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CASE REPORT

A Case of Antinuclear Antibody-Negative Dermatomyositis with Interstitial Lung Disease

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SUMMARY

Background: **Antinuclear antibody (ANA) is important for the diagnosis of autoimmune diseases. When ANA is positive, further specific autoantibody tests are needed to make a definite diagnosis.**
Methods: **This article reports a case of a patient with an autoimmune disease who had inconsistent results in the detection of antinuclear antibodies by indirect immunofluorescence assay (IIFA) and linear immunoblotting assay (LIA).**
Results: **This patient presented with negative ANA and positive anti-SSA/Ro52.**
Conclusions: **IIF-ANA negative and LIA-ANAS positive exists in clinical tests. The combination of IIFA and LIA is important.**
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KEYWORDS

ANA, anti-SSA/Ro52, dermatomyositis, IIFA, LIA

CASE PRESENTATION

A 69-year-old woman was diagnosed with "Sjogren syndrome and interstitial pneumonia" in Shaoxing People's Hospital in 2016 and returned to the outpatient clinic regularly.
She was admitted to hospital on October 14, 2022 mainly due to “arthralgia, dry mouth, dry skin, shortness of breath”. CT showed interstitial pneumonia in both lungs, and the tear secretion test was positive. ANA.IgG was positive (cytoplasmic granular type ++), anti-SSA/Ro52KD was positive (++), anti-PL-12 was positive (++). Sjogren syndrome, antisynthase syndrome, and interstitial lung disease were diagnosed. Later, the patient was admitted to the hospital several times due to fatigue, cough, arthralgia, dry mouth, xerosis cutis, and shortness of breath. In September 2023, she was diagnosed with dermatomyositic pulmonary interstitial, pulmonary fungal infection, and pulmonary infection in Shanghai Renji Hospital. 2023.09.19 - 2023.11.08 in the respiratory Department of Shaoxing People's Hospital, she was diagnosed as invasive pulmonary aspergillosis,

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severe pneumonia, dermatomyositis interstitial pneumonitis, epilepsy, diabetes mellitus type 2 and discharged from hospital after treatment.
Outpatient follow up was done regularly. ANA.IgG was negative (-), anti-SSA/Ro52 was positive (++) on March 18, 2024. ANA.IgG was negative (-), anti-SSA/Ro52 was positive (+) on June 29, 2024.

DISCUSSION

ANA is a general term for autoantibodies targeting various components of eukaryotic cells [1]. ANA is the most common type of autoantibody in patients with autoimmune disease. Traditionally, the target antigens of ANA are the nuclear components of eukaryotic cells. The current understanding of ANA is no longer limited to nuclear components, but generally refers to autoantibodies against all antigenic components in the cell, including nucleus, cytoplasm, cytoskeleton, cell division cyclin, etc. [2].
Anti-SSA antibody and anti-SSB antibody are specific antibodies for the diagnosis of Sjogren's syndrome. Anti-SSA antibody has the highest positive rate, while anti-SSB antibody is a marker antibody. The specificity of anti-SSA/Ro52 was less than that of anti-SSA/Ro60 in patients with Sjogren's syndrome. Anti-SSA antibodies are also myositis-associated antibodies, which often indicate myositis combined with other connective tissue diseases such as SLE, SS, etc. It has been noted that anti-SSA/Ro52 is present in 30% of patients with idiopathic inflammatory myopathy and is the most common autoantibody in this disease [3]. The Ro52 antigen is known as the 52kDa protein and belongs to the TRIM protein family [4]. Ro52 is designated as TRIM21 among 68 TRIM proteins [5]. The members of the TRIM protein family are involved in a wide variety of cellular processes, including cellular proliferation, differentiation, development, apoptosis, and tumorigenesis [6]. Ro52 has E3 ubiquitin ligase activity and plays an important role in the process of cellular protein ubiquitination. Anti-SSA/Ro52 can be found in a variety of autoimmune diseases and is generally not used as a diagnostic indicator. This antibody is associated with poor prognosis in autoimmune myositis-related interstitial lung disease and systemic sclerosis [7].
ANA can be detected by a variety of methods, including indirect immunofluorescence assay (IIFA), enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), etc. [8], which differ in sensitivity and specificity. IIFA using Hep-2 cells as the experimental substrate is the reference method and the preferred method for ANA detection. It is the most widely used autoantibody screening test at present, and it can reflect the negativity and positivity, titer, and fluorescence models of ANA. The antigen spectrum of HEp-2 cell is rich, more than 100 kinds of autoantibodies can be detected.
Linear immunoblotting assay (LIA) is one of ANA specific autoantibody confirmation assays. Further detection of specific autoantibodies is usually performed by LIA when IIF-ANA is positive. LIA can simultaneously detect a variety of specific autoantibodies in patients due to a variety of highly purified or recombinant antigenic substances. In general, LIA has higher specificity than HEp-2 IIFA [9]. LIA has low sensitivity due to the limitation of antigens. HEp-2 IIFA has a rich antigenic spectrum that allows comprehensive screening for antinuclear antibodies, but there are some antibodies that cannot be detected at some times. Ho [10] reported that among 291 ANA negative samples, 4.1% (12/291) were IIFA negative but LIA positive, including antibodies SSA/Ro60, Ro52, SSB, RNA-A, RNP-C, RNP-70, smD, Scl-70, and Jo-1.
IIFA negative and LIA positive may be due to the fact that SSA/Ro antigen is highly soluble, and is prone to decrease, lose, disperse and loss of immune activity during the preparation process [11]. A decrease of this antigen leads to a decrease in the sensitivity of this assay. It has been shown that transfection of HEp-2 with SSA/Ro60 cDNA significantly improved the sensitivity and specificity of IIFA [12]. Another explanation is that this autoantibody primarily recognized denatured epitopes in the antigenic leucine zipper domain, which is almost absent in the HEp-2 IIFA. In addition, the binding between the antibody and the natural epitope is blocked. Autoantibodies to 52kd Ro/SSA are specific to the denatured 52kd Ro/SSA. Samples with only anti-Ro52 reactivity tend to be negative in HEp-2 IIFA or do not show reliable nuclear or cytoplasmic fluorescence patterns [13].

CONCLUSION

In summary, the use of a single method to detect antinuclear antibodies has limitations. Neither IIFA nor LIA detection of autoantibodies can identify all patients due to the different detection principles. Different assay can complement each other. In daily work, the laboratory physician should actively communicate with the clinician and provide reasonable interpretation of the test results.

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Declaration of Interest:
The authors declare that there is no conflict of interest.

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