Research Paper

Soluble Antigen Antibodies Detected by Immunodot and Autoimmune Pathologies Associated: The experience of the Immunology Laboratory, University Hospital Hassan II, Fez

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ABSTRACT *Background: Soluble anti-antigen antibodies (Anti-ENA) represent a specificity of anti-nuclear auto-antibodies (ANAs) which are directed against nuclear particles. The aim of this study is to retrospectively analyse the positive serum for Anti-ENA antibodies and the pathologies associated with them. Materials and methods: During a 2 years period, 821 serum samples, addressed for the ANAs test, were studied in the Immunology department. The indirect immunofluorescence method (IFI) was used to detect the ANAs in the serums. In case of a positive result, an identification of the antigenic targets is performed by the Immunodot technique. Results: Eighty-eight positive serum for anti-ENA antibodies were found. The average age in our series was 41 ±15.5 years. There were 80 women and 8 men with a sex ratio F/M of 10. The results show a strong association of the anti-ENA antibodies positivity with Lupus and Gougerot-Sjögren syndrome (GSS). Conclusion: Anti-ENA antibodies are immunological markers for Lupus and GSS.*
KEYWORDS: Anti-ENA antibodies; Gougerot-Sjögren syndrome; Lupus

I. INTRODUCTION

Anti-ENA antibodies are a variety of anti-nuclear antibodies (ANAs) directed against antigenic epitopes composed of small RNA and proteins [1]. These autoantibodies are found in certain autoimmune diseases (ADs), most frequently in Systemic Lupus Erythematosus (SLE) and Gougerot-Sjögren syndrome (GSS) [1-2]. The detection of these diseases is based on a screening strategy which starts with an initial search by indirect immunofluorescence (IFI). In case of positive screening, an identification of antigenic targets should be performed. This study's aim is analysing retrospectively positive serums for anti-ENA antibodies and the pathologies associated with them.

II. MATERIALS AND METHODS

We measured ANAs in 821 patients. The study was conducted up on a sample of a serums received for anti-nuclear antibodies analysis at the Immunology Department, Central Laboratory of Medical Analysis, University Hospital Hassan II - Fez, Morocco. ANAs detection was realised by IFI method on HEp-2 cell lines, followed by an identification of antigenic targets using Immunodot technique according to the manufacturer's protocol (D-tek).

The data was collected and analysed by SPSS version 26 software at the Epidemiology Laboratory of the Faculty of Medicine, Dentistry and Pharmacy of Fez. The results were expressed as an average ± standard deviation for the quantitative variables, percentages for the quantitative variables, and illustrated by tables and graphs.
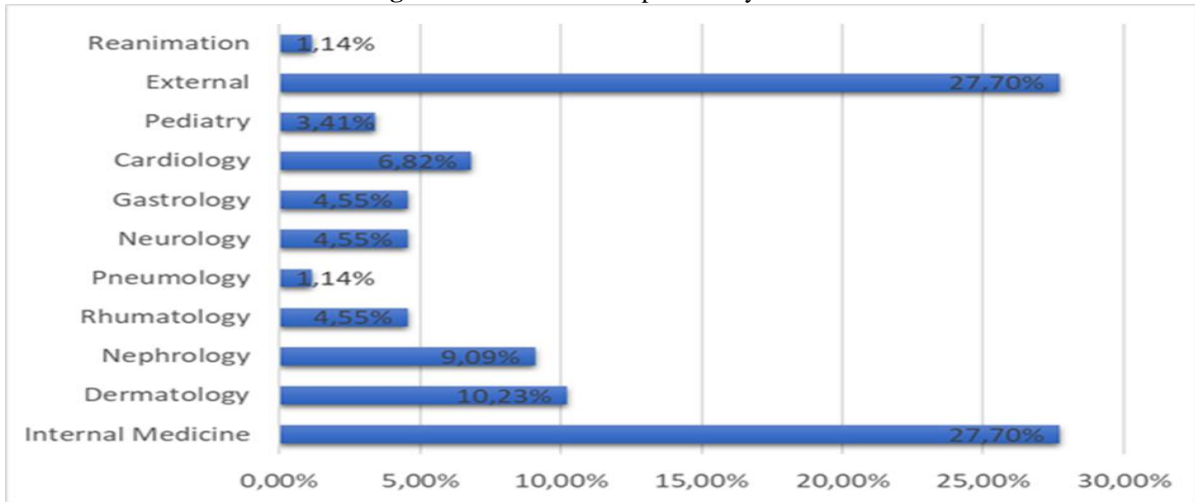
III.RESULTS

Of all the suspected cases for anti-nuclear antibodies, eighty-eight serums containing anti-ENA antibodies were found. The average age in our series was 41 ± 15.6 years. There were 80 women and 8 men with a sex ratio F/M of 10 (Table1).

Table 1: Gender distribution of patients.

	Number	Percentage
Women	80	90.9%
Men	8	9.1%

**Figure 1:** Distribution of patients by service.

In our series, indirect immunofluorescence test for ANAs was positive in 21,8% of cases, with a predominance of the speckled appearance (88.5%), followed by the homogeneous appearance (5%), then the nucleolar appearance (1.1%), and And lastly by the centromeric appearance (0.2%).

Anti-Sm antibodies, anti-RNP and anti-Sm/RNP were positive in 18.1%, 12.5%, and 26.1% of cases respectively. Furthermore, positive anti-Sm and anti-RNP antibodies were mainly associated with SLE (39%), followed by scleroderma in 21% of cases. However, 51.1% of positive anti-SSA/Ro antibodies were found, with the presence of SSA/Ro 60 kD specificity in 80% of cases and SSA/Ro 52 kD in 48.8% and both specificities are associated in 26.6% of cases.

Anti-SSA/Ro antibodies were associated in the majority of cases studied with SLE and GSS. Thus, anti-SSB antibodies were positive in 11.3% of the cases and associated with anti-SSA/Ro antibodies in 70% of cases (Figure 2).

Figure 2: Distribution of anti-ENA identified by Immunodot
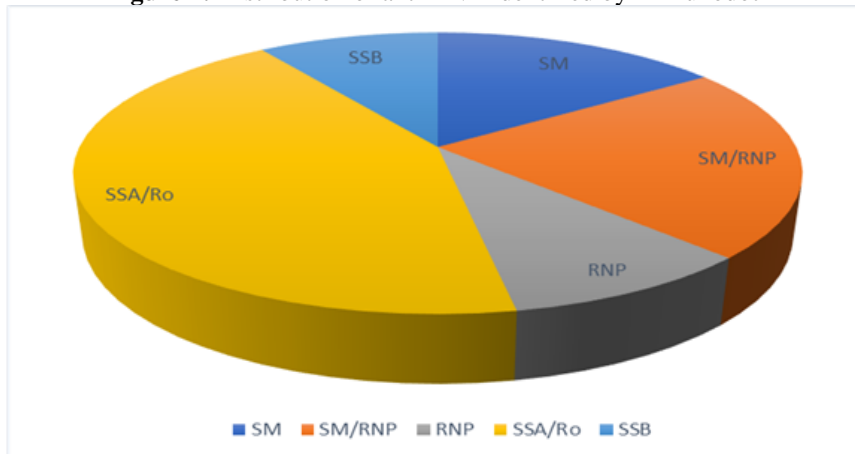
IV. DISCUSSION

In our series, the significant female and average adult age predominance are in agreement with the literature data, which may be related to hormonal factor incrimination in women with a pathophysiology of autoimmune diseases [3]. In fact, various studies have shown this clear predominance of ANAs in women and in 40+ years old [4].

In our study, the search for ANAs on Hep-2 cells was positive in 21.8%, which agrees with several studies [5].
The appearance of fluorescence is suggestive of specific antibodies. Our results show the
predominance of the speckled appearance, which is related to the predominance of anti-ENA
antibodies [4-6].

The amount of ANAs is important, indeed, a correlation between high amounts and the presence of autoimmune disease was noted. In our series, 1/160 was the most frequently observed (76.7%), which is consistent with the literature [7].

Anti-ENA antibodies are diagnostic markers for several non-organ-specific autoimmune diseases principally SLE, Gougerot-Sjögren's syndrome, systemic scleroderma (SSC), CREST syndrome, Sharp's syndrome, polymyositis (SP) and dermatomyositis (DM) [5-8].

Anti-SSA antibodies are associated with autoimmune diseases at variable frequencies depending on the studied populations and the used techniques: 50% with Lupus, 80% with Gougerot-Sjögren's syndrome [11-12], 20% with Scleroderma [11] and about 10% with rheumatoid arthritis and polymyositis [9-10].

In our study, anti-SSA 52 and 60Kd are frequently associated with Lupus and GSS. This association has been reported by previously described works [13-15].

Anti-RNPs are associated with Lupus in 85% of cases [9]. In our series, these autoantibodies were present in 39% of Lupus patients. These results are in agreement with Margaux and Co. [9].

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 [17].

V.CONCLUSION

Anti-ENA antibodies are important immunological markers for Lupus and GSS. However, the presence of certain isolated antigenic specificities does not necessarily indicate the presence of an autoimmune pathology and must be reassessed later.

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