Brief report

Antineutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in a patient with anti-glomerular basement membrane disease

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Abstract

An unusual case is described of a patient with both anti-glomerular basement membrane (GBM) disease and anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity (MPO-ANCA) presenting with acute renal failure. Four years before a seronegative arthritis of the left wrist was diagnosed. Tests for ANCA by indirect immunofluorescence were repeatedly negative. The diagnosis was made by renal biopsy and by testing the serum with specific enzyme-linked immunosorbent assays (ELISA) for MPO-ANCA and anti-GBM antibodies. To our knowledge, this is the first patient presenting with such findings in the Dutch literature.

Keywords: Acute renal failure; Anti-neutrophil cytoplasmic antibody; Anti-GBM antibodies; Crescentic glomerulonephritis; Seronegative arthritis

1. Introduction

In anti-glomerular basement membrane (GBM) disease autoantibodies are found in serum directed against an epitope within the globular domain of type IV collagen of glomerular, distal tubular, and alveolar basement membranes (Goodpasture antigen). Most patients present with signs and symptoms of a rapidly progressive glomerulonephritis, sometimes accompanied by pulmonary haemorrhage (Goodpasture syndrome). The occurrence of anti-GBM disease is rare: e.g., in Great Britain the incidence is estimated at 0.5 new cases per million inhabitants every year [1]. In recent studies the coexistence of anti-GBM and anti-neutrophil cytoplasmic antibodies in patients presenting with rapidly progressive glomerulonephritis has been shown with possible prognostic implications with regard to recovery of renal function [2–9]. In this report we describe a patient presenting with acute renal failure and both anti-GBM and anti-neutrophil...
cytoplasmic autoantibodies with myeloperoxidase specificity in her serum.

2. Case report

A 49-year-old woman was admitted to hospital with a 4-week history of progressive malaise, nausea, vomiting and haematuria. There was also continuous retrosternal pain, not related to physical exercise or posture. Four years before admission a chronic monarthritis of the left wrist developed. Two years later a synovectomy with resection of the distal ulna was performed, showing a chronic aspecific synovitis. Two months before admission laboratory tests showed normal renal function (serum creatinine 75 µmol/l; normal 60–100 µmol/l). Six weeks before admission treatment with sulphasalazine 1000 mg 2dd was started because of an arthritis of the right ankle. One week later she noticed haematuria, with normal urinary output. Respiratory tract complaints (sinus pain, nasal discharge, cough, haemoptysis) were absent. She smoked 5 cigarettes a day. No history of familial renal or rheumatological disease was present. Physical examination revealed a pale woman with a blood pressure of 150/90 mmHg. Neck veins were not distended; the lungs were clear. Pericardial rubbing was present on cardiac examination. Abdominal examination revealed no abnormalities.

Laboratory tests showed an erythrocyte sedimentation rate > 140 mm, a haemoglobin concentration of 5.7 mmol/l, thrombocytosis of 329 × 10^9/l and a normal white cell and differential count. The urinary protein excretion was 1.1 g/24 h. The sediment contained > 100 erythrocytes, many rust-cylinders and a few erythrocyte-cylinders, but no leucocytes. Urine culture was negative. The serum creatinine concentration had risen to 866 µmol/l; serum urea concentration was 24.7 mmol/l. The calculated endogenous creatinine clearance was 3 ml/min. Serum sodium, potassium, blood glucose, liver functions and LDH were normal. Serum calcium was 2.53 mmol/l, phosphorus 1.69 mmol/l and serum albumin 34 g/l. Protein electrophoresis revealed no paraproteins. Tests for cryoglobulins, rheumatoid factors (Waaler-Rose and latex), antinuclear and antiperinuclear antibodies were negative. Complement factors C3 (1.4 g/l) and C4 (0.31 g/l) were normal, while C3d was elevated (285%).

Tests for anti-neutrophil cytoplasmic antibodies by indirect immunofluorescence using a routine procedure (assay previously described by van de Woude et al. [10]) were negative twice (performed at Groot Ziekenhuis and at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam), as were tests performed on a serum sample drawn 2 weeks later (during treatment). However, the sandwich (catching) ELISA (as previously described by Goldschmeding et al. [11], results expressed as (arbitrary) ELISA units (EU), cut-off level 35 EU) with monoclonal antibodies, used for testing the specificity of antibodies, was positive twice (215 and 217 EU), with specificity for anti-myeloperoxidase. A test performed on a serum sample drawn 2 weeks later (during treatment) was also positive for MPO-ANCA, with a decrease in antibody level (54 EU). All samples were also positive for MPO-ANCA in a direct ELISA using plates coated with purified antigen, thereby excluding false-positive results based on cross-reaction with monoclonal antibodies used in the catching ELISA (tests performed at CLB).

Cardiac sonography showed some pericardial effusion, due to pericarditis. Abdominal sonography showed normal-sized kidneys (length right kidney 11 cm, left kidney 10 cm), with increased echographic density of the renal cortex, equal to that of the adjacent liver and spleen. There was no evidence of hydronephrosis. X-thorax was normal. Percutaneous renal biopsy showed in nearly all glomeruli a recent extracapillary proliferation mixed with fibrin deposits (Fig. 1). One glomerulus showed recent destruction of loops, while total glomerular destruction with a granulomatous reaction occurred in another. Some Bowman's capsules and tubular basement membranes were disrupted, with extensive necrosis of tubular epithelium. Intracapillary proliferation was not present. No signs of vasculitis could be found. The biopsy material for immunofluorescence did not contain glomeruli.

By indirect immunofluorescence on frozen
normal human renal tissue it was found that the IgG's of the patient's serum were bound in a linear way to the glomerular and distal tubular basement membranes. The diagnosis of anti-GBM nephritis was confirmed by ELISA (antigen M2 sub-unit of type IV collagen, kit by Euro-Diagnostica, assay previously described by Wieslander et al. [12]) by which procedure circulating anti-GBM antibodies directed to the Goodpasture antigen were found in a titre of 33 U/ml. The titre of the antibodies in this test is expressed as arbitrary units (U/ml). A level < 10 U/ml is considered negative, a level > 10 and < 20 U/ml is dubious, and a level > 20 U/ml is regarded as positive. Sensitivity and specificity of this assay have been reviewed previously [12].

The patient was treated with combination therapy consisting of daily haemodialysis, plasma-

pheresis of 2–4 l/day, cytostatic (cyclophosphamide 150 mg dd) and corticosteroid (prednisone 50 mg dd) drug therapy for 3 weeks. No improvement in renal function was observed. At this point, because of developing thrombocytopenia (down to 99 × 10^9/l) and continuing anuria cyclophosphamide was stopped. Also plasmapheresis was stopped and the prednisone dose was reduced to 20 mg dd. The patient is still on haemodialysis 3 times weekly. No pulmonary symptoms have occurred so far.

3. Discussion

This patient with a history of seronegative destructive arthritis presented with acute renal failure due to a diffuse crescentic glomerulonephritis with both circulating anti-GBM antibodies and anti-neutrophil cytoplasmic antibodies with myeloperoxidase specificity. To our knowledge, this is the first case so far presenting with such findings to be reported in the Dutch literature. Cohen Tervaert et al. [13] studied the association of anti-MPO antibodies with different forms of vasculitis. They found a 26% sensitivity rate for MPO antibodies in patients with active systemic necrotizing vasculitis of the polyarteritis group, Wegener's granulomatosis or idiopathic crescentic glomerulonephritis. The specificity of MPO antibodies for these diseases was 99%.

There are several studies reporting on the coexistence of ANCA and anti-GBM antibodies in patients with rapidly progressive glomerulonephritis [2–9]. In two studies by Bosch et al. [3,9] ANCA were present in about 30% of patients with anti-GBM disease, although with a positive indirect IF assay. Almost all were directed against myeloperoxidase, possibly suggesting a coexisting systemic vasculitis. In our patient the presence of anti-MPO antibodies was demonstrated by catching as well as by direct ELISA. O'Donoghue et al. in a prospective study on patients with serologically and biopsy-proven anti-GBM disease found 11 out of 64 patients to be positive for ANCA antibodies (by indirect IF), all directed against myeloperoxidase [4]. The apparent discrepancy of a negative indirect IF test

Fig. 1. Light microscopy shows extensive extracapillary proliferation with collapse of the glomerular tuft (methenamine silver staining; × 400).
with positive anti-MPO ELISA has been described before: Weber et al. [5] detected low titres of anti-MPO antibodies in 7 out of 23 patients with Goodpasture's syndrome or anti-GBM glomerulonephritis. In these patients indirect IF for ANCA was negative as in ours.

A possible explanation for the simultaneous production of both types of antibodies might be that anti-GBM antibody production starts after injury to the GBM by ANCA-associated glomerulonephritis. The age of our patient, being nearer to that of patients with ANCA alone (sixth decade) as opposed to patients with anti-GBM alone (third decade), would support this hypothesis [3,8]. This is also in accordance with the study of O'Donoghue et al. [4] who reported a strong association of double positives with female sex and (higher) age as opposed to patients with anti-GBM disease alone. In our patient the titre of anti-GBM antibodies was rather low. This is in accordance with Bosch et al. who detected an inverse relationship between anti-GBM antibodies and ANCA titres [3].

As mentioned above, the assay for ANCA by indirect immunofluorescence in our patient was negative twice. In the study by Bosch et al. [3] a diffuse nuclear immunostaining pattern with additional endonuclear bands was seen in the indirect IF tests of nearly all patients with anti-MPO antibodies. The most probable explanation for this discrepancy is a difference in the indirect immunofluorescence detection technique for ANCA. IIF tests in our patient were performed on ethanol-fixed granulocytes. It has been shown that anti-MPO positive sera produce a perinuclear staining pattern on ethanol-fixed granulocytes and a cytoplasmic staining pattern on formalin-fixed granulocytes [14]. Moreover, it has been demonstrated that anti-MPO sera that are negative on ethanol-fixed granulocytes produce a cytoplasmic staining pattern on granulocytes when cross-linking fixatives are used [15]. Therefore a probable explanation for the negative IIF test in our patient could be the loss of antigen and/or relevant epitopes during ethanol fixation, in which case a IIF test using formalin-fixed granulocytes might be a more reliable alternative.

It has been concluded that in anti-GBM disease ANCA may represent a serological marker of good (renal) prognosis. In the study by Bosch et al., 4 out of 11 patients (36%) with both ANCA and anti-GBM antibodies initially on dialysis recovered satisfactory renal function. Three patients died (27%). In contrast, all 13 survivors with anti-GBM antibodies alone still required dialysis support after treatment, while 9 patients (41%) died [3]. In our patient there is no indication for recovery of renal function. This is in accordance with the study by O'Donoghue et al. [4] who found a poor outcome in the double positive group (11 patients) with significant mortality (4 patients, 36%) and irreversible renal failure (100% requiring dialysis with 9% independent renal function at 3 months). Of 53 patients with anti-GBM alone 34 (64%) required dialysis, 23 (44%) having independent renal function at 3 months. The differences in renal prognosis in the afore-mentioned studies might at least partly be due to differences in age between the study populations, the patients of Bosch (mean age 59 years) being considerably younger than those of O'Donoghue (mean age 73 years). Weber et al. also reported a poor renal prognosis for patients with combined MPO-ANCA and anti-GBM similar to that for patients without ANCA antibodies [5].

In our patient because of developing thrombocytopenia combination therapy was stopped after 3 weeks. Since patients with rapidly progressive glomerulonephritis may respond after 4 weeks [16], it cannot be excluded that renal recovery might have occurred if treatment with cyclophosphamide and/or plasmapheresis could have been continued. However, Herody et al., among others, concluded in their study of patients with anti-GBM disease that creatininaemia over 600 \( \mu \text{mol/l} \) and circulating anti-GBM antibodies detected by immunofluorescence were features which indicated an unfavourable renal course [1].

More extensive extrarenal features in patients with combined ANCA and anti-GBM antibodies have been reported. The development of pulmonary disease seems significantly associated with smoking [1,4]. As for the interpretation of our patient's arthritis, it is known that rheumatic manifestations often precede a fulminant course
in systemic vasculitis [17]. Furthermore, ANCA occur in rheumatoid arthritis (RA): Cambridge et al. detected anti-MPO antibodies in 12% of patients with RA [18]. We were not able to find references pointing to a possible association between anti-GBM disease and arthritis in the literature. Therefore, at present it can neither be demonstrated nor ruled out that the seronegative arthritis in our patient is an extrarenal manifestation of anti-MPO disease.

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References


