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Original Article

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Anti-glomerular basement membrane antibodies in the diagnosis of Goodpasture syndrome: a comparison of different assays

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Abstract

Background. The role of anti-glomerular basement membrane (GBM) antibodies in the pathogenesis of Goodpasture syndrome (GPS) is firmly established. Untreated, the disease may follow a fulminating course. Early identification of patients has important implications in terms of management and prognosis. Therefore, a diagnostic test for the determination of circulating anti-GBM antibodies, of very high sensitivity and specificity, is necessary. A number of assays, using different antigenic substrates, are available, but studies comparing the 'performances' of the different tests are scarce.

Methods. The aim of our work was to evaluate the sensitivity and specificity of four immunoassay-based anti-GBM antibodies kits. Thirty-four serum samples from 19 GPS patients, 41 pathological and 28 normal controls were studied retrospectively (the follow-up samples were not included in the analysis of performance data). Cut-off limits were derived from receiver operating characteristics curve analysis.

Results. All the assays showed a comparable good sensitivity (between 94.7 and 100.0%), whereas specificity varied considerably (from 90.9 to 100.0%). The better performance in terms of sensitivity/specificity was achieved by a fluorescence immunoassay which utilizes a recombinant antigen.

Conclusion. All the assays have a good performance, with high sensitivity; however, the specificity may vary considerably.

Keywords: anti-glomerular basement membrane (GBM) antibodies; anti-GBM disease; ELISA; Goodpasture syndrome; NC1 portion of the α3 chain of type IV collagen

Introduction

Anti-glomerular basement membrane (GBM) antibody disease is a rare autoimmune disorder characterized by crescentic rapidly progressive glomerulonephritis [1]. When pulmonary haemorrhage is also present, this condition is usually named Goodpasture syndrome [1]. Tissue injury is mediated by anti-GBM antibodies that bind glomerular (and alveolar) basement membranes. The target autoantigen has been identified as the α3(IV) collagen chain and is found only in basement membranes in the kidney, lung, cochlear and eye [2].

Untreated, the disease follows a progressive, often fulminant, course [3]. The use of plasma exchange in association with corticosteroids and cyclophosphamide has dramatically improved outcome [4]. However, patient and renal survival still depend very much on the degree of renal failure at presentation [3]. Therefore, an early diagnosis is essential for patient survival and to recover renal function.

The diagnosis of anti-GBM disease is traditionally based on the demonstration of linear deposits of immunoglobulins along the glomerular basement membrane by direct immunofluorescence microscopy. However, a kidney biopsy cannot always be easily and/or promptly performed in such ill patients.

Different methods and techniques have been developed to detect circulating anti-GBM antibodies. Anti-GBM antibodies can be demonstrated by indirect immunofluorescence on normal human or primate kidneys, but this method is not quantitative and sensitive enough [5]. Solid phase assays [radioimmunoassay and enzyme-linked immunosorbent assay (ELISA)], using whole solubilized GBM, purified α3(IV) collagen chain and, more recently, recombinant Goodpasture antigen, have been shown to be sensitive and specific and are commercially available. However, studies comparing the 'performances' of the different assays are scarce. Therefore, the aim of our work

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was to evaluate the sensitivity and specificity of four commercially available immunoassay-based anti-GBM antibody kits.

Patients and methods

Patients

In total, 103 serum samples from the following groups were studied retrospectively: (i) 34 serum samples from 19 patients with anti-GMB disease (12 with pulmonary involvement); (ii) 41 serum samples from 41 disease controls (22 with Wegener's granulomatosis, 15 with microscopic polyangiitis, one with Churg–Strauss syndrome, two with systemic lupus erythematosus, one with idiopathic pulmonary fibrosis); and (iii) 28 serum samples from blood donors.

The patients with anti-GBM disease had a clinical picture of rapidly progressive glomerulonephritis, and the diagnosis of anti-GBM disease was confirmed in all cases by the detection of linear deposits of IgG along the GBM [1,3].

The patients with ANCA-associated systemic vasculitis (AASV) were classified using the names and definitions adopted by the Chapel Hill Consensus Conference [6]. Wegener's granulomatosis and microscopic polyangiitis were diagnosed according to the EUVAS criteria [7] and Churg–Strauss syndrome according to the ACR criteria [8].

All the serum samples, with the exception of those from normal controls, were retrieved from the serum bank of the Department of Nephrology and Immunology, where they were sent for anti-GBM antibody and/or anti-neutrophil cytoplasmic antibody (ANCA) testing.

Methods

Sera were tested for the detection of anti-GBM antibodies by specific immunoassay using the following kits: (i) anti-GBM Immunoscan Euro-Diagnostica (Malmö, Sweden), which utilizes as antigen the M2 subunit from the non-collagenous (NC1) domain of type IV collagen; (ii) anti-GBM antibodies Wielisa-kit from Wieslab (Lund, Sweden), which utilizes as antigen the extracted purified human α3 chain of type IV collagen; (iii) Varelisa GBM antibodies from Pharmacia Diagnostics (Freiburg, Germany), which utilizes as antigen a human recombinant α3 chain of collagen type IV expressed in insect cells (SF9/baculovirus); and (iv) EliA GBM antibodies from Pharmacia Diagnostics (Freiburg, Germany), which utilizes as antigen a human recombinant α3 chain of collagen type IV expressed in insect cells (SF9/baculovirus).

The first three assays are standard ELISAs, whereas the latter uses single polystyrene wells coated with the antigen, which are automatically dispensed and processed in the UniCAP 100 instrument.

Anti-GBM antibodies were also detected using indirect immunofluorescence on normal primate kidney (Euroimmun, Germany).

All procedures were followed precisely according to the product insert.

ANCAs were detected using indirect immunofluorescence on ethanol-fixed granulocytes and antigen-specific, proteinase 3 (PR3) and myeloperoxidase (MPO) ELISA, as previously described [9,10].

Statistical analysis

Statistical analysis has been carried out using the MedCalc statistical software (Mariakerke, Belgium). Cut-off limits were derived from ROC curve analysis comparing anti-GBM disease patients with pathological (and normal) controls. For this purpose, only the first serum sample was considered.

Results

Sensitivity was found to be quite comparable for all the assays, ranging from 94.7 to 100.0% (Table 1). Specificity *vs* normal controls was 100.0%, whereas specificity *vs* disease controls varied from 90.9 to 100.0% according to the different assays. Positive and negative likelihood ratios were satisfactory for all the tests (Table 1).

A good agreement was found between the different assays, with correlation coefficients ranging from 0.8285 to 0.9065 ($P < 0.0001$ for all the comparisons) (Table 2).

Four serum samples from disease control patients were found to be positive in one or more assays (Table 3). Two samples were positive in a single assay. One was from a patient with ANCA-positive microscopic polyangiitis, characterized by a pulmonary–renal syndrome, and the other from a patient with Wegener's granulomatosis with lung nodules and renal involvement. Both patients had histologically proven

Table 1. Sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value of the different assays

	Varelisa (nv ≤7 AU)	EliA (nv ≤8 AU)	Wielisa (nv ≤10 AU)	Immunoscan (nv ≤15 AU)
Sensitivity (95% CI)	94.7 (73.9–99.1)	94.7 (73.9–99.1)	94.7 (73.9–99.1)	100.0 (82.2–100.0)
Specificity (95% CI)	94.9 (82.6–99.2)	100.0 (91.3–100.0)	97.4 (86.5–99.6)	90.9 (75.6–98.0)
LH+	18.47	38.84	36.95	11.00
LH−	0.06	0.05	0.05	0.00
PPV	90.0	100.0	94.7	86.4
NPV	97.4	97.6	97.4	100
AUC (95% CI)	0.973 (0.892–0.997)	0.991 (0.923–0.995)	0.955 (0.866–0.991)	0.953 (0.855–0.991)

nv, normal value; AU, arbitrary units; CI, confidence interval; LH+, positive likelihood ratio; LH−, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve calculated from receiver operating characteristics (ROC) curves.

Table 2. Correlation coefficient (with 95% confidence interval) between the different assays

	Varelisa	EliA	Wielisa	Immunoscan
Varelisa	–	0.9065* (0.8543–0.9406)	0.8287* (0.7389–0.8896)	0.8539* (0.7705–0.9086)
EliA	0.9065* (0.8543–0.9406)	–	0.8401* (0.7555–0.8972)	0.8285* (0.7335–0.8917)
Wielisa	0.8287* (0.7389–0.8896)	0.8401* (0.7555–0.8972)	–	0.8829* (0.8144–0.9271)
Immunoscan	0.8539* (0.7705–0.9086)	0.8285* (0.7335–0.8917)	0.8829* (0.8144–0.9271)	–

*$P < 0.0001$.

Table 3. Diagnosis, clinical features and anti-GBM antibody results in the different assays of 'false-positive' serum samples

Patient number	Diagnosis	Clinical features	Varelisa (nv ≤7 AU)	EliA (nv ≤8AU)	Wielisa (nv ≤10 AU)	Immunoscan (nv ≤15 AU)	IIF
1	CSS	NCGN, asthma, purpura, neuropathy, MPO-ANCA+	**28**	1	10	**300**	Neg
2	WG	NCGN, pulmonary nodules, PR3-ANCA+	7	3	**110**	15	Neg
3	IPF	Pulmonary haemorrhage, no kidney involvement	**15**	7	10	**185**	Neg
4	MPA	NCGN, pulmonary haemorrhage, MPO-ANCA+	6	4	10	**52**	Neg

nv, normal value; AU, arbitrary units; IIF, indirect immunofluorescence on primate kidney; CSS, Churg–Strauss syndrome; WG, Wegener's granulomatosis; IPF, idiopathic pulmonary fibrosis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NCGN, pauci-immune necrotizing crescentic glomerulonephritis; PR-3, proteinase 3. Abnormal value are in bold.

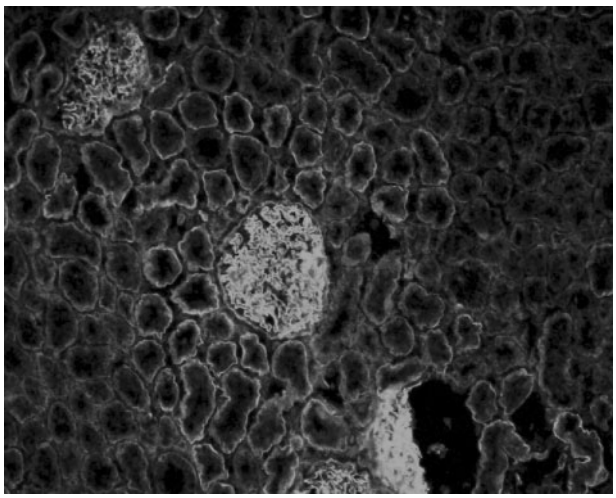

Fig. 1. Indirect immunofluorescence on primate kidney: linear deposits of IgG along the glomerular basement membrane and tubular basement membrane using a serum samples from a patient with Goodpasture syndrome.

pauci-immune necrotizing crescentic glomerulonephritis (no linear immune deposits on kidney biopsies). Two additional samples were positive in two assays. One was from a patient with idiopathic pulmonary fibrosis (with no renal involvement), and the other from a patient with Churg–Strauss syndrome. All these samples were negative for anti-GBM antibodies by indirect immunofluorescence on normal primate kidney sections (Figure 1 and Table 3). 'False-positive' serum samples were analysed a second time by all the assays with comparable results (data not shown).

Since anti-GBM antibody level monitoring is considered useful to guide treatment (in particular, plasma exchange), the sensitivity of the different assays was also recalculated including follow-up samples, collected during the acute phase of the disease. Sensitivity varied from 81.8 to 97.0% (Figure 2 and Table 4) according to the various immunoassays, and the area under the curve was significantly different for some of the kits.

Discussion

Our study demonstrates that various commercially available immunoassay kits for the detection and measurement of anti-GBM antibodies have an overall good sensitivity (~95% or more) for anti-GBM disease. Specificity, however, can vary considerably among the different immunoassays.

Anti-GBM disease, and in particular Goodpasture syndrome, if untreated or if treated with delay, has a fulminant course. In a recent survey, 6 months after a sample with a positive anti-GBM test was drawn, 35% of the patients had died, 40% were on renal replacement therapy and only 25% were alive with a functioning kidney [11].

Since an early diagnosis is essential to allow survival and renal function recovery in anti-GBM disease, a sensitive and specific assay for the detection of anti-GBM antibodies should be available when a clinical suspicion is raised.

Until a few years ago, the diagnosis of anti-GBM disease was based on the demonstration of linear deposits of immunoglobulins along the GBM on kidney biopsies [5]. However, kidney biopsy cannot always be promptly performed in such critically ill patients. Alternative and subsequent methods for the detection of anti-GBM antibodies were indirect immunofluorescence on normal human or primate

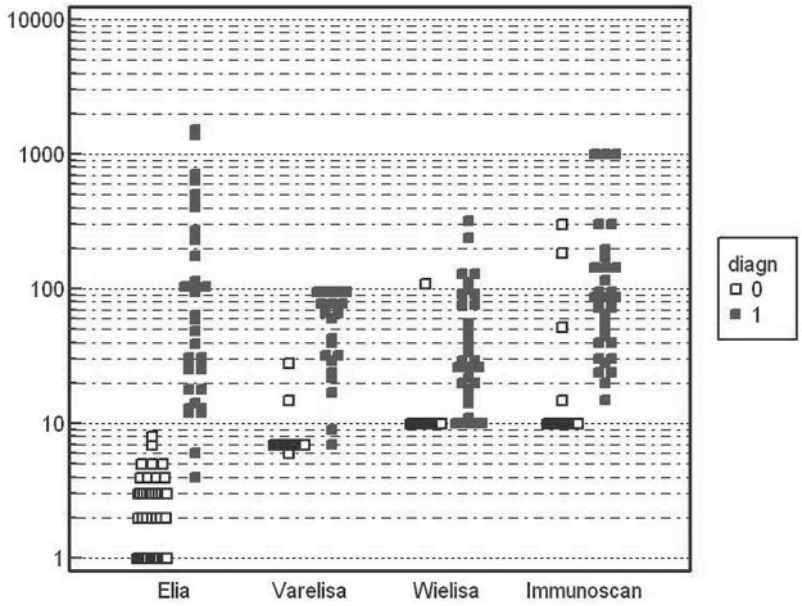

Fig. 2. Anti-GBM antibodies in serum samples (all samples) from anti-GBM disease patients (diagn 1) and pathologic controls (diagn 0) measured with the different kits. The results are expressed as the logarithm of ELISA units.

Table 4. Sensitivity of the different assays for all the serum samples from anti-GBM disease patients

	Varelisa (nv ≤7 AU)	EliA (nv ≤8 AU)	Wielisa (nv ≤10 AU)	Immunoscan (nv ≤15 AU)
Sensitivity (95% CI)	97.0 (84.2–99.5)	94.1 (80.3–99.1)	81.8 (64.5–93.0)	97.0 (84.2–99.5)
AUC (95% CI)	0.980* (0.909–0.997)	0.991[§] (0.928–0.995)	0.885 (0.782–0.951)	0.937 (0.847–0.982)

nv, normal value; AU, arbitrary units; CI, confidence interval; AUC, area under the curve.
*$P = 0.006$ *vs* Wielisa; [§]$P = 0.006$ *vs* Wielisa and $P = 0.067$ *vs* Immunoscan.

kidney sections and radioimmunoassay with collagenase-digested GBM. The first assay is not quantitative and sensitive enough, and is subjective; the latter was available only in selected research laboratories [4,5].

Recently, the more precise identification of the target antigen of anti-GBM antibodies has led to the development of rapid, sensitive and quantitative immunoassays [12]. However, there are only a few studies which have evaluated and compared the diagnostic performance of anti-GBM antibody immunoassays [13,14].

Previous studies have shown an excellent sensitivity (~100%) but a wide range of specificity (68.0–96.0%) of the different immunoassays evaluated [13,14]. Jaskowski *et al.* [14] compared four enzyme immunoassays from Scimedx Corporation (Neville, NJ), INOVA (San Diego, CA), Binding Site (Birmingham, UK) and Wieslab (Lund, Sweden), showing variable (81.0–95.2%) agreement with indirect immunofluorescence. In our experience, the performance of the four tested immunoassays, including two of those analysed in the previous studies, was better in terms of specificity with a comparable sensitivity.

There may be several explanations for the slightly different results. First of all, to define the true positive samples, we used the clinical and immunohistological diagnosis of anti-GBM disease, confirmed by the demonstration of linear immunoglobulin G deposits by direct immunofluorescence on kidney biopsies, and not a formula based on assay results. Secondly, we have not used the normal range suggested by the manufacturer but the cut-off limits were calculated using ROC curves.

Since anti-GBM antibody levels can be used to monitor treatment, we wanted to evaluate, in addition to the diagnostic sensitivity of the different assays, the overall sensitivity of the various kits. While the diagnostic sensitivity was quite comparable, the overall sensitivity varied from 81.8 to 97.0%.

The fact that three of four 'false-positive' samples were from patients with AASV is worthy of note. It is well known that a significant percentage of patients with anti-GBM disease (10–38%, 22% in our series, data not shown) also have ANCAs in their serum, usually with specificity for MPO [15–18], whose clinical significance is uncertain [15–19]. In contrast, a smaller percentage of patients (usually <10%) with AASV have also been found to be anti-GBM antibody positive [16–19]. While the target antigen of anti-GBM antibodies, in patients with Goodpasture syndrome, is the

NC1 domain of GBM, irrespective of the co-existence of ANCAs or not, the fine specificity of anti-GBM antibodies in AASV is still debated [17–20].

All of these patients had a necrotizing crescentic pauci-immune (no linear immune deposits) glomerulonephritis, which would suggest that these anti-GBM antibodies are not pathogenic, and might be directed against other antigenic determinant(s).

The fact that these 'false-positive' results were especially found with an ELISA, which utilizes an extractive antigen, would suggest a possible contamination of the antigen preparation, but other possibilities cannot be ruled out.

Two kits, using the same recombinant antigen, gave slightly different results which can, however, be explained by the different assay conditions: one is a classical ELISA while the other is an automated processed test.

In conclusion, our data suggest that commercially available kits for the detection of anti-GBM antibodies have a very good comparable diagnostic sensitivity whereas specificity can vary widely. The performance of the different assays for monitoring antibody titre may also vary among the different kits.

Conflict of interest statement. R.A.S. and A.R. are consultants for Menarini Diagnostics, Florence, Italy.

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