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Clinical Report

A case of triple pathology: seronegative anti-glomerular basement membrane antibody-mediated glomerulonephritis and membranous nephropathy in a patient with underlying diabetic kidney disease

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Abstract

In diabetic patients with acute kidney injury (AKI), kidney biopsy often reveals non-diabetic kidney pathology. This case describes a patient with known Type 1 diabetes who presented with AKI, nephrotic syndrome and haematuria. Combination pathology of seronegative anti-glomerular basement membrane antibody-mediated glomerulonephritis (anti-GBM GN), membranous nephropathy (MN) and diabetic nephropathy (DN) was demonstrated. Strong linear GBM IgG-staining on biopsy with crescentic GN and clinical AKI led to a diagnosis of anti-GBM GN, although serum antibodies were not detectable. Features of DN, Kimmelstiel–Wilson nodules and albumin staining were also present, along with features of MN, such as subepithelial deposits on electron microscopy. Despite treatment with immunosuppression and plasmapheresis, there was no recovery of kidney function. Coexisting anti-GBM GN and MN is well recognized, but the concurrent diagnosis with DN has not been described.

Keywords: anti-glomerular basement membrane (GBM) disease; diabetic nephropathy; membranous nephropathy; seronegative disease

Background

Non-diabetic kidney disease in patients with diabetes is identified in biopsy-based studies with frequencies ranging 10–60% [1–3]. Indications for kidney biopsy in patients with diabetes are the presence of features atypical for diabetic nephropathy (DN); glomerular haematuria, acute kidney deterioration or severe proteinuria [2, 3]. Membranous nephropathy (MN) and IgA nephropathy (IgAN), superimposed on DN, are most prevalent in communities with a relatively high incident of disease, respectively [1, 2]. MN alongside of DN can present with severe proteinuria and progress quickly to dialysis-dependent end-stage kidney disease (ESKD) [4]. Concomitant anti-glomerular basement membrane (GBM) glomerulonephritis (GN) and DN is rare, with poor kidney outcomes reported [5–8]. The simultaneous or sequential occurrence of anti-GBM GN and MN is well recognized [9]. However, this is the first report of concurrent seronegative anti-GBM GN, MN and DN.

Case report

A 22-year-old Caucasian male with Type 1 diabetes mellitus (T1DM), presented with acute kidney injury (AKI), serum creatinine (SCr) 387 μmol/L and nephrotic syndrome (timed urinary protein excretion 14 g/day, serum albumin 23 g/L (35–50 g/L), underwent a diagnostic kidney biopsy. Clinical assessment 1 year prior indicated minimal proteinuria (albumin-to-creatinine ratio 1.5 mg/mmol) and normal kidney function (eGFR [10] >90 mL/min/1.73 m^2) at that time. Antineutrophil cytoplasmic, extractable nuclear antigen (ENA) and anti-GBM antibodies were undetected. Urine studies showed protein-to-creatinine ratio 1.133 g/mmol, dysmorphic erythrocytes (>1000 × 10^6/L) and lipiduria.

T1DM was diagnosed at age 2 and insulin therapy delivered via insulin pump. Glycosylated haemoglobin (HbA1c) at presentation was 7.2%. The patient weighed 100 kg and had smoked 10 cigarettes per day for the past 7 years. There was no history of retinopathy or hypertension, illicit or non-steroidal anti-inflammatory drug use or alcohol dependence. He had been commenced on frusemide and irbesartan/hydrochlorothiazide 3 weeks earlier to treat oedema.

The kidney biopsy demonstrated a focal segmental necrotizing GN with cellular and fibrocellular crescents involving 29 of 40 glomeruli; another 6 glomeruli were sclerosed. Glomeruli had moderate nodular mesangial expansion and hypercellularity with several showing

Kimmelstiel–Wilson nodules. Numerous tubular red blood cell casts were present. There was moderate interstitial fibrosis and tubular atrophy with a patchy interstitial mixed chronic inflammatory cell infiltrate (see Figures 1A and B and 2A and B). Immunofluorescence showed strong linear GBM staining for IgG. Weaker anti-albumin staining localized in a linear fashion to the GBM, Bowman's capsule (BC) and tubular basement membrane (TBM) staining (Figure 3A and B). Under oil immersion (×1000 magnification), a dual pattern of linear and granular glomerular peripheral capillary wall staining was seen. This was confirmed by confocal microscopy (Figure 4A–C). Electron microscopy showed Stage 1 MN with small subepithelial electron-dense 'immune-type' deposits with early GBM spike formation (Figure 5A and B). The overall diagnosis was that of anti-GBM GN and Stage 1 MN superimposed on Class III DN with Kimmelstiel–Wilson lesions.

The simultaneous diagnosis of seronegative anti-GBM GN, MN and DN was consistent with clinical presentation of proteinuria, haematuria and AKI, although unexpected, as anti-GBM antibody serology was negative (Orgentec, Mainz, Germany). A more sensitive biosensor assay was unavailable. The patient's human leucocyte antigens (HLA) were: A1, 24; B8, 39; DR4, 16. Screening for serum anti-secretory phospholipase A2 receptor (PLA2R) antibodies was not detected using an indirect immunofluorescence test (Euroimmun AG, Lubeck, Germany) [11]. In addition, PLA2R was not detected by immunofluorescence in the paraffin-embedded kidney biopsy using anti-rabbit PLA2R antibody (Atlas Antibodies).

Treatment included corticosteroids (3× intravenous methylprednisolone 1 g/day then 75 mg/day oral prednisolone), oral cyclophosphamide (2 mg/kg/day) after fertility protection measures and 10 alternate-daily plasmapheresis treatments (3 L volume exchanges, replacement albumin 4%). He was discharged with mildly improved stable kidney function, with SCr 253 μmol/L.

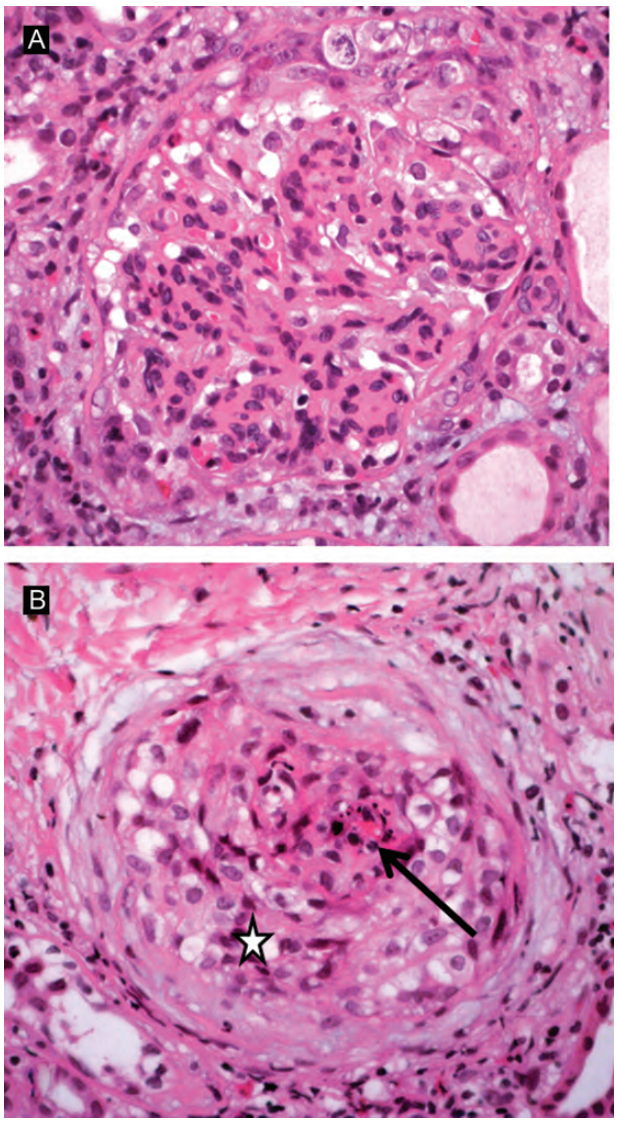

Fig. 1. (**A**) The glomerulus shows nodular mesangial expansion and hypercellularity. (**B**) This glomerulus shows segmental necrosis (arrow) and large cellular crescent (asterisk). Haematoxylin and eosin stain. Original magnification ×400.

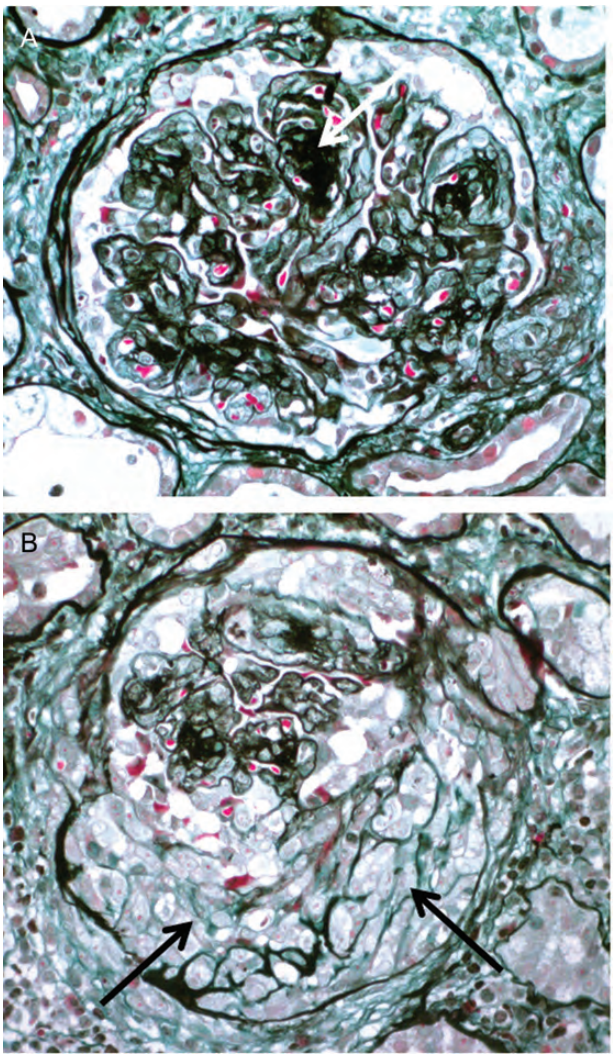

Fig. 2. (**A**) This glomerulus shows a Kimmelstiel–Wilson nodule (arrow). (**B**) This glomerulus shows a large cellular crescent (arrows). Periodic acid Silver stain. Original magnification ×400.

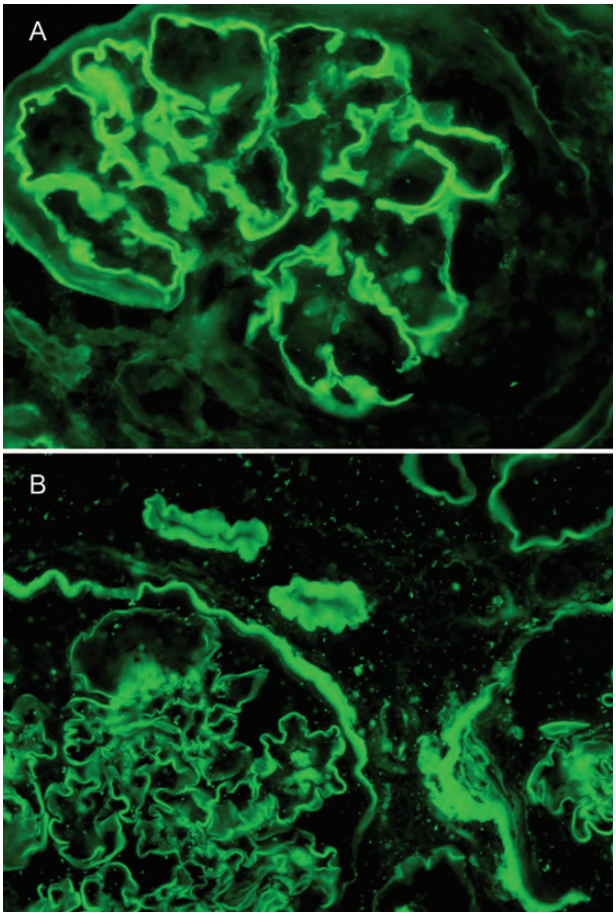

Fig. 3. (**A**) Direct immunofluorescence shows strong linear GBM staining for IgG. BC and tubular BM are negative. (**B**) In contrast, albumin shows strong staining of GBM, BC and TBM. Original magnification ×400.

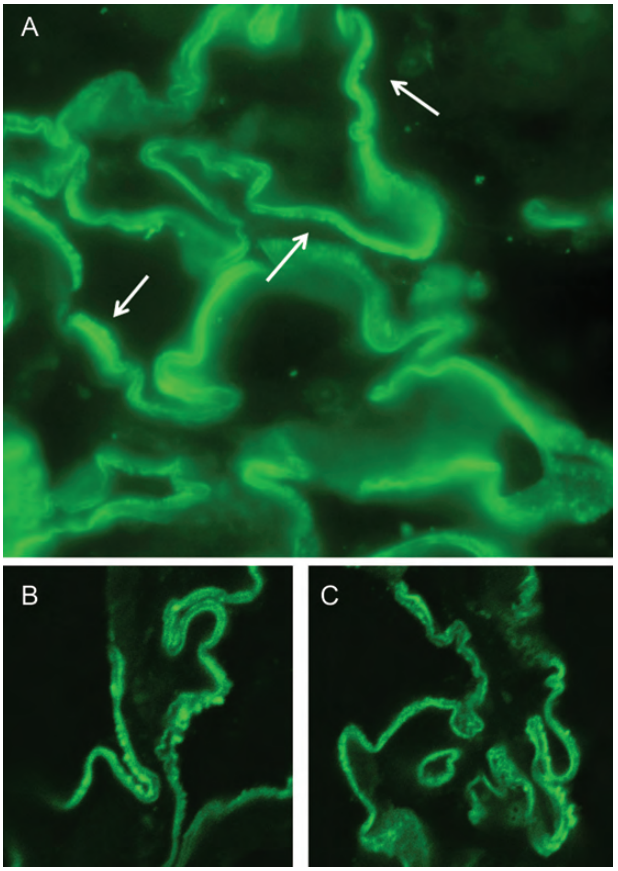

Fig. 4. (**A**–**C**) Direct immunofluorescence at a higher magnification shows a dual pattern of IgG immunofluorescence with linear GBM staining together with granular staining on the outside or urinary space aspect of the GBM (arrows). Images (B) and (C) were acquired on a Nikon A1Rsi confocal microscope with a Z-slice of 0.6 μm.

One week later, he developed small-volume haemoptysis that settled with oral antibiotics, cessation of smoking and cyclophosphamide reduction (1.5 mg/kg/day). Significant oedema persisted despite maximal oral diuretic therapy. Five weeks later, he developed neutropaenia necessitating withdrawal of cyclophosphamide. Prednisolone 60 mg/day was continued in light of further deterioration in kidney function, with SCr 383 μmol/L and worsening proteinuria at 19 g/day.

Another nine alternate-daily plasmapheresis treatments were initiated once neutropaenia resolved, attempting to salvage kidney function. The renal function remained severely impaired and proteinuria worsened to 21 g/day. His clinical course was complicated by *H. Zoster* reactivation (T7 dermatome) that resolved with valaciclovir treatment. Anti-GBM serology remained undetectable throughout.

Progressive renal disease and inability to monitor disease activity serologically led to a repeat kidney biopsy, which showed segmental sclerotic lesions and fibrous crescents in 13 of 22 glomeruli and another 3 glomeruli sclerosed. No segmental necrotizing lesions or cellular crescents were seen. Immunofluorescence showed a strong dual pattern of staining for IgG as described in the first biopsy (not illustrated). The overall diagnosis of the subsequent biopsy was inactive anti-GBM GN and MN superimposed on DN. Persistent strong linear GBM staining for IgG despite lack of active lesions in this biopsy.

The patient commenced maintenance haemodialysis 14 weeks after initial presentation when refractory fluid overload ensued, coupled with no renal recovery. Immunosuppression was discontinued. Currently, 5 months after presentation, he is training for home haemodialysis and suitability for combined kidney–pancreas transplant is being assessed.

Discussion

This is an unusual report of the simultaneous diagnosis in a kidney biopsy of seronegative anti-GBM GN, MN and DN. Kidney biopsy was undertaken in this patient with longstanding diabetes and clinical renal parameters not typical of DN. He presented with nephrotic range proteinuria, an active urinary sediment and AKI. Pulmonary involvement from anti-GBM disease was uncertain, as haemoptysis was minimal, settled with antibiotics and occurred in the context of smoking and recent immunosuppression. Absence of serum anti-GBM antibodies made serological assessment of disease activity difficult.

Anti-GBM kidney disease, characteristically a rapidly progressive crescentic GN associated with pulmonary haemorrhage and circulating anti-GBM antibodies directed against the NC1 domain of type IV collagen α3 chain (α3(IV)NC1) [12], is commonly diagnosed through a

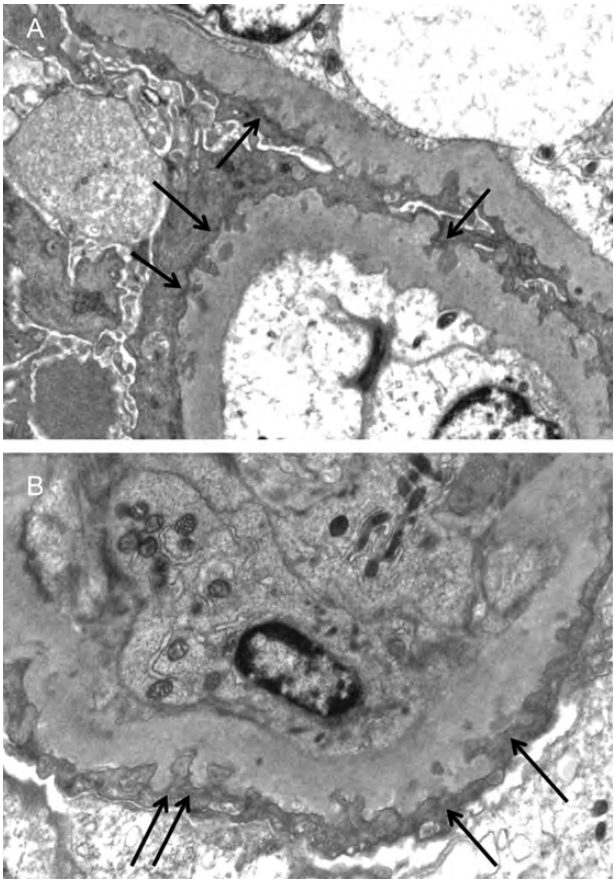

Fig. 5. (**A** and **B**) Transmission electron microscopy shows GBM thickening with small subepithelial electron-dense deposits (arrows) with early GBM spike formation (double arrows). Original magnification ×10 000.

combination of clinical, histological and serological findings [13]. Seronegative anti-GBM disease occurs in ∼2–3% of cases, where antibodies not detected by ELISA methods are found with other biosensor techniques [14]. Jia *et al*. [15] suggest that circulating anti-GBM antibodies undetectable by ELISA may recognize cryptic and highly conformation-dependent epitopes restricted on α3(IV) NC1. They used indirect immunofluorescence for antibody detection. However, the gold standard for anti-GBM GN diagnosis is strong linear GBM histological staining for IgG.

Occurrence of crescentic GN with DN is uncommon, with five previous reports of anti-GBM GN superimposed on DN [5–8], and only one subject recovered kidney function [6]. Three patients had SCr >880 μmol/L prior to treatment initiation. Poor prognostic factors for kidney recovery described in anti-GBM GN include oligoanuria, SCr >600 μmol/L, high serum anti-GBM antibodies (ELISA) levels and a high percentage of crescents on biopsy [16].

In biopsy-based studies, MN is a relatively common non-diabetic kidney pathology in diabetic populations [1, 2]. Though, severe proteinuria as biopsy indication suggests selection bias, as an autopsy study investigating kidney pathology in diabetes demonstrated no MN cases and only one IgAN in 210 subjects [17]. Outcomes in those with combined MN and DN are poor, both contributing to worsening proteinuria and acceleration of kidney injury [18].

Conversely, associations between MN and anti-GBM GN are well recognized, and were first described in 1974 [19]. Two recent reviews identified ∼30 cases, sequential occurrence in one-third (either anti-GBM GN following MN or vice versa), and simultaneous diagnosis in the remainder [9, 20]. Reported outcomes varied with kidney recovery in one-third, progression to ESKD in the majority and death in four patients. Some have speculated that initial alteration in basement membrane permeability allows further disruption leading to heightened immune system exposure and development of additional disease [9, 21]. Seronegative anti-GBM GN and MN have not been reported together, although seronegativity itself is rare.

All three diseases, in part at least, implicate autoimmunity. Anti-GBM disease is one of the few human autoimmune diseases where the autoantigen is known [22] with multiple HLA-positive associations, namely *HLA-DR15* and *HLA-DR4* [23, 24]. HLA typing in our patient was positive for *HLA-DR4* and *-DR16* but negative for *-DR15*. More recently, primary MN has been linked with the *HLA-DQA1* allele on chromosome 6p21 [25], and PLA2R identified as a major autoantigen [26]. Circulating autoantibodies against M-type PLA2R in the podocyte are present in 70% of idiopathic MN patients [26]. Serum PLA2R antibody was negative in this patient and PLA2R was not detected in the glomeruli on biopsy. Hence, the features do not support diagnosis of idiopathic MN. Rather secondary MN subsequent to GBM/podocyte injury caused by anti-GBM GN and/or DN is postulated. T1DM, long considered a chronic autoimmune disease, results from anti-islet autoimmunity developing in genetically susceptible individuals [26], where major susceptibility loci map to *HLA-DRB1* and *HLA-DQB1*, also located on chromosome 6p21 [27]. Immunological tolerance mechanisms prevent these diseases in healthy individuals and occurrence of all three in our patient suggests such tolerance may have broken down.

Presence of DN can complicate kidney biopsy interpretation of other pathology. In diabetes, the biopsy shows weak to moderate linear staining of GBM, BC and TBM for IgG and albumin [28]. However, in this case, there was very strong linear GBM staining for IgG with negligible staining in BC and TBM, a pattern diagnostic of anti-GBM GN. In contrast, albumin showed diffuse moderate staining of GBM, BC and TBM (illustrated in Figure 3A).

With triple pathology, kidney recovery seemed unlikely despite reasonable prognostic markers at presentation, SCr <600 μmol/L and urine output >2 L/day. Progression of disease despite aggressive immunosuppression prompted repeat kidney biopsy to guide treatment. Subsequent complications, neutropaenia and opportunistic infection, in the setting of worsening histology with less overall activity, resulted in immunosuppression cessation and dialysis commencement, with a view to future combined kidney–pancreas transplantation.

The kidney biopsy in glomerular disease is invaluable, providing diagnosis and important disease information whilst aiding management decisions [13]. Despite advances with serological testing [13], this case continues to illustrate the importance of the biopsy. Such as in this instance, kidney biopsies of patients with diabetes, particularly those with clinical features not typical of DN, may reveal other potentially treatable kidney pathology.

Conflict of interest statement. None declared.

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