NDT Plus (2010) 3: 253–256
doi: 10.1093/ndtplus/sfq003
Advance Access publication 30 March 2010

NDT PLUS
Nephrology Dialysis Transplantation

Teaching Point
(Section Editor: A. Meyrier)

Antibody-negative Goodpasture's disease

Mordecai Stolk[1], Daniel Carl[2] and H. Davis Massey[3]

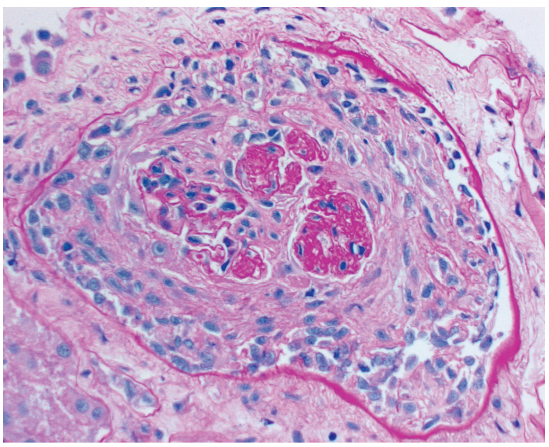
[1]Department of Nephrology, [2]Internal Medicine, Division of Nephrology and [3]Department of Pathology, Virginia Commonwealth University, Richmond, VA, USA

Correspondence and offprint requests to: M. Stolk; E-mail: mordecaistolk@hotmail.com

Keywords: glomerulonephritis; Goodpasture's disease; immunology; kidney biopsy

Introduction

Goodpasture's syndrome is a rare, life-threatening pulmonary-renal syndrome characterized by the production of autoantibodies to a component of the glomerular basement membrane (GBM). The syndrome involves an autoimmune response to the non-collagen domain 1 of the human alpha-3 chain of type IV collagen, α3(IV)NC1. The term Goodpasture's syndrome is often used to refer to the constellation of a rapidly progressive (crescentic) glomerulonephritis, pulmonary haemorrhage and, by definition, detection of circulating anti-GBM antibody. Many clinicians rely on commercially available assays to detect anti-GBM antibodies in the clinical setting of rapidly progressive glomerulonephritis; however, there are few case reports of Goodpasture's syndrome in the absence of these antibodies. We report here an unusual case of a patient with clinical and histological evidence of anti-GBM antibody mediated Goodpasture's syndrome with positive anti-neutrophil cytoplasm antibodies (ANCA) but without evidence of circulating anti-GBM antibody.



Case report

A 55-year-old African American male presented to our hospital with shortness of breath and haemoptysis lasting 3 weeks. The patient had a past medical history significant for coronary artery disease, mixed connective tissue disease (MCTD) with a remote history of prednisone use and hypertension which was well controlled on metoprolol, fosinopril and hydrochlorothiazide. He denied any fevers, night sweats, weight loss, exposure to any sick contacts, nausea/vomiting or urinary complaints. Upon arrival, his serum creatinine level was 1.44 mg/dL, which rapidly increased to 5.21 mg/dL over 72 h. Urinalysis revealed >100 red blood cells per high-powered field, and 2+ proteinuria without red blood cells or granular casts. Chest computed tomography (CT) revealed ground glass opacities consistent with diffuse alveolar haemorrhage, which prompted a bronchoscopy yielding a diagnosis of diffuse alveolar haemorrhage. Serologic studies including HIV antibody, hepatitis B and C panels, serum cryoglobulins, anti-nuclear antibodies (ANA), cytoplasmic anti-neutrophil cytoplasm antibodies (c-ANCA), complement levels (C3, C4) and anti-GBM antibody performed by enzyme-linked immunosorbent assay (ELISA) were negative, while the only positive result was a positive perinuclear ANCA at a dilution of 1:160.

Initial transjugular right kidney biopsy yielded two fragments of renal cortex with only eight glomeruli. On light microscopy, there were only two glomeruli available for review, of which neither demonstrated crescents. The preliminary diagnosis from the serologies and inadequate transjugular renal biopsy was a pauci-immune glomerulonephritis. The consulting nephrology team was not comfortable with the diagnosis in light of the patient's clinical presentation, and the decision to perform a CT-guided renal biopsy was made 2 days later.

The second kidney biopsy yielded three cores of renal cortex with 25 glomeruli per tissue level. Light microscopy revealed that six glomeruli (24%) had cellular crescents, three glomeruli (12%) were globally sclerotic, and four glomeruli (16%) contained foci of fibrinoid necrosis. There

was no evidence of GBM reduplication or spike formation on silver stain, nor was arteritis identified. Immunofluorescence microscopy showed weak linear staining of the glomerular basement membrane and some vessel walls with antisera to C3. Antisera to IgG strongly stained the GBM in a linear fashion. Antisera to IgM stained with weak intensity in a granular pattern primarily in the mesangium. Antisera to C1q and IgA failed to stain the tissue. Antisera to κ and λ light chains weakly stained the GBM in a linear fashion.

The patient responded to induction therapy using methylprednisolone 250 mg IV every 6 h with cyclophosphamide 2 mg/kg PO for 3 days, followed by a reduction in immunosuppression to prednisone 1 mg/kg PO twice daily and cyclophosphamide 1 mg/kg PO daily. The patient continued to improve clinically and biochemically as he received chemotherapy and dialysis three times a week. He was discharged on 1 mg/kg of oral cyclophosphamide a day for 6 months, 1 mg/kg of prednisone twice a day with a prolonged taper over 12 months and continued to receive dialysis thrice weekly for a total of 3 weeks until his native renal function returned. More than 12 months have now passed, and he remains dialysis independent with a serum creatinine of 1.57 mg/dL and negative serologies while receiving maintenance immunosuppression of azathioprine (100 mg/day) and low-dose prednisone (1 mg/day).

Discussion

Collagen is a major building block of the basement membrane for all epithelial cells. Collagen type IV is made of six distinct α-chains [α1(IV)–α6(IV)] and is the most common protein present in basement membranes of humans. Collagen α1(IV) and α2(IV) are ubiquitously expressed in the basement membrane of most organ systems, whereas the limited presence of α3(IV) through α6(IV) chains belies their highly specialized functions. The α3(IV) chain's presence is confined to the kidney, lung, cochlea, Bruch's membrane of the retina and the testis. The Goodpasture antigen is the non-collagenous domain of the α3 chain of type IV collagen located near the C-terminus and is usually hidden from immune surveillance through interactions with other non-collagenous domains of the triple helical promoter α3α4α5(IV). The specialized GBM is essential to the proper function of the kidney as witnessed by patients with Alport's syndrome. Homozygotes lacking both α3(IV) chains develop progressive renal failure and sensineuronal deafness, while heterozygotes traditionally develop thin basement membrane disease [1]. Alport's patients who receive renal allografts are also at increased risk of de novo anti-GBM antibodies [2].

The histology in Goodpasture's disease is characterized by a linear distribution of immunoglobulin within the GBM. Promptly diagnosing anti-GBM disease is vital to the patient with acute glomerulonephritis, as early treatment of Goodpasture's disease leads to improved recovery of renal function and mortality [3]. Additionally, the majority of patients will not recover renal function if treatment is initiated when their serum creatinine is >5.7 mg/dL [4,5]. In light of these data, rapid detection of circulating anti-GBM antibodies is a crucial part of the diagnostic armamentarium.

Multiple assays have been used to determine the presence of circulating anti-GBM antibodies. The first of these assays employed indirect immunofluorescence of normal human kidney tissue; however, this method was plagued by a lack of quantification (with an inherently lower sensitivity) and consequently fell into disvafour [6]. These assays were supplanted by ELISA using human purified or recombinant Goodpasture antigen. Currently, there are many different commercial anti-GBM antibody assays produced using different antigenic substrates claiming to have excellent sensitivity and specificity. The anti-GBM antibody used at our institution is the LabCorp Binding Site assay with a 'manufacturer-claimed diagnostic sensitivity of 100%'. Sinico *et al*. compared the performance of four antibody kits using the sera of 103 subjects: 34 serum samples from 19 Goodpasture patients, 41 samples from disease controls and 28 serum samples from healthy donors. The authors found that the sensitivity of all four assays was comparable, ranging from 94.7% to 100%, while the specificity varied from 90.9% to 100% [7].

Although there is a paucity of literature discussing the diagnosis of Goodpasture's disease in the absence of a positive ELISA test, there has been a preponderance of research devoted to identifying the epitopes that elicit anti-GBM antibody production. In 1999, Hellmark *et al*. published a paper documenting a series of crucial amino acid residues near the N-terminal portion of the α3(IV)NC1 protein, residues 17, 18, 19, 21, 24, 27, 28, 31 and 57, which they labeled S2, that could elicit a pathogenic autoantibody response [8]. Netzer *et al*., using epitope mapping of 14 different α1α3 (IV)NC1 chimeric proteins, identified two immunodominant regions at amino acid residues 17–31 and 127–141, which they named Ea and Eb, respectively [9]. Additional evidence suggests that the Ea epitope is the antigen most commonly associated with clinical Goodpasture's disease [10].

Furthermore, the clinical significance of the Goodpasture's disease conformational epitopes is obfuscated by the simultaneous presence of ANCA's. In the largest biopsy series to date, Yang *et al*. took 205 samples of anti-GBM antibody-positive sera and analysed their specificity and absorbance values. The results were fascinating in that 63 of the 205 (30.7%) serum samples were doubly positive for anti-GBM antibody and ANCA (61 +MPO-ANCA, 6 +PR3-ANCA, and 4 triple positive) [11]. These results were echoed in other research which showed that the presence of ANCA in anti-GBM disease ranged from 10% to 38% of cases [12–15]. In 2007, Yang *et al*. demonstrated that the immune response is different in patients positive for both ANCA and anti-GBM (double-positive patients) compared to patients expressing only the anti-GBM antibody. Anti-α3(IV)NC1 antibody levels and epitope recognition defined by absorbance values to epitopes Ea, Eb and S2 were all lower in double-positive patients as compared to patients with anti-GBM antibody alone. The lower affinity or lower absolute concentrations of the anti-GBM autoantibodies in these double-positive patients may have arisen because of a lower specificity for the normal immunogenic antigen in Goodpasture's disease or because the

immune response developed a broader spectrum of autoantibodies [11]. Such lower affinity antibodies to the GBM epitope may account for the negative ELISA results in our patient. The standard anti-GBM ELISA utilized Fe-specific alkaline phosphatase-conjugated goat antihuman anti-IgG immunofluorescent antibodies to visualize absorbance. In this proposed mechanism, the antibody's binding to the GBM during the ELISA procedure lacks the sufficient affinity to remain attached during the washout step. In a similar mechanism, Salama *et al*. [16] described three patients with IgG, IgA and IgM antibody-negative Goodpasture's disease using standard ELISA techniques. All three diagnoses were confirmed by renal biopsy, which demonstrated linear IgG deposition along the GBM with crescent formation. However, when the authors utilized a highly sensitive biosensor system with real-time antibody–antigen recognition, the pathogenic antibody was subsequently detected. Another possible explanation for the negative anti-GBM ELISA assay can be due to different immunoglobulin classes such as an IgA anti-GBM antibody to induce clinical Goodpasture's disease [17].

Animal models of experimental autoimmune glomerulonephritis provide reasonable evidence that Goodpasture's disease can be antibody negative. This T-cell-mediated process has been shown to occur in the absence of any immunoglobulins and directly involves CD4+ T cells and macrophages [18]. Robertson *et al*. not only demonstrated the presence of a single T-cell epitope in the N-terminal area of α3(IV)NC1 construct Ea, but also only three amino acid residues within this construct (at positions 17, 19 and 20) were essential to generate a cell-mediated immunologic response [19]. Wu *et al*. were able to demonstrate that transferring α3(IV)NC1-specific T cells alone was able to induce glomerulonephritis in animal models without the production of an anti-GBM antibody [20]. Another possibility is that this patient may have had an autoantibody to an epitope other than α3(IV)NC1 which elicited an immune response to the GBM. For example, either the non-collagenous domains of α4 or α5 type IV collagen chains could be the pathogenetic epitopes. This seems unlikely since previous reports have shown that 60–80% of Goodpasture's patients with antibodies to α3(IV)NC1 also have antibodies to other α(IV)NC1 domains [11,21,22], while there are no reports demonstrating the converse. Given that our patient had strong linear staining of IgG along the GBM on renal biopsy and these animal models inherently show negative immunofluorescence, this mechanism cannot fully explain the reason for a lack of a positive IgG anti-GBM antibody.

We believe that the diagnostic distinction between Goodpasture's disease and other etiologies is important to this case, and we feel that the patient was best served by repeating the native renal biopsy. The diagnostic distinction between ANCA-associated systemic vasculitis and Goodpasture's disease is important clinically with respect to patient prognosis. Recovery of renal function is rare for Goodpasture's patients presenting with severe renal insufficiency [4]. The largest retrospective review of 71 patients receiving standard treatment with plasma exchange, corticosteroids and cyclophosphamide showed that patients presenting with a serum creatinine of 5.7 mg/dL or greater, but not requiring dialysis, had a 1-year renal and patient survival of 82% and 83%, respectively. However, patients presenting with dialysis-dependent renal failure had a worse prognosis with renal and patient survival being 8% and 65% at 1 year and 5% and 36% at 5 years, respectively [3]. Long-term data for Goodpasture's patients is not currently available. In comparison, patients with Wegener's granulomatosis have a more benign prognosis in regards to being dialysis dependent. In a retrospective analysis of 108 patients, 5-year renal survival was placed at 75% [23], and 40–70% of Wegener's patients presenting with dialysis-dependent renal failure still had functioning kidneys 3 years after presentation [24]. For patients presenting with both anti-GBM antibodies and positive ANCAs, studies have shown either a better prognosis for these double-positive patients [25,26] or a similar prognosis [27,28] compared to patients with only a positive anti-GBM antibody.

The issue of diagnostic accuracy is also relevant to treatment resistance, relapse rates and alternate treatment regimens. In a large cohort of 334 patients with AASV, 23% of treated patients became treatment resistant [29]. Patients with AASV-induced end-stage renal disease who are fortunate enough to receive a kidney transplant still have a risk of recurrence [30]. In comparison, the risk of recurrence of Goodpasture's disease after treatment is rare [31] with only five cases of recurrent GBM disease in renal transplant patients [32]. Alternative treatments are also available for persisting, relapsing or refractory disease in AASV but are nonexistent in Goodpasture's disease.

Many physicians rely on the validity of a negative anti-GBM antibody ELISA assay. However, this method of antibody detection has its limitations, and the natural history of Goodpasture's disease dictates that any false negative result and resultant error in diagnosis is severely detrimental to both patient and renal survival. As demonstrated by the present case, a kidney biopsy remains the best method of diagnosing anti-GBM disease in the appropriate clinical context. Furthermore, it is important for clinicians to understand the limitations of current assays used in the laboratory work-up of anti-GBM disease.

Teaching points

(1) Goodpasture's disease and ANCA-associated vasculitis have a serologic overlap which may be clinically relevant.

(2) Animal models using immunologic intricacies of Goodpasture's disease support the idea that the disease can be present without a pathogenic autoantibody.

(3) No single test can replace the accuracy of a good-quality kidney biopsy and a nephrologist's clinical acumen in diagnosing Goodpasture's disease.

References

1. Lemmick HH, Schröder CH, Monnens LA *et al*. The clinical spectrum of type IV collagen mutations. *Human Mutations* 1997; 9: 477–499

2. McCoy RC, John HK, Stone WJ *et al*. Absence of nephritogenic GBM antigen(s) in some patients with hereditary nephritis. *Kidney Int* 1982; 21: 642–652
3. Levy JB, Turner AN, Rees AJ *et al*. Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med* 2001;1033–1042
4. Daly C, Conlon PJ, Medwar W *et al*. Characteristics and outcome of anti-glomerular basement membrane disease: a single center experience. *Renal Failure* 1996; 18: 105–112
5. Herody M, Bobrie G, Gourain C *et al*. Anti-GBM disease: predictive value of clinical, histological and serological data. *Clin Nephrol* 1993; 40: 249–255
6. Wilson CB, Dixon FJ. Diagnosis of immunopathologic renal disease. *Kidney Int* 1974; 5: 389–401
7. Sinico RA, Radice A, Corace C *et al*. Anti-glomerular basement membrane antibodies in the diagnosis of Goodpasture's syndrome: a comparison of different assays. *Nephro Dia Transplant* 2006; 21: 397–401
8. Hellmark T, Burkhardt H, Weislander J. Goodpasture disease: characterization of a single conformational epitope as the target of pathogenic autoantibodies. *J Biol Chem* 1999; 274: 25862–25868
9. Netzer KO, Leinonen A, Boutard A *et al*. The goodpasture autoantigen. Mapping the major conformational epitope(s) of alpha residues 17–31 and 127–141 of the NC1 domain. *J Biol Chem* 1999; 274: 11267–11274
10. Borza DB, Netzer KO, Leinonen A *et al*. The Goodpasture autoantigen: identification of multiple cryptic chain. *J Biol Chem* 2000; 275: 6030–6037
11. Yang R, Hellmark T, Zhao J *et al*. Antigen and epitope specificity of anti-glomerular basement antibodies in patients with goodpasture cytoplasmic antibodies. *J Am Soc Nephrol* 2007; 18: 1338–1343
12. O'Donoughe DJ, Short CD, Brenchley PEC *et al*. Sequential development of systemic vasculitis with anti-neutrophil cytoplasmic membrane disease. *Clin Nephrol* 1989; 32: 251–255
13. Jayne DRW, Marshall PD, Jones SJ *et al*. Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 1990; 37: 965–970
14. Weber MFA, Andrassy K, Pullig O *et al*. Anti-neutrophil cytoplasmic antibodies and anti-glomerular basement membrane antibodies in Goodpasture's syndrome and in Wegener's granulomatosis. *J Am Soc Nephrol* 1992; 2: 1227–1234
15. Hellmark T, Niles JL, Collins AB *et al*. Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol* 1997; 8: 376–385
16. Salama AD *et al*. Goodpasture's disease in the absence of circulating anti-glomerular basement membrane antibodies as detected by standard techniques. *Am J Kidney Dis* 2002; 39: 1162–1167
17. Shaer AJ, Stewart LR, Cheek DE *et al*. IgA antiglomerular basement membrane nephritis associated with Crohn's disease: a case report and review of glomerulonephritis in inflammatory bowel disease. *Am J Kidney Dis* 2003; 41: 1097–1109
18. Dean EG, Wilson GRA, Li M *et al*. Experimental autoimmune Goodpasture's disease: a pathogenetic role for both effector cells and antibody in injury. *Kidney Int* 2005; 67: 566–575
19. Robertson J, Wu J, Arends J *et al*. Characteriztion of the T-cell epitope that causes anti-GBM glomerulonephritis. *Kidney Int* 2005; 68: 1061–1070
20. Wu J, Hicks J, Borillo J *et al*. CD4(+) T cells specific to a glomerular basement membrane antigen mediate glomerulonephritis. *J Clin Invest* 2002; 109: 517–524
21. Hellmark T, Johansson C, Wieslander J. Characteriztion of anti-GBM antibodies involved in Goodpasture's syndrome. *Kidney Int* 1994; 46: 823–829
22. Yang RY, Cui Z, Hellmark T *et al*. Natural anti-GBM antibodies from normal human sera recognize α3(IV)NC1 restrictively and recognize the same epitopes as anti-GBM antibodies from patients with anti-GBM disease. *Clin Immun* 2007; 124: 207–212
23. Aasarod K, Iversen BM, Hammerstrom J *et al*. Wegener's granulomatosis: clinical course in 108 patients with renal involvement. *Nephrol Dial Transplant* 2000; 15: 611–618
24. Geffriaud-Ricouard C, Noël LH, Chauveau D *et al*. Clinical spectrum associated with antineutrophil cytoplasmic antibodies of defined antigen specificities in 98 selected patients. *Clin Nephrol* 1993; 39: 125
25. Bosch X, Mirapeix E, Font J *et al*. Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in anti-glomerular basement membrane disease. *Clin Nephrol* 1991; 36: 107–113
26. Segelmark M, Hammad T, Wieslander J. The prognostic significance in Goodpasture's disease of specificity, titer, and affinity of anti-glomerular basement membrane antibodies. *Nephron Clin Pract* 2003; 94: c59–c68
27. Levy JB, Hammad T, Coulthart A *et al*. Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int* 2004; 66: 1535–1540
28. Rutgers A, Slot M, van Paasen P *et al*. Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCAs in crescentic glomerulonephritis. *Am J Kidney Dis* 2005; 46: 253–262
29. Hogan SL, Falk RJ, Chin H *et al*. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Annals of Int Med* 2005; 143: 621–631
30. Nachman PH, Segelmark M, Westman K *et al*. ANCA-associated small vessel vasculitis after transplantation. *Kidney Int* 1999; 56: 1544–1550
31. Levy JB, Lachmann RH, Pusey CD. Recurrent Goodpasture's disease. *Am J Kidney Dis* 1996; 27: 573–578
32. Khandelwal M, McCormick BB, Lajoie G *et al*. Recurrence of anti-GBM disease 8 years after renal transplantation. *Nephrol Dial Transplant* 2004; 19: 491–494

Received for publication: 18.7.09; Accepted in revised form: 8.1.10