Clin. Lab. 2025;71:XXX-XXX

CASE REPORT

False Positive C-ANCA Caused by Antinuclear Antibody: a Case Report and Literature Review

Hong-Gang Sun [1], Li-Qin He [2]

[1] *Department of Medical Laboratory, Shaoxing People's Hospital, Shaoxing, Zhejiang Province, China*
[2] *Department of Geriatrics, Shaoxing People's Hospital, Shaoxing, Zhejiang Province, China*

SUMMARY

Background: **Antineutrophil cytoplasmic antibody (ANCA) is an autoantibody against the cytoplasmic components of neutrophils and monocytes. ANCA related vasculitis includes granulomatous polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatous polyangiitis (EGPA). The detection of ANCA has important clinical significance for the diagnosis, differential diagnosis, classification, condition monitoring, and prognosis of these diseases. The treatment and prognosis of ANCA associated vasculitis are closely related to the titer and activity of ANCA, so the detection of ANCA has important clinical application value. The interference factors of ANCA detection include antinuclear antibody (ANA), which may affect the accuracy of ANCA detection results.**

Methods: **Antineutrophil cytoplasmic antibody and antinuclear antibody were detected by indirect immunofluorescence, and the related antibodies in antinuclear antibody were detected by western blotting. In the diagnosis of GPA, C-ANCA test results should be interpreted in combination with the clinical manifestations of patients, and interference should be excluded. C-ANCA is positive and MPO is negative. It should be considered that antinuclear antibody (ANA) may interfere with ANCA test results.**

Results: **The patient was positive for C-ANCA (+++) and negative for MPO antibody. The patient showed no symptoms related to vasculitis. Further detection of antinuclear antibodies and indirect immunofluorescence results showed that the nuclear homogeneous type and specific ds-DNA antibody were positive, suggesting the presence of systemic lupus erythematosus or another autoimmune disease with a high specificity for these autoantibodies.**

Conclusions: **Antinuclear antibody test showed that ana positive and ds-DNA antibody positive indirect immunofluorescence results were nuclear homogeneous type. Therefore, the patient was considered as a false positive of C-ANCA caused by antinuclear antibody.**

(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.241036)

Correspondence:
Li-Qin He
Department of Geriatrics
Shaoxing People's Hospital
Shaoxing
Zhejiang Province, 312000
China
Phone: +86 0575-88559463
Email: xiaoshi606@163.com

Case Report accepted October 23, 2024

KEYWORDS

C-ANCA, interference, antinuclear antibody

INTRODUCTION

C-ANCA (Cytoplasmic Anti-Neutrophil Cytoplasmic Autoantibody) is an autoantibody directed against specific antigens in the cytoplasm of neutrophils, with the primary target antigen being proteinase-3 (proteinase-3, PR3) [1]. C-ANCA is associated with a variety of vasculitis diseases, particularly granulomatosis with poly-

angiitis (GPA), formerly known as Wegener's granulomatosis [2]. The positive rate of C-ANCA in patients with GPA is very high, usually exceeding 95% [3], while it is lower in patients with microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA).

CASE PRESENTATION

The patient, a 70-year-old female, suffered from depigmentation of forehead and face skin with partial pigmentation without obvious inducement for more than one year, without rash and itching, skin redness and swelling, and pain. At that time, she did not pay attention to it and did not see a doctor. One year ago, the patient had stiff skin on both hands without obvious inducement, unable to hold hands tightly, numb fingers in her right hand, no morning stiffness, no white hair and purple hair when her hands were cold, and he was not treated at that time. Posterior depigmentation with partial pigmentation gradually involved the chest and back. More than one month ago, the patient had swelling and pain in wrist joint, metacarpophalangeal joint, proximal interphalangeal and distal interphalangeal joints without obvious inducement. Laboratory test showed that C-ANCA was positive (+++), MPO was negative. After communicating with the clinician, the patient had no symptoms related to vasculitis. Considering the interference, antinuclear antibody was detected. The indirect immunofluorescence results showed nuclear homogeneity, and the specific ds-DNA antibody was positive.

DISCUSSION

C-ANCA testing typically includes two steps: first, the presence of antinuclear cytoplasmic antibodies is detected by indirect immunofluorescence (IIF), and if the result is positive, further enzyme-linked immunosorbent assay (ELISA) is used to detect the presence and titer of antibodies against PR3 [4]. In the diagnosis of GPA, the results of C-ANCA testing should be interpreted in conjunction with the patient's clinical manifestations. In patients with active systemic disease, a high titer of C-ANCA has a sensitivity and specificity of over 98% for the diagnosis of GPA. However, in patients with atypical disease manifestations or those who do not exhibit all the manifestations of GPA, the sensitivity may drop to 60 - 70% [5,6].

It is worth noting that an increased titer of C-ANCA is not an accurate indicator of disease activity. Even in patients during remission, the titer of C-ANCA may remain elevated, and most of these patients will not develop a relapse in the future. Conversely, a rapid decrease in the titer of C-ANCA may imply ongoing residual lesions. Therefore, changes in the titer of C-ANCA can serve as an indicator to increase vigilance during patient follow-up, helping to maintain a high suspicion of relapse in patients with persistently elevated levels. However, the titer of C-ANCA has no role in guiding immunomodulatory treatment or providing prognostic information to patients.

Overall, C-ANCA is a key biomarker for GPA and plays an important role in diagnosis and treatment monitoring. However, the positive rate of C-ANCA testing in patients with systemic lupus erythematosus (SLE) varies widely, which may be related to interference from ANA. The titer of ANA in SLE patients is often high, so testing with ethanol-fixed neutrophil substrate alone may be insufficient, and positive nuclear peripheral fluorescence staining may lead to difficulties in distinguishing from pANCA. Therefore, it is recommended to use a combination of HEp-2 cells and formaldehyde-fixed neutrophil substrate for testing to improve the accuracy of the test.

The influence of anti-nuclear antibody (ANA) on the detection of antineutrophil cytoplasmic antibody (ANCA) is mainly reflected in the following aspects:

1) Interference of ANA: Studies have shown that homogeneous ANA can interfere with the detection of ANCA, which may lead to false positive results in ANCA testing. Especially when using formaldehyde and ethanol-fixed neutrophils as the detection matrix, the interference of ANA is particularly significant.

2) Specific ANA impact: Among the 115 subjects, the study found that anti-nucleosome antibody (ANuA) was the most common interference to ANCA test results, accounting for 67%, followed by anti-double-stranded DNA (ds-DNA) antibody and anti-histone antibody (AHA), accounting for 58.3% and 41.7% respectively [7]. In 27.8% of serum samples, there were positive anti-dsDNA antibodies, ANuA, and AHA antibodies simultaneously, while the proportion of other antibody positives was only 15.7% [8].

3) Method for eliminating ANA interference: Combining the detection results of anti-dsDNA antibodies, ANuA, and AHA in patients can help eliminate the interference of ANA on the detection results of ANCA [9]. Therefore, when performing ANCA testing, if the anti-nuclear antibody spectrum testing is also performed at the same time, the ANCA results can be more accurately determined [10].

4) Adjustment of dilution: In cases where it is difficult to identify the interference of antinuclear antibodies on ANCA results, increasing the dilution can help testers eliminate the interference of antinuclear antibodies.

5) Selection of detection methods: Different detection methods have an impact on the consistency of ANCA detection results. For example, the combination of indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) can improve the accuracy of detection, while using one method alone may lead to some false negative results.

6) Diversity of ANCA target antigens: In addition to the common target antigens of myeloperoxidase (MPO) and proteinase 3 (PR3), ANCA also targets human leukocyte elastase (HLE), lactoferrin (LF), lysosome (LYS),

cathepsin G (Cath G), and bactericidal/permeability-increasing protein (BPI) [11]. The diversity of these target antigens also has an impact on the results of ANCA testing.

CONCLUSION

The patient had no vasculitis related symptoms. Considering the interference, antinuclear antibody detection was carried out. The indirect immunofluorescence result was nuclear homogeneity, and the specific ds-DNA antibody was positive. The patient was considered to have false positive C-ANCA caused by antinuclear antibody.

Source of Support:
This work was supported by the Shaoxing City Science and Technology Bureau Grant (2023A14022).

Declaration of Interest:
All authors declare that they have no conflict.

References:

1. Weidebach W, Viana VS, Leon EP, et al. C-ANCA-positive IgG fraction from patients with Wegener's granulomatosis induces lung vasculitis in rats. Clin Exp Immunol 2002;129(1):54-60. (PMID: 12100022)

2. Sanchez A, Lozier M, Adkinson BC, Ilaiwy A. c-ANCA vasculitis after initiation of denosumab. BMJ Case Rep 2019;12(3): e228336. (PMID: 30826782)

3. Hellmich B, Sanchez-Alamo B, Schirmer JH, et al. EULAR recommendations for the management of ANCA-associated vasculitis: 2022 update. Ann Rheum Dis 2024;83(1):30-47. (PMID: 36927642)

4. Goto A, Ando M, Usagawa Y, Kadota JI. PR3-ANCA-positive Pulmonary Cryptococcosis. Intern Med 2017;56(23):3265-6. (PMID: 29021464)

5. Jadhav AP. Vascular Myelopathies. Continuum (Minneap Minn) 2024;30(1):160-79. (PMID: 38330477)

6. Schneider SA, Tschaidse L, Reisch N. Thyroid Disorders and Movement Disorders-A Systematic Review. Mov Disord Clin Pract 2023;10(3):360-8. (PMID: 36949803)

7. Zeng Y, Xiao Y, Zeng F, et al. Assessment of anti-nucleosome antibody (ANuA) isotypes for the diagnosis and prediction of systemic lupus erythematosus and lupus nephritis activity. Clin Exp Med 2023;23(5):1677-89. (PMID: 36385418)

8. Bizzaro N, Villalta D, Giavarina D, Tozzoli R. Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis. Autoimmun Rev 2012;12(2):97-106. (PMID: 22810055)

9. Zahid S, Anwar Z, Qadeer K, et al. Ascorbic acid assisted photodegradation of methylcobalamin using corrective irrelevant absorption spectrophotometric assay: A kinetic study. Spectrochim Acta A Mol Biomol Spectrosc 2024;323124902. (PMID: 39126865)

10. Castello A, Frances F, Verdu F. Bleach interference in forensic luminol tests on porous surfaces: more about the drying time effect. Talanta 2009;77(4):1555-7. (PMID: 19084679)

11. Vamvakopoulos J, Savage CO, Harper L. ANCA-associated vasculitides-lessons from the adult literature. Pediatr Nephrol 2010; 25(8):1397-407. (PMID: 20358231)