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Profile of Autoantibodies and Clinical Symptoms in Guinean Patients with Connective Tissue Diseases

Mariama Sadjo Diallo[1,2,3*#], Doufin Traore[1,2#], Adame Diouf[4], Djiba Kaba[1,2], Issiaga Diallo[1,2], Doudou Georges Massar Niang[4], Alimou Camara[1], Oumar Mouctar Diallo[1,2], Boh Fanta Diane[1,5], Abdoulaye Barry[1,6], Ouo-Ouo Yaramon Kolie[2], Moustapha Mbow[4], Aly Badra Kamissoko[1,6], Djibril Sylla[1,7], Babacar Mbengue[4], Mohamed Cisse[1,5]

[1]Faculté des Sciences et Techniques de la Santé, Université Gamal Abdel Nasser de Conakry, Conakry, Guinée
[2]Laboratoire de Biologie Moléculaire Nestor BANGOURA/Helene LABROUSSE, Hôpital de jour Donka, CHU Donka, Conakry, Guinée
[3]Centre d'Excellence Africain Pour la Prévention et le Contrôle des Maladies Transmissibles, Conakry, Guinée
[4]Laboratoire d'Immunologie, Université Cheikh Anta Diop, Dakar, Sénégal
[5]Service de Dermatologie-Vénérologie, CHU Donka, Conakry, Guinée
[6]Service de Rhumatologie, Hôpital National Ignace Deen, Conakry, Guinée
[7]Service de Médecine Interne, CHU Donka, Conakry, Guinée
Email: *nenettediallo@yahoo.fr

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Abstract

Connective tissue diseases (CTDs) are Autoimmune diseases (AIDs) characterized by the appearance of autoantibodies, which are diagnostic markers. Investigations of these autoantibodies play a major role in the management of several autoimmune diseases. The objective of this study was to describe the profile of anti-ENA antibodies according to the clinical symptoms of mixed CTDs in Conakry teaching Hospital. We performed a cross-sectional study during six months. A total of 20 patients was recruited and we measured antibodies using the ELISA technique. The mean age of our patients was 36.5 years, with a predominance of females. Cutaneous and rheumatological signs were the main clinical manifestations. SLP was the most frequent CTDs; the threshold of ENA antibodies positivity was higher in scleroderma with and SLP. Anti-ENA identification reveals the frequency of anti-SSA (83.33%), anti-U1RNP (66.66%) and anti-histone (50%) antibodies. Antinuclear antibodies (ANA) react with various components of the cell nucleus. Their detection is of major interest in the diagnosis of CTDs. Our results highlight the importance of determining the specificity of these antibodies to guide differential diagnosis.

*Corresponding author.
#Worked as first author.

Keywords

Autoantibodies, Extractible Nuclear Antigen (ENA), Connective Tissue Diseases, ELISA

1. Introduction

Autoimmune diseases (AIDs) affect 5% to 10% of the global population, with a predominance of women [1] [2]. They represent the third leading cause of morbidity in developed countries, after cardiovascular diseases and cancers [3]. Connective tissue diseases (CTDs) are AIDs with the appearance of autoantibodies. In various CTDs, autoantibodies were considered as the primarily diagnostic markers [1] [2]. Mostly detected with indirect immunofluorescence on HEp-2 cells, Autoantibodies were difficultly detected. They do not appear in any CTD diagnostic criteria and depending on geographic factors, several variations were previous described. Many articles have focused on describing relationships between clinical manifestations and antibodies profiles in Caucasian patients. Recently, several publications suggested a real prognostic interest of autoantibodies, especially during inflammatory myopathies, systemic lupus erythematosus (SLE), systemic scleroderma, primary Gougerot-Sjögren's syndrome and so-called mixed CTDs [1] [2]. Although their pathogenic role of autoantibodies is debatable, they are widely explored in management and results need to be interpreted considering the clinical data [4] [5]. The production of autoantibodies is secondary to the activation of auto-reactive B lymphocytes, linked to a disturbance in the self recognition process, and thus to a breakdown in immune tolerance [6]. In addition to immunological factors, genetic, hormonal and environmental factors are thought to be associated with the occurrence of CTDs [7] [8] [9]. The detection of antibodies may precede the appearance of clinical signs and serve as a prognostic factor. In Sub-Saharan Africa, very few studies were published and some particularities can be founded according to.

The general objective of our study was to determine the profile of anti-ENA antibodies following the clinical signs of mixed CTDs, in Guinean patients recruited from the Departments of Dermatology-Venerology, Internal Medicine at the CHU de Donka and Rheumatology at the Hôpital National Ignace Deen in Conakry (Guinea).

2. Material and Methods

2.1. Study Design, Patients, Samples and Recruitment

This work is a prospective, cross-sectional study conducted over 6-month period from April 1 to October 15, 2022 in the Dermatology-Venerology and Internal Medicine departments of the Donka University Hospital and the Rheumatology Department of the Ignace Deen University Hospital in Conakry (Guinea). The study involved patients hospitalized or followed up on an outpatient basis for

autoimmune diseases. We collected 5 ml of blood from each patient and sera were separated and frozen at - 80˚C until the date of analysis. Samples were collected from patients clinically diagnosed for CTDs clinically and biologically confirmed. Patients with clinically detected signs of immune deficiency and infection were not included.

2.2. Ethics Statement and Procedure

This study was performed in the Dermatology-Venerology and Internal Medicine departments of the Donka University Hospital and the Rheumatology Department of the Ignace Deen University Hospital in Conakry (Guinea). In this study, all the Immunological assays were performed in the Immunology Laboratory of the Faculty of Medicine of Cheikh Anta Diop University in Dakar. Sample collection and monitoring were done in collaboration with the clinicians in Conakry. From each participant and/or relatives, informed consent was obtained before inclusion, after providing written or verbal information in their native language. The protocol was approved by the Research Committee of the University Gamal Abdel Nasser (Conakry, Guinea) and performed following the Declaration of Helsinki.

2.3. Antibodies Detection and Identification

Autoantibodies were detected by using two commercially kits from Human® (Berlin, Germany):

- IMTEC-ENA Screen® (for the global detection of extractable anti-nuclear antibodies (ENA) by ELISA for the qualitative determination of autoantibodies, which only detects their presence but does not determine their type. Positive and negative controls were used according to the manufacturer's instructions and in appropriate wells. For each sample, 50 µl per well (duplicate) was added and the plate was incubated under agitation at 400 rpm for one hour. After washing 100 µl of HRP-conjugated detection antibodies were added to each well before 1-hour incubation with agitation at 400 rpm. Tetra-methyl benzidine (TMB) was used as a liquid substrate (100 µl added) and incubated for 30 minutes in the dark, at room temperature before the addition of 100 µl of Stop Solution (2N, H_2SO_4). Absorbance at 450 nm (with a reference wavelength of 620 nm) was measured on a microplate reader (Multiskan FC, Thermo Scientific®). The results are expressed as positive or negative. As recommended by the manufacturer, samples with absorbance ≥ 0.118 are considered as positive using the software Analyzer (Human® Berlin, Germany).
- IMTEC-ENA Profile® (Human® Berlin, Germany) for the simultaneous determination of autoantibodies types: anti-SSA, anti-SSB, anti-Sm, anti-RNP, anti-Scl70, anti-J01 and anti-centromeres by LIA. Line Immuno Assay test is based on the immobilization of purified ENA Ags on nitrocellulose bands. After the incubation with sera, fixation of patients' antibodies on nitrocellu-

lose's band is detected with a secondary antibody conjugated to peroxidase enzyme (Human® Berlin, Germany). The result was read with the scanner associated to the Software Analyzer (Human® Berlin, Germany).

2.4. Data Collection and Statistical Analysis

Clinical and paraclinical data were collected using a pre-established data processing form, completed based on interviews. Data were analyzed with Statview® version 5.1 software. The non-parametric Mann-Whitney and Kruskal-Wallis tests were used for the different comparisons between patients groups. Relationships between variables were assessed using the Spearman rank test. A value was considered statistically significant when $p < 0.05$.

3. Results

3.1. Demographic Characteristics of Study Population

Twenty (20) patients suffering from various Connective tissue diseases were selected. This study involved 4 men and 16 women, *i.e.* an F/M sex ratio of 4. The mean age was 36.5 years with extremes from 14 to 68 years. In addition, 60% of the patients were younger than 40 years of age at the time of diagnosis.

3.2. Clinical Characteristics of Patients

As shown in Table 1, Physical asthenia was the most frequent general sign found in 85% of cases (17 patients), followed by weight loss in 55% (11 patients). Erythema was the main cutaneous-mucosal manifestation in 50% of cases (10 patients), followed by alopecia and photosensitivity with 45% each symptom (9 patients), and the main rheumatological manifestation was polyarthritis with 65% (13 patients).

Table 2 shows that patients presented different types of connective tissue diseases (CTDs), with a predominance of systemic lupus erythematosus (SLE).

Table 1. Repartition of Clinical symptoms in the study population.

Clinical symptoms	Numbers of patients	%
Physical asthenia	17	85
Weight loss	11	55
Alopecia	9	45
Erythema	10	50
Photosensitivity	9	45
Malar rash	8	40
Erosion/ulceration	7	35
Polyarthritis	13	65
Joint stiffness	8	40
Skin sclerosis	1	5

Indeed, seven patients (35%) showed SLE, and Behçet's disease was observed in 20% of the study population. However, some CTDs combinations were found and they concerned: Dermatomyositis associated to SLE, Rheumatoid Arthritis associated to Gougerot-Sjögren syndrome and Sarcoidosis associated to Polyarthritis. For each CTDs combination, we observed 1 patient affected (5%). Moreover, the only case found for sarcoidosis is the one associated to polyarthritis.

3.3. Serological Characteristics of Patients

Table 3 summarizes the main immunological abnormalities and the frequency of the various autoantibodies that we observed in our patients. Among the 20 patients in whom Anti-ENA antibodies were measured using the Screen test, 8 patients were positive, corresponding to 40%. Specifically, the profile test was performed in 6 of the 8 positive patients. The highest rate of antibodies was

Table 2. Distribution of patients according to connective tissue diseases.

Diagnosed CTDs	Numbers of patients	%
Dermatomyositis	1	5
Systemic lupus erythematosus (SLE)	7	35
Behçet's disease	4	20
Rheumatoid Arthritis (RA)	2	10
Scleroderma	1	5
Gougerot-Sjögren syndrome (GSS)	2	10
Dermatomyositis + SLE	1	5
RA + GSS	1	5
Sarcoidosis + Polyarthritis	1	5
Total	20	100

Table 3. Distribution of different specific autoantibodies in six (6) anti-ENA positive patients.

Anti-ENA antibodies	Numbers n = 6	%
SSA	5	83.33
SSB	1	16.66
SmD1	2	33.33
RNP	4	66.66
Histone	3	50
Centromere	3	50
SL70	1	16.66
Jo1	3	50

Legend: SSA (anti–Sjögren's-syndrome-related antigen A), SSB (Anti-Sjögren's syndrome type B), RNP: Ribonucleoprotein, SL70 (anti-topoisomerase I).

Table 4. ENA antibodies responses according to cutaneous and arthritic symptoms.

Symptoms	Total (N = 20)	Negative (N = 12)	Positive (N = 8)	p-value
Erythema	50% (10)	25% (3)	87.5% (7)	0.020
Alopecia	45% (9)	25% (3)	75% (6)	0.065
Ulceration	35% (7)	25% (3)	50% (4)	0.4
Oral aphta	45% (9)	41.6% (5)	50% (4)	>0.9
Skin sclerosis	5.0% (1)	0% (0)	12.5% (1)	0.4
Photosensitivity	45% (9)	41.6% (5)	50% (4)	>0.9
Polyarthritis	65% (13)	75% (9)	50% (4)	0.4
Myalgia	40% (8)	41.6% (5)	37.5% (3)	>0.9

noted for anti-SSA (83.33%), followed by anti-RNP and anti-histone in 66.66% and 50% respectively. Anti-centromere antibodies were found in 3 patients (50%), but only one patient had showed anti-SSB and anti-SL70 antibodies.

3.4. Variation in ENA Antibodies Responses According Cutaneous and Arthritic Symptoms

Table 4 shows that the ENA antibodies response is significantly higher in patients with cutaneous erythema (p = 0.020). Conversely, for the other *cutaneous and arthritic symptoms*, we don't observe a significant variation of ENA antibodies. Furthermore, our results showed a statistical tendance with a high prevalence of alopecia in ENA antibodies positive group.

3.5. Comparison in ENA Autoantibodies According to Clinical Diagnosis

The detection absorbance of ENA autoantibodies was highest in scleroderma with an absorbance of 1.24, followed by systemic lupus with an absorbance of 0.90. Ac responses were stronger in both types of disease (Figure 1).

4. Discussion

Connective tissue diseases (CTDs) are diseases characterized by inflammatory and immunological damage to connective tissue. They are autoimmune affections presenting a great clinical and biological variability and characterized by the appearance of autoantibodies which constitute diagnostic markers. The aim of this study was to determine the profile of anti-NA antibodies following the clinical signs of mixed CTDs, in Guinean patients from the Dermatology-Venerology and Internal Medicine Departments of the CHU de Donka and the Rheumatology Department of the Hôpital National Ignace Deen in Conakry (Guinea).

The limitation of this study was its small sample size. Two explanations can be

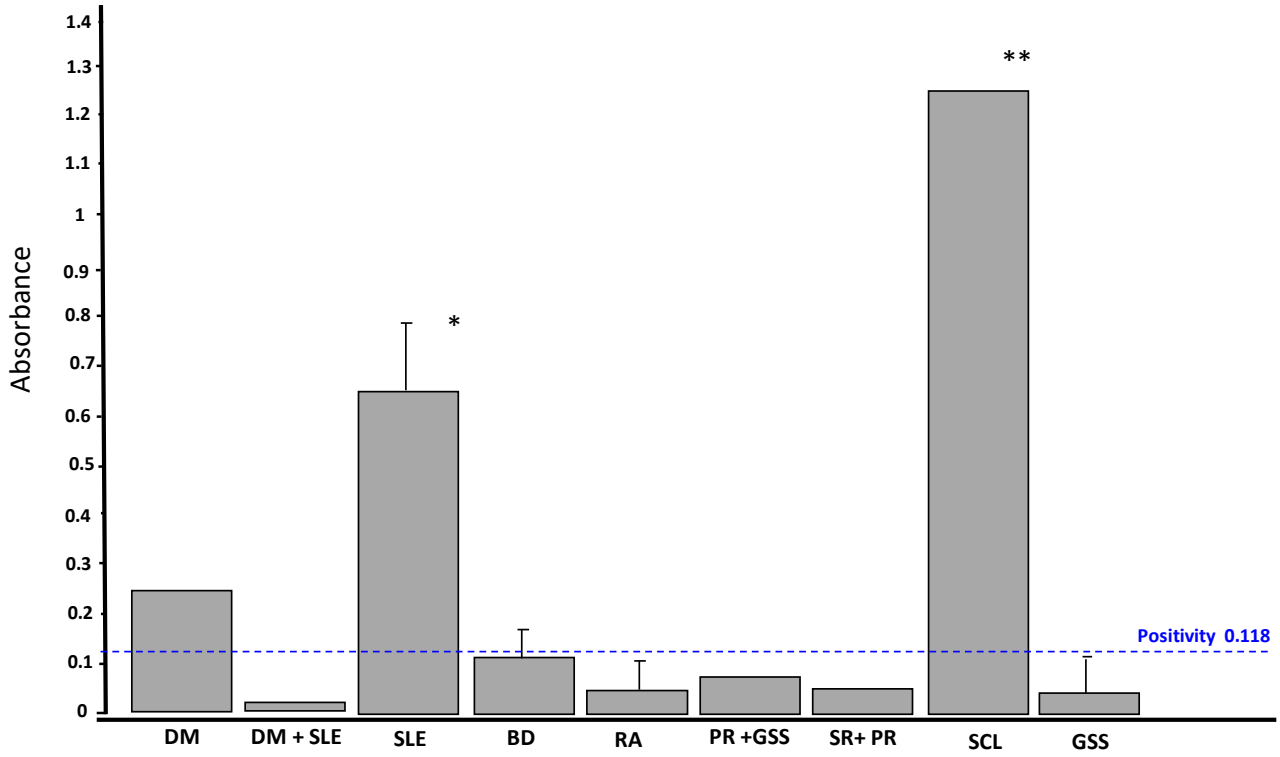

Figure 1. Variations in ENA absorbance according to clinical diagnosis.

provided, firstly this work is a dynamic study and secondly the CTDs are uncommon autoimmune diseases affecting 5% to 10% of the global population.

We collected 20 patients with clinically suspected CTDs. Blood samples were taken, and isolated sera analyzed at the Immunology Laboratory of the Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University, Dakar, Senegal.

We observed a predominance of women, with a sex ratio (F/H) of 4. This predominance was previously reported in several studies [10] [11] [12] [13], and can be considered as a confirmation that women are more affected by autoimmune diseases related to hormonal factors. Classically, the role of sex hormones in the immune response is described as a result of various interaction between oestrogens and nuclear receptors expressed by immune cells [10] [11] [12] [13].

As reported is previous studies, CTDs are complex and various diseases found in young adult, with mean ages of 37.28 and 37.66 years [14] [15]. These mean ages are similar to our finding: 36.5 years.

In our patients, rheumatological symptoms are the most frequent clinical manifestations (65%), followed by erythema (50%). These results confirm the clinical polymorphism of CTDs as previously described in common studies [11] [16]. However, compared to results from Abidjan [15], we found high prevalence (44.4% versus 65%).

SLE is the most represented CTDs (35%) in the present work. We observed 7 cases of SLE all of them are women. This prevalence of SLE is lower than that described by Diousse P. *et al*. in Senegal, who reported 65.2% SLE. The differ-

ence between results can be related to Senegalese study was carried out over 7 years compared to the duration of our study (6 month), with a large sample size than ours (20 samples). In addition, the predominance of SLE in CTDs has been widely reported in several studies [15] [17] [18].

Eight patients (40%) were positive for anti-nuclear antibodies (ENA). Among ENA, anti-SSA antibodies appear as the most abundant autoantibodies (83.3%). This result is comparable to that reported by Michel K *et al.* Thus, they found that anti-SSA was the most expressed antibody [15]. For anti-RNP antibodies, 66.66% of our patients tested are positive, a similar result was reported in our previous study [10]. The high rate of anti-RNP antibodies may be explained by the fact that they are specific for Sharp's syndrome (a CTDs combination), which may initially be mistaken for SLE. The prevalence of anti-SSB antibodies was 16.33%, close to that reported by Haddouk *et al.* 14.3% [19]. In our series, the threshold of ENA positivity is higher in scleroderma followed by SLE. This could be explained by a delay in diagnosis, as the course is often progressive and chronic. The Antibodies is more prevalent in SLE patients than the other cases of CTDs, which may be explained by the existence of epitope spreading in SLE.

5. Conclusion

This study confirms the predominance of CTDs in the young women with mean age of 36.5. The main clinical symptoms were Rheumatological and mucocutaneous manifestations. SLE was the most frequent CTDs with high expression of anti-SSA and anti-RNP antibodies. For the future, investigations in more population can provide data to confirm our results, this proposed study will by conducted according to genetic, environmental and clinical factors.

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Authors' Contribution

Contributed to data collection: MSD, DT, DK, OMD, DS, ABK, AB, OYK, ID, AC.

Contributed to participant recruitment and evaluation: DT, MSD, AD, DGMN, BM.

Contributed to study design: MSD, BM, DT, DK, MC.

Contributed to data analysis: MSD, DT, DK, DGMN, BM.

Wrote the paper: MSD, DT, DK, ID, BM.

Contributed to editing the paper: All. All authors have read and agreed to the published version of the manuscript.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. The

protocol was approved by the R.C of the University Gamal Abdel Nasser (Conakry, Guinea) and performed following the Declaration of Helsinki.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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