

LABORATORY TECHNIQUES FOR DIAGNOSIS OF AUTOIMMUNE DISEASES

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Abstract: *The Fluorescence Immunoassay and Enzyme-linked-immunosorbent-assay (ELISA) can be used as an aid in the diagnosis in certain autoimmune diseases, being extremely important in medical practice. The study was conducted on a total of 75 patients registered between 11.11.2011-16.02.2012, in Clinical Emergency Hospital of Brasov. The aims of this paper are highlighting the importance of titration in patients with antinuclear antibodies presents and the importance of ELISA technique for diagnosis of autoimmune diseases. Fluorescence can be used as a screening method for determination of antinuclear antibodies. A positive titer accompanied by the appearance pattern orients us on correct and fast diagnosis. Both fluorescence and ELISA are important for diagnosing and monitoring of autoimmune diseases.*

Key words: *fluorescence, ELISA, autoimmune diseases, ANA, TPO.*

1. Introduction

An alternative to classical methods represents immunochemical techniques which have developed serological and nonserological techniques for detection of infectious and noninfectious diseases.

These methods use specifically interaction between an antigen and an antibody, in order to obtain an immunocomplex that can be qualitative and quantitative detected in direct or indirect way [2].

Fluorescence is the phenomena in which absorption of light by a fluorochrome (fluorescein isothiocyanate, dansyl

chloride, methyl umbelliferone, ethyl eosin) is followed by the emission of light at longer wavelength [3]. Fluorescence techniques have advantages over other light-based methods: noninvasive, high speed and sensitivity, low costs.

Immunofluorescence methods combine high sensitivity of fluorescence with specificity of antigen-antibody interactions, and examination is performed using fluorescent microscope [1].

Immunofluorescence is a screening method, and can be used as imagistic (immunohistochemistry) or analytical (immunoassay) techniques.

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ELISA (Enzyme linked immunosorbent assay) is based on action of enzyme (use as marker) from immune conjugate, against a specific substrate, which change its structure and ability to absorb radiation in the visible (VIS) or ultraviolet (UV) spectrum. The difference of absorption between specific substrate and their enzymatic reaction product can be spectrophotometric measured for direct or indirect detection of antigen-antibody [5].

2. Material and Methods

The study was conducted on a total of 75 patients registered between 11.11.2011-16.02.2012, to Clinical Emergency Hospital of Brasov.

The assay procedure for determination of antinuclear antibodies (ANA), was according to the insert "NOVA Lite™

HEp-2 ANA kits/Substrate Slides" and this is an indirect immunofluorescent assay. We use the fluorescence microscope, type ECLIPSE 200, product by NIKON-Japan [6].

For determination of thyroid peroxidase autoantibodies (TPO) in human serum, we used the assay procedure according to the "QUANTA Lite® TPO ELISA". We determined the TPO with automatically ELISA reader, type PR 1100, product by SANOFI/PASTEUR/BIORAD-France [4].

3. Results and Discussions

After determination of ANA antibodies we obtained, 37.33 % of the patients had a negative ANA test reaction and 62.67 % a positive one (anti-nuclear antibodies detected) which demonstrates a high incidence of autoimmune diseases (Fig.1).

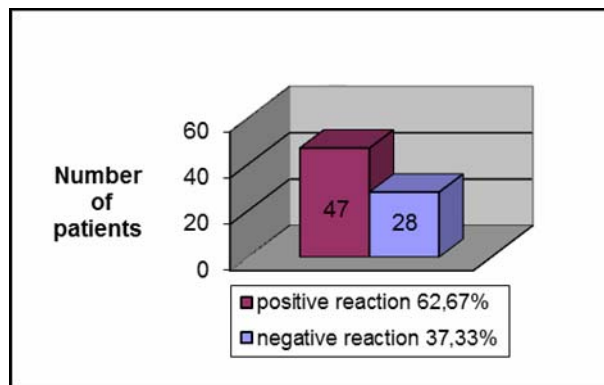


Fig.1. ANA Test - distribution of patients.

According to the staining, most of the patterns have speckled (31.91 %) or homogeneous (27.66 %) aspects. Even knowing this, the full diagnosis can be

made only after specific testing of the auto-antibodies (Fig.2).

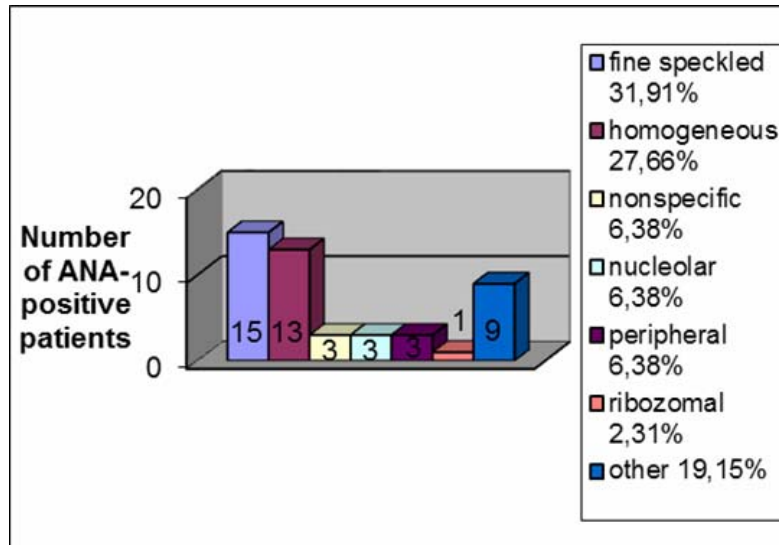


Fig.2. ANA Test – types of antibodies.

Therefore, from patients with ANA – autoantibodies against specific nuclear antigens (SS-A, SS-B, Sm, Scl-70, Jo) positive reaction, 59.57 % were also positive at typing-autoimmunity screening (ImmunoDOT™ -test for detection of

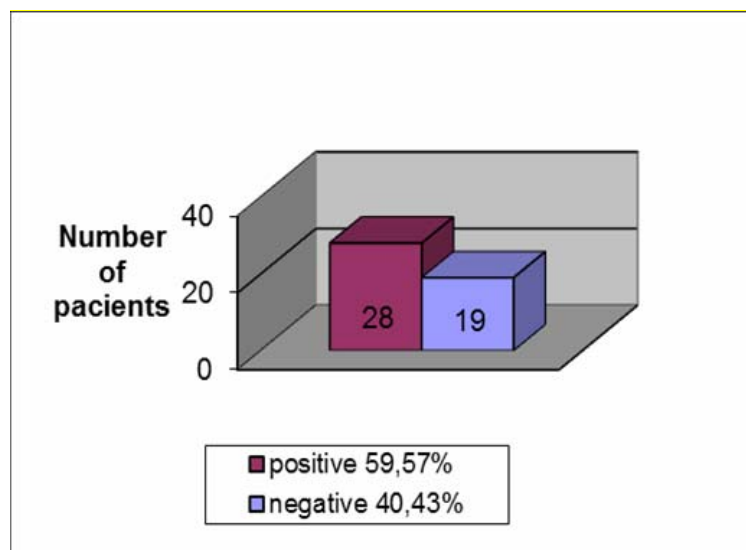


Fig.3. Distribution of patients with positive reaction at typing (ImmunoDOT).

After typing ANA antibodies we obtained the following types of extractable antigens, which oriented us to several autoimmune diseases, particularly systemic lupus erythematosus (SLE) because of a

higher incidence for extractable antigens (ENA) Sjögren's Syndrome-antigen A (SSA/Ro) and Sjögren's Syndrome-antigen B (SSB/La) (Fig. 4).

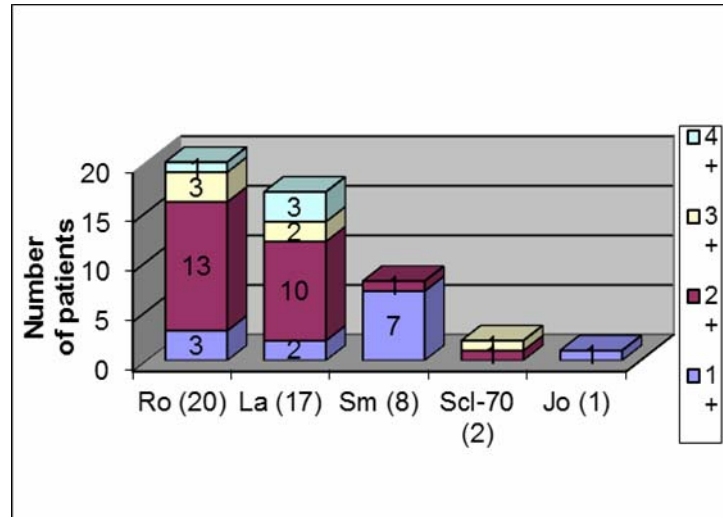


Fig. 4. Distribution of extractable antigens at ImmunoDOT™.

SSA/La-Sjögren's Syndrome-antigen A.
SSB/Ro- Sjögren's Syndrome-antigen B.
Sm-Smith ENA.
Scl-70 –Scleroderma antigen-70kDa.
Jo-ENA –histidyl-tRNA synthase.

From initial 75 patients, for 56 patients we have determined antibodies TGO (ELISA), and got positive reaction to 32.14 % and 67.86 % negative reaction (Fig.5).

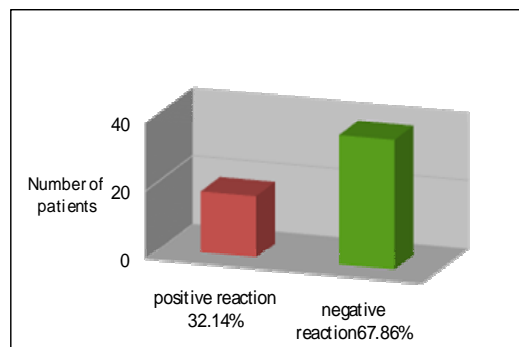


Fig.5. Distribution of thyroid peroxidase autoantibodies (TPO).

We analyzed ANA and TPO antibodies, and saw that there was correlations which oriented to a diagnosis of autoimmune thyroiditis or might be a cross reactivity

because of immunosuppressive medication (nonspecific reaction) which has been administered (Fig. 6).

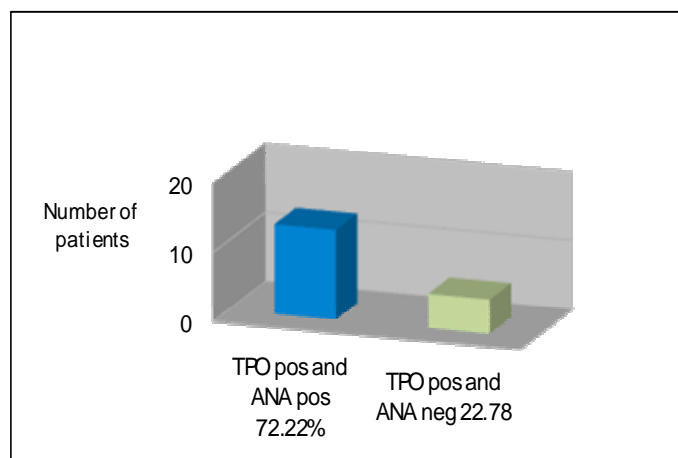


Fig. 6. *Distribution of the patients with TPO positive and ANA negative or positive.*

4. Conclusions

From the survey, from those 62.67 % of patients who had anti-nuclear antibodies detected in serum, 59.57 % had also positive reaction at typing, most of them with anti-Ro and anti-La antibodies. The high percentage of speckled aspect of the pattern is caused by the fact that this is the most common staining for a large number of diseases, while the other aspects of staining can be referred to some specific autoimmune diseases.

After determination the antibodies we observed the importance of testing the patients with ANA positive and TPO positive and the importance to detect such autoimmune diseases: autoimmune thyroid diseases.

Acknowledgement

We wish to thank to Clinical Emergency Hospital Laboratory of Brasov, for the scientific instruments and material supports.

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