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Research Paper

Descriptive profile of anti-nuclear antibodies in the Fez region-Morocco: Experience of the Immunology Laboratory- Hassan II University Hospital

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ABSTRACT: *Introduction: Anti-nuclear antibodies (ANAs) are a group of antibodies directed against a wide variety of autoantigens, mainly against the nucleus. Their aspect and titer make them useful for the diagnosis and prognostic of autoimmune diseases (AIDs). Their detection is based on multiple techniques, in which indirect immunofluorescence (IIF) on HEp-2 cells remains the recommended reference technique. In this context, the aim of this study is to describe the profile of ANAs in the region of Fez, Morocco.*
Materials and methods: *Retrospective study of sample serums for ANAs analysis sent to the Immunology Department, Central Laboratory of Medical Analysis, Hassan II University Hospital, Fez, was done over a 20-month period.* ***Results:*** *This study included Eight hundred and twenty-one patients. The average age in our series was 38.5 ±19.8 years. We observed a female predominance with a sex ratio of F/M=2.6. ANAs were positive in 21.8% of cases, with 1/160 being the most frequent titer (76.7%). The speckled pattern represented the highest percentage (88.5%). The identification of antigenic targets showed a positivity of 20.83% for anti-SSA/Ro antibodies.* ***Conclusion****: ANAs are an important test for any suspected connectivity. The result must be interpreted according to the clinical context, hence the need for a good collaboration between the prescribing physician and the Immunologist.*
KEYWORDS: *Anti-nuclear antibody; Indirect immunofluorescence; Autoimmune disease.*

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I. INTRODUCTION

Anti-nuclear antibodies (ANAs) are a group of auto-antibodies directed against a wide variety of autoantigens, mainly against the nucleus (nucleic acids, proteins and nucleoprotein complexes). The presence of ANAs is non-specific and may be related to many non-autoimmune affections, such as cancer, infection, drugs, certain treatments, etc. As a result, an ANAs positive frequency in healthy population in more than 20% has been reported [1]. Although, a higher ANAs titer is strongly associated with higher risk of emergence of autoimmune diseases (AIDs)[1,2,3].The detection of ANAs is based on several techniques, in which indirect immunofluorescence (IIF) remains the most recommended reference technique [4]. The fluorescence pattern and titer of ANAs represent a diagnosis and prognosis element in AIDs [4]. The interpretation of ANAs result should be based on the clinical and biological context of the patient [5]. In this context, the aim of this study is to describe the profile of ANAs in the region of Fez, Morocco.

II. MATERIALS AND METHODS

This is a monocentric retrospective study, with a descriptive and analytical aim on requests for ANAs analysis addressed to the Immunology Department, Central Laboratory of Medical Analysis, Hassan II University Hospital of Fez, done over a period of 20 months. The ANAs screening was performed by the IIF technique on Hep2 cells. If it is positive, the antigenic targets are determined based on the aspect noticed at the IIF, by an immuno-enzymatic technique (ELISA), and/or by an Immunodot technique, according to the instructions of the suppliers (BIORAD, D-tek).

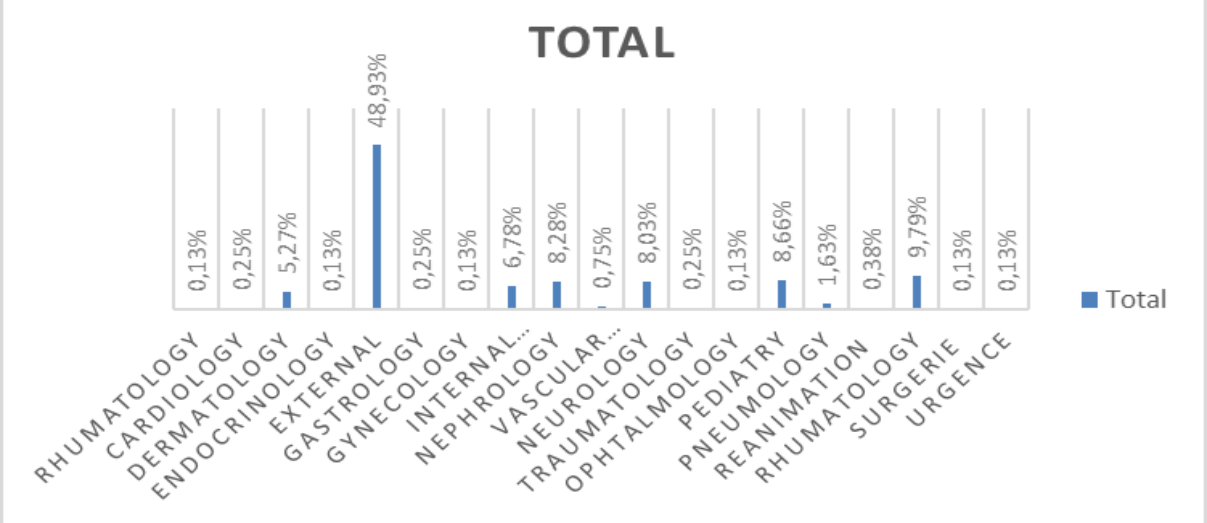
Patient's data (age, sex, prescribing department, clinical information, requested examinations) was collected and saved in a Microsoft EXCEL database.

Statistical analysis was performed using SPSS version 26 software at the Epidemiology Laboratory of the Faculty of Medicine, Dental and Pharmacy of Fez.

The results are presented as an average ± standard deviation for quantitative variables, and proportions for qualitative variables, and illustrated in tables.

III. RESULT

The mean age of our series was 38.5 ± 19.8 years, with a sex ratio F/M = 2.6. The patients in our series are followed up on in consultation at the department depicted in graph 1.

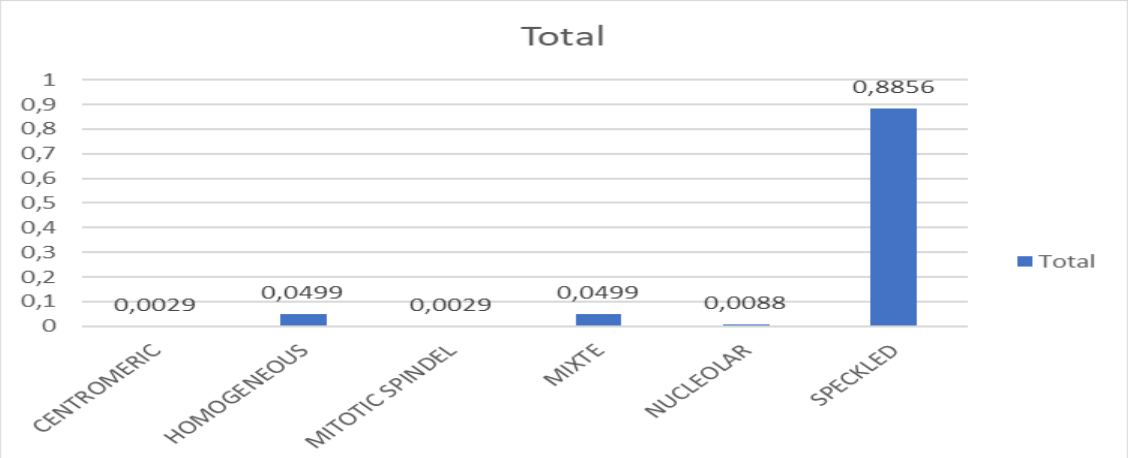


Graph 1: Distribution of patients according to department.

ANAs were positive in 21.8% of cases, of which 1/160 was the most frequent titer (76.7%). The speckled pattern represented 88.5%, the mixt and homogeneous patterns 4,9% (Table 1,2 and Graph 2).

Table 1: IIF results of ANAs in our series.

Results	Frequence	Percent
Negative	464	56,5%
Positive	179	21,8%
Weak positive	178	21,7%



Graph 2 : Results of the different aspects of ANAs at the IIF in our series.

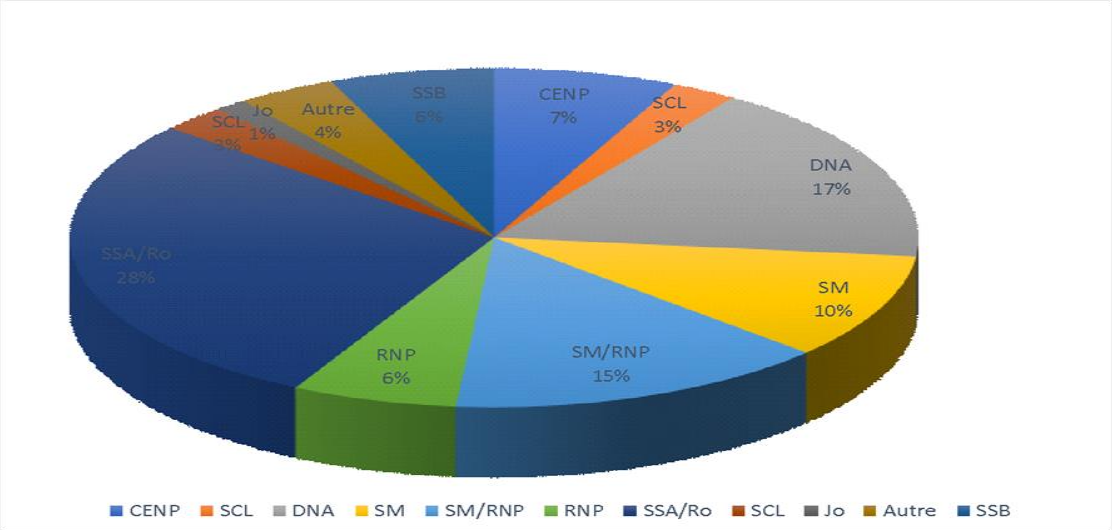
Table 2 : Results of the distribution of ANAs titer at the IIF in our series.

Titer	Frequence	Percent
1/160	244	76,7%
1/320	32	10,1%
1/640	20	6,3%
1/1280	22	6,9%
Total	318	100%

Anti-SSA/Ro was detected in 20.83% of patients, with anti-SSA 60 kDa specificity positive in 60% of cases and anti-SSA 52 kDa specificity positive in 40% of cases. These antibodies were associated with Lupus in 18.2% and with SGS in 27%. However, anti-SSB was positive in 11.3% of cases. These antibodies are associated with anti-SSA/Ro antibodies in 70% of cases.

In our series, anti-DNA was positive in 17% of cases. These antibodies were identified in 42.85% of Lupus patients. Anti-Sm was positive in 18.1%, anti-RNP in 12.5%, and anti-SM/RNP in 26.1%, they were associated with Lupus in 39% of cases and with Scleroderma in 21% of cases.

Anti-CENP-A and anti-CENP-B antibodies were discovered in 5 cases, anti-CENP-B isolated in 4 cases, and anti-CENP-A/-B antibodies were observed in 2 cases. Anti-SCL were found in 5 of the cases. In 60% of cases, they were linked to Scleroderma (Graph 3).



Graph 3: Distribution of antigenic targets identified by Immunodot.

VI. DISCUSSION

Antinuclear antibody testing is required for individuals with clinical symptoms of systemic inflammatory autoimmune disease [6]. The aim of this study is to describe the profile of ANAs in the region of Fez, Morocco.

The prevalence of ANAs positive on Hep-2 cells in this study was 21.8% and less than those obtained in Rabat-Morocco (34.2%) by Missoum et al. [7]. It is also lower than that reported by Koubi et al. [8] who reported a positivity of 22.3% in Marseille (France). Furthermore, our results are higher than those of Satoh et al. [9] which estimated the prevalence of ANAs to be 13.8% among 4758 participants in the US. Eleven percent of patients presenting to an obstetric gynecology service in Bloemfontein, South Africa, were positive according to the study of Afman et al. [10].

In our study, women are the most affected and this is consistent with the literature. Guo et al [2] indicate that the ratio of females to males in the ANAs positive is almost 4:1, except in the 80-year-old group and in girls (8.2%) was higher than in boys (2.5%). Similarly, it was higher in women than in men (17.8 % vs 9.6 %) in the US [9]. This ratio (F vs. M) was 17.8% vs. 9.6% in the US. Furthermore, the gender difference in the prevalence of ANAs observed in our study may support the role of hormones in the pathogenesis of AIDs in women [8,11]. Indeed, the occurrence of some AIDs flare-ups has been described during pregnancy, peri- and postpartum and with the use of estrogen-progestin contraceptives [12-15].

In our series, the ANAs screening predominated in patients with an average age of 38.5 years. According to previous studies, a study carried out in Rabat-Morocco[16] found an age range of 20-30, 44.5 years was the average age in Tunisia [16], between 40 and 49 years in the USA [9], and a range of 20-30 and 40-50 years in China [2].

The fluorescence pattern is suggestive of specific antibodies. The present study shows the predominance of the speckled pattern, which is in agreement with a study conducted in Marrakech in 2018, that revealed a prevalence of 55.6%. This predominance may be proportional to the frequency of Lupus and GSS in the study population which is related to the predominance of ANAs antibodies [18, 19].

The amount of ANAs is important, indeed, a correlation between high amounts and the presence of AIDs was noted. In our series, the group of patients with an ANAs titer of 1/160 was the most frequent (76.7%), this result is close to those of Kang et al. [20] where a titer of (1/640) was important but their percentage in the study group was 33%. While Feki et al. [17] report the titer of 1/320 as the most abundant.

In our series, anti-SSA/Ro were, in the majority of cases, associated with Lupus and GSS, which agrees with the literature [21,22] These antibodies can be associated with Scleroderma in 20% of cases [15], with rheumatoid arthritis and polymyositis in 10% of cases [19-21]. These figures tend to increase in recent decades, perhaps with the biotechnical developments.

Early studies of anti-SSA/Ro antibodies noted a frequent association of the two antigenic specificities in Lupus and GSS, with a significant association between the diagnosis of GSS and the presence of isolated anti-SSA 52 kDa antibodies on the one hand, and the diagnosis of Lupus and the existence of isolated anti-SSA 60 kDa antibodies on the other hand [14,23,24] then it appeared that, depending on the techniques used, and on the populations studied, the results had to be nuanced [19–25]. Our results are close to these last authors with a predominance of anti-SSA antibodies 60 kDa during GSS. Moreover, the positivity of anti-SSA 52 kDa antibodies in this work are different from those of the authors mentioned above [19–25], nevertheless is in agrees with Saint Clair [26] and Tsuzaka [19] related to the association with Lupus and SGS,

In our work, the prevalence of these anti-DNA antibodies was 17%. These antibodies are specific for Lupus [2, 27]. Anti-Sm autoantibodies were present in 18.1% of cases and are very specific for Lupus. This result is in agreement with the literature where anti-Sm antibodies are detected in 5-30% of lupus patients [28,19]. We also found that anti-RNP antibodies were positive in 12.5% of cases. This percentage is slightly lower (9.02%) in the investigated series of Chakar [16].

Anti-RNPs are associated with Lupus in 85%of cases [29]. In our series, these autoantibodies were present in 39% of Lupus patients. These results are in agreement with Margaux and Co. [29].

In this study, anti-Sm was present in 18.1% of cases. These auto Abs are very specific for Lupus. This result is in agreement with the literature which reports that anti-Sm is detected in 5-30% of Lupus patients [30].

In the first studies [31-36], CENP antibodies emerged as a marker of CREST syndrome, a mild form of Sclerosis. The prevalence of these antibodies varies from one series to another, it is all the higher the more systemic scleroderma is localized (57-82% of CREST syndromes) [37]. However, these antibodies are found more rarely in other connective tissue diseases, which gives them an important diagnostic value. They are associated with the risk of pulmonary arterial hypertension (PAH). In a short consecutive series, Gonzalez et al. [37] found 100% oesophageal involvement in patients with positive CENP antibodies. In fact, the presence of CENP antibodies in a patient with systemic sclerosis was considered a carcinogenic risk factor [39].

V.CONCLUSION

Antinuclear antibodies are essential immunological markers of certain AIDs, mainly of connectivity. The interpretation of the result of their search must be done according to the clinical context, hence the need for collaboration between the prescribing physician and the Immunologist.

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