Clin Kidney J (2012) 5: 591–594
doi: 10.1093/ckj/sfs106
Advance Access publication 19 October 2012

CKJ

Teaching Point
(Section Editor: F.P. Schena)

Renal injury due to anti-glomerular basement membrane antibody-mediated glomerulonephritis without circulating antibody

Aruna Dash[1], Huma Fatima[1], Mandeep Grewal[1], Claude Galphin[2] and Paisit Paueksakon[1]

[1]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA and [2]Nephrology Associates, Chattanooga, TN, USA

Correspondence and offprint requests to: Paisit Paueksakon; E-mail: paisit.paueksakon@vanderbilt.edu

Keywords: anti-glomerular basement membrane antibody disease; crescentic glomerulonephritis; nodular glomerulosclerosis

Introduction

Patients with anti-glomerular basement membrane (GBM) antibody-mediated GN usually present with rapidly progressive glomerulonephritis (GN) [1]. Patients may have isolated renal disease or may also have pulmonary symptoms [1]. The disease is caused by antibodies to the non-collagenous-1 (NC1) domain of the α3 chain of collagen IV, termed Goodpasture's antigen, in the majority of cases [2].

The antibody may cross-react in some patients with alveolar basement membranes and cause pulmonary hemorrhage (Goodpasture's disease) [1]. It is a rare cause of acute kidney injury, with an incidence of 0.5–1 cases per million populations [3]. Diagnosis may be based on the detection of circulating antibodies by immunoassays, and is usually confirmed by a renal biopsy. A renal biopsy shows linear staining of immunoglobulin (Ig) along the GBM by immunofluorescence. The autoantibody is generally IgG, although other Ig classes may be co-deposited. There are isolated reports of IgA or IgM alone being deposited on the GBM and also detected in the circulation [4]. The specificity of the antibody can be confirmed by western blotting [3]. False-negative serology antibody results may occur, generally in patients with isolated mild pulmonary disease [3]. False-positive results can occur in certain assays when circulating antibodies are generated to GBM antigens other than the NC1 domain of the α3 chain of collagen IV or in patients with polyclonal immune stimulation [3]. We report a patient who underwent a renal biopsy because of poorly controlled hypertension, nephrotic range proteinuria and rapidly progressive renal failure. The renal biopsy showed necrotizing crescentic GN superimposed on idiopathic nodular glomerulosclerosis (ING) and linear staining of IgG along the glomerular capillary walls. Circulating anti-GBM antibodies were found to be absent by standard immunoassays. This case illustrates the complexity of renal lesions that may coexist. Implication for establishing the diagnosis of anti-GBM-mediated GN and the need for renal biopsy in making specific diagnosis are also discussed.

Case history

A 79-year-old man presented with complaint of persistent lower extremity edema, not relieved by diuretics. He had a history of poorly controlled hypertension, nephrotic range proteinuria and rapidly progressive renal failure with rising of serum creatinine from baseline 150.0 to 266.0 mmol/L within 2 months. His past clinical history was significant for hyperlipidemia, chronic obstructive pulmonary disease, benign prostatic hyperplasia, osteoarthritis and hyperparathyroidism. The remaining review of systems was negative. There was a history of smoking for 40 years. There was no history of diabetes mellitus or glucose imbalance.

On physical examination, he appeared acutely ill. His blood pressure was 203/90 mmHg, respiratory rate 15/min, pulse rate 79/min and temperature 36.1°C. There was a 2+ pitting edema of the lower extremities. The remaining physical examination was unremarkable.

Laboratory studies demonstrated blood glucose levels to be 6.0 mmol/L, sodium 144 mmol/L, potassium 4.4 mmol/L, chloride 107 mmol/L, bicarbonate 29 mmol/L, calcium 2.0 mmol/L, phosphorus 1.7 mmol/L, blood urea nitrogen 7.6 mmol/L, creatinine 266.0 μmol/L and total serum proteins 53.0 g/L with gamma globulin 30.0 g/L. Hemoglobin A1c was 0.06 hemoglobin fraction. His lipid profile was within the normal range. Serum anti-nuclear antibody was negative, anti-double stranded DNA was <20 IU/mL, anti-neutrophil cytoplasmic antibody (ANCA) was negative, anti-GBM antibody test by ELISA was <5 kU/L, C3 1.3 g/L, C4 0.3 g/L and serum protein electrophoresis showed no M-spike. Urine dipstick was positive for 3+ blood and 3+ protein. The urine protein:creatinine ratio was 8470 mg/mg (normal range 0–200). A renal ultrasonography showed the right kidney measuring 9.1 cm and left kidney 11.1 cm in length. A percutaneous renal biopsy was performed to assess the cause of acute kidney injury.

Renal biopsy findings. The renal biopsy specimen included two pieces of cortex containing 17 glomeruli, 4 of which were globally sclerosed. Six glomeruli showed crescents, one

cellular and five fibrocellular, and three glomeruli had focal fibrinoid necrosis (Figure 1) and one also had segmental endocapillary proliferation. The GBM revealed segmental double contours but there were no spikes, holes or corrugation. In addition, there was a moderate increase in mesangial matrix and mild increase in cells with focal nodular expansion with mesangiolysis and microaneurysmal dilatation of the capillaries with fragmented red blood cells within the mesangial nodules (Figure 2). The mesangial nodules stained positive with Jones' silver stain and periodic acid Schiff stain. There was ~30% patchy, well-delineated interstitial fibrosis with proportional tubular atrophy and moderate lymphoplasmacytic infiltrate. There were no features suggestive of obstruction such as dilated Bowman's spaces or tubular dilatation. There were no fractured casts, crystals or polarizable materials. Arterioles showed moderate hyalinosis, but not demonstrated to involve both afferent and efferent arterioles. Arteries showed moderate intimal fibrosis without fibrinoid necrosis or vasculitis.

Three of seven glomeruli examined by immunofluorescence also showed fibrocellular crescents. There was 3+ (0 to 3+ scale) diffuse linear staining along the GBM for IgG (Figure 3), and 2+ equal staining for kappa and lambda light chains. There was discontinuous, trace to 1+ linear staining of GBM for C3. There was no glomerular staining for albumin, IgA, IgM and C1q. There was no tubular basement membrane (TBM) staining for any antisera.

By electron microscopy, GBM showed normal thickness in areas away from increase in lamina rara interna with an average of 450 nm (average GBM thickness for adult man in our laboratory 370 ± 42 nm) (Figure 4). There was a diffuse increase in lamina rara interna. There were no immune complex deposits. There were no fibrin tactoids or reticular arrays. There was a moderate increase in mesangial matrix. The foot processes were ~90% effaced. There were no TBM deposits.

The final morphologic diagnosis was anti-GBM antibody-mediated necrotizing crescentic GN, superimposed on changes consistent with ING, and moderate arterionephrosclerosis.

Clinical course. The patient received solumedrol, 3 g i.v., cytoxan 1 g i.v. and plasmapheresis ×14. He subsequently was transitioned to oral prednisone 60 mg daily and oral cytoxan 100 mg daily. He developed end-stage renal

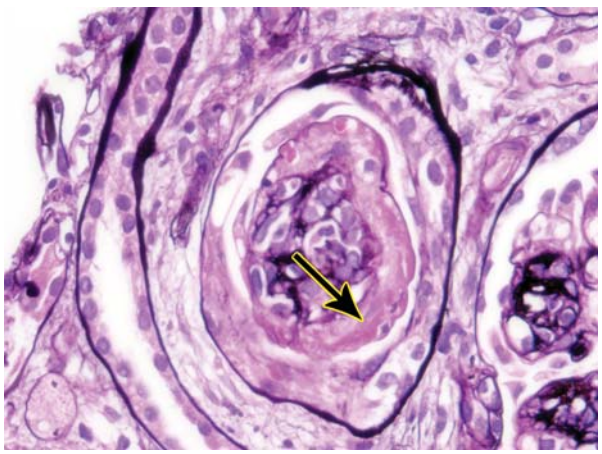

Fig. 1. A glomerulus with nodular sclerosis and segmental fibrinoid necrosis (arrow) (Jones' silver stain, ×400).

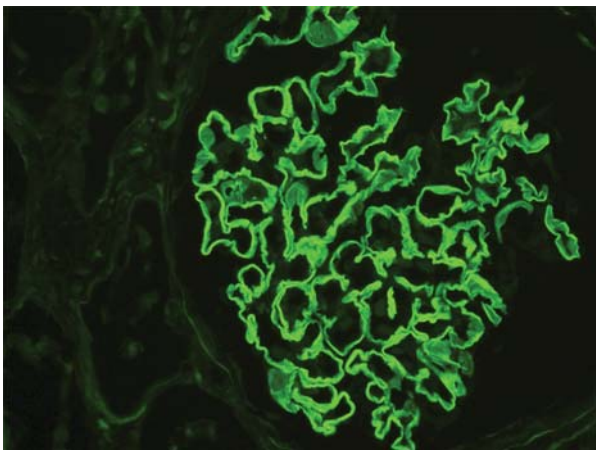

Fig. 3. Linear staining of the glomerular capillary wall for IgG (anti-IgG immunofluorescence, ×400).

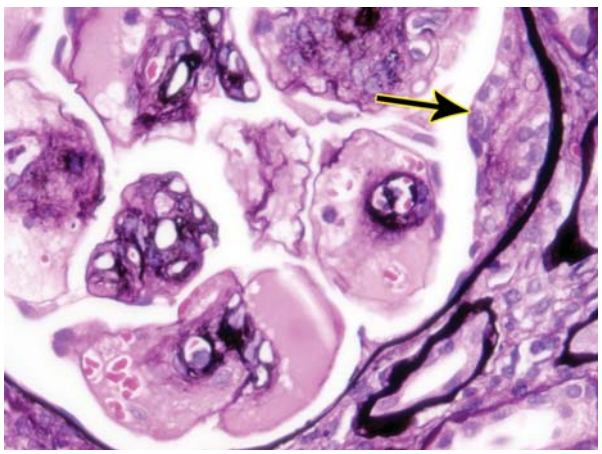

Fig. 2. A glomerulus with nodular sclerosis with mesangiolysis and microaneurysmal formation with fragmented red blood cells within the mesangial nodules associated with a small cellular crescent (arrow) (Jones' silver stain, ×400).

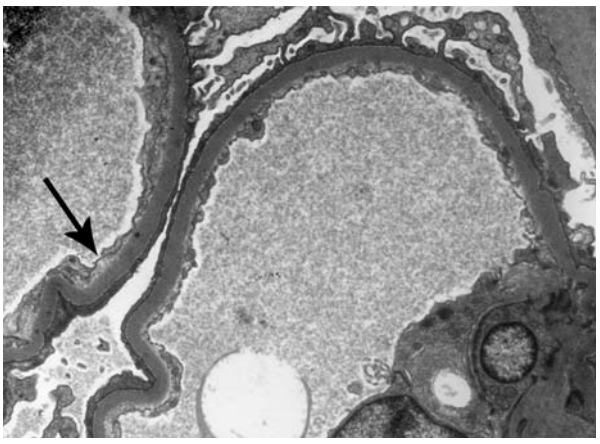

Fig. 4. Electron microscopy shows normal thickness of lamina densa with a diffuse increase in lamina rara interna (arrow) and extensive foot process effacement (transmission electron microscopy, ×5200).

disease and has remained on thrice weekly hemodialysis. Repeat laboratory testing for serum anti-GBM antibody by ELISA following the renal biopsy diagnosis was negative.

Discussion

This biopsy showed complex findings with necrotizing crescentic GN with linear staining of GBM for IgG and nodular sclerosis. Nodular sclerosis may be present in diabetic nephropathy and have linear GBM accentuation [5]. However, our patient did not have diabetes and there was no history of glucose intolerance, and electron microscopy showed normal GBM thickness, thus not indicative of diabetic nephropathy. Additional possibilities of nodular sclerosis include diverse conditions such as idiopathic and secondary membranoproliferative GN (MPGN) Type I and II, amyloidosis, monoclonal Ig deposition disease, fibrillary GN, immunotactoid glomerulopathy, cryoglobulinemia and ING. In this case, there were no immune complex deposits by immunofluorescence or electron microscopy to suggest MPGN Type I or II. There were no fibrillary deposits to suggest amyloidosis or fibrillary GN, and no microtubular or other deposits to suggest cryoglobulinemia or immunotactoid glomerulopathy. There were no monoclonal Ig deposition disease type deposits and no TBM deposits. Therefore, these findings are indicative of ING, a diagnosis of exclusion [6–8]. The linear GBM staining then must be reconsidered, and integrated with the remaining findings, namely necrotizing crescentic GN. This patient had negative ANCA and negative anti-GBM antibody testing. However, the renal biopsy findings indicate likelihood of anti-GBM-induced necrotizing crescentic GN.

Anti-GBM antibody-mediated GN, characterized by the deposition of anti-GBM antibodies in a linear fashion along the GBM, results in necrotizing crescentic GN, leading to acute and often irreversible kidney injury [9]. It accounts for 5–10% of cases of acute kidney injury presenting with rapidly progressive GN [10]. Making the diagnosis of anti-GBM antibody-mediated GN as the cause of acute kidney injury is vital because early treatment may be lifesaving and allow preservation of renal function [9]. Rarely, anti-GBM disease occurs in combination with other diseases. The most common concurrent process includes ANCA-associated crescentic GN and membranous glomerulopathy [11, 12]. Rarely, anti-GBM disease has also been reported in association with diabetes [13]. However, our review of the medical literature did not reveal any cases of ING with anti-GBM antibody positivity.

Anti-GBM antibodies are usually IgG, mostly of the IgG1 subclass [14]. These are directed against Goodpasture's antigen (NC1 domain of the α3 chain of collagen IV). Anti-GBM antibodies can be detected by ELISA and western blotting methods [3]. The rapid diagnosis of anti-GBM disease is increasingly reliant on ELISA techniques to detect circulating anti-GBM antibodies [3]. However, false-positive and false-negative results occasionally may occur using this method. The former generally results from sera containing high levels of polyclonal Ig and can be excluded by both appropriate negative controls and use of the more sensitive and specific western blotting technique, only accessible in reference laboratories [3]. False-negative results are less common, but may occur in 2–3% of cases because of the low range of anti-GBM antibody level or non-IgG (e.g., IgA) causing disease, which may not be detected in standard anti-GBM ELISAs [3]. A few cases have been reported in which the only anti-GBM antibody detectable in serum and along the GBM was IgA or IgM class [4, 15]. Such cases were only identified after ELISA techniques were modified or renal biopsy material was analyzed. In this patient, anti-GBM antibodies were negative by ELISA, and the renal biopsy was the only test detecting anti-GBM mediated GN. These biopsy results must be interpreted in light of the clinical setting, serological results and histopathological findings. Salama *et al.* demonstrated that highly sensitive biosensor techniques could detect circulating anti-GBM antibodies despite negative ELISA and western blot results [3]. These studies suggested that ELISA- and western blot-negative anti-GBM disease may occur in patients with anti-GBM antibody-mediated GN. However, this technique is not currently available for routine clinical assays. Therefore, in patients with rapidly progressive GN and negative serological test results, an early renal biopsy may allow the diagnosis to be made and effective treatment to be initiated.

The term 'idiopathic nodular glomerulosclerosis' was first introduced by Herzenberg *et al.* to describe renal findings that closely resemble nodular diabetic glomerulosclerosis, but occurring in non-diabetic patients [8]. The present case appears to be one such example. Markowitz *et al.* reported 23 cases of ING in which other possible entities that can produce nodular sclerosis were excluded [6]. They found that the patients with ING are typically older (mean age 68.2 years), Caucasian (73.9%), men (78.3%) with a history of long-standing (mean 15.1 years) hypertension (95.7%) and smoking. Clinical presentation included renal insufficiency (82.6%) and nephrotic range proteinuria (69.6%). All these clinical features were present in this patient and, although not specific, support this diagnosis.

Immunofluorescence studies in ING may show linear GBM staining which is invariably accompanied by equally bright linear staining for albumin just like diabetic nephropathy [8, 13]. In contrast to anti-GBM disease, the staining for C3 is absent [8]. In this case, albumin stain was negative and there was discontinuous linear staining for C3 along the GBM. Therefore, the strong linear GBM staining for IgG in the presence of necrotizing crescentic GN in this case is indicative of anti-GBM antibody-mediated disease.

Conclusion

We report a case of anti-GBM antibody-mediated GN superimposed on ING in the absence of circulating anti-GBM antibodies by ELISA. This false negative result occurs only in 2–3% of patients. This low range of anti-GBM antibody level or non-IgG (eg, IgA) causing disease may not be detected in standard anti-GBM ELISAs. In this reported case, the renal biopsy was the only test detecting anti-GBM antibody-mediated GN.

Teaching points

(i) Serum anti-GBM antibodies by ELISA are not entirely sensitive in making a diagnosis of anti-GBM disease.
(ii) A negative test for the serum anti-GBM antibodies test does not absolutely rule out renal disease due to such antibodies.
(iii) A renal biopsy is the gold standard in establishing a diagnosis of anti-GBM antibody-mediated GN.

Acknowledgements. We thank Professor Agnes Fogo for helpful advice.

Conflict of interest statement. None declared.

(See related Editorial comment by A.S. Bomback. Anti-glomerular basement membrane nephritis: why we still 'need' the kidney biopsy. *Clin Kidney J* 2012; 5: 496–497)

References

1. Wilson CB, Dixon FJ. Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int* 1973; 3: 74–89
2. Pedchenko V, Bondar O, Fogo AB *et al*. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. *N Engl J Med* 2010; 363: 343–354
3. Salama AD, Dougan T, Levy JB *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
4. Fivush B, Melvin T, Solez K *et al*. Idiopathic linear glomerular IgA deposition. *Arch Pathol Lab Med* 1986; 110: 1189–1191
5. Ainsworth SK, Hirsch HZ, Brackett NC Jr *et al*. Diabetic glomerulonephropathy: histopathologic, immunofluorescent, and ultrastructural studies of 16 cases. *Hum Pathol* 1982; 13: 470–478
6. Markowitz GS, Lin J, Valeri AM *et al*. Idiopathic nodular glomerulosclerosis is a distinct clinicopathologic entity linked to hypertension and smoking. *Hum Pathol* 2002; 33: 826–835
7. Muller-Hocker J, Weiss M, Thoenes GH *et al*. A case of idiopathic nodular glomerulosclerosis mimicking diabetic glomerulosclerosis (Kimmelstiel-Wilson type). *Pathol Res Pract* 2002; 198: 375–379
8. Herzenberg AM, Holden JK, Singh S *et al*. Idiopathic nodular glomerulosclerosis. *Am J Kidney Dis* 1999; 34: 560–564
9. 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; 134: 1033–1042
10. Salama AD, Levy JB, Lightstone L *et al*. Goodpasture's disease. *Lancet* 2001; 358: 917–920
11. Jennette JC, Lamanna RW, Burnette JP *et al*. Concurrent antiglomerular basement membrane antibody and immune complex mediated glomerulonephritis. *Am J Clin Pathol* 1982; 78: 381–386
12. Jayne DR, Marshall PD, Jones SJ *et al*. Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 1990; 37: 965–970
13. Nair R, Said M. Diabetic woman with massive proteinuria and acute renal failure. *Am J Kidney Dis* 2005; 46: 362–366
14. Segelmark M, Butkowski R, Wieslander J. Antigen restriction and IgG subclasses among anti-GBM autoantibodies. *Nephrol Dial Transplant* 1990; 5: 991–996
15. Border WA, Baehler RW, Bhathena D *et al*. IgA antibasement membrane nephritis with pulmonary hemorrhage. *Ann Intern Med* 1979; 91: 21–25

Received for publication: 25.1.12; Accepted in revised form: 17.7.12