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Original Article

Antineutrophil Cytoplasm Antibody: Positivity and Clinical Correlation☆

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ABSTRACT

Objective: To determine positivity and clinical correlation of anti-neutrophil cytoplasmic antibodies (ANCA), taking into account the interference of antinuclear antibodies (ANA).
Material and methods: A prospective study was conducted in the Laboratory of Immunology of the National Cuban Center of Medical Genetic during one year. Two hounded sixty-seven patients with indication for ANCA determination were included. ANCA and ANA determinations with different cut off points and assays were determined by indirect immunofluorescense. Anti proteinase 3 and antimyeloperoxidase antibodies were determined by ELISA.
Results: Most positivity for ANCA was seen in patients with ANCA associated, primary small-vessel vasculitides, rheumatoid arthritis and systemic lupus erythematosus. Presence of ANCA without positivity for proteinase 3 and myeloperoxidase was higher in patients with ANA and little relation was observed between the perinuclear pattern confirmed in formalin and specificity by myeloperoxidase. Highest sensibility and specificity values for vasculitides diagnostic were achieved by ANCA determination using indirect immunofluorescense with a cut off 1/80 and confirming antigenic specificities with ELISA.
Conclusion: ANCA can be present in a great number of chronic inflammatory or autoimmune disorders in the population studied. This determination using indirect immunofluorescence and following by ELISA had a great value for vasculitis diagnosis. Anti mieloperoxidasa assay has a higher utility than the formalin assay when ANA is present.

Anticuerpos contra el citoplasma del neutrófilo: positividad y correlación clínica

Palabras clave:
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Proteinasa 3
Mieloperoxidasa
Anticuerpos antinucleares
Valor diagnóstico

RESUMEN

Objetivo: Determinar la positividad y la correlación clínica de los anticuerpos contra el citoplasma del neutrófilo (ANCA), teniendo en cuenta la interferencia de los anticuerpos antinucleares (ANA).
Material y método: Se realizó un estudio prospectivo en el Laboratorio de Inmunología del Centro Nacional de Genética Médica de Cuba durante un año. Se incluyó a 267 pacientes con indicación de ANCA. Las determinaciones de ANCA a diferentes puntos de corte y de ANA se realizaron mediante inmunofluorescencia indirecta. Los anticuerpos antiproteinasa 3 y antimieloperoxidasa fueron determinados mediante ELISA.
Resultados: Nuestro estudio mostró que la mayor positividad de ANCA fue vista en pacientes con vasculitis asociadas a ANCA, artritis reumatoidea y lupus eritematoso sistémico. Fue superior la presencia de ANCA sin especificidad por la proteinasa 3 o la mieloperoxidasa en pacientes con ANA y se observó poca relación entre el patrón perinuclear confirmado en formalina y la presencia de anticuerpos frente a la

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mieloperoxidasa. Los mayores valores de sensibilidad y especificidad para el diagnóstico de las vasculitis se alcanzaron para la determinación de ANCA mediante inmunofluorescencia indirecta a un valor de corte de 1/80 y confirmando la especificidad antigénica mediante ELISA.
Conclusiones: Los ANCA pueden estar presentes en un amplio número de enfermedades asociadas a estados inflamatorios y autoinmunes en la población estudiada. Su determinación mediante inmunofluorescencia indirecta seguida de la determinación mediante ELISA tiene gran valor para el diagnóstico de las vasculitis. La determinación de anticuerpos antimieloperoxidasa tiene mayor utilidad que el ensayo en láminas de formalina cuando hay ANA.

Introduction

Anti-neutrophil cytoplasm antibodies (ANCA) are a group of autoantibodies directed against the cytoplasmic constituents of neutrophils and monocytes. Their determination constitutes a diagnostic test for small-vessel vasculitis associated with ANCA (AAV) including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA) and vasculitis limited to the kidney.[1]

The presence of ANCA is determined by indirect immunofluorescence (IIF) from fixed neutrophils on glass slides and fluorescence positivity must be confirmed by the enzyme-linked immunosorbent assay enzyme (ELISA) to determine the antigenic specificity, although some authors suggest that the simultaneous use of both methods has shown greater diagnostic value.[2]

There are two main patterns of fluorescence depending on the purpose of the ANCA. The cytoplasmic pattern (cANCA) refers to the pattern with which the cytoplasm stains under immunofluorescent microscope when neutrophils are fixed with ethanol or acetone. The main cANCA antigen is proteinase 3 (PR3). The perinuclear pattern (pANCA) refers to the staining pattern of neutrophils, which occurs only when they are fixed in ethanol or acetone, to permeabilize the membrane of the cytoplasmic granules, and allows highly cationic proteins, such as myeloperoxidase (MPO), to exit and bind to the negatively charged nuclear membrane. When neutrophils are fixed in formalin, this pANCA pattern is observed as cANCA because the attraction effect of cationic proteins to the nucleus[1–3] is reduced.

Over 90% of patients with active typical GPA have identifiable antibodies against PR3, with a sensitivity of 28%–92%, depending on the expression of the disease, and a specificity of 80%–100%; while between 80% and 70% of patients with PAM and between 70% and 85% of EGPA patients have identifiable MPO antibodies.[4,5]

It has been confirmed that the pANCA pattern can be observed when the antibodies react with antigens other than MPO, something called an atypical pANCA pattern. Perinuclear fluorescence without nuclear extension is also known as atypical pANCA.[6,7]

Some authors have also suggested that there is a cANCA atypical pattern combining perinuclear fluorescence with nuclear extension and cytoplasmic fluorescence. This pattern can be produced by the interference of antinuclear antibodies (ANA).[6,7]

Hence the importance of the simultaneous determination of ANA and ANCA determination, although there are few studies on the prevalence of ANCA patterns by IIF in different diseases, differentiating the presence or absence of ANA.

Atypical patterns may have antigenic specificities against nuclear, cytosolic or granular components and they include elastase, cathepsin G, bactericidal permeability enhancer (BPE), betaglucoronidase, lysozyme, lactoferrin, catalase, alpha enolase, actin, histone, among others.[7,8]

Some authors suggest the usefulness of ANCA is not only to diagnose AAV, but also to diagnose and assess the prognosis of other inflammatory or autoimmune disorders, where the pattern of associated IIF is usually described as pANCA, although there have been cANCA or atypical patterns.[1,7,9,10] ANCA positivity has also been described in infectious and malignant hematological diseases.[1,11,12]

The objective of this study was to determine the positivity and clinical correlation of ANCA, taking into account the interference of ANA in patients treated at the Laboratory of Immunology at the National Centre for Medical Genetics (NCMG) of Cuba in 2012.

Materials and Methods

A prospective study was performed with patients referred to the Laboratory of Immunology of the NCMG of Cuba in 2012.

Patients

The sample consisted of 267 patients with indications for ANCA determination: 35 with suspected systemic lupus erythematosus (SLE), 60 with confirmed SLE, 17 with suspected AAV,13 with AAV, 91 with rheumatoid arthritis (RA), 10 with viral hepatitis, 14 with scleroderma, 8 with mixed connective tissue disease, 4 with Sjögren's syndrome, five with autoimmune hepatitis (AIH) type I, 3 with ulcerative colitis (UC), 3 with Raynaud's phenomenon, 2 with dermatomyositis, one with primary biliary cirrhosis and one with spondyloarthropathy. The diagnosis of AAV was performed according to the criteria established by the Conference of Chapell Hill.[13–15] The diagnosis of other patients was performed by clinicians and immunologists with criteria for each disease.

Methods

ANA determination was performed by IIF, considering as positivity titers starting at 1/80 and using a commercial assay (ORGENTEC, Germany). The determination of ANCA was performed by IIF on ethanol-fixed and formalin human neutrophils, considering positivity from 1/20[16]; determinations of anti-PR3 and anti-MPO were performed by ELISA (ORGENTEC, Germany) to patients who were positive for ANCA by IIF, using as cutoff 5 IU / mL. 2 IIF patterns were identified: cANCA (cytoplasmic fluorescence), pANCA (perinuclear fluorescence without nuclear extension).ANCA positive samples were titrated by IIF. The titer was considered as the highest dilution at which the ANCA pattern was clearly observed. Sensitivity and specificity of ANCA determination by IIF was determined using different cutoffs and confirming the antigenic specificities by ELISA.

For statistical analysis we used the Statistica 7.0 and 3.1 EPIDAT programs. To analyze the influence of ANA positivity in determining ANCA, the χ^2 statistic was calculated as well as odds ratios (OR) and the magnitude of association with their respective confidence interval of 95%. Different cutoffs were compared in terms of their discrimination capability, using the area under the curve (AUC) of the receiver operating curve characteristics (ROC curves).

Research fulfilled the Declaration of Helsinki of the World Medical Association criteria, which establishes the ethical principles for medical research involving human subjects.[17] The study was approved by the Ethics Committee of the NCMG.