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Prevalence and Factors Associated with Positivity of Antinuclear Antibodies (ANA) Patterns, Native Anti-DNA and Extractable Nuclear Antigens (ENA) Antibodies: Experience from a Laboratory in Dakar

Diop Abdou[1], Diallo Thierno Abdoulaye[1], Ndiaye Babacar[1], Mahou Chantal[1], Diop Marième Gaye[1], Dubrous Phillippe[1], Seck Abdoulaye[1,2]

[1]Medical Biology Laboratory, Pasteur Institute of Dakar, Dakar, Senegal
[2]Cheikh Anta Diop University of Dakar, Dakar, Senegal
Email: diopabdou03@yahoo.fr, babacar.ndiaye@pasteur.sn, thierno.diallo@pasteur.sn, chantal.mahou@pasteur.sn, phillippe.dubrous@pasteur.sn, mariemegaye.diop@pasteur.sn, abdoulaye.seck@pasteur.sn

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Abstract

Background: Diagnosis of autoimmune diseases (AID) is challenging, due to overlapping features with other non-immune disorders. Anti-nuclear antibodies (ANA) are sensitive screening tests but anti-deoxyribonucleic acid-antibody (anti-DNA), and anti-extractable nuclear antigens (anti-ENA) are specific for AIDs. We aimed to look at ANA patterns in our patients and correlated them with anti-ENA for proper interpretation and better patient management cost-effectively. **Methods:** A retrospective study was conducted over 1 year from January to December 2022 who were tested for ANA at biology medical laboratory of Pasteur Institute of Dakar. Anti-ENA and anti-DNA results were also analyzed for ANA-positive patients. Statistical analysis was performed using STATA 14.0, $p < 0.05$ was considered statistically significant. **Results:** 216 patients were analyzed. Women predominated at 79.2% and mean age was 48 years [CI 95%, 46 - 50], with extremes of 10 and 89. Most represented age group was [41 - 60] with 38%. ANA was positive in 27 (12.5%) of patients, 59.2% of whom were strongly positive (titer of 1/1000, 1/3200 or 1/6400). The most common pattern was nuclear speckled, which was found in 77.8% of samples. Anti-ENA and anti-DNA positivity in ANA-positive patients was found respectively in 63% (17/27) and 1.4% (3/27) of the samples analyzed. Most commonly identified anti-ENA was anti-Sm 29.6%, anti-SSA 29.6%, anti-Ro-52 25.9%, anti-RNP 18.5% and anti-SSB 14.8% which was associated with speckled pattern. Association results indi-

cated a significant relationship between both tests and between ANA titer in the anti-ENA- and ANA-positive patients ($p < 0.001$). **Conclusions:** ANA, Anti-ENA and anti-DNA antibodies are essential for AIDS diagnosis. However, the testing repertoire should follow an algorithm comprising of clinical features, followed by ANA results with nuclear, mitotic, and cytoplasmic patterns, anti-ENA, and anti-DNA for a more meaningful, and cost-effective diagnostic approach.

Keywords

Antinuclear Antibodies, Extractable Nuclear antigen, Autoimmune Disease, Indirect Immunofluorescence

1. Introduction

Multiple factors cause autoimmune diseases (AID) and involve a wide variety of genes and environmental factors, such as stress, age, sex, hormones, and infection exposure [1]. Autoimmune diseases are characterized by auto aggression of the immune system against constitutive antigens of an individual via the production of autoantibodies, which exhibit clinical significance when associated with other disease manifestations [2] [3]. They are rare diseases, affecting 5% to 10% of the world's population (rare diseases being defined by a frequency of occurrence of less than 1/2000). In 80% of cases, these pathologies are predominantly female. They are the third leading cause of morbidity in developed countries, after cardiovascular disease and cancer. There are currently around 80 autoimmune diseases. Epidemiological values that can be found in the literature are extremely variable, underlining important genetic and environmental features [4] [5].

Detection of antibodies against cellular antigens (AACA) in HEp-2 cells, also known as antinuclear antibodies (ANA), using indirect immunofluorescence (IIF) is the methodology of choice for screening and identification of various autoantibodies [6] [7]. ANA assay detects a range of antibodies that react with antigens in the nucleus, nucleolus, cytoplasm, and mitotic cellular apparatus [8]. However, this test should be complemented by the research and identification of autoantibodies and specific autoantigens, many of which exhibit great clinical utility and may play roles as diagnostic markers, prognostic indicators, or for monitoring of autoimmune diseases [9] [10] [11]. In addition, the presence of positive ANA does not necessarily indicate a disease state, because low levels of ANA are detected in 30% of healthy individuals [12] [13].

Most frequent antigens described in autoimmune diseases exhibit a nuclear localization and are called extractable nuclear antigens (ENA) [14]. Anti-ENA research is used to identify a group of specific autoantibodies, including anti-SSA/Ro, anti-SSB/La, anti-RNP, anti-Sm, anti-Scl-70, anti-Jo-1, anti-CENP-B, anti-NUC, and anti-DNA.

These autoantibodies are detected using several methodologies, such as im-

munoblot, counter-immunoelectrophoresis, immunodiffusion, enzyme-linked immunosorbent assay (ELISA), and hemagglutination. However, variations in results can be found because these techniques differ in sensitivity and specificity [15] [16] [17]. DNA as an antigen is not included as ENA by some authors, because anti-DNA test is commonly performed with another methodology (IIF with Crithidia as antigen). There is no clear consensus on the prevalence of AID in Senegal, as the epidemiology of AID is not accurately documented in this region. As such, it is crucial to conduct further research on the disease to accurately determine its epidemiology. The aim of this work was to document autoimmune serological profiles of patients suspected of having autoimmune diseases by precisely determining frequencies of ANA, anti-DNA and anti-ENA antibodies in these patients.

Secondarily correlation between ANA tests, titles obtained with the positivity of anti-ENA-ECT was studied.

2. Material and Methods

It was a descriptive and retrospective study, carried out on a retrospective setup over 1 year from January to December 2022 at medical biology laboratory of Pasteur Institute of Dakar. Patients referred to our laboratory by their doctors for immunofluorescence detection for ANA, anti-native DNA and anti-ENA-ECT were included in the study.

2.1. ANA and Anti-ENA Detection by Immunofluorescence Technique

Assessment of ANA and anti-DNA patterns and titers was carried out with EUROIMMUN Medizinische Labordiagnostika AG (Lübeck, Germany), which is a semi-automated high-throughput system. The kit consists of glass microscope slides that are coated with tissue sections or HEp-2 cells. HEp-2 is a human epithelium cell, cultivated from the tissue of a patient suffering from carcinoma of the larynx. After the dilution of samples, conjugation with fluorescein-labeled antihuman antibody conjugate was done. A specific green-colored, fluorescent staining pattern of antigen-antibody complexes was visualized with the aid of a fluorescent microscope under 10× and 40× objectives. The slides were evaluated in comparison with positive and negative controls provided in the manufacturer kit. Qualified laboratory consultants assessed these slides. A titer of ≥1:100 was used as a cutoff for ANA positivity as recommended by the manufacturer of this HEp assay.

2.2. Detection of Anti-ENA Antibody Types

Qualitative determination of human autoantibodies of the immunoglobulin class IgG in serum or plasma was carried out using the EUROLINE® Immunoblot EUROIMMUN Medizinische Labordiagnostika AG (Lübeck, Germany) for specific autoantibodies, which include anti-Smith (Sm) antigen,

anti-ribonucleoprotein (RNP), anti-Sjogren's syndrome type A (SSA), anti-recombinant Ro52 (Ro52), anti-Sjogren's syndrome type B (SSB), anti-scleroderma/topoisomerase (Scl-70) and anti-histidyl-tRNA (Jo1).

2.3. Data Analysis

Patients were classified according to age, gender, anti-ENA and ANA test results and ANA titer. An association between ANA and anti-ENA tests, the relationship between ANA titer and anti-ENA positivity were tested for anti-ENA positive patients was investigated.

Data were analyzed using STATA version 14.0 (STATA Corp, USA). For categorical data, a chi-square test was done. A p-value of less than 0.05 was taken as significant.

3. Results

Overall, 216 patients were analyzed. Women predominated at 79.2% (4 women for 1 man). The mean age was 48 years [CI 95%, 46.11 - 50.73], with extremes of 10 and 89. The most representative age groups were [41 - 60] with 37.9% (Table 1).

Among these samples, 27 (12.5%) were ANA positive by IIF method. Out of these ANA positives, the proportion of weakly positive (titer of 1/100), moderately positive (titer of 1/320), and strongly positive (titer of 1/1000, 1/3200 or 1/6400) were 14.8%, 26%, and 59.2%, respectively.

Four (4) patterns of nuclear fluorescence were noted. In these fluorescence-positive samples, the speckled pattern was the most common pattern seen in 77.8% of cases, followed by homogenous and nucleolar patterns at 7.4% each, cytoplasmic and nucleolar patterns at 3.7% each. The various ANA patterns seen in the IIF-positive samples are shown in Table 2.

Anti-ENA and anti-DNA positivity in ANA-positive patients was found respectively in 63% (17/27) and 1.4% (3/27) of the samples analyzed. The most commonly identified anti-ENA were anti-Sm 29.6%, anti-SSA 29.6%, anti-Ro-52 25.9%, anti-RNP 18.5%, and anti-SSB 14.8% which was associated with a speckled pattern.

The association results indicated a significant relationship between ANA positivity and ANA titer in anti-ENA-positive patients ($p < 0.001$) by chi-square test (Table 3). Table 3 shows patients with positive ANA tests that most (8/9) anti-ENA-negative patients exhibited a lower (1/100) or intermediate (1/320) titer range. However, Table 3 shows that (15/18) of the samples were obtained from anti-ENA-positive patients in the highest range (titer of 1/1000, 1/3200 or 1/6400).

Table 1. Socio-demographic characteristics of the study population.

	Number	%
Gender		
Women	171	79.2

Continued

Men	45	20.8
Sex ratio M/W	0.78	
Mean age (Extremes)	48 ans [CI 95%, 46.11 - 50.73] (10 - 89 years)	
Age group		
[0 - 20]	12	5.6
[21 - 40]	62	28.7
[41 - 60]	82	37.9
[>60]	60	27.8
Total	216	100

Table 2. ANA positivity and fluorescence staining patterns were observed in this study population.

ANA patterns	Quantity	Proportion	p
Positive	27	12.5	<0 .001
Negative	189	87.5	
Cytoplasmic	1	3.7	
Nuclear Dots	1	3.7	
Homogenous	2	7.4	<0 .001
Speckled	21	77.8	
Nucleolar	2	7.4	

Table 3. Relationship between ANA, ANA titre in positive patients and anti-ENA-ECT.

Characteristics	ENA-ECT					
	Negative	%	Positive	%	Total	p-value
ANA						
Negative	167	77.3	22	10.2	187	p < 0.001
Positive	10	4.6	17	7.9	27	
Total	177	81.9	37	18.1	216	
ANA Titre						
1/6400	0	0.0	1	3.7	1	
1/3200	1	3.7	13	48.1	14	
1/1000	0	0.0	1	3.7	1	p < 0.001
1/320	5	18.5	2	7.4	7	
1/100	3	11.1	1	3.7	4	
Total	9	33.3	18	66.7	27	

4. Discussion

Numerous studies have demonstrated that a positive ANA test is a strong indi-

cator of an autoimmune disease, and this test is a good methodology to extensively screen for autoimmunity. However, progressive and vigorous improvements in the technology of various elements composing assay, including quality of HEp-2 cell slides, fluorescent conjugates, and fluorescence microscopes, have revised this concept [18] [19].

These technological improvements greatly increased test sensitivity and current tests detect antibodies at lower serum levels and less avidity than earlier assays. Therefore, the screening for antibodies against cellular antigens also exhibits a lower specificity [20]. In addition, the prescription of ANA test started to be made by a broad spectrum of medical specialists, which was once primarily prescribed solely by rheumatologists. Therefore, the pretest probability of auto-immunity was high and favored the diagnostic performance of the test [18] [19].

Therefore, chance for positive results in healthy individuals or individuals with less expressive clinical presentations is greater [21] [22] [23]. This increase shows the importance of requesting tests for the identification of specific autoantibodies after receiving a positive ANA test.

Several studies related to ANA prevalence worldwide have been published, but insufficient research has been conducted in this field, especially in our part of the world.

We showed that 79.2% were women and only 28.8% were men. Parks et al [24]. stated that estrogen is a modifier of autoimmunity, and childbearing may have a role in initial antigen stimulation or reducing tolerance to self-antigens. Mean age in our study was 48 [CI 95%, 46.11 - 50.73]. However, there was a difference in mean age worldwide. Studies conducted by Satoh *et al.* [25]. and Prapinjumrune *et al.* [26] reported high prevalence in the older age group, while Guo *et al.* [27], Minz *et al.* [28], and Mengeloglu *et al.* [29] reported prevalence in 32, 42, and 43 years, respectively.

ANA positivity was 12.5%. Different studies from other countries such as Japan by Hayashi *et al.* (9.5%) [30], Turkey by Mengeloglu *et al.* (15.8%) [29] and USA by Satoh *et al.* (13.8%) [25] showed positivity which was comparable with our study. However, few studies have been conducted in India by Gupta *et al.* [31] from Rajpur, Sebastian *et al.* [32] from Bangalore, and Minz *et al.* [28] from Chandigarh, which showed ANA prevalence of 33%, 38.2%, and 18.9%, respectively.

Akmatov *et al.* [33] from Germany and Prapinjumrune *et al.* [26] from Thailand have also reported frequencies of 33% and 39.6%, respectively, higher positivity compared to our study. In this study, the most common pattern was speckled 77.8% followed by homogenous pattern 7.4%, and nucleolar pattern 7.4%. Peene *et al.*, 2001 reported that the most prevalent fluorescence pattern was speckled (42.5%), followed by homogeneous (41.4%) and nucleolar (10.6%) in their study [34]. The speckled pattern, frequently identified ANA pattern in this study showed an association with Sm, RNP, SSA/Ro or SSB/La. Thus, with a speckled pattern, one could project further that the serum had antibodies against anti–Sm, RNP, SSA or SSB. Mutasim and Adams also reported a similar associa-

tion between speckled patterns and various ribonucleoprotein in their study [35].

Regarding anti-ENA antibodies in ANA positive patients, commonest reactivity was against Sm and SSA where 29.6% were positive each, followed by Ro-52 (25.9%), RNP (18.5%) and anti-SSB (14.8%). Reactivity against SSA is the most common finding in patients of AI disorders as determined by Peene *et al.* [34]. Jo-1 and Scl70 were not present in these patients. Studies carried out in China and Japan also reported that anti-Scl-70 was the least common antibody.

Our results showed that of the anti-ENA-positive patients were in the highest ANA titer range (15/18). Our present data are consistent with past studies of laboratory series which showed that overall anti-ENA frequency increased with ANA level (measured using HEp-2 cells), whether reported as titer [36] [37] [38] [39] or as fluorescence intensity [34] [40]. This result is very similar to that reported by Jeong [41], who used an anti-ENA test and found 83.9% positivity in ANA and anti-ENA tests in a cohort of Asian patients with AD. A study in Bangladesh [42] showed autoimmune diseases in 85.5% of double positivity (ENA and ANA tests), working with a dot-blot methodology for ENA detection. This demonstrates that the association of these two tests (ANA for screening and anti-ENA to confirm) is essential for the diagnosis of AD [12]. Anti-ENA-positive with ANA-negative was found in 22 (10.2%) patients, which was unexpected because ANA tends to have a higher sensitivity than immunoblot tests. However, the occurrence is not uncommon [14] [21] [43] and some of these ANA-negative results could be patients in immunosuppressive therapy [44]; a revision of their medical records should be necessary to clarify this point.

There are certain limitations in this study. It is a retrospective study, due to which factors responsible for the causation of autoimmune diseases cannot be identified or studied. It is a pilot study with data not taking into consideration various factors such as genetic predisposition, clinical diagnosis, metabolic disorders, cardiovascular diseases, occupation, or biochemical factors. An extensive and detailed large-scale study is required in the future, especially from Senegal, to determine the relationship of ANA with various etiologic and biochemical factors. However, the strong point of this study is that it provides an overview of ANA positivity and autoimmunity status.

5. Conclusion

Anti-ENA antibodies along with ANA results (ANA titers and patterns) and anti-DNA are required to make a final diagnosis of systemic AID. However, an algorithm should be followed comprising of clinical features, ANA as an initial screening test with nuclear, mitotic and cytoplasmic patterns, anti-ENA, and anti-DNA for a more meaningful, and cost-effective diagnostic approach. In this study, we did not find ANA patterns as a gating strategy to decide on possible anti-ENA reactivity in patients' samples. A future study on a larger cohort of patients including results of anti-ENA in samples with all cellular patterns includ-

ing nuclear, mitotic, and cytoplasmic, and with clinical correlation will be more informative. There is also a need to do a more expanded analysis of clinical features in correlation with autoantibodies and rarer cellular patterns. Accordingly, using more expanded anti-ENA patterns will help us to understand the pathogenesis of the disease for targeted therapies.

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Authors' Contributions

All authors contributed to the drafting of the manuscript. All have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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