Pulmonary-Renal Syndrome with a Focus on Anti-GBM Disease

Jan-Stephan F. Sanders, M.D., Ph.D.,1 Abraham Rutgers, M.D., Ph.D.,2 Coen A. Stegeman, M.D., Ph.D.,1 and Cees G.M. Kallenberg, M.D., Ph.D.2

ABSTRACT

Pulmonary-renal syndrome is a potentially life-threatening combination of pulmonary hemorrhage and acute renal failure. Several pathological entities can cause this syndrome. This review discusses the diagnostic strategy required to initiate appropriate therapy. Rapid serological testing and appropriate interpretation can be of great additive diagnostic value. Also discussed are the pathogenesis, therapy, and outcome of anti–glomerular basement membrane disease, one of the pathological entities that can cause pulmonary-renal syndrome.

KEYWORDS: Anti-GBM, Goodpasture, ANCA, rapidly progressive glomerulonephritis, vasculitis

Goodpasture syndrome is the eponym for pulmonary-renal syndrome and can be caused by several diseases. One of these clinical entities is Goodpasture disease, which is characterized by lung hemorrhage and rapidly progressive glomerulonephritis associated with linear deposition of antibodies along the glomerular basement membrane (GBM).

In 1919 the American pathologist Dr. Goodpasture attempted to define the specific pathological features of influenza infection in the lung.1 However, one of his patients had a systemic disease with both pulmonary and renal involvement, resulting in a pulmonary-renal syndrome.

In 1958 the clinical picture of pulmonary-renal syndrome [ie, diffuse alveolar hemorrhage and rapidly progressive glomerulonephritis (RPGN)], was described by Stanton and Tange and named after Dr. Goodpasture.2 A major breakthrough was the subsequent identification of anti-GBM antibodies in 1967.3 With this discovery the diagnosis and definition of Goodpasture disease, also called anti-GBM disease, could be established and further progress was made regarding therapy in this specific condition. This article presents an overview of the diagnostic considerations in pulmonary-renal syndrome and subsequently focuses on therapy and outcomes for anti-GBM disease.

PULMONARY-RENAL SYNDROME

The strict definition of pulmonary-renal syndrome is the combined clinical picture of rapidly progressive glomerulonephritis and pulmonary capillaritis that requires histological confirmation. However, the clinical setting is that of a patient presenting with acute renal failure and pulmonary infiltrate(s) with suspected pulmonary bleeding. This is also called Goodpasture syndrome. Goodpasture syndrome can be caused by several different diseases and is thus by definition not a final diagnosis.
Table 1 Differential Diagnosis in Patients Presenting Clinically with Pulmonary-Renal Syndrome

- NECROTIZING SMALL-VESEL VASCULITIS
  * PR3- and MPO-ANCA associated (MPA, WG, CSS)
  * Anti-GBM disease
  * Other vasculitides (Henoch-Schönlein purpura, SLE, cryoglobulinemia, drug induced)
- CATASTROPHIC ANTI-PHOSPHOLIPID SYNDROME
- RENAL FAILURE WITH VOLUME OVERLOAD / CARDIAC FAILURE
  * Chronic/acute glomerulonephritis, diabetes
  * Atherosclerosis/hypertensive nephrosclerosis
  * Microangiopathic renal failure/hemolytic uremic syndrome
- RENAL FAILURE ASSOCIATED WITH PULMONARY INFECTION
  * Legionella, mycoplasma, streptococcus
  * Hemorrhagic fever with renal syndrome (eg, Hantavirus)
- ENDOCARDITIS
- SIRS/SEPSIS WITH MULTIORGAN FAILURE
- CARDIOVASCULAR (eg, renal artery stenosis)

Table 2 Diagnostic Policy in Pulmonary-Renal Syndrome

- URINARY SEDIMENT
  * Glomerular hematuria
- CHEST X-RAY
- RENAL SONOGRAPHY
  * Urological problem(s)
  * Renal size
- MICROBIOLOGICAL TESTS (culture of urine, blood, sputum)
- COMPLEMENT C3, C4 LEVELS
- SSS: SPEEDY SPECIFIC SEROLOGY
  * ANCA
  * Anti-GBM
- RENAL BIOPSY
  * Immunohistochemical staining

ANCA, anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane.

Because the term Goodpasture syndrome can be confusing in combination with the clinical application of the term Goodpasture disease, this article uses the terms pulmonary-renal syndrome and anti-GBM disease where appropriate.

Pulmonary-renal syndrome can rapidly lead to severe pulmonary and renal failure and should thus be considered a medical emergency. A considerable number of patients will require admission to an intensive care unit (ICU) for renal replacement therapy or mechanical ventilation, and mortality both from primary disease and treatment-related morbidity is significant. 4 Therefore it is of utmost importance to establish the correct diagnosis and initiate the appropriate therapy as soon as possible. Several diseases can cause pulmonary-renal syndrome; a differential diagnosis is shown in Table 1.

DIAGNOSTIC STRATEGY

Essential diagnostic tests in the clinical workup of patients with pulmonary-renal syndrome are a blood count, biochemistry including serum creatinine, chest radiograph, urinary sediment, renal ultrasonography, and subsequent specific serology (Table 2). Collection of samples for microbiological analysis is required to rule out infectious causes of disease. Diffuse alveolar hemorrhage manifests bilateral, diffuse parenchymal or more patchy pulmonary infiltrates on chest radiographs. 5 Unfortunately, these chest radiographic abnormalities are not specific for alveolar hemorrhage. Further, the occurrence of hemoptysis is neither sensitive nor specific. Additional pulmonary diagnostic procedures such as computed tomographic (CT) scans and bronchoscopy with bronchoalveolar lavage (BAL) might be needed. 6 The urinary sediment will provide clues to detect glomerular disease by glomerular hematuria. In skilled hands the finding of red cell casts is highly sensitive for glomerular disease. 7 In addition to or in the absence of red cell casts acanthocytes are of diagnostic value for identifying glomerular hematuria. In glomerulonephritis (GN) some proteinuria will be present, but rarely in the nephrotic range (>3.5 g/24 hours). The potential value of renal ultrasonography is to exclude postrenal problems and measure renal size, with small kidney size and loss of renal cortex suggesting more longstanding renal disease. 8

For an optimal diagnostic strategy in patients presenting with pulmonary-renal syndrome it is important to realize that some diseases are highly prevalent in these patients. In the literature many case reports and several series of patients with the pulmonary-renal syndrome have been described, with differences in frequency of reported diagnoses. In three publications including 142 patients, 87 had microscopic polyangiitis or Wegener granulomatosis (WG), and 22 had anti-GBM disease (Table 3). 4,9,10 Despite the very low incidence of these diseases in the total population (10 to 20 per million per year), as many as 60% of patients presenting with the pulmonary renal syndrome will be diagnosed with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) or anti-GBM disease.

Because microscopic polyangiitis, WG, and anti-GBM disease are often associated with specific serologic features it is of major diagnostic utility to rapidly obtain specific serology of ANCA and anti-GBM antibodies. These serological tests are discussed in more detail following here. An additional useful biochemical screening test is the level of complement proteins C3 and C4. Several diseases, such as systemic lupus erythematosus...
ethanol-fixed neutrophils. In patients with vasculitis, CAs are detected by indirect immunofluorescence on cytoplasmic antibodies (ANCAs). Conventionally, ANCA and anti-GBM antibodies are associated with antibodies against components of neutrophilic granulocytes, also called anti-neutrophil cytoplasmic antibodies (ANCA). These specific antibodies can be detected by enzyme-linked immunosorbent assay (ELISA). In screening for ANCA, the indirect immunofluorescence test is the method of choice. When results are positive, it is essential to determine antigen specificity by specific ELISAs for both PR3- and MPO-ANCA. In clinical practice PR3-ANCA are predominantly associated with WG, whereas MPO-ANCA are more often associated with MPA, CSS, and NCGN. However, in WG MPO-ANCA can be present, and PR3-ANCA can be present in MPA and NCGN. Finally, a small number of patients with WG, MPA, and NCGN will present without detectable ANCA at diagnosis or during follow-up. Anti-GBM antibodies were first described in 1967 by Lerner et al and are specifically associated with anti-GBM crescentic glomerulonephritis, anti-GBM alveolitis, and the combination. The auto-antigen for the anti-GBM antibodies has been identified as the noncollagenous part of the α3 chain of type IV collagen. The detection of anti-GBM antibodies is highly specific for anti-GBM antibody-mediated disease. The antibodies can be detected in situ by direct immunostaining of a kidney biopsy or in serum (detected by ELISA). There are few reports on the sensitivity of anti-GBM antibodies. However, there are case reports of patients having histopathological proof of anti-GBM antibody-mediated disease without detectable circulating anti-GBM antibodies, suggesting absorption of the circulating antibodies by the diseased tissue. Thus negative serology should be interpreted with caution. Approximately 30% of patients with anti-GBM disease are also positive for MPO-ANCA; this specific subgroup will be discussed in more detail.

Recently, new diagnostic tests for rapid determination of both ANCA and anti-GBM antibodies have been developed. In a retrospective study of patients with biopsy-proven NCGN an ANCA-GBM dot-blot when compared with a specific ELISA yielded a sensitivity of 92 to 95% for PR3-ANCA (n = 36) and 80 to 86% for MPO-ANCA (n = 32), with a specificity of 100%. In patients with biopsy-proven anti-GBM nephritis (n = 9) anti-GBM was detected by both ELISA and dot-blot. The specificity of the anti-GBM dot-blot was 91 to 94%. The authors concluded that the ANCA-GBM dot-blot in comparison to ELISA is a useful screening tool, but

### Table 3 Reported Diagnosis in Pulmonary-Renal Syndrome

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Age (range)</th>
<th>M/F</th>
<th>Dialysis dependent (range)</th>
<th>Ventilatory support (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA/WG</td>
<td>87</td>
<td>60 (19–84)</td>
<td>60%</td>
<td>60%–40%</td>
<td>40%–86%</td>
</tr>
<tr>
<td>Anti-GBM</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>33</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

**Serological findings**

<table>
<thead>
<tr>
<th>PR3/MPO-ANCA*</th>
<th>94</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-GBM*</td>
<td>22</td>
</tr>
</tbody>
</table>

**Note:** ANCA, anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; WG, Wegener granulomatosis.

* 7 patients were both MPO-ANCA and anti-GBM positive.

### ANCA AND ANTI-GBM ANTIBODIES

WG, MPA, i (idiopathic)NCGN, and CSS are frequently associated with antibodies against components of neutrophilic granulocytes, also called ANCA. Conventional ANCA are detected by indirect immunofluorescence on ethanol-fixed neutrophils. In patients with vasculitis, three distinct patterns can be distinguished: a granular cytoplasmic neutrophil fluorescence with central interlobular accentuation (C-ANCA), a perinuclear pattern (P-ANCA), and an atypical pattern. Approximately 90% of sera that produce a C-ANCA pattern contain antibodies that are directed against the antigen perinuclear pattern 3 (PR3), a component of neutrophil granules. In contrast, P-ANCA represent a less specific finding, in ~70% of patients suspected of vasculitis. This pattern is associated with antibodies directed against the enzyme myeloperoxidase (MPO), also a component of neutrophil granules. These specific antibodies can be detected by enzyme-linked immunosorbent assay (ELISA).
negative MPO-ANCA results should be interpreted with caution, and positive anti-GBM results need additional confirmation by ELISA. Subsequently we used the dot-blot test prospectively in our center; this resulted in excellent test data. In 50 out of 51 tests the dot-blot and ELISA produced the same result, with only one patient sample scored as negative in the MPO-ANCA dot-blot and positive in the MPO-ANCA ELISA (unpublished data, Table 4). In this study six patients were double positive in MPO-ANCA ELISA and dot-blot, nine patients were positive in PR3-ANCA ELISA and dot-blot, four were positive in both anti-GBM ELISA and dot-blot, and 31 were negative in both dot-blot and ELISA.

Thus the dot-blot is a valuable addition for rapid serological testing in pulmonary-renal syndrome. However, results should be confirmed according to the approach recommended in the guidelines with indirect immunofluorescence followed by ELISA. New assays are available or under development, and future research and use in clinical practice might lead to new diagnostic approaches for serological testing and incorporation in guidelines.24,25

### PATHOGENESIS OF ANTI-GBM DISEASE

Anti-GBM disease is caused by the presence of anti-GBM antibodies. Transfer of these antibodies from the kidney of a patient with anti-GBM disease into two monkeys resulted in the development of GN in the monkeys.27 Additional animal studies demonstrated that anti-GBM antibodies were pathogenic.28 The pathogenicity of these autoantibodies has been further strengthened by a positive correlation between autoantibody avidity and titers with disease severity.27 As mentioned previously the auto-antigen for the anti-GBM antibodies was identified as the noncollagenous part of the a3 chain of type IV collagen.29 This molecule is expressed in the basement membranes of the kidney, alveoli, ear, and eye.28 Normally the antigen is hidden from the immune system, and it is thought that a first hit to the GBM or alveolar BM is necessary to open up the epitope for immune recognition. Anti-GBM antibodies bind the glomerular basement membrane and activate complement; this initiates an inflammatory pathway that results in disruption of the filtration barrier. Inflammatory mediators will recruit macrophages and neutrophils to enter the Bowman space and stimulate epithelial proliferation resulting in crescent formation. For the development of severe crescentic GN cell-mediated mechanisms are important. Besides the antibodies autoreactive T lymphocytes are also directed against the same a3 chain of type IV collagen.30 These T cells are key mediators for the development of severe crescentic GN. In rats the transfer of T cells reacting specific against the a3 chain of type IV collagen was sufficient to induce anti-GBM nephritis.30 However, in normal individuals T cells reacting against the a3 chain of type IV collagen are present as well.31 Anti-GBM disease is a so-called monophasic disease, and relapses are highly uncommon. Apparently during the course of disease self-tolerance is restored. Regulatory CD4+ CD25+ T cells may play a major role in this restoration of tolerance. When CD4+ CD25+ regulatory T cells were transferred before onset of experimental disease in murine crescentic GN, glomerular injury was significantly reduced.32 More data about the exact role of T cell immunity in anti-GBM disease will be necessary to understand the role of various subsets of T cells in this disease.

Finally, genetic factors have been implicated in the pathogenesis of anti-GBM disease. Anti-GBM disease has strong positive HLA (human leukocyte antigen) associations. More than 80% of patients carry the HLA alleles DR15 or DR4, whereas the alleles DR7 and DR1 are rarely found.33

### THERAPY AND OUTCOME IN ANTI-GBM DISEASE

The identification of anti-GBM antibodies led to therapeutic interventions to remove these pathogenic antibodies. Before availability of current therapy and renal replacement therapy, mortality exceeded 90%. Current therapies employing plasmapheresis plus corticosteroids and cyclophosphamide have reduced mortality to less than 20% prednisolone.34–36 Since its introduction as a therapeutic option for anti-GMB disease in the mid-1970s, plasmapheresis was quickly adopted worldwide and has been incorporated in all clinical trials. Because of the rarity of anti-GBM disease, only one randomized trial has compared immunosuppressive therapy with the combination of immunosuppressive therapy plus plasma exchange (PE).34 In that study, 17 patients with anti-GBM disease were randomized to either PE plus cyclophosphamide (CYC) and prednisone (n = 8) or CYC and prednisone without PE (n = 9). Plasmapheresis was associated with more rapid disappearance of anti-GBM antibody and improved renal function compared with
immunosuppressive agents alone. Analysis of clinical and pathological values at study entry, however, indicated that the percent of crescents on initial renal biopsy and entry serum creatinine correlated better with outcome than did the therapeutic modality. Patients with low crescents (less than 30%) and well preserved function did well with either treatment, while patients with severe crescentic involvement and impaired glomerular filtration rate did poorly. Although the incremental value of PE over immunosuppressive therapy alone has not been elucidated, PE is accepted as a cornerstone of therapy for anti-GBM disease (together with immunosuppressive agents to inhibit antibody production). Some investigators have employed azathioprine in place of CYC, but studies comparing these agents have not been performed. Most investigators favor CYC over azathioprine. Nowadays treatment consists of oral steroids (1 mg/kg/day of prednisolone, maximum 60 mg), and oral CYC (2 to 3 mg/kg). Daily plasmapheresis (50 mL/kg, maximum 4L) should be instituted for a total of 14 sessions or until the anti-GBM antibodies are undetectable. Plasma is replaced with 4.5% human albumin solution, or, when active pulmonary bleeding is present, fresh frozen plasma. This immunosuppressive regimen is based upon expert opinion and has not been tested in a randomized controlled trial.

Levy et al studied long-term outcome of 71 patients with anti-GBM disease treated with this regimen of PE and immunosuppression. Patients presenting with a creatinine concentration less than 500 μmol/L had a renal survival (ie, did not need dialysis) of 95% after 1 year. When a patient presented with dialysis-dependent renal failure, renal survival was only 8% at 1 year after diagnosis. Patients with pulmonary involvement had higher early mortality, but, possibly because of earlier presentation, better long-term renal survival. Pulmonary hemorrhage is an important early cause of death, but it also responds well to plasma exchange. The data of Levy et al are interesting regarding allocation of intensive therapy, as one might question the value of intensive immunosuppressive therapy in a patient with anti-GBM disease presenting with isolated renal insufficiency. Hardly any study has reported on patients presenting with serum creatinine above 600 μmol/L who recovered renal function. This differs strikingly from the better chance of renal recovery in patients with ANCA-associated renal vasculitis presenting with renal insufficiency. Nevertheless, a small proportion of patients with anti-GBM disease might recover and thus benefit from intensive therapy. Thus an individualized assessment of risk-benefit should be made for a patient presenting with isolated renal failure. In general, CYC should be continued for 2 to 3 months; steroids should be slowly tapered over 6 to 9 months. As discussed previously anti-GBM disease is generally a monophasic disease; recurrences are unusual and there is no indication for long-term maintenance therapy. When patients survive the first critical months and recover renal function, reported long-term renal and patient survival is good.

As an alternative to plasma exchange several patients have been effectively treated by immunoadsorption, which effectively removes anti-GBM antibodies; however, it is unclear whether this is a more effective treatment than plasma exchange. In a case of refractory anti-GBM disease rituximab, a chimeric anti-CD20 monoclonal antibody, has been used resulting in clinical improvement. However, to our knowledge, no other cases of anti-GBM disease treated with rituximab have been published. Finally, in another case of refractory Goodpasture disease, switching CYC to high-dosage nycophenolate mofetil resulted in clinical improvement. This suggests that nycophenolate mofetil might be an alternative for CYC.

For dialysis-dependent patients renal transplantation is not associated with extra risks and has good outcome if antibodies are not detectable at transplantation. However, when transplantation is done in the presence of anti-GBM antibodies the disease will recur. Late recurrence of anti-GBM disease after renal transplantation is rare but has been reported. In ~25% of patients anti-GBM antibodies and MPO-ANCAs coexist. Previously, it was suggested that double-positive patients might have a better renal outcome. However, in a retrospective analysis of 69 patient Rutgers et al found a renal survival of 10% in double-positive patients versus 15% in anti-GBM anti-
body positive patients (Fig. 1). Similarly, Levy et al found poor renal prognosis in double-positive patients presenting with severe renal disease. In conclusion double-positive patients seem to have similar renal and patient survival as other patients with anti-GBM disease, although they are generally older.

**CONCLUSION**

Pulmonary-renal syndrome is a potentially life-threatening combination of pulmonary hemorrhage and acute renal failure. Several pathological entities can cause this syndrome. It is of utmost importance to rapidly establish a diagnosis and promptly initiate appropriate therapy. Rapid serological testing and interpretation can be of great additive diagnostic value. When anti-GBM disease is diagnosed therapy should consist of CYC, steroids, and plasmapheresis. If the patient with anti-GBM disease presents with dialysis-dependent renal failure recovery of renal function is unlikely even with appropriate therapy.

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