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Circulating Anti-Glomerular Basement Membrane Antibodies with Predominance of Subclass IgG4 and False Negative Immunoassay Test Results in Anti-GBM Disease

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Abstract

Autoantibodies against a constituent of the glomerular basement membrane (GBM), the α 3-chain of type IV collagen, can cause both rapidly progressive glomerulonephritis and alveolar hemorrhage, referred to as anti-GBM disease or Goodpasture's disease. Anti-GBM antibodies are generally of immunoglobulin G subclass 1 (IgG1) and can in most cases readily be detected in the circulation using enzyme linked immunosorbent assays (ELISA).

Here we report 4 cases where anti-GBM ELISA yielded negative or borderline results despite life-threatening disease. All four patients were positive in IgG4 anti-GBM ELISA and all were ANCA negative. All cases were confirmed with kidney biopsy. Two of the patients exhibited higher results in anti-GBM ELISA when using a non-denaturing coating buffer. All four were young women with severe alveolar hemorrhage and favorable renal outcome suggesting that patients with predominance of IgG4 autoantibodies may constitute a distinct subgroup of anti-GBM disease.

We conclude that patients with idiopathic alveolar hemorrhage can have anti-GBM disease detected only by IgG subclass specific tests or by kidney biopsy.

Introduction

Anti-glomerular basement membrane (anti-GBM) disease, also called Goodpasture's disease, is characterized by auto-antibodies specific for the $\alpha 3$ -chain of type IV collagen¹. It usually presents as rapidly progressive glomerulonephritis, with or without alveolar hemorrhage (AH)^{2,3}. The relative severity and the temporal relationship between renal and pulmonary disease varies among patients. A small minority are diagnosed with normal kidney function despite severe AH⁴.

Anti-GBM antibodies can normally be detected in the circulation by enzyme linked immunosorbent assay (ELISA)⁵. They are mostly of subclass IgG1, though other IgG subclasses can be found⁶⁻⁹. We here report 4 young women with severe AH and anti-GBM antibodies predominantly of subclass IgG4, who exhibited low or negative results in regular anti-GBM ELISA.

Case 1

A 43 year-old woman, current smoker, presented with acute respiratory insufficiency. Four years earlier she had been treated at another hospital under the diagnosis of atypical pneumonia with hemolytic anemia. She had anemia, cough, hemoptysis and microscopic hematuria, which all resolved during treatment with antibiotics, steroids and azathioprine.

At the present admission C-reactive protein (CRP) was 51 $\mu\text{g/mL}$ and hemoglobin (Hb) 10.7 g/dL. Chest X-ray demonstrated profuse alveolar alterations. A day oxygen saturation (SaO_2) fell to 86 % and chest X-ray showed progression. Creatinine remained normal, but Hb decreased to 6.8 g/dL and her clinical condition continued to worsen. Bronchoscopy revealed profuse peripheral airway bleeding. Screening ELISA was positive for anti-GBM, however, quantitative ELISA was negative¹⁰. To sort out the discrepancy, more detailed investigations were done. IgM and IgA against GBM were negative along with IgG1, IgG2 and IgG3; however, anti-GBM of subclass IgG4 was positive⁷. When a physiological non-denaturing coating buffer (phosphate buffered saline) was used instead of the standard denaturing guanidine buffer the IgG4 reactivity increased substantially. Kidney biopsy

showed normal histology, but immunofluorescence displayed linear staining for IgG and C3. Her condition improved and after a month she was discharged. Intravenous cyclophosphamide was followed by oral azathioprine for more than 2 years.

Case 2

A 21 year-old woman, current smoker, sought medical attention at multiple occasions because of persistent cough with blood tinted expectorate. Treatment with antibiotics had no effect. At her third visit Hb of 7.7 g/dl and creatinine of 15.1 mg/dl was detected. A CT scan indicated AH. Screening ELISA was positive for anti-GBM, but quantitative ELISA was negative. Extended anti-GBM analysis was negative for IgA, IgM, IgG2 and 3; positive for IgG1 and strongly positive for IgG4. Kidney biopsy showed cellular crescents in 18 out of 28 glomeruli and linear immunofluorescence for IgG.

Two weeks after start of treatment her condition suddenly deteriorated, with acute dyspnea and SaO₂ 62%. Bronchoscopy samples were positive for influenza virus B and zanamivir was given. Her condition stabilized, kidney function improved and peritoneal dialysis was stopped after 3 months.

Case 3

A 22 year-old woman sought medical attention because of progressive fatigue. Blood tests revealed Hb 5.6 g/dL. After a work-up including renal ultrasound, gastroscopy and colonoscopy, a diagnosis of iron deficiency was made and she was discharged with supplement.

A month later she was re-admitted because of nausea, episodes of macroscopic hematuria and creatinine of 1.92 mg/dL. ELISA was positive for anti-GBM and 2 days later she developed hemoptysis. When standard ELISA turned negative plasmapheresis was discontinued. However, her kidney function continued to deteriorate. After two months a second kidney biopsy showed fresh cellular crescents (Figure 1). Determination of IgG subclasses of anti-GBM revealed positive results for IgG4, but negative results for all other subclasses. Plasmapheresis was restarted and creatinine peaked at 5.4 mg/dL. At discharge creatinine was 2.8 mg/dL.

Case 4

An 18 year-old non-smoking woman was admitted because of hemoptysis. One month earlier she had received antibiotics due to dyspnea and subfertility. On admission tests revealed Hb 7.9 g/dL, CRP 102 µg/mL, creatinine 0.92 mg/dL and dipstick hematuria. A thoracic CT scan showed extensive bilateral symmetric nodular infiltrates. ELISA for anti-GBM was slightly above the detection limit.

Plasmapheresis and treatment with cyclophosphamide pulses and steroids was started. A repeated analysis of anti-GBM antibodies 2 days after diagnosis was negative but anti-GBM IgG4 was positive. When using a non-denaturing coat an increase in reactivity was seen. The AH ceased and IgG4-antibody tests turned negative. Proteinuria peaked at 0.5g/24 hours. Kidney biopsy showed focal necrotizing lesions in 5 out of 50 glomeruli. Immunofluorescence was positive for IgG in a linear pattern. Six months later s-creatinine was 0.69 mg/dl and albuminuria within normal range, but microhematuria persisted.

Despite ongoing treatment with azathioprine, the patient relapsed with AH accompanied by mild urinary abnormalities 15 months after the initial diagnosis. All antibody tests were negative including IgG subclasses of anti-GBM. Immunosuppressive treatment was restarted, AH resolved and renal function remained normal.

Discussion

These four patients demonstrate that predominance of subclass IgG4 of anti-GBM can be a cause for false negative tests. Cases of anti-GBM disease without detectable circulating antibodies have been seen in many series⁵. There are several possible reasons for negative test results. The half-life of kidney bound antibodies is longer than the half-life for those in the circulation, and thus circulating antibodies may have disappeared when the serum sample is drawn. However, Salama and co-workers were able to demonstrate circulating antibodies in two patients using biosensor technique, even though both ELISA and Western blot analysis were negative¹¹. Another possible explanation for

false negative results is autoantibodies reacting with a different antigen or epitopes as compared with “regular” anti-GBM antibodies, that all react with two well defined epitope regions of the NC1-domain of Type IV collagen α 3-chains¹². Such findings were recently described in four Chinese patients with biopsy proven anti-GBM disease, and have been described in patients with IgA anti-GBM¹³⁻¹⁵. A better reactivity when using the non-denaturing coating conditions in two of the present cases indicate that epitope differences do contribute to the low test results in our patients. However, the major reason for the false negative results is most probably a reduced ability of some antisera to recognize the human γ 4-chain. If so the problem to detect IgG4 autoantibodies may not be restricted to anti-GBM testing.

There are other common features between these four cases, raising the possibility that anti-GBM disease with IgG4 predominance constitutes a distinct clinical subgroup, as suggested by Cui et al¹⁶. All of them were relatively young women, they had severe pulmonary disease, all were ANCA negative, all had preserved kidney function on follow-up and two have experienced relapses.

In the early case series as well as in recent Chinese studies there is a strong male preponderance^{17,18}. Other recent series tend to show a more even sex distribution^{2,19}. In our 1991 study on anti-GBM subclasses we had no patients with predominance of IgG4, but we found that high IgG4 was more common in women⁷. This finding was, however, not confirmed by Cui et al⁸. Anti-GBM disease has been shown to have a bimodal age distribution, with one peak around 20 and a second peak between 60 and 70¹⁹. In our study the median age at diagnosis was 60 years, considerably higher than the age of the present cases². ANCA-positivity is more common among elderly anti-GBM patients²⁰.

All the present cases had life-threatening AH. In our Swedish series we found overt hemoptysis in only 23 %², but other studies report higher figures^{3,21,22}. Smoking has repeatedly been linked to pulmonary engagement in anti-GBM disease²³ and three of our patients were current smokers, but the severity of the lung disease in the current cases is intriguing. The limited capacity of IgG4 to bind

complement and engage Fc receptors on neutrophils raises questions regarding their pathogenic potential. Bowman et al reported on a patient where no clinical symptoms were seen when IgG4 anti-GBM antibodies reappeared four years after diagnosis⁶. However, recently there has been a growing interest in IgG4 related autoimmune syndromes²⁴. Other autoantibodies of subclass IgG4 in organ specific autoimmune diseases, such as bullous pemphigoid and membranous nephropathy, have been shown to harbor pathogenic potential^{9,25,26}. Beck et al have shown that IgG4 anti-PLA2R activates the lectin pathway of complement due to defects in glycosylation²⁷.

All patients in the present series had functioning native kidneys. This was the case in only 20 % of our Swedish series and even less in the studies from Beijing^{2,28}. Interestingly the case with IgG4 anti-GBM reported by Cui also had mild kidney disease¹⁶; and in a French study²³ 5 of 13 cases with alveolar hemorrhage and normal kidney function had no detectable anti-GBM in the circulation. It is possible that differences in epitope specificity and/or in engagement of inflammatory effector systems account for the differences in phenotype between our patients with IgG4 anti-GBM and patients with “regular” IgG1 anti-GBM. Two the of the four patients have relapsed, late relapses are rare in anti-GBM disease, we found 1 case out of 81 while Levy et al found 2 among 71^{22,29}.

In summary our cases demonstrate that negative testing for circulating anti-GBM antibodies do not rule out the possibility of anti-GBM disease and that IgG4 autoantibodies is a possible cause of false negative testing. Whether this finding is more common in patients with the combination of severe lung disease and mild kidney disease remains to be determined.

Table 1. Selected clinical data from 4 patients with anti-GBM disease and autoantibodies predominantly of subclass IgG4.

Patient	1	2	3	4
Sex	F	F	F	F
Age (years)	43	21	22	18
Max creatinine (mg/dL)	1.0	21.9	5.4	0.92
Creatinine at 6 month (mg/dL)	0.90	0.85	2.5	0.69
Min Hb g/dL	6.8	7.7	5.6	7.9
Lung hemorrhage	+++	+++	+++	+++
Kidney biopsy	Normal glomeruli	Crescentic GN 18/28 glomeruli	Crescentic GN 25/25 glomeruli	Focal GN 5/50 glomeruli
Immunofluorescence	Linear IgG	Linear IgG	Linear IgG	Linear IgG
Total IgG-anti-GBM **	3	12	32/0*	4
IgG1-anti-GBM**	0	7	2/0*	0
IgG2-anti-GBM**	0	0	3/0*	0
IgG3-anti-GBM**	0	0	5/0*	0
IgG4-anti-GBM**	33	28	100/14*	9
MPO-ANCA	neg	neg	neg/neg*	neg
PR3-ANCA	neg	neg	neg/neg*	neg
Treatment	PLEX, CYC iv, pred***	PLEX, CYC iv, pred***	PLEX, CYC iv, pred***	PLEX, CYC iv, pred***

* Sample sent to the reference laboratory at the time of the second kidney biopsy

**Normal ranges in the assays are: total IgG <10; IgG1 <3; IgG2<8; IgG3 <27; IgG4<7 ELISA units.

*** PLEX = plasma exchange, CYC iv = inter mittent intravenous cyclophosphamide, pred = prednisolone

Figures legend

Figure 1

- PAS stained light microscopy sample from the second biopsy of patient 3, showing crescentic nephritis.
- Immunoperoxidase staining of the same biopsy using anti-IgG, showing linear fluorescence.

A.

B.

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