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The Effect of Antinuclear Antibody Patterns on Antineutrophil Cytoplasmic Antibody Test

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Abstract : Antineutrophil cytoplasmic antibody (ANCA) is used to diagnose and monitor the activity primary systemic small vessel vasculitides. Serum samples with antinuclear antibody (ANA) interfere with the reading of neutrophil indirect immunofluorescence. The question arises, will various ANA patterns affect the results of ANCA test differently? To answer this question, documents were retrospectively reviewed for the criteria of ANCA test with positive ANA from January 1 to December 31, 2000. Of 137 serum specimens with ANCA and ANA tests, 58 were positive for ANA test. The result showed that the number of positive P-ANCA with homogeneous pattern of ANA (88.9%) was statistically higher than that with speckled pattern (38.78%). In conclusion, there is a different effect of various ANA patterns on ANCA results. A further prospective study is required to confirm this result.

เรื่องย่อ : ผลกระทบจากชนิดของ Antinuclear Antibody ต่อการทดสอบ Antineutrophil Cytoplasmic Antibody

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Antineutrophil cytoplasmic antibody (ANCA) ถูกนำมาใช้เพื่อการวินิจฉัยและติดตามอาการของโรค primary systemic small vessel vasculitides ตัวอย่างน้ำเหลืองที่มี antinuclear antibody (ANA) อยู่จะรบกวนการอ่าน immunofluorescence ของเม็ดเลือดขาวชนิด neutrophil ปัญหาที่ต้องการทราบคือลักษณะรูปร่างของ ANA ที่ต่างกัน จะมีผลกระทบต่อผลการทดสอบ ANCA แตกต่างกันหรือไม่ เพื่อตอบปัญหานี้ได้รวบรวมข้อมูลการทดสอบ ANCA และ ANA ที่ให้ผลบวกย้อนหลัง ตั้งแต่ 1 ม.ค. 2543 ถึง 31 ธ.ค. 2543 จาก 137 รายที่ทำการทดสอบ ANCA และ ANA พบว่ามี 58 รายให้ผลบวกต่อ ANA การศึกษานี้แสดงให้เห็นว่าความสัมพันธ์ของ P-ANCA กับ ANA ชนิด homogeneous (88.9%) มีค่าสูงกว่าความสัมพันธ์ของ P-ANCA กับ ANA ชนิด speckled (38.78%) สรุปได้ว่าลักษณะรูปร่างของ ANA ที่ต่างกันจะมีผลกระทบต่อผลการทดสอบ ANCA ควรมีการศึกษาเพิ่มเติมเพื่อยืนยันผลการศึกษานี้

INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against cytoplasmic constituents of neutrophil and monocyte.¹⁻³ The most common reasons for requesting an ANCA test are to diagnose and monitor the activity of primary systemic small vessel vasculitides, namely Wegener's granulomatosis, microscopic polyangiitis and its renal-limited form, pauciimmune segmental necrotizing glomerulonephritis, and Churg-Strauss syndrome.⁴⁻⁶ The most common methods used to detect ANCA are indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assays (ELISA). Serum samples from patients with primary systemic small vessel vasculitis produce 2 neutrophil IIF patterns. These are cytoplasmic fluorescence with central interlobular accentuation (C-ANCA) which usually occurs with proteinase 3 (PR3) specificity, and perinuclear fluorescence often with nuclear extension (P-ANCA) that occurs with myeloperoxidase (MPO) specificity. Serum samples with P-ANCA together with an antinuclear antibody (ANA) interfere with the reading of neutrophil IIF. We sought to determine whether various patterns of ANA have different effects on ANCA results or not.

MATERIALS AND METHODS

This study was conducted in the laboratory of Siriraj Hospital. Documents were retrospectively reviewed for a period from January 1 through

December 31, 2000. The criteria for reviews included serums tested for both ANCA and ANA excluding negative results for ANA.

ANCA Test

Ethanol-fixed neutrophil preparations of a commercial kit from Binding Site Ltd. were used to perform routine laboratory tests as manufacturer's instruction. A negative control, a positive C-ANCA control and a positive P-ANCA control were similarly added to neighboring wells at the same dilution (1:20) for comparison with patient samples. Cytoplasmic fluorescence with central interlobular accentuation and perinuclear fluorescence often with nuclear extension were considered as positive for C-ANCA and P-ANCA respectively.

ANA Test

The first test used for ANA screening was the ELISA technique (Bio-Rad Diagnostic Group). If the results became positive, then IIF technique was further performed to confirm the positive results. In-house rat kidney preparations were used for the ANA confirmatory tests and were considered as positive when a titer of 1:40 or more was reported. Hep 2 cell preparations from DiaSorin S.r.l were performed to confirm the pattern of ANA.

Statistical Analysis

Fisher's exact probability test was used to compare the difference between the two proportions.

A p value of less than 0.05 was considered to be statistically significant. The program of SPSS version 9.0 was used for statistical analysis.

RESULTS

One hundred and thirty seven serum specimens were sent to the laboratory for ANCA and ANA test. Of 137 serum specimens, 58 were positive for ANA. These were from 13 male and 45 female patients. The mean and median ages were 41.12 (S.D.

19.33 years) and 41.5 years respectively. Their ages ranged from 2 to 85 years old. The association between ANCA patterns and ANA patterns is shown in table 1. Eight out of nine serum specimens with positive homogeneous pattern gave P-ANCA patterns whereas 19 out of 49 serum specimens with speckled patterns were also positive for P-ANCA. Homogeneous and speckled pattern of ANA were associated with 88.9% and 38.78% positive results for P-ANCA respectively. The difference between these two proportions was statistically significant.

Table 1. The association between ANCA patterns and ANA patterns.

ANCA patterns	ANA patterns	
	Homogeneous (%)	Speckled (%)
P-ANCA	8 (88.9)*	19 (38.78)
C-ANCA	1 (11.1)	8 (16.33)
Negative	0	22 (44.89)
Total	9 (100)	49 (100)

*P value <0.01

DISCUSSION

This study showed that two patterns of ANA had different effects on ANCA results. Positive P-ANCA tests were associated less often with speckled patterns of ANA (38.78%) than with homogeneous patterns (88.9%). Because different antigens are associated with these two patterns,^{7,8} they interfere with the reading of neutrophil IIF differently. The important antigens for the homogeneous pattern are DNA and histone that can also be found in the nucleus of neutrophil whereas some, but not all, antigens for speckled patterns may be found in the neutrophil nucleus because various antigens have been found for this pattern.^{7,8} However, the true positive results of P-ANCA had not been proved due to lack of confirmatory test recorded in documents. Further study is required to confirm this result. Recently, in

routine laboratory practice we have performed this test with ethanol-fixed neutrophil preparations for initial screening followed by ELISA technique or formalin-fixed neutrophil preparations to confirm positive P-ANCA results. This study suggests that the serum specimens that are positive for ANA with homogeneous pattern should be performed with formalin-fixed neutrophil preparations first followed by ethanol-fixed neutrophil preparations or ELISA to confirm positive test results. For speckled patterns, further study is required to determine the cost-effectiveness and performance of these two preparations for ANCA screening.

In conclusion, various ANA patterns had different effects on the interpretation of the ANCA test, particularly with homogeneous pattern which was associated with a rate of 88.9% positive P-ANCA.

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Table 1. The association between ANCA patterns and ANA patterns

ANA patterns	Homogeneous (%)	Speckled (%)
P-ANCA	8 (88.9)*	10 (55.7)
C-ANCA	1 (11.1)	8 (44.2)
Negative	0	23 (44.8)
Total	9 (100)	41 (100)

*P value < 0.01

DISCUSSION

This study showed that two patterns of ANA had different effects on ANCA results. Positive P-ANCA tests were associated less often with speckled patterns of ANA (88.9%) than with homogeneous patterns (88.9%). Because different antigens are associated with these two patterns,¹⁴ they interfere with the loading of neutrophil IgG differently. The important antigens for the homogeneous pattern are DNA and histone that can also be found in the nucleus of neutrophils whereas some, but not all, antigens for speckled pattern may be found in the neutrophil nucleus because various antigens have been found for this pattern.¹⁵ However, the true positive results for P-ANCA had not been proved due to lack of confirmatory test described in document. Further study is required to confirm this result. Recently, it

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In conclusion, various ANA patterns had different effects on the interpretation of the ANCA test, particularly with homogeneous pattern which was associated with a test of 88.9% positive P-ANCA.