

HOW THE SEROLOGICAL CORRELATION BETWEEN ANA AND dsDNA CAN ENHANCE COST EFFECTIVENESS

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The term "antinuclear antibodies" (ANA) is a nomenclature used to describe autoantibodies against a wide variety of cellular nuclear proteins.^{1,2} The presence of such autoantibodies in the serum correlates positively with different autoimmune illnesses.¹⁻³ Some of these immunological parameters have diagnostic and/or prognostic value.^{2,4} Currently, the ANA test is recognized as a screening tool for unraveling connective tissue disorders.^{4,5}

In clinical practice, antibodies to double-stranded deoxyribonucleic acid (dsDNA) are among the commonly used ANA antibodies.^{6,7} The dsDNA continue to show a high disease correlation with patients suffering from systemic lupus erythematosus (SLE), and it is possible that they play a role in its pathogenesis.⁸⁻¹⁰ Additionally, they have also been used clinically to monitor disease activity.^{10,11}

Unfortunately, in many local hospital laboratories, dsDNA tests have been performed routinely in most of the ANA requested tests, irrespective of their value. Furthermore, requests for this test have been made frequently by some doctors even in ANA-negative cases. This is a practice that has long ceased in international laboratories. This is an indication of the inappropriate use and lack of understanding of proper autoantibody testing in the assessment of autoimmune disease.¹²

Because of the financial impact of these immunological tests on hospital budgets, continuous monitoring is necessary to ensure cost-effective optimal patient care. The overall cost does not only comprise the test cost per patient, but also the time and effort spent by laboratory technicians. In order to determine the laboratory expenditure, we attempted to explore the possible cost effectiveness of dsDNA test in ANA cases through investigating their serological correlation in the clinical immunology laboratory of King Abdulaziz University Hospital (KAUH), Jeddah.

Materials and Methods

The study period lasted from January 1997 until January 1998. Samples of sera sequentially sent to the KAUH laboratory for ANA and dsDNA tests were included in the study. These tests were requested on patients suspected to have rheumatological diseases or other autoimmune disorders. The medical files of these patients were reviewed for demographic data and clinical diagnosis. The dsDNA tests were done routinely in all cases with positive ANA test. Additionally, dsDNA test was also performed on 30 sequentially selected cases with negative ANA test, which were used as controls.

ANA tests were performed by indirect immunofluorescence (IIF) technique, utilizing human epithelial cells (Hep-2) fixed on glass slides which were commercially prepared (INOVA System Quanta Lite™ ANA Kit, INOVA Diagnostic Inc., San Diego, CA). Briefly, patients' sera were diluted with phosphate-buffered saline (PBS) in a 50 µL aliquot, and were overlaid in a well on the Hep-2 substrate slide. The slides were placed in a humid chamber, incubated for 20 minutes at room temperature, followed by washing over 10 minutes in two changes of PBS on a reciprocating shaker. The substrate was then covered with approximately one drop of the conjugate solution. After the incubation for 30 minutes at room temperature, the slides were then washed in PBS as before and were immediately covered with glycerol-PBS (mounting medium) and viewed with a standard immunofluorescence microscope (Olympus, Japan). The fluorescence strength depends on sample titration, based on 1:40 dilutions. Zero titer refers to absent ANA on immunofluorescence (ANA negative).

Antibodies to dsDNA were performed by the enzyme-linked immunosorbent assay (ELISA) technique using the same INOVA System Quanta Lite™ Ds-DNA Kit. Briefly, patients' sera were diluted with an ELISA sample diluent and added to separate wells of microwell plate. The strips were covered and incubated for 30 minutes at room temperature. Then conjugate was added to each well and incubated for 30 minutes and washed as before. Then the substrate was added to each well and incubated for 30 minutes at room temperature. An ELISA stopping solution was added to each well, and the plates were read at 450 nm using an ELISA reader (Dyntech, USA).

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FIGURE 1. Distribution of dsDNA levels among different ANA titers.

TABLE 1. General characteristics of the group.

Clinical data	Positive ANA cases	Negative ANA cases	Total
Total number of cases	211	33	244
Age (years)	3-70	10-66	
Mean	31.4±SD14.6	32.8±SD15.9	
Sex			
Male	36 (17.1%)	15 (45.5%)	51
Female	175 (82.9%)	18 (54.5%)	193
Diagnosis			
Rheumatological	145 (68.7%)	27 (81.1%)	172 (71%)
SLE	70 (33.2%)	0	70 (28.7%)
Rheumatoid arthritis	18 (8.5%)	0	18 (7.4%)
Osteoarthritis	11 (5.2%)	6 (18.1%)	17 (7%)
Other rheumatological	13 (6.2%)	0	13 (5.3%)
Undiagnosed joint pain	33 (15.6%)	23 (63.1%)	54 (22.1%)
Other autoimmune	24 (11.4%)	0	16 (6.6%)
Recurrent abortion	16 (7.6%)	3 (9.1%)	27 (11.1%)
Miscellaneous	26 (12.3%)	3 (9.1%)	29 (11.9%)

TABLE 2. Levels of dsDNA and means among different positive ANA titer groups.

Positive ANA cases	dsDNA levels				Positive dsDNA (>200 IU/L)		
	Time	Freq	%	Range	Mean±SD	Freq	%
1:40	80	32.8	60-337	132±54	6	7.9	7.5
1:80	69	28.3	61-1314	216±218	19	25	27.5
1:160	47	19.3	99-2264	840±633	39	51.3	83
1:320	15	6.1	104-3923	1600±1130	12	15.8	80
Total	244	100			76	100	

* Percent within each ANA titer group.

The data were entered into a personal computer. Frequency tables, correlation analysis by Pearson's test and analysis of variance were performed by using SPSS statistical program (version 7.5).

Results

Two hundred and eleven cases of positive ANA test sequentially sent to the immunology laboratory were studied. Females were predominant and constituted 83% of cases. Rheumatological disorders were the most common in 145 cases (69%). Other general characteristics of the studied group with the controls are shown in Table 1.

Low ANA titers of 1:40 (weakly positive) were predominant and were found in 80 cases (33% of the studied group), as shown in Table 2. Levels of dsDNA ranged from 60 to 3923 IU/L, with a mean of 403 (SD±610). Different dsDNA levels with their ranges among different ANA titers are demonstrated in Table 2. Positive dsDNA test, which is an abnormal dsDNA level of more than 200 IU/L, was found in 76 (36%) of the ANA-positive cases. Most of the dsDNA-positive cases (92%) were found at ANA titers equal or above 1:80. Only six positive dsDNA cases were found at weakly positive ANA titers (1:40), and they were of low abnormal dsDNA levels (<337 IU/L). Their clinical diagnoses were systemic lupus erythematosus (SLE) in two cases, recurrent abortion in three cases, and osteoarthritis in one case. The dsDNA range in the negative ANA cases (control group) was 75-152 IU/L (mean of 117±24), and there was no single case of positive dsDNA test among them.

The distribution of dsDNA levels among different ANA titers is shown in Figure 1. Levels of dsDNA showed a significant moderate positive correlation with increasing ANA titers ($R=0.62$, $P<0.001$), and there was also a high linear association between them ($P<0.001$).

Discussion

Autoantibodies towards dsDNA are among the commonly used ANA in the evaluation of several autoimmune disorders. Since the serological correlation between them is an area of controversy, we tried to explore this correlation and its possible cost effectiveness.

In the ANA cases performed at KAUH immunology laboratory, female predominance was expected, since they are more prone to autoimmune diseases.^{5,13} As rheumatological disorders are characterized by several autoantibodies, they were the predominant illnesses among the cases needing ANA test. Of these, SLE was the most common and had the highest DNA levels, as has been reported in the literature.^{9,11,13} Interestingly, recurrent abortions accounted for 11% of the studied ANA-positive cases, which can be attributed to the increased awareness of the role of autoimmune mechanisms in some abortions.^{14,15}

This study has demonstrated the positive serological correlation between ANA and dsDNA levels. This correlation has been noticed previously but little importance had been attached to it. The present study

showed that high dsDNA levels (>200 IU/L) were mainly found at ANA

titers equal to or higher than 1:80, and that in only six cases were there weakly positive ANA titers at 1:40. In all six cases, dsDNA levels were of low concentration (<337 IU/L). This data advocates performing dsDNA tests only if clinically indicated and when ANA titers are equal to or higher than 1:80. Abnormal dsDNA levels were rarely found in weakly positive ANA titer and were not seen at all in any ANA negative cases. This is also a caution against the overuse of the dsDNA test in ANA negative cases. Moder recently stated that lower titer values (<1:160) are often of little clinical significance and may not be related to the patient's symptoms.¹⁶

Antinuclear antibody testing is a useful way of confirming the diagnosis of SLE when the clinical suspicion is high, or to exclude it in cases when it is in the differential diagnoses, but the likelihood of it is low to moderate. Because the test is very sensitive and yet not specific for lupus, an inappropriately ordered ANA test with a positive result can cause diagnostic confusion and unnecessary anxiety for the patient and the physician.¹⁷ So, unless there is a high index of clinical suspicion of related illness, avoiding performing dsDNA test in weakly positive ANA cases will lead to some saving in laboratory-allocated expenditures. Eventually, incorporating such guidelines in immunology laboratories might enhance the cost effectiveness of dsDNA tests. Savings in such tests will lead to improved laboratory productivity and future development.

Low-positive dsDNA levels are rare in any clinically active rheumatological disease, and may hint at the possibility of an ongoing low level of autoimmunity. In such cases, recommending further tests in a few months may be more useful.¹⁸ Additionally, low dsDNA levels have been used in monitoring disease activity, particularly in SLE cases going into remission.^{13,16,19} Interestingly, low-positive dsDNA levels that were found in a few cases with recurrent abortion might be due to the presence of cross-reacting autoantibodies or clinically inactive autoimmune disorder. Further research is still needed to confirm the importance of these findings.

Finding a positive ANA test result in the face of normal dsDNA level (64% of studied cases) may be due to the presence of other types of antinuclear antibodies, such as extractable nuclear antigens (ENA).^{4,5} In rare cases, some patients might demonstrate positive ANA due to cross-reacting (i.e., heterophil) antibodies.^{1,4,5} Clinically important ENA antibodies include anti-Sm (Smith), anti-RNP, anti-Ro (SS-A), and anti-La (SS-B). These have been frequently reported in several rheumatological disorders.^{4,5,13} The search for ENA autoantibodies is recommended in ANA-positive cases, particularly those with the speckled pattern on immunofluorescence.^{6,13}

Positive ANA tests have also been reported in some healthy individuals, particularly the elderly population and pregnant women.^{13,14,20}

False-positive ANA reading can occur when the washing of the slides is inadequate or when there is a high background of fluorescence (INOVA System Quanta Lite™ ANA Kit). Appropriate quality control methods should be strictly followed according to the manufacturer's recommendations. In this study, the indirect immunofluorescence (IIF) technique was used for the detection of ANA. More recently, some laboratories have advocated the use of the ELISA technique to measure ANA level, similar to the dsDNA test.^{20,21} Further studies are needed to explore the correlation between ANA by ELISA and dsDNA and their cost effectiveness.

In conclusion, this study showed that there is a highly positive serological correlation between ANA and dsDNA, and that abnormal dsDNA levels (>200 IU/L) are mainly found when ANA titers are \geq 1:80 titer. These data advocate that dsDNA test should be performed essentially if clinically indicated in ANA titers \geq 1:80. Abnormal dsDNA found at weakly positive ANA are usually of low levels and have limited clinical significance. We recommend abandoning dsDNA tests in ANA negative cases. Eventually, adopting these guidelines in immunology laboratories will lead to better costing of dsDNA tests.

References

1. Peter JB, Dawkins RL. Evaluating autoimmune diseases. *Diagn Med* 1979;2:68-76.
2. Peter JB, Dawkins RL. The value of immunology tests. *Diagn Med* 1979;2:79-88.
3. Van Venrooij WJ, Charles P, Maini RN. The consensus workshops for the detection of autoantibodies to intracellular antigens in rheumatic diseases. *J Immunol Methods* 1991;140:181-9.
4. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989;44:93-151.
5. Mongey A-B, Hess EV. Antinuclear antibodies and disease specificity. In: Stollerman GH, LaMont JT, Leonard JL, Siperstein MD, editors. *Advances of Internal Medicine*. St Louis: Mosby Year Book 1991;36: 151-69.
6. Reichlin M. ANA and antibodies to DNA: their use in clinical diagnosis. *Bull Rheum Dis* 1993;42:3-5.
7. Hang L, Nakamura R. Current concepts and advances in clinical laboratory testing for autoimmune diseases. *Crit Rev Clin Lab Sci* 1997;34:275-311.
8. Nakamura RM. Clinical and laboratory evaluation of systemic rheumatic diseases. 19th edition. In: Henry JB, editor. *Clinical Diagnosis and Management by Laboratory Methods*. Philadelphia: WB Saunders, 1996:1013-24.
9. Von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum* 1995;24:323-58.
10. Moder K. Use and interpretation of rheumatologic tests: a guide for clinicians. *Mayo Clin Proc* 1996;71:391-6.
11. Moder K, Mason T. The current use and interpretation of rheumatologic tests. *Adolesc Med* 1988;9:25-34.
12. Suarez-Almazor ME, Gonzalez-Lopez L, Gamez-Nava JJ, Belseck E, Kendall CJ, Davis P. Utilization and predictive value of laboratory tests in patients referred to rheumatologists by primary care physicians. *J Rheumatol* 1998;25:1980-5.
13. Thomas C, Robinson J. The antinuclear antibody test. When is a positive result clinically relevant? *Postgrad Med* 1993;94:55-66.

14. Ogasawara M, Aoki K, Kajiura S, Yagami Y. Are antinuclear antibodies predictive of recurrent miscarriage? *Lancet* 1996;347:1183-4.
15. Ogasawara M, Aoki K, Katano L, Aoyama T, Kajiura S, Suzumori K. Prevalence of autoantibodies in patients with recurrent miscarriages. *Am J Reprod Immunol* 1999;41:86-90.
16. Moder KG. Immunological tests in rheumatology. *Ann Allergy Asthma Immunol* 1999;81:539-47.
17. Illei GG, Klippel JH. Why is the ANA result positive? *Bull Rheum Dis* 1999;48:1-4.
18. Radic MZ. Initiation of systemic autoimmunity and sequence specific anti-DNA autoantibodies. *Crit Rev Immunol* 1999;19:117-26.
19. Spronk PE, Bootsma H, Kallenberg CG. Anti-DNA antibodies as early predictor for disease exacerbations in SLE. Guidelines for treatment? *Clin Rev Allergy Immunol* 1998;16:211-8.
20. Xavier RM, Yamauchi Y, Tanigawa Y, Ishikura T, Kobayashi S.
21. Antinuclear antibodies in healthy aging people: a prospective study. *Mech Ageing Dev* 1995;78:145-54.
22. Jitsukawa T, Nakajima S, Usui J, Watanabe H. Detection of anti-nuclear antibodies from patients with systemic rheumatic diseases by ELISA using Hep-2 cell nuclei. *J Clin Lab Anal* 1991;5:49-53.
23. Alem M, Moghadam S, Malki J, Zaid A, Nayak N, Li T. Detection of autoantibodies to nuclear antigens by EIA and IF techniques. *Allerg Immunol (Paris)* 1997;29:191-4.