MPO-ANCA-positive crescentic glomerulonephritis superimposed on a mesangial proliferative glomerulonephritis in a patient with mixed cryoglobulinemia due to hepatitis C infection: a case report

ABSTRACT

Renal involvement of antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis typically occurs in the form of a pauci-immune crescentic glomerulonephritis. The finding of some immune complex deposits in these glomerulonephritis is not unusual, but the cases with greater than mild deposition suggest the presence of a second and immune complex related glomerular disease. The significance of the immune complex deposits and their role in ANCA associated vasculitis is still a topic of debate, but higher levels of proteinuria and worse prognosis with respect to renal function have been described in these patients.

We report a case of a patient with a known mesangial proliferative glomerulonephritis related to a mixed cryoglobulinemia secondary to a chronic hepatitis C infection that later developed a rapidly progressive glomerulonephritis due to a superimposed anti-myeloperoxidase ANCA positive glomerulonephritis.

Key-words: ANCA-associated vasculitis; immune complex deposits; mesangial proliferative glomerulonephritis; rapidly progressive glomerulonephritis.
INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis have similar characteristics on renal histology and usually present as a pauci-immune crescentic glomerulonephritis (GN). The finding of some immune complex (IC) deposits in these GN is not unusual, and the majority are typically mild (≤ 2+ on a 0 to 4+ scale). The cases of ANCA-associated GN with greater than mild IC deposits suggest the presence of a second and IC-related glomerular disease. IgA nephropathy, post-infectious GN, lupus nephritis, hepatitis C virus (HCV) associated glomerulopathy and membranous nephropathy have already been described in situations with a superimposed ANCA GN. The significance of the IC deposits and their role in ANCA associated vasculitis is still a topic of debate.

We report the case of a patient presenting with a rapidly progressive (RPGN) MPO-ANCA-positive GN superimposed on a known mesangial proliferative GN related to a mixed cryoglobulinemia secondary to a chronic HCV infection.

CASE REPORT

A 35-year-old Caucasian man was admitted to our hospital’s emergency room due to marked asthenia and polyarthralgias of inflammatory rhythm affecting the shoulders, elbows, wrists, metacarpophalangeal and proximal interphalangeal joints, knees and ankles with a few weeks of evolution. The patient also complained of diminished urinary output and slight oedema of his lower limbs. He had a known history of chronic HCV infection, type III mixed cryoglobulinemia, hypertension (known for ten years) and additive symmetric polyarthritis (known for 2 years) being treated by a rheumatologist with 500 mg of naproxen and 5 mg of prednisone daily. During the initial investigation of this polyarthritis, an active urinary sediment was found with microscopic haematuria (437.99 erythrocytes/μL with dysmorphic features) and 24 hour proteinuria of 870 mg. By that time, additional laboratory results showed negative anti-nuclear antibodies, negative anti-double-stranded DNA (anti-dsDNA), negative ANCA and normal C3 and C4. This led to the execution of a renal biopsy six months prior to his admission. Light microscopy (LM) showed 13 glomeruli with diffuse mesangial matrix expansion and hypercellularity. The interlobular arteries showed some intimal hyalinization and the interstitium was normal. The silver methenamine–stained section showed a normal glomerular basement membrane. Immunofluorescence (IF) microscopy contained 13 glomeruli with diffuse mesangial matrix expansion and hypercellularity. The interlobular arteries showed some intimal hyalinization and the interstitium was normal. The silver methenamine–stained section showed a normal glomerular basement membrane. Immunofluorescence (IF) microscopy contained ten glomeruli showing strong diffuse and segmental staining in the mesangium and less on the capillary walls of IgG (3+ on a 0 to 4+ scale), IgM (2+) and C3 (3+) and C1q (1+) staining. The final histopathological diagnosis was a mesangial proliferative GN in the context of a type 3 mixed membranous nephropathy.
cryoglobulinaemia associated with a HCV infection. The patient had also known significant alcoholic habits (heavy drinking, usual consumption of more than 15 units of alcohol per week) and a high tobacco exposure with 20 pack-years of smoking. Despite these addictions, he denied recent alcohol abuse or previous consumption of recreational drugs. He mentioned a car accident when he was 13 years old with a resultant exposed tibia fracture and the need for a blood transfusion (before 1992), the probable cause of his chronic HCV infection.

On admission, physical examination revealed a reasonable performance status, (karnofsky performance status scale of 80 %) with apparent age slightly superior to real age, a body mass index of 26.1 Kg/m2, and an elevated blood pressure (150/100 mmHg). A slight pain on active and passive movement of his shoulders and metacarpophalangeal joints was perceived, but with no signs of stiffness, tenderness, swelling or deformity of these or other affected joints. No purpura, petechiae, ecchymoses or other dermatologic signs were observed. His neurologic, cardiac, pulmonary and abdominal examinations were normal. He had a slight pitting oedema on both ankles and his urinary output in the first 24 hours under observation was of 40 ml/h. Laboratory results at admission were as follows: haemoglobin 9.9 g/dL (normal range, 12.5-17.0 g/dL), mean corpuscular volume 92 fl (normal range, 80–100 fl), white blood cell count 5.9 x 109/L (normal range, 9.9-10.5 x 109/L), platelets 207 x 103 per mm3 (normal range, 150-300 103 per mm3), prothrombin time and partial thromboplastin time within the normal range, serum creatinine (Scr) 7.2 mg/dL (normal range, 0.72-1.18 mg/dL), blood urea nitrogen 110 mg/dL (normal range, 7.94-29.9 mg/dL), potassium 6.1 mmol/L (normal range, 3.5-5.1 mmol/L), albumin 3.8 g/dL (normal range, 3.5-5.2 g/dL), total cholesterol 177 mg/dL, HDL cholesterol 22 mg/dL, triglycerides 163 mg/dL, aspartate aminotransferase 23 U/L (normal range, < 38 U/L), alanine aminotransferase 18 (normal range, <41 U/L), alkaline phosphatase 62 U/L (normal range, 30-120 U/L), gamma-glutamyl transpeptidase 62 U/L (normal range, < 38 U/L) and C-reactive protein 2.39 mg/dL (normal range, < 0.05 mg/dL). The urinary sediment analysis showed 12.8 leucocytes/µL and 547 erythrocytes/µL with dysmorphic features. The spot urine protein-to-creatinine ratio was 2190 mg/g. A renal ultrasound was performed revealing normal sized kidneys with good corticomedullary differentiation and increased parenchymal echogenicity. His chest radiography was normal. There was deemed no need for emergency haemodialysis. He was admitted to our nephrology department with the diagnosis of a non-oliguric severe acute kidney injury and further investigation ensued. We obtained routine blood biochemistry from the patient, dated 3 months before this episode, that showed normal renal function and an active urinary sediment (9.5 leucocytes/µL and 79 erythrocytes/µL with dysmorphic features) and mild proteinuria (urine protein-to-creatinine ratio of 470 mg/g) with no other significant abnormalities. Additional diagnostic tests included: C3 1.45 g/L (normal range, 0.9-1.8 g/dL), C4 0.31 g/L (normal range, 0.1-0.4 g/dL), perinuclear ANCA (p-ANCA) strongly positive (++++) with a myeloperoxidase (MPO) titer of > 600 U/ml. His anti-nuclear antibodies were slightly positive (+) with a diffuse granular pattern, the anti-dsDNA antibodies had a level of 3.9 IU/ml (normal range < 4.2 U/ml) and the rheumatoid factor, anticyclic citrullinated peptide (CCP), anti-Smith (Anti-Sm), anti-ScI-70, anti-Ro/SS-A, anti-La/SS-B, and anti-GBM antibodies all were negative. The hepatitis C antibody (Anti-HCV) was positive but HCV-RNA was undetectable in the serum. The remaining viral screening for hepatitis B and the human immunodeficiency virus (HIV) 1 and 2 was negative. The known type III mixed cryoglobulinaemia was once again found (total concentration of polyclonal cryoprecipitate IgG, IgM and IgA: 0.028 g/L). The HCV-RNA in the cryoprecipitate was undetectable. The serum protein electrophoresis (SPEP) showed a slightly elevated level of the alpha-2 zone and no monoclonal spike. The immunoglobulin levels were normal and the serum and urinary immunofixation found no monoclonal protein.

A second renal biopsy was performed and two fragments were collected for LM and IF. The LM showed a cortico-medullary fragment with sixteen glomeruli. Twelve glomeruli (75 %) showed extra-capillary proliferation with fibroepithelial crescents (Fig. 1). A diffuse mesangial matrix expansion and hypercellularity was also present. The silver methenamine–stained section showed a normal glomerular basement membrane, with no double contours, spikes or subendothelial deposits (Fig. 2). Moderate chronic tubulointerstitial nephritis was present. The interlobular arteries showed lesions of atherosclerosis with intimal hyalinization and irregular narrowing of
their lumen. The IF fragment contained ten glomeruli and revealed strong diffuse and segmental staining in the mesangium and capillary walls of IgG (3+), IgA (3+) and C3 (3+), with weaker IgM (1+) and C1q (1+) staining.

On the second day of admission, the patient started treatment for a crescentic GN with 3 pulses of 500 mg of methylprednisolone followed by 150 mg of oral cyclophosphamide (2 mg/Kg per day) and 80 mg of prednisone (1 mg/Kg/dia). Prophylaxis against opportunistic infections was used with daily 480 mg trimethoprim-sulfamethoxazole. During the following days, his renal function progressively improved and the sCr stabilized at 3.5 mg/dL. He was discharged on the tenth day with significant clinical improvement and good blood pressure control with an angiotensin-converting-enzyme (ACE) inhibitor. At this point, the urinary sediment showed signs of lesser activity (110 erythrocytes/µL), but significant proteinuria was still present (spot urine protein-to-creatinine ratio: 3910 mg/g). One month later, in a routine follow-up consultation, the patient had no complaints, showed stable renal function and a MPO titer of 85 U/ml.

Three months after discharge, the patient was diagnosed with pneumonia and severe leucopenia and readmitted to the nephrology department. Oropharyngeal candidiasis was present and reinforced the idea of a high immunosuppression state. A chest computed tomography scan (CT scan) showed consolidation areas in the posterior segment of the right superior lobe and on both lower lobes. Cyclophosphamide was stopped and the patient remained on 20 mg of prednisone. A multi-drug-resistant *Pseudomonas aeruginosa* was isolated from a bronchoalveolar lavage (BAL) and the patient was started on intravenous (IV) colistin, which he maintained for ten consecutive days. Renal function remained stable. Twenty days after being discharged, he was admitted with a recurrence of the pneumonia. Severe respiratory insufficiency developed and orotracheal intubation was required for the institution of mechanical ventilation. A new CT scan showed various areas of consolidation with cavities in both lungs. The biggest cavitation was found in the right superior lobe and was 7.8 cm in diameter. A multi-drug-resistant *Pseudomonas aeruginosa* was again isolated, this time from a sputum culture and a BAL. Blood cultures were negative. After five days in an intensive care unit, he was again transferred to our department. Due to recurrent fever and other signs of treatment resistance, amikacin was associated with colistin for the approach of the isolated multi-drug-resistant *Pseudomonas aeruginosa*. An upper gastro-intestinal endoscopy was performed due to epigastric pain and dyspepsia showing extensive esophageal candidiasis, which was treated with fluconazol. A multi-disciplinary approach to this clinical
picture was used and an infectologist, a pneumologist and pulmonary surgeon were consulted. The hypothesis that the pulmonary disease was ANCA-associated was considered and two lung biopsies of the cavitations were performed: a transbronchial biopsy with flexible bronchoscopy and a second one with thoracoscopy. Both showed unspecific inflammatory findings without signs of small vessel vasculitis or granulomatous inflammation in the surrounding tissues. Despite his severe inflammatory state his renal function remained stable. The ANCA-MPO titer was repeated and a level of 25 U/ml was found. His clinical condition progressively deteriorated with multi-organ failure and the patient died six months after the diagnosis of RPGN was made.

**DISCUSSION**

Our patient presented a typical RPGN overlapping with a known mesangial proliferative GN. The RPGN is a syndrome currently categorised into one of four disorders, which reflect different mechanisms of glomerular injury: Anti-glomerular basement membrane (GBM) antibody disease (Type 1), immune complex deposition (Type 2), pauci-immune (Type 3) and double-antibody positive disease (Type 4), which has features of both types 1 and 39. Looking at the differential diagnosis of our case we would essentially have to consider either a type 3 RPGN with pauci-immune ANCA with associated vasculitis or a type 2 RPGN with a cryoglobulinemic associated vasculitis. The histological and laboratory findings suggest a MPO-ANCA-positive GN superimposed on a mesangial proliferative cryoglobulinemic GN. Renal involvement in type III mixed cryoglobulinemia is variable and usually non-specific, contrary to the type II mixed cryoglobulinemia, in which the IgMk monoclonal component is related to a specific well-characterised pattern of a glomerular disease, the membranoproliferative GN. [10-12] Our patient’s history of active urinary sediment and mild proteinuria together with his chronic complaints of arthralgias, especially those affecting the metacarpophalangeal, knees and ankles, are common manifestations of type III mixed cryoglobulinemia13. We believe that the acute crescentic activity in this RPGN is in association with the ANCA. The high ANCA-MPO titer associated with normocomplementemia at diagnosis and the simultaneous fall in the ANCA-MPO titer with treatment and clinical improvement favour this hypothesis. Chronic kidney insufficiency, significant proteinuria and microscopic haematuria ensued despite this improvement in renal function. This is in agreement with the trend towards a worse outcome with respect to renal function, higher serum creatinine levels at the time of biopsy, greater percentage of crescents and higher levels of proteinuria, as described by Neumann et al. and Haas et al. in their studies concerning IC deposits in ANCA-associated crescentic GN2-3. Pulmonary involvement is common in ANCA associated vasculitis and our CT scan findings with areas of consolidation and cavitations are, therefore, not an unusual type at presentation14,15. Despite this, we consider that the aggravated immunosuppression status of the patient and the persistent isolation of *Pseudomonas aeruginosa* manifestly point to a central infectious explanation for these radiologic findings.

The superimposition of the MPO-ANCA-positive crescentic GN on a mesangial proliferative GN may be coincidental or reflect a common pathophysiological process in both diseases. Some authors suggest an interaction between IC and ANCA in producing GN, especially in the beginning of the pathological process2,16-18. An animal model developed to produce ANCA GN and vasculitis showed that the process was not truly pauci-immune and that the IC played a role in its pathophysiology19. There is also experimental evidence suggesting that the alternative pathway of complement is involved in the pathogenesis of ANCA-associated glomerulonephritis and that its inhibition might be a potential therapeutic approachforexample20-22.

Our case report illustrates a unique MPO-ANCA-positive GN superimposed on a mesangial proliferative cryoglobulinemic GN with two renal biopsies performed at distinct times. Of interest is the absence of IgA deposition on the first biopsy and strongly positive on the IF of the second one, relating to the ANCA associated vasculitis. The RPGN with positive MPO ANCA associated IgA nephropathy was already described by other authors and the role of the IC deposition is still to be determined.4-23 We hypothesise that a conjugation of factors took place in the pathophysiological subset of the ANCA GN of this patient, including a pre-existing pro-inflammatory environment, the glomerular IC deposition and a possible subsequent complement activation.

Conflict of interest statement: None declared.
References


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