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ANTINUCLEAR ANTIBODIES IN THE DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMASIS

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Abstract: Systemic Lupus Erythematosus (SLE) is an autoimmune disease involving multiple organs, characterized by a large amount of autoantibodies, particularly antinuclear antibodies (ANAs). Its diagnosis is based on clinical and laboratory criteria manifested over time, with a wide differential diagnosis, especially in the early stages. The classic symptomatology is cutaneous lesions in young women, in the fertile period, but there is an immense syndromic variety. The purpose of this article is to report on the importance of the ANA test in the diagnosis of SLE. One of the most valuable tests in the screening for SLE is the ANA, which uses an indirect immunofluorescence technique using cells of the HEp-2 lineage, derived from human epithelial tumor cells, as a substrate. Depending on the specificity of the antibody present, different stains are found in specific structures of the cell, giving rise to different patterns of cellular fluorescence. The reagent ANA test reveals the presence of serum antibodies that bind to components of the cell nucleus. The ANA titer is the maximum dilution of serum that still produces detectable nuclear staining. Therefore, although the ANA test has a high reactivity, making it highly sensitive for SLE, it does not have high specificity, due to the fact that these antibodies are detected in other autoimmune and infectious diseases, or even in elderly people.
Keywords: Lupus, antinuclear antibodies, immunofluorescence.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune inflammatory disease that can present in a variety of clinical forms. The pathogenesis of SLE is still unknown, but it seems to be the result of multiple factors such as genetic, environmental (post-infections, pollutants, tobacco, diet and stress), hormonal, nutritional and immunological, dendritic cell dysfunction, B cells, T cells, production of autoantibodies and immune complexes (activating the complement system) and reactive T cells (LIMA et al., 2018; MOK, 2018).

Regarding the etiology of genetic predisposition to SLE, several genes seem to be associated with the deficiency of complement system proteins - mainly C1q and C4, polymorphisms in TREX1 and DNAse1, mutations in alleles of the major histocompatibility complex (MHC) class II, genes linked to innate immunity, such as STAT4, IRF5 and TLR7 and some linked to signaling and production of IFN-α, whose expression is increased in about 60-80% of patients. There is also the hormonal relationship of estrogenic synergism with the production of autoantibodies. This hormone favors the adhesion of mononuclear cells to the vascular endothelium, stimulates the secretion of some cytokines, such as interleukin-1 and expression of adhesion molecules and MHC. Estrogens also reduce the apoptosis of autoreactive cells and accelerate their maturation, especially in B cells with high DNA affinity (NETO et al., 2021).

UV radiation, which is related to the environmental etiology of SLE, causes DNA damage, with the formation of photoproducts such as cyclobutane pyrimidine dimers, seems to be one of the main triggers. (SANDERS et al., 2003).

Exposure to ultraviolet light (UV) causes apoptosis of keratinocytes and expression of molecules that act in the amplification of the immune response, activation of macrophages and antigen processing, resulting in a systemic inflammatory response. Viruses can also be stimulants in the activation of the immune response, as in infection by *Epstein-Barr virus* (EBV), cytomegalovirus (CMV) and Mycobacterium tuberculosis have already been linked to SLE. The use of medications,

especially hydralazine and isoniazid, and smoking can also act as a trigger for manifestations of the disease. (NETO, et al., 2021).

The *American College of Reumatology*, together with the European League of Rheumatology (EULAR), advocates the importance of clinical and laboratory findings. One of the laboratory entry criteria for the evaluation of SLE in patients is the reactive ANA test. (ARINGER; JOHNSON, 2020; NARVÁEZ, 2020).

There are at least four clinical and laboratory criteria for the diagnosis of SLE, the most common being: kidney injury, arthritis, malar erythema, neurological alterations and serositis. Such criteria aim to standardize the scientific studies of the disease in addition to assisting in the diagnosis, although, rarely, there are patients who do not present four of the 11 classification criteria, as shown in Table 1 (BORBA, et al., 2008).

SLE can affect all ages, but is more common between 15 and 55 years of age, with a predominance of women (9:1 ratio). According to the Brazilian Society of Rheumatology (SBR), one in every 1,700 women in Brazil is believed to have the disease. (SOCIEDADE BRASILEIRA DE REUMATOLOGIA, 2019).

Treatment must be individualized, specialists and generalists must work together, and active involvement of patients and their families in the overall therapeutic plan must be emphasized, the therapeutic goal must be to achieve and maintain remission or low disease activity once the diagnosis is made. for as long as possible. Standard treatments include antimalarials, corticosteroids (CS) and immunosuppressants.

Despite a better understanding of the disease process, there is still a significant and unmet need for new treatment due to the continued high risk of mortality and progression of organ damage. Furthermore, the chronic burden of symptoms and the toxicity of immunosuppressive therapies also have a significant impact on the patient's quality of life (MERRILL et al., 2018). Among the most used medications, we can mention hydroxychloroquine, which acts by inhibiting the B cell receptor and signaling; cyclophosphamide, a B and T cell annihilating agent and suppressor of antibody production; rituximab, an anti-CD20 monoclonal antibody that leads to the depletion of peripheral B cells; belimumab, responsible for reducing circulating B cells; calcineurin inhibitors such as cyclosporine and immunosuppressants such as azathioprine, mycophenolate and tacrolimus. In view of the above, it is evident that SLE is a disease that negatively affects the quality of life of affected individuals, bringing unfavorable symptoms to their survival, in addition to not presenting a definitive cure (FAVA, PETRI, 2018).

METHODOLOGY

This work was based on a literature review in Google Scholar, Lilacs, Scielo, EBSCO databases, with the descriptors: SLE, diagnosis, autoantibodies.

RESULTS

In the physiological phenomenon of autoimmunity, there is a reaction of antibodies with self-antigens, normal cellular proteins or a complex of proteins, which are mistakenly attacked by the immune system of healthy individuals. In this system, characteristic of systemic lupus erythematosus (SLE), a large number of autoantibodies, particularly antinuclear antibodies (ANAs), result in injury, essentially by deposition of immune complexes and binding of antibodies to various cells and tissues (FORTE, et al., 2003; BERBERT, MANTESE, 2005).

Antinuclear antibodies are naturally present in the immunological pathways, constituting

MANDATORY ENTRY CRITERIA
Antinuclear factor (ANA) with titer ≥ 1:80 in HEp-2 cells or positive equivalent test.

If absent, do not classify as LES. If present, apply additional criteria.

ADDITIONAL CRITERIA Within each domain, only the highest scoring criterion is counted towards the total score. The occurrence of a criterion on only one occasion is sufficient. The criteria need not occur simultaneously
Do not consider a criterion if there is a more likely explanation than SLE.

Clinical domains and criteria	Score	Immunological domains and criteria	Score
Constitutional Fever	2	**antiphospholipid antibodies** Anticardiolipin or anti-BGP1 Or lupus anticoagulant	2
Hematological leukopenia thrombocytopenia autoimmune hemolysis	 3 4 4	**Complement** C3 or C4 low Low C3 and C4	 3 4
Neuropsychiatric *Delirium* Psychosis Epileptic seizure	 2 3 5	**SLE-specific antibodies** Anti-dsDNA or anti-Sm	6
Mucocutaneous non-scarring alopecia oral ulcers Subacute or discoid cutaneous lupus Acute cutaneous lupus	 2 2 4 6		
Serosite Pleural or pericardial effusion Acute pericarditis	 5 6		
Skeletal muscle joint involvement	 6		
Renal Proteinuria > 0,5 g/24 h Class III or IV lupus nephritis Class III or IV lupus nephritis	 4 8 10		

TOTAL SCORE

Classify as systemic lupus erythematosus if score >10

Table 1: ACR/EULAR 2018 criteria for SLE classification (NETO, et al, 2021).

a group innately capable of defending against usual pathogens and physiologically as auxiliaries in the opsonization of apoptotic remains. Its high entitlement is characteristic of systemic autoimmune diseases, including Systemic Lupus Erythematosus (SLE), Sjögren's Syndrome (SS), Rheumatoid Arthritis (RA), Progressive Systemic Sclerosis, Polymyositis, and Mixed Connective Tissue Disease (DMTC) (LARA, NEVES, 2004; PASSOS, 2008).

Among the main autoantibody tests, the one with the greatest value in screening for SLE is the antinuclear antibody (ANA) test, making this test the most sensitive for lupus, but not the most specific, due to the fact that these antibodies can be detected. in other autoimmune, infectious diseases, or even in elderly people. The search for antibodies such as anti-native DNA, anti-Sm and antinucleosome help for better laboratory identification of the condition (BERBERT, MANTESE, 2005; BORBA, et al., 2008).

In 1957, the indirect immunofluorescence technique (IFI) was developed, whose principle was to bind antibodies to antigenic epitopes of cells, in which there is detection by a second antibody labeled with fluorescent substances and analyzed under a fluorescence microscope (HOLBOROW, et al., 1957). Nuclear fluorescence has four known reading patterns:

1) Speckled, considered the most frequent and most unspecific. Characterized by the presence of nuclear antibody systems such as nuclear ribonucleoprotein (nRNP), detected in patients with mixed connective tissue disease, rheumatoid arthritis and progressive systemic sclerosis; and Sm (designated by the initials of the name "Smith", the first patient from which this antigen was extracted), highly specific for SLE. The presence of the speckled pattern may denote the presence of antibodies against the so-called nuclear extraction antigens (ENA), which include antibodies against RNP, Sm, Ro and La (BERBERT, MANTESE, 2005),

2) Peripheral, highly specific for SLE, however found in patients with other collagen vascular diseases. Shows antibodies against native DNA. Its presence is associated with an increased risk of kidney disease (NIEBOER, 1986).

3) Homogeneous, observed in patients who have antibodies against the nucleoprotein, responsible for the LE phenomenon.

4) Nucleolar, occurring in about 50% of patients with progressive systemic sclerosis, being rare in SLE. Complement dosage in SLE is an important indicator of disease activity, and the presence of hypocomplementenemia is a strong indication of renal injury (NIEBOER, 1986; VONFELDT, 1995).

The uniform, speckled, and nucleolar immunofluorescence patterns in the nuclei of these cells, when incubated with sera from patients with a variety of rheumatic diseases, were demonstrated after using histological sections of rat liver (BECK JS, 1961).

The use of human cell lines as a substrate in IFI became popular and among the various cultures tested, HEp-2 cells dominated and are now the standard of excellence worldwide (HUMBEL, 1993; EARNSHAW,1986).

HEp-2 are immortalized cells, derived from human carcinoma, grown in monolayers with a growth cycle of approximately 36 hours, allowing cells in various phases of the cell cycle to be observed on the same slide. Even antigens that are preferentially identified in cell division are easily characterized, such as centromeric and mitotic spindle-associated antigens (ANDRADE, et al., 1996)

The cell line used in IFI allowed the detection of autoantibodies against nucleus, nuclear membrane, nucleolus and other antigens located in the cytoplasm. Furthermore, HEp-2 cells recognize autoantibodies against protein structures expressed in the mitotic

spindle and nucleus in different phases of the cell cycle (TAN, 1997). Depending on the specificity of the antibody present, different stains are found in specific structures of the cell, giving rise to different patterns of cellular fluorescence. A positive ANA test reveals the presence of serum antibodies that bind to components of the cell nucleus. The test is performed with different dilutions of the patient's serum in a monolayer of human cells placed on a glass slide. A second fluorescently labeled anti-IgG antibody is then added and the cells are examined with a fluorescence microscope to detect whether any of the serum antibodies have bound to the nucleus. The ANA titer is the maximum dilution of serum that still produces detectable nuclear staining. (KUMAR, et al., 2016, ABBAS; LICHTMAN; PILLAI, 2017).

The IIF technique also started to be used in the search for autoantibodies in sera from patients with other autoimmune diseases, being considered the universal method of initial screening of patients' serum samples. The laboratory test has been called HEp-2 anti-nuclear factor (ANA or HEp-2 ANA) or, more recently, HEp-2 anti-nuclear antibody (HEp-2 ANA) test (FRANÇA, 2011).

In the investigation of ANA by IIF in HEp-2 cells, the cells are previously fixed and incubated for screening with 1/80 patient sera in phosphate buffered saline pH 7.2 (PBS) for 30 minutes in a humid chamber at room temperature. The slides are then washed twice for 10 minutes in PBS and incubated for 30 minutes with fluorescein isothiocyanate (FITC)-conjugated anti-human antimaglobulin secondary antibody in a dark chamber at room temperature. After incubation the slides are washed in PBS and mounted with buffered glycerin and a coverslip. The reading is performed in a fluorescence microscope, model Olympus BX 50 under 50x magnification. Sera with a reagent result of 1/80 are titrated at 1:80, 1:160, 1:320, 1:640, 1:1280, in the same buffer under the same conditions described above. To perform the tests, positive control sera and negative control sera are used, provided by the ANA kit (Wama Diagnótica, Brasil) (ANDRADE, et al., 1996).

The HEp-2 ANA test provides three types of information:

1) the presence or absence of autoantibodies;

2) the concentration of the autoantibody in the serum, represented by the titer, which corresponds to the highest dilution of the serum that still results in a positive reaction;

3) the immunofluorescence pattern, which, although still little appreciated, is of great clinical relevance. The latter can help in the proper valuation of a positive test, as some patterns are closely related to certain autoimmune diseases, while others are found with some frequency in healthy individuals. (MARIZ et al., 2011).

The use of the ANA test as a diagnostic method, associated with the clinical criteria, in patients suspected of having SLE is useful, since the reaction is positive in 98% of the cases, except in cases in which the ANA test can be negative if the patient takes concomitant use of corticosteroids or immunosuppressants, or due to the presence of other autoantibodies that can be detected due to the high sensitivity of the method. (NAKAMURA, MUHLEN, 2002; QUEIROZ, et al, 2003).

This diagnostic method can be used in the diagnosis of SLE, and its significantly positive result confirms the condition. Furthermore, a negative result does not exclude the disease, since anti-nDNA antibodies are present in proportions of 50% to 83% of patients with this condition. (MUTASIM, ADAMS, 2000).

Monitoring ANA titers can be helpful in assessing the therapeutic response, together with the clinical picture and complement titers (C2), for example. Patients who progress

to lupus nephritis present alterations on urinalysis, persistently high anti-DNA titers, and/or decreased complement, deserving special attention. (BORBA, et al., 2008).

CONCLUSION

Therefore, the ANA test has a high positivity, which makes this test the most sensitive for SLE, but not the most specific, due to the fact that these antibodies are detected in other autoimmune and infectious diseases, or even in elderly people. Its negative result does not exclude the disease, but its positivity confirms the patient's condition in a high percentage. Clinical laboratory correlation is necessary, even with a positive ANA test, since it is sensitive to other diseases. Even so, ANA titers can be used in the follow-up of patients with SLE.

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