The Incidence of ANA and ETI-dsDNA Detected by Enzyme Immunoassays and Indirect Immunofluorescence Assay (IFA)

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ORIGINAL PAPER

SUMMARY

While the SLE (Systemic Lupus Erythematosus) specificity of ANA is low, that of anti-dsDNA autoantibodies is high. The DNA used in the assay must be double stranded: autoantibodies to single –stranded (ss) DNA exist in many diseases and specific to none. The prevalence (70%) of anti-dsDNA autoantibodies is much higher in SLE, giving a higher diagnostic sensitivity than the similarly disease-specific anti-Sm autoantibodies (30%). Anti-dsDNA autoantibodies are usually detected by very analytically sensitive techniques, such as ELISA (Enzyme Linked Immunosorbent Assay). Within SLE, ds-DNA autoantibodies tend to associate with the presence of glomerulonephritis. Their levels are used to monitor disease activity. We suggest the use of ds-DNA to find the difference between SLE patients with benign variants and classical syndrome of severe skin and renal disease.

Keywords: SLE, systemic lupus erythematosus, ANA, anti-nuclear antibodies, ds-DNA, double-stranded DNA antibodies, IFA, immunofloures-cence assay, ELISA, enzyme linked immunosor-bent assay

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1. INTRODUCTION

The sera-immunological hallmark of SLE is anti-nuclear antibody (ANA). In the absence of ANA, the diagnosis of SLE is put into question. So, the ANA is very sensitive test for SLE, being present in virtually all patients and frequently at high titers. Its disease specificity is relatively low since it is frequently found in other rheumatic diseases, as well as in autoimmune liver disease, during viral infection, and , occasionally, at low titers, in normal subjects. It has tendency to increase in prevalence with age in healthy adults.

While the SLE (Systemic Lupus Erythematosus) specificity of ANA is low, that of anti-dsDNA autoantibodies is high. The DNA used in the assay must be double stranded: autoantibodies to single –stranded (ss) DNA exist in many diseases and specific to none (1). The prevalence (70%) of anti-dsDNA autoantibodies is much higher in SLE, giving a higher diagnostic sensitivity than the similarly disease-specific anti-Sm autoantibodies (30%). Anti-dsDNA autoantibodies are usually detected by very analytically sensitive techniques, such as ELISA (Enzyme linked immunosorbent assay) (1). Within SLE, ds-DNA autoantibodies tend to associate with the presence of glomerulonephritis. Their levels are used to monitor disease activity.

2. MATERIAL AND METHODS

During the period of a year (January 2008-January 2009), we have analyzed 2132 serum specimens using IF method and 1188 sera specimens using ELISA method for ANA and ds-DNA respectively. The most commonly used method for ANA testing is Immunofluorescence Assay (2, 3). The basic principle of the procedure is using slides with epithelial cells (Hep-2 cells) as substrate that is incubated in few steps with diluted serum. The unbound material is removed by aspirating and washing. The drop of the fluorescence conjugate (anti human IgG fluorescein labeled containing Blue dye and 0.099 sodium azid) is added (4). Depending on the amounts of autoantibodies in specimens, using IF microscope, it is possible to detect different intensity degree of apple-green fluorescence light. Fluorescence grade is determined as: 4+; 3+; 2+ and +. The ELISA kits are solid-phase enzyme immunoassays. Antigen-precoated microplate wells are incubated with calibrators, controls and serum specimens. During the incubation, antibody present in the test sample binds to the coated wells. Horseradish peroxides-conjugated anti-human IgG is incubated in the wells to recognize the autoantibodies bound to the coated wells. Chromogen is added and autoantibodies are measured using a spectrophotometer plate reader. At end of each incubation, the unbound material is removed by aspirating and washing. When ANA is considered the positive result value is above 23 while the positive result value for ds-DNA is above 60. Results in range of 20 to 40 are considered negative, while results in range value of 40 to 60 are considered limited positive.

3. RESULTS

During the period of a year we have analyzed 2132 serum specimens using IF method. 924 specimens have been analyzed for ANA antibodies, while 1208 specimens have been analyzed for ds-DNA. All results were interpreted using determined fluorescence grade as 4+; 3+; 2+; + and negative.

Among those 924 specimens analyzed for ANA antibodies the following result were obtained:

- 74% (683) specimens were negative
- 13% (118) specimens were +
- 9% (84) specimens were 2+
- 3% (29) specimens were 3+
- 1% (10) specimens were 4+.

Results are presented on Graph 1.

The same IF method has been used for ds-DNA detection. 1208 specimens were analyzed for ds-DNA antibodies and following results were obtained:

- 94% (1131) specimens were negative
- 2% (25) specimens were +
- 3% (42) specimens were 2+
- 1% (10) specimens were 3+.

Results are presented on Graph 2.

During the same period 1188 serum specimens were analyzed using ELISA method. 745 serum specimens were analyzed for ANA antibodies and results were interpreted as negative if <23 and positive if >23. Results obtained were:

58% (435) specimens were negative
42% (310) specimens were positive

Results are presented on Graph 3.

For ds-DNA using ELISA method were analyzed 443 serum specimens. Results in range of 20 to 40 are considered negative, while results in range value of 40 to 60 are considered limited positive. Results above 60 were positive.

Results obtained were:

- 68% (299) specimens were negative
- 9% (41) specimens were limited positive
- 23% (103) specimens were positive

Results are presented on Graph 4.

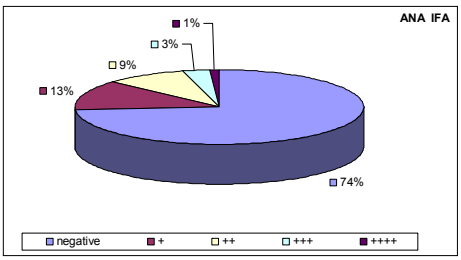
4. DISCUSSION

Immunofluorescence assay of ANA showed 74% negative results and 23% positive, either 2+, 3+ or 4+. ELISA tests for ANA antibodies showed 58% negative results and 42% positive results – that in comparing with results achieved using IF –ANA seems to be large number of positive patients, but considering practicing and quality of ELISA it could be expected. Some studies demonstrated that the commercially available ANA-EIA kits show different levels of sensitivity and specificity (4, 5). On the other hand, at increased temperatures dsDNA denaturizes into ss-DNA that results in false negative results using IF method (6, 7). That is the reason why ELISA should be used as confirmation method in those cases. Positive ANA test is not specific for SLE and can be associated with many illnesses as: RA, Sjorgen's syndrome, scleroderma and infectious diseases as mononucleosis, autoimmune thyroid and liver disease. Furthermore, certain medications can cause positive ANA; many healthy people with no expressed illness have a positive ANA (4, 8).

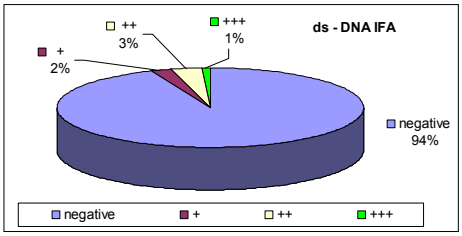
Antibodies to dsDNA (anti-ds-DNA, dsDNA-Ab) are frequently found in systemic lupus erythematosus, especially during active disease and differ with respect to immunoglobulin classes and avidity (9). Immunofluorescence assay of dsDNA showed 94% negative specimens and 6% of positive specimens either +, 2+ or 3+. Using ELISA method 68% of specimens were negative, 9% were bordered positive and 23% were positive. Detection of anti-dsDNA may precede the diagnosis of SLE by more than a year. Fluctuations in the level of anti-ds-DNA in an individual patient may give important information on the clinical status of the patient (9).

Most of commercial ELISA test systems have great advantages in routine laboratory testing but often detect dsDNA-Ab which are not specific for SLE and therefore give false positive results for non-SLE patients (10, 11).

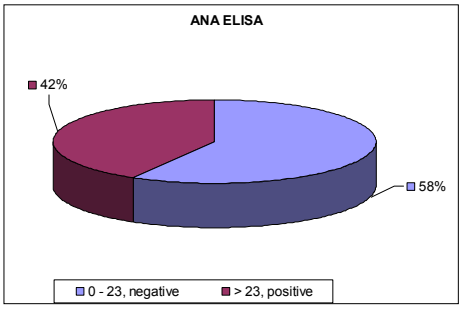
Anti-dsDNA antibody test incorporated in criteria for the classification of SLE needs updating to reflect current insights and technical achievements, including allowance for the presence of non-pathological anti-dsDNA antibodies (12). Anti-DNA of low avidity occurs in rheumatic diseases other than SLE as well, making detection of such antibodies of less diagnostic value (13). It has often been tried to discriminate between clinical subsets of this heterogeneous disease by studying differences within the population of anti-dsDNA antibodies (14). Immunospecificity, complement-fixing ability, avidity, immunoglobulin (sub) class composition have all been the subject of different studies, yet conclusions are contradictory and it has not been elucidated (14, 15).



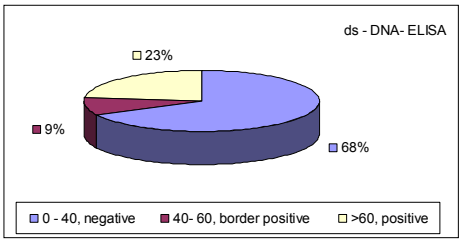
GRAPH 1. Detection of ANA-antibodies using IFA method



GRAPH 2. Detection of Ds-DNA using IFA method



GRAPH 3. Detection of ANA-antibodies using ELISA method



GRAPH 4. Detection of Ds-DNA antibodies using ELISA method

5. CONCLUSION

The most precise method used for SLE detection and keeping up with petition status is ELISA anti-dsDNA, still needs updating. Furthermore, in the presence of antibodies ANA should no longer be considered a valid criterion.

This strategy might ultimately facilitate the difference between SLE patients with benign disease variants and classical syndrome of severe skin and renal disease in pathogenic anti-ds-DNA antibodies.

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