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Clinical Relevance of Anti-PR3 Capture ELISA in Diagnosing Wegener's Granulomatosis

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Wegener's granulomatosis (WG) is one type of systemic small vessel vasculitis and antineutrophil cytoplasmic antibodies (ANCA) have become an established diagnostic tool for systemic vasculitis. The sensitivity and specificity of anti-PR3 (proteinase 3) capture enzyme-linked immunosorbent assay (ELISA) in diagnosing WG were investigated, as well as the correlation with the indirect immunofluorescence test (IIFT). Sera from 72 patients with WG, 100 disease controls, and 206 healthy blood donors were investigated for anti-PR3 and cytoplasmic ANCA (cANCA) by anti-PR3 classic ELISA, anti-PR3 capture ELISA, and IIFT. The sensitivity of anti-PR3 classic ELISA and capture ELISA in diagnosing WG was 74% and 87.5% separately. The specificity of the two ELISA was identical (100%). For the combination of IIFT with anti-PR3 capture ELISA, the sensitivity for WG patients was up to 91.6%. The sensitivity of anti-PR3 capture ELISA is superior to anti-PR3 classic ELISA, and the correlation between anti-PR3 capture ELISA and IIFT is also more superior. For suspected WG, anti-PR3 capture ELISA and IIFT should be applied in parallel. J. Clin. Lab. Anal. 22:73–76, 2008.

Key words: capture ELISA; classic ELISA; anti-proteinase 3; Wegener's granulomatosis

INTRODUCTION

Wegener's granulomatosis (WG) is one type of systemic small vessel vasculitis, characterized by necrotizing granulomatous inflammation of the upper and lower respiratory tract with pauci-immune necrotizing crescent glomerulonephritis and vasculitis of predominantly small blood vessels. Antineutrophil cytoplasmic antibodies (ANCA) have become an established diagnostic tool for systemic vasculitis (1). Early diagnosis is of paramount importance, since systemic small vessel vasculitis often has a progressive course that rapidly results in an increased extent and severity of organ involvement. Cytoplasmic ANCA (cANCA) has a close correlation with WG and the specific target antigen has been defined as an azurophilic neutral serine proteinase, named proteinase 3 (PR3) (2). Anti-PR3 antibody has clinical importance in diagnosis, monitoring disease activity, and predicting relapse of WG.

A recommended strategy to determine these antibodies is combining indirect immunofluorescence test (IIFT) and monospecific Anzyme-linked immunosorbent assay (ELISA) to increase the serological hit rate of WG. However, the results obtained by IIFT and by ELISA for cANCA demonstration do not always correlate. Some patients can be positive by IIFT and negative by anti-PR3 classic ELISA. To avoid this, Euroimmun Medizinische Labordiagostika AG (Seekamp, Germany) developed an anti-PR3 capture ELISA to increase the sensitivity for determining anti-PR3 and reduce the discrepancy between IIFT and ELISA. The aim of this study is to compare anti-PR3 classic ELISA with the newly developed anti-PR3 capture ELISA in sensitivity and specificity for diagnosing WG as well as the correlation with IIFT.

MATERIALS AND METHODS

Samples

Serum samples from 278 individuals were divided into three groups. Group 1, samples from 72 patients who were diagnosed as having WG according to the

Abbreviations: WG, Wegener's granulomatosis; ANCA, antineutrophil cytoplasmic antibodies; cANCA, cytoplasmic ANCA; PR3, proteinase 3; IIFT, indirect immunofluorescence test; MP, microscopic polyangiitis.

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American College of Rheumatology 1990 criteria (3). Renal involvement was 100%, including 54 with crescentic glomerulonephritis, eight with necrotizing glomerulonephritis, and 10 with proliferative glomerulonephritis. Because ANCA serology may become negative after the initiation of immunosuppressive therapy, all sera were drawn before therapy. Group 2, samples from 100 disease controls, including secondary vasculitis, various kinds of glomerulonephritis, or granulomatous disease. The diagnoses in these patients was based on solid clinical evidence, preferably with histology. Group 3, samples from 206 healthy blood donors.

Reagents and Apparatus

The ANCA IIFT kit (antigen substrates: ethanol-fixed granulocytes/formaldehyde-fixed granulocytes/HEp-2 cells/primate liver), anti-PR3 classic ELISA, and anti-PR3 capture ELISA were produced by Euroimmun Medizinische Labordiagostika AG. Optic density was measured with the absorption photometer Anthos 2001 (Anthos Labtec Instruments, Wals, Austria). The fluorescence was read by a fluorescence microscope (model BX60; Olympus, Tokyo, Japan).

IIFT

IIFT was performed on ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, and HEp-2 cells/primate liver. Patient sera diluted 1:10 in phosphate buffered saline (PBS) were incubated for 30 min at room temperature and bound immunoglobulin G (IgG) was detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG. The fluorescence was examined by using fluorescence microscope. Negative and positive controls were always included in every test. Control serum was added directly to the corresponding reaction field of the reagent tray. Titer of antibody $1:<10$ in sample was considered as negative and 1:10 as positive, $1:\geq 100$ was considered as strong positive.

ELISA

The procedures for the two ELISA were similar. Patient sera were incubated for 30 min at room temperature and bound IgG was detected by enzyme-conjugated goat anti-human IgG. The intensity of the formed color is proportional to the concentration of anti-PR3 antibody. Negative and positive controls and a series of calibrations were always included in every test. The results were obtained from standard curve. Reference interval was <20 RU/mL (relative units per milliliter).

Statistical Analysis

The sensitivity for WG of anti-PR3 classic ELISA and anti-PR3 capture ELISA was compared using χ^2 test and Spearman's rank correlation was used for calculation of the correlation coefficient between IIFT and ELISA. The P values were two-sided, and the term statistically significantly implies a P value <0.05. All data were analyzed using the Statistical Program for Social Sciences 10.0 software program (SPSS, Inc., Chicago, IL).

RESULTS

Results Obtained by IIFT, Classic ELISA, and Capture ELISA

The results detected by IIFT and by classic and capture ELISA are presented in Table 1. A total of 63 out of the 72 patients with WG were positive for anti-PR3 capture ELISA, and the sensitivity was 87.5%. The sensitivity of classic ELISA and IIFT cANCA was 74% and 84.7%, respectively. There was significantly higher sensitivity of the anti-PR3 capture ELISA compared to the anti-PR3 classic ELISA ($P<0.05$).

Of the disease controls, four (4%) patients were positive for the cANCA pattern (two systemic lupus erythematosus (SLE) patients, two ulcerative colitis patients, three Crohn's disease patients, and one mixed cryoglobulinemia patient). A total of four patients had positive anti-PR3 classic ELISA (two SLE patients, one rheumatoid vasculitis patient, and one ulcerative colitis patient). A total of five patients were positive for anti-PR3 capture ELISA (two SLE patients, two rheumatoid vasculitis patients, and one IgA nephropathy patient). Of the healthy control sera, 0% were positive for the cANCA pattern, anti-PR3 classic ELISA, and anti-PR3 capture ELISA. Then, the specificity of the IIFT was 92% toward disease controls and 100% toward healthy

TABLE 1. Anti-PR3 and cANCA result in WG, disease controls, and healthy controls

Results	Group 1 n = 72 (%)	Group 2 n = 100 (%)	Group 3 n = 206 (%)
Positive in IIFT for cANCA	61 (84.7)	8 (8)	0 (0)
Positive in anti-PR3 classic ELISA	53 (74)	4 (4)	0 (0)
Positive in anti-PR3 capture ELISA	63 (87.5)	5 (5)	0 (0)

TABLE 2. Correlation between anti-PR3 ELISA and IIFT

IIFT titer	n	Classic ELISA	Capture ELISA
1:10	13	9	12
1:32	17	14	15
1:≥100	31	26	31
Total	61	49	58

controls. The specificity of classic ELISA and IIFT cANCA was 96% and 95% toward disease controls, respectively.

Correlation Between Anti-PR3 Capture ELISA, Classic ELISA, and IIFT

As shown in Table 2, there is a somewhat better correlation between the results obtained by anti-PR3 capture ELISA and cANCA IIFT ($r = 0.9172$, $P < 0.001$) than between classic ELISA and cANCA ($r = 0.8269$, $P < 0.001$). In this study it was also found that the correlation between anti-PR3 classic ELISA and IIFT increased with cANCA titer, but there was no statistical significance.

Combined Anti-PR3 Capture ELISA and cANCA IIFT to Diagnose WG

Using anti-PR3 capture ELISA or IIFT alone, the sensitivity for WG patients was 87.5% and 84.7%, respectively. For the combination of cANCA IIFT with anti-PR3 capture ELISA, the sensitivity was up to 91.6%, but there was no statistical significance for this increase. The specificity toward disease in the control patients decreased to 90%, toward healthy controls to 100% after combining the IIFT with capture ELISA.

DISCUSSION

ANCA have been widely used as diagnostic markers for several forms of primary systemic vasculitis, such as WG and microscopic polyangiitis (MP) (1). An IIFT with ethanol-fixed neutrophils could be used to discriminate a cytoplasmic (cANCA) and a perinuclear (pANCA) pattern. The major target antigens for ANCA in primary vasculitis has been characterized as enzymes present in the granules of the neutrophil. The cANCA pattern was strongly associated with antibodies against PR3 (2), and the pANCA pattern with a number of enzymes: myeloperoxidase (MPO), lactoferrin, elastase, and cathepsin G, etc. A disadvantage of the IIFT for ANCA detection was that the assay was not antigen-specific. Characterization of the target molecules of ANCA made antigen-specific detection in solid phase assays possible. It has been reported that the specificity of cANCA was 90% for WG, whereas the specificity of anti-PR3 ELISA was above 95% (4). Besides, determination of ANCA by IIFT was often interfered with antinuclear antibodies (ANA). We know that ANA can produce staining that cannot be distinguished from ANCA. When the result of ANA testing was a high titer homogenous pattern, it was very difficult to distinguish if ANCA was present with ANA, and this sometimes caused false-positive results. Therefore, it was recommended to determine ANCA by IIFT in parallel with ELISA. The strategy was also recommended by an international group to ensure optimal sensitivity and specificity (5).

In classic ELISA test kits, the highly-purified PR3 antigen, isolated from human neutrophils, was directly coated onto the solid phase by passive absorption. During this process, the antigen epitopes could be hidden or destroyed (6). It was reported that functional antigen was only 5% among the total antigens coated onto the solid phase. To solve the problem, antiserum, streptavidin-avidin system, and protein A were ever used to coat antigen onto solid phase indirectly. The antigen faces almost the same orientation and distributes evenly on solid phase. The functional antigen was up to 60%, about 10 times higher than directly coating. Therefore, analytical sensitivity was increased (7–9). For the anti-PR3 capture ELISA adopted in this study, a plate precoated with a monoclonal antibody was used to capture the antigen. The results of our study indicate that the sensitivity of anti-PR3 capture ELISA for WG was superior to anti-PR3 classic ELISA, and the correlation between IIFT and anti-PR3 capture ELISA was also more superior.

For suspected primary systemic vasculitis patients, ANCA-IIFT and target antigen-specific ELISA should be applied in parallel to increase the sensitivity for ANCA-associated vasculitis, including WG. It was reported that about 15% of patients with positive anti-PR3 ELISA were negative for the IIFT test or only positive at the dilution below 1:10 (1:3.2). Among the 72 sera from patients with WG investigated in this study, five sera were cANCA-negative by IIFT, but positive by anti-PR3 capture ELISA. Using IIFT or anti-PR3 ELISA alone, the sensitivity for WG patients was 87.5% and 84.7%, respectively, and the sensitivity was up to 91.6% after combining the IIF test with capture ELISA, which was similar to Hagen et al.'s report (10).

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