allow us to exclude the presence of other connective tissue diseases?”

In a more in-depth evaluation of ANuA specificity, we have stratified ANuA positivity in the different pathology groups and in healthy subjects. While in healthy subjects the specificity is nearly absolute (99.5%), MCTD and SSc are the two diseases with the highest rate of ANuA positivity (25.3% and 14.9%, respectively). For MCTD a positive ANuA result may be expected, as MCTD is a typical overlap syndrome which may include clinical and immunological features of SLE, SSc and polymyositis. For SSc, the problem is more complex and relates to the characteristics of the nucleosome antigens used in the diagnostic test.

4.3. H1-stripped versus entire nucleosomes

ELISA techniques for ANuA detection differ in terms of the antigen preparation used. Some methods use nucleosomal particles obtained by adding native DNA to the histone core or to histone dimers; others use purified chromatin generally obtained by digestion with micrococcal nuclease and subsequent removal of histone H1 by 0.5 M NaCl extraction at a neutral pH. Because it was demonstrated that the presence of the H1 histone in the antigenic preparation of nucleosomes was a cause of “false positive reactions” (i.e., positive results in subjects with diseases other than SLE), many manufacturers modified the antigenic substrate

Table 1

Prevalence of anti-nucleosome antibodies (ANuA) in autoimmune diseases other than SLE and in healthy subjects.

Disease	No. of patients	ANuA pos no.	Specificity (%)	References
MCTD	198	50	74.7	[24,32,35,36,41]
Systemic sclerosis	838	125	85.1	[13–15,19–22,24,26,28–32,34–36,41,42,45]
Sjögren's syndrome	653	47	92.8	[13,14,21,23,24,26,29–32,34–36,41,42,45]
Rheumatoid arthritis	736	57	92.3	[13,20,22–25,27,29,31,32,35,36,42,44,45]
Myositis	219	14	93.6	[13,19,21,31,32,36,41,42]
pAPS	157	16	89.8	[13,26,32,41]
Vasculitis	221	2	99.1	[13,14,23,32,41,48]
Viral infections	512	6	92.8	[12,13,23,24,29,34,36,44,45]
Other diseases	574	67	88.3	[13,23–25,32,36,42,46]
Healthy subjects	2559	13	99.5	[16–22,25,26,29,31,33–39,41–48]
Total	6667	397	94.0	

(MCTD, mixed connective tissue disease; pAPS, primary antiphospholipid syndrome).

Table 2
Results of pooled estimates of H1-stripped versus intact nucleosome assays (41 studies).

	H1-stripped nucleosomes		intact nucleosomes	
	%	(95%CI)	%	(95%CI)
Sensitivity	61.4	(59.7–63.1)	59.5	(55.9–63.0)
Specificity	95.7	(95.1–96.2)	87.5	(85.3–89.5)
Positive likelihood ratio	14.7	(10.4–26.5)	5.1	(3.3–11.1)
Negative likelihood ratio	0.3	(0.3–0.4)	0.4	(0.2–0.6)
Summary Diagnostic Odds Ratio	42.8	(30.4–76.1)	14.5	(4.7–44.4)

Table 3
Results of pooled estimate comparison of ANuA with anti-dsDNA antibody assay (33 studies).

	ANuA		anti-dsDNA	
	%	(95%CI)	%	(95%CI)
Sensitivity	59.9	(58.2–61.6)	52.4	(50.7–54.0)
Specificity	94.9	(94.3–95.4)	94.2	(93.5–94.9)
Positive likelihood ratio	14.3	(8.7–23.4)	12.7	(7.9–20.6)
Negative likelihood ratio	0.39	(0.33–0.46)	0.47	(0.40–0.55)
Summary Diagnostic Odds Ratio	41.0	(23.8–70.8)	27.8	(16.7–46.1)

by eliminating the H1 histone from the nucleosomal complex [53]. In a separate analysis, we compared the data collected from the methods that use H1-stripped nucleosomes with those that use whole nucleosomes. In effect, with parity of sensitivity (61% vs 59%), the specificity results were much better (95.7%) for methods using H1-stripped nucleosomes than for methods using whole nucleosomes (87.5%).

This aspect was shown clearly by the study of Suer et al. [24] in which it was demonstrated that, with parity of sensitivity, in 424 controls inserted in the study the specificity was 84.7% with whole nucleosomes, while it was 99.8% with H1-stripped nucleosomes. The most important aspect is that 95% of the false positive results were found in patients with SSc (62/119) while with H1-stripped nucleosomes not one patient with SSc came out ANuA positive.

The low specificity of the test with whole nucleosomes in subjects with SSc was also confirmed by the data obtained by Sato [22] which found 25% of false positives in subjects with this disease. Still, the problem of low specificity of ANuA in SSc cannot be considered completely resolved by the use of H1-stripped nucleosomes. In effect, Ticky, who used an H1-stripped method, found ANuA positives in 5 of his 30 patients (16%) with SSc and no positives in the 27 patients with rheumatoid arthritis or in the 30 healthy controls [25]. Because in a subgroup of patients with SSc the antibodies are typically directed to topoisomerase I, which is a protein associated with

Table 4
Results of pooled estimate comparison of ANuA with anti-dsDNA antibody assay in children (3 studies).

	ANuA		anti-dsDNA	
	%	(95%CI)	%	(95%CI)
Sensitivity	66.5	(59.7–72.9)	64.1	(57.2–70.6)
Specificity	98.8	(96.5–99.7)	97.1	(94.2–98.8)
Positive likelihood ratio	43.3	(15.2–123.1)	17.4	(7.7–39.2)
Negative likelihood ratio	0.30	(0.16–0.55)	0.36	(0.25–0.52)
Summary Diagnostic Odds Ratio	146.1	(41.2–518.0)	51.5	(16.0–165.4)

DNA, it is possible that, in some patients with SSc, there are present contemporaneously both anti-topoisomerase I and ANuA, and that therefore in these patients ANuA do not represent analytic false positives but probably a diagnostic false positive.

The third question was, perhaps, the most relevant from the practical point of view: “Is the ANuA test comparable from the diagnostic and prognostic point of view to the test for anti-dsDNA antibodies?”

4.4. ANuA versus anti-dsDNA antibody assay

In the comparative analysis with anti-dsDNA antibodies, among the 44 studies on ANuA, the 33 that also presented data on anti-dsDNA were chosen. The analyses showed that ANuA have greater diagnostic sensitivity (60% vs 52%) and a specificity only slightly superior (94.9% vs 94.2%) to anti-dsDNA. The probability that a subject with positive ANuA would have SLE was 41 times greater than for those who do not have positive ANuA, while for anti-dsDNA this probability was 28 times.

Even in children, ANuA displayed a more relevant predictive value than anti-dsDNA antibodies, as shown by the much higher positive likelihood ratio (43 vs 17) and DOR (146 vs 51). Although only three articles could be included in this sub-metanalysis and the low number of studies confronted may account for the observed differences, SLE in children is a rare disease and those three studies are therefore of value.

A marginal aspect in this study, but one of certain interest, is that of the diagnostic performance of the three methods used for anti-dsDNA research. The IIF on *Crithidia luciliae* proved to be more specific but also in the absolute less sensitive. To this method, therefore, we must ascribe the low global value of sensitivity of the anti-dsDNA antibodies compared to the ANuA. The Farr technique and the ELISA methods have in fact demonstrated performance results superimposable on those of ANuA. Because the IIF method is largely used as a screening test in

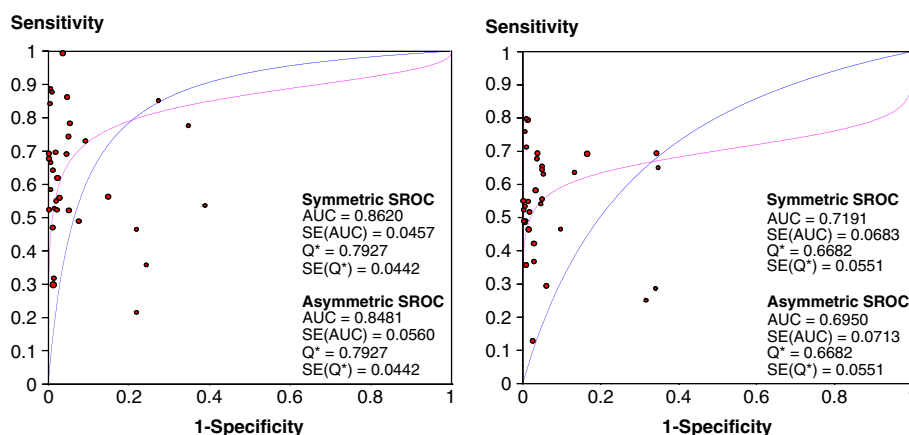


Fig. 5. sROC curves comparing ANuA (left) and anti-dsDNA antibody (right) assays in the 33 studies that assayed the two tests in the same series of patients. The blue lines show the symmetric (conventional) curves, while the red lines represent the asymmetric curves. The sROC curves of the two tests show a higher AUC and a lower threshold effect (the interpolated curve is less asymmetric) for the ANuA assays than for anti-dsDNA assays.

Table 5

Correlation results of ANuA and anti-dsDNA antibody assays with nephropathy and disease activity (25 studies).

Reference	No. of SLE patients	ANuA		anti-dsDNA	
		Correlation with disease activity	Correlation with active renal disease	Correlation with disease activity	Correlation with active renal disease
Amoura et al. [15]	58	$p > 0.05$	–	–	–
Amoura et al. [16]	120	$p < 0.0001$	$p = 0.004$	–	–
Braun et al. [17]	78	$p = 0.0001$	$p > 0.05$	–	–
Bruns et al. [27]	136	$p < 0.0001$	$p < 0.02$	–	–
Campos et al. [50]	74	$p < 0.001$	$p > 0.05$	$p = 0.001$	–
Cervera et al. [29]	100	$p = 0.01$	$p < 0.01$	–	$p < 0.001$
Cortés-Hernández et al. [30]	199	$p = 0.01$	$p = 0.04$	$p = 0.02$	$p = 0.0001$
Düzgün et al., [31]	131	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Ghirardello et al. [32]	101	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Julkunen et al. [34]	296	–	$p = 0.006$	–	–
Min et al. [19]	129	$p < 0.001$	$p > 0.05$	–	–
Pútová et al. [37]	74	–	$p < 0.001$	–	–
Quattrocchi et al. [38]	47	$p > 0.05$	$p > 0.05$	–	–
Ravirajan et al. [20]	33	$p = 0.022$	$p = 0.4$	–	–
Saisoong et al. [40]	65	$p = 0.007$	$p < 0.05$	$p = 0.002$	$p > 0.05$
Sallai et al. [21]	105	$p < 0.01$	$p > 0.05$	–	–
Schett et al. [39] (a)	73	$p < 0.001$	–	$p = 0.02$	–
Schett et al. [39] (b)	73	$p = 0.001$	–	–	–
Shabana et al. [23]	38	$p < 0.001$	$p < 0.01$	–	–
Simón et al. [44]	73	$p < 0.0001$	$p = 0.01$	$p = 0.03$	–
Su et al. [45]	233	$p < 0.001$	$p = 0.048$	–	–
Tikly et al. [25]	86	$p = 0.024$	$p > 0.05$	–	–
Wu et al. [51] (a)	30	$p < 0.0001$	$p > 0.05$	$p > 0.05$	$p < 0.001$
Wu et al. [51] (b)	29	$p = 0.047$	$p > 0.05$	$p > 0.05$	–
Navarra et al. [36]	232	–	$p = 0.028$	–	–
Total	2613	19/22 ($p < 0.0001$)	13/22 ($p = 0.365$)	6/9 ($p = 0.344$)	4/6 ($p = 0.562$)

anti-dsDNA antibody research, the insufficient sensitivity shown by the results of this study would indicate that it should be abandoned as a screening test and be used only as a confirmation test.

4.5. Correlation with nephropathy and disease activity

There is evidence in experimental models that ANuA play a part in the pathogenesis of SLE, and especially in the development of nephritis, a major complication of the disease. Studies considered in the systematic review have confirmed the correlation between ANuA and the activity of the disease, measured according to internationally validated global score systems (e.g., SLAM, SLEDAI, ECLAM, BILAG), but not between ANuA and kidney involvement.

With regard to anti-dsDNA autoantibodies, the selected studies have not shown a significant correlation, either with renal damage or with the activity of the disease. The dissimilarity of results obtained by the various studies—many of which were cross-sectional—may find explanation in differences occurring at the time of collection of the blood sample or in the differences in the IgG isotypes found (for example, Amoura et al. [16] showed a correlation between ANuA and disease activity only for the sub-class IgG3), as well as in the IgG/IgM ratio (not investigated in this review), since the IgM-class anti-dsDNA antibodies seem to have a protective role for nephropathy [54,55].

In any case, the limited number of studies examined, especially with respect to anti-dsDNA antibodies, does not allow us to track definitive conclusions on the correlation of ANuA and the anti-dsDNA with nephropathy and disease activity. Additional primary prospective studies will be necessary, therefore, on a broad sample base of SLE, in order to have material available that can be used to answer this query.

5. Conclusions

In conclusion, even with the typical limitations of a systematic review due to the heterogeneity of the studies, the ANuA test on the whole appears to have an adequate level of diagnostic accuracy for

diagnosing SLE. In case of ANuA positivity, the specificity of the test does not allow for excluding the presence of other connective tissue diseases. The ANuA test is superior for diagnostics than the test for anti-dsDNA antibodies. These data give renewed vigor to the hypotheses posed 5 years ago by Isenberg [56] that probably the end of anti-dsDNA antibody assays is rapidly approaching.

Take-home messages

- Data from the metanalysis have shown that anti-nucleosome antibodies are a highly accurate diagnostic marker for SLE.
- Anti-nucleosome antibodies have equal specificity but higher sensitivity, positive likelihood ratio and diagnostic odds ratio than anti-dsDNA antibodies for the diagnosis of SLE. Hence, anti-nucleosome antibody testing may represent a reliable alternative to tests for anti-dsDNA antibodies.
- Measuring anti-dsDNA antibodies with the IIF method must now be considered obsolete due to its low diagnostic sensitivity and the difficulty of interpretation—a trait common to all IIF tests.

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