THE ROLE OF ANCA AND ANTI-GBM ANTIBODIES IN PULMONARY-RENAL SYNDROM DUE TO WEGENER’S GRANULOMATOSIS

Pulmonary-renal syndrome (PRS) is defined as a diffuse alveolar hemorrhage and rapidly progressive glomerulonephritis. We present a retrospective study of 22 consecutive patients with Wegener’s granulomatosis (WG). Logistic regression analysis and a Wilcoxon test were included in the statistics. Survival time death risk were assessed using the Kaplan-Meier estimator and the Cox proportional hazard model. At recognition, the median Birmingham Vasculitis Activity Score for Wegener’s Granulomatosis (BVAS/WG) was 30.0 (23.0-32.5), PO₂ on air was 5.8 ±0.5 kPa, creatinine level was 7.2 ±1.4 mg/dl. Fifteen patients were PR3 positive, among them 4 patients were also positive for anti-glomerular basement membrane antibodies (anti-GBM). Renal biopsy was performed in 16 patients. Histological examination reviled segmental necrotizing crescentic GN in 15 patients. Thirteen patients were initially dialysis-dependent, and 7 required ventilatory support. All patients were treated with methylprednisolone and cyclophosphamide (pulses). The patients were followed up for 24 ±8 months. Of the survivors, 55% and 31% were alive after 1 and 2 years. Early recognition and proper treatment may improve outcome in PRS.

Key words: pulmonary-renal syndrome, Wegener granulomatosis

INTRODUCTION

Pulmonary-renal syndrome (PRS) is defined as a diffuse alveolar hemorrhage and rapidly progressive glomerulonephritis occurring as the presenting manifestation of the underlying, multisystem autoimmune disease. It represents
a medical emergency with a high mortality which necessitates rapid diagnosis and institution of therapy (1).

The major causes of PRS are anti-glomerular basement membrane antibody (anti-GBM)-associated disease (16%) and anti-neutrophilic cytoplasmic antibodies (ANCA)-associated systemic vasculitis (62%). PRS is an important presentation of vasculitis. Diffuse alveolar hemorrhage is found in 45-50% of patients with Wegener’s granulomatosis (WG) and in 29% of patients with microscopic polyangiitis, and the incidence of diffuse alveolar hemorrhage rises with the severity of renal involvement. In fact PRS has a wide spectrum, including, on the one hand, those patients presenting with rapidly progressing renal failure and absent or mild pulmonary disease and, on the other hand, patients who have substantial pulmonary involvement but mild renal symptoms or even normal renal function.

 Constitutional symptoms of malaise, fatigue, night sweats, fever together with more specific symptoms such as myalgias, polyarthralgias, episcleritis or purpuric rash precede clinical presentation by an average of 3 months. A remarkable feature of PRS is a rapid progression from a cough with hemoptysis to hypoxic respiratory failure over a few hours or days. Hemoptysis is common but not invariable, and its absence may delay the diagnosis. The usual presentation of renal disease in PRS is rapidly progressive glomerulonephritis, hematuria and crescentic, necrotizing glomerulonephritis. The presence or absence of immune deposits aids diagnosis. Non-immune deposit or ‘pauci-immune’ glomerulonephritis is typical for primary vasculitis (1, 2).

 Those cases of PRS that are not related to Goodpasture’s syndrome usually have clinical features suggesting such diagnoses as vasculitis, connective tissue diseases or multiplex mononeuritis. In such cases, it may be useful to detect ANCA antibodies or cryoglobulins, since none of them are usually found in Goodpasture’s syndrome.

 Approximately 30% of all anti-GBM positive sera are also ANCA positive, usually myeloperoxidase (MPO-ANCA) rather than proteinase 3 (PR3-ANCA), and in PRS the proportion is higher at almost 50%. Such cases with both autoantibodies - ANCA and anti-GBM - represent a genuine overlap with clinical and histological features of both diseases which probably have a more severe clinical presentation.

MATERIAL AND METHODS

Patients

The study was approved by a local Ethics Committee. Informed consent was obtained from all patients examined. The examination was performed in patients hospitalized in the Department of Internal Medicine, Warsaw Medical University in Warsaw, Poland. Patients who remain under observation by the authors are also treated in the Systemic Vasculitis Outpatient Clinic,
Czerniakowski Hospital in Warsaw, and in the Department of Internal and Metabolic Diseases, Warsaw Medical University in Warsaw, Poland. The study relied on a prospective observation of disease outcomes in 22 patients: 16 women and 6 men of the mean age 44.5 (32-67), with documented diagnosis. Wegener’s granulomatosis had been established between 1995 and 2006 on the grounds of usually standing criteria, i.e., the assessment of the disease process progress, serological assessment (ANCA antibodies titers), and histopathological examinations. The serological test for detection of ANCA antibodies was performed using the indirect immunofluorescence method and ELISA. The treatment was introduced after obtaining a consent. The patients were treated with immunosuppressive drugs (methylprednisolone pulses for 3 consecutive days, prednisone orally and cyclophosphamide both orally and intravenously). Each patient was assessed with respect to early death factors.

The patients enrolled into the present study consisted of those who had generalized disease, rapidly progressive glomerulonephritis, and diffuse alveolar hemorrhage. On the grounds of physical examination, anamnesis, assessment of results of additional, biochemical and imaging examinations it was concluded that they had a kidney involvement characterized by abnormal results of the urine analysis (proteinuria, erythrocyturia, erythrocytic, and granular casts) and accompanied by rapidly progressive renal failure in the disease course with concomitant diffuse alveolar hemorrhage.

An analysis of the correlation between the parameters known at the time of diagnosis, such as the duration of disease signs and symptoms (months), the necessity for dialysis, the hemoglobin concentration (g%), and the maximum creatinine concentration and the incidence of early death (less or equal to 12 months from diagnosis) was conducted.

Clinical analysis was based on the disease extent index (DEI) and BVAS/WG index (Birmingham Vasculitis Activity Index for Wegener’s Granulomatosis).

Statistical analysis

Values are given as means ±SE. A logistic regression analysis was used to investigate the relationship between the parameters known at the time of diagnosis establishment and the early death risk. For assessment of survival time the Kaplan-Meier estimator and the Cox proportional hazard model were used. The relationship between quantitative and qualitative variables was assessed using a Wilcoxon test.

RESULTS

At recognition, the median BVAS/WG index was 30.0 (23.0-32.5) and the median DEI index was 14.0 (13.0-15.0). The mean duration of the prodromal symptoms characterized by weight loss, lethargy, and arthralgias was 2.6 ±0.3 months. Fifteen patients were PR3 positive (68%), 3 patients were MPO positive (14%), and among them there were 4 patients also positive for anti-GBM anybodies (18%). Thirteen patients were initially dialysis-dependent (59%), and 7 required ventilatory support (32%). The creatinine level was 7.2 ±1.4 mg/dl, GFR 32 ±11.2 ml/min, and PO₂ was 5.8 ±0.5 kPa. Clinical features at presentations are listed in Table 1. Biochemical and serological features at presentations are listed in Table 2.
Renal biopsy was performed in 16 of the 22 patients (73%). Histological examinations (light microscopy) revealed segmental necrotizing crescentic GN in 15 patients and membranous GN in 1 patient with positive ANCA titers, 68% and 5%, respectively. Fourteen of the 15 specimens (64%) showed no evidence of immune complex deposition, confirming a renal pauci-immune rapidly progressive glomerulonephritis.

The patients were treated with corticosteroids at a mean staring dose of 58.0 ±4.4 mg of prednisolone. Nineteen (86%) of the 22 patients received methylprednisolone (pulses), 20 patients (91%) received cyclophosphamide (pulses) and 2 patients received cyclophosphamide daily, and 8 of the 22 patients patients (36%) underwent plasma exchange. Four of the latter 8 patients were administered sequential intravenous immunoglobulin after plasma exchange. Cyclophosphamide treatment was planned to be continued for 3 to 6 months, and the patients had a dose reduction or were maintained on azathioprine therapy (5 patients). The white blood cell count were monitored weekly. Cytopenia was mainly associated with the treatment used. Neutropenia (neuthrophil count <3x10^9/L) was seen in 4 of the 22 patients. En early reduction in the dose of cyclophosphamide due to neutropenia was required in 4 patients. The patients were followed up for 24 ±8 month. Six patients died in the first month. Of the survivors, 55% and 31% remained alive after 1 and 2 years of the completed follow-up: 73% and 55% of those were dialyzed, respectively. The outcome data at 1 month, 6 months, 1 year , and 2 years are listed

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<th>Table 1. Clinical features.</th>
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<td>Age</td>
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<td>BVAS-WG</td>
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<tr>
<td>DEI</td>
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<tr>
<td>Dialysis depedence (%)</td>
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<tr>
<td>Assisted ventilation (%)</td>
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<td>Breathing rate (min)</td>
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BVAS/WG - Birmingham Vasculitis Activity Index for Wegener’s Granulomatosis; DEI - disease extent index.

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<th>Table 2. Biochemical and serological features.</th>
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<tr>
<td>Hemoglobin concentration</td>
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<td>Serum creatinine (mg/dl)</td>
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<td>Glomerular filtration rate (ml/min)</td>
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<td>PO_2 (kPa)</td>
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<td>PO_2 on max oxygen therapy (kPa)</td>
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<td>c-ANCA / PR3-ANCA positivity (%)</td>
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<td>p-ANCA / MPO-ANCA positivity (%)</td>
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<td>Double p-ANCA/MPO-ANCA and anti-GBM positivity (%)</td>
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in Table 3 and Fig. 1. Main causes of death in the first year were: sepsis - 3 patients (14%), pulmonary embolism - 2 patients (9%), and a massive pulmonary hemorrhage - 1 patient. All patients who did not achieve remission died. We observed that remission seemed to have been likely within a year after recognition in 40% of the patients, whereas after three years’ observation remission will had been achieved in 7% patients. We also noted a significant (P<0.05) decline in remission appearance with the passage of years since disease recognition. A median time to remission was 18.4 ±2.1 months.

Relapses were seen in three patients (14%). One relapse presented as diffuse alveolar hemorrhage and a deterioration in renal function at 9 months in a patient who was initially C-ANCA positive and had undergone a 6-month course of cyclophosphamide therapy, being next switched to azathioprine therapy. The relapse was successfully treated by plasma exchange, intravenous immunoglobulin and cyclophosphamide pulses. A second relapse presented as diffuse alveolar hemorrhage at 10 months in a patient who was initially perinuclear anti-neutrophilic cytoplasmic antibodies (p-ANCA) positive, shortly after cessation of cyclophosphamide therapy; the relapse was successfully treated with cyclophosphamide (pulses) and plasma exchange. A third relapse presented as a gastrointestinal tract vasculitis and central nervous system vasculitis at 7

<table>
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<th>Follow-up</th>
<th>Survivals (%)</th>
<th>Dialysis depedence (%)</th>
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<tr>
<td>1 month</td>
<td>73</td>
<td>59</td>
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<tr>
<td>6 months</td>
<td>68</td>
<td>49</td>
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<td>1 year</td>
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<td>73</td>
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<td>2 years</td>
<td>31</td>
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Table 3. Outcome data.

Fig. 1. Patients survival.
months in a patient who was initially p-ANCA positive. The relapse also appeared shortly after cessation of cyclophosphamide therapy and was successfully treated with intravenous methylprednisolone, cyclophosphamide (pulses), and plasma exchange.

**DISCUSSION**

Recognition of pulmonary-renal syndrome is established by clinical presentation, serological and histological results and may present practical difficulties in a critical care setting. The occurrence of prodromal illness of significant duration in most cases indicates a need for earlier diagnosis with the use of serological tests (1, 2).

 Constitutional symptom of malaise, fatigue, night sweats, fever together with more specific symptoms such as myalgias, polyarthralgias, episcleritis or purpuric rash precede clinical presentation by an average of 3-6 months (3, 4). In the present study that encompassed 22 patients with Wegener’s granulomatosis, the mean duration of the prodromal symptoms characterized by weight loss, lethargy, and arthralgias was 2.6 ±0.3 months, which is similar to the 2.9 ±0.4 months reported in a study of Gallagher et al (2). The duration of prodromal illness and degree of renal dysfunction, as assessed by the serum creatinine level and GFR also were similar to that reported in earlier studies (Saxena et al (5), Niles et al (6), and Maaten et al (7)). The group of patients analyzed in the present study was quite homogenous in that all of them had a rather short prodromal period, sudden onset, and a dynamic course of disease.

In the Gallagher et al (2) retrospective study, 14 patients were enrolled of a mean age of 65.0 ±2.1 years, 13 with systemic vasculitis and one patients with systemic lupus erythematosus. Of the 14 patients 7 were women. Our patients had a mean age considerably younger (44.5 years), and this could have contributed to a better outcome than that seen in the previous studies (2, 5, 7). The degree of pulmonary dysfunction in the patients of the current study was likely to be a less significant determinant of outcome, 32% required ventilatory support for massive pulmonary hemorrhage, representing an occurrence of acute respiratory failure in excess of that previously documented for PRS (7). The intensity and degree of pulmonary hemorrhage in group presented, assessed by a mean hemoglobin level was similar to that in the Gallagher et al (2) study. In our study, renal replacement therapy was required in 59%. This percentage was lower in a study of Lanque et al (8), amounting to 25%, but those patients were of younger age.

The proportion of patients in the present study with both ANCA and anti-GBM positivity was similar to that observed in other studies (2, 5, 7, 9). There is a strong association of PRS with the presence of ANCA, which is believed to be pathognomonic on the ground of both association of ANCA titer with disease activity and induction of generalized pulmonary tissue injury.
Remission is considered as the absence of disease activity in any organ system (8-10). Once the disease has been controlled by the initial treatment regimen, which is dictated by the degree of disease, severity, the focus of therapy shifts to maintaining disease remission, often with medications less toxic than those used to induce a remission (11-13). The two principal aims in the treatment of Wegener’s granulomatosis are to limit the extent and severity of permanent organ damage by controlling the disease promptly, and to minimize the short – and long-term morbidity that often results from therapy. The “remission-induction” phase of treatment often involves high doses of corticosteroids and cytotoxic agents (e.g., cyclophosphamide for periods of 3 to 6 months) (14, 15). In “remission-maintenance”, lower doses of corticosteroids and less toxic alternatives to cyclophosphamide, such as methotroxate or azathioprine, are employed for periods lasting from 12 to 18 months or longer. The proper treatment in most patients leads to remission (16-20). The incidence of immunosupresion-related adverse effects in the present study was remarkably high as was in the Gallagher et al (2) study as well. Cyclophosphamide-associated neutropenia and infections were significant contributors to four of the six deaths, two patients died of active vasculitis, but in the Gallagher et al (2) study cyclophosphamide-associated neutropenia and infections were contributors to six of the seven deaths. Patients in our study survived the acute presentation in 55% and 31% after 1 and 2 years, respectively. This observation is consistent with other reports suggesting the survivors of the acute phase of PRS have a better prognosis -75% in the Falk et al (9) study and 36% in the Gallagher et al (2) study.

Relapses of disease represents a major problem in Wegener’s granulomatosis, since they are frequent and difficult to predict, and their management is largely undefined. Relapse is defined as the reappearance of activity of the vasculitic disease, with elevated inflammatory activity and with rapid deterioration of organ function in a patient who had achieved remission. In the Gallagher et al (2) study, five relapses were seen in four patients. The remarkable efficiency of cyclophosphamide to induce remission is, overshadowed by a high rate of relapses. Other drugs are studied to identify more efficient therapy, which would be able to both induce remission and prevent relapses, but reliable data are still missing to determine the best therapeutic regimen. In the present study, relapse analysis was limited to the patients who survived the first year of observation since recognition of disease. Relapse hazard was 20% per year and was minimally changing after 2 years’ observation. The mean time till relapse was 25.9 ±3.7 months.

The number of involved organs and permanent organ damage in Wegener’s granulomatosis determine the clinical disease course and its complications, especially renal and respiratory tract involvement. Duration of immunosuppressant treatment had to be long enough, usually ≥12 months, till complete remission is achieved. When the treatment starts too late, is not properly modified, or is to short both survival and achieving remission is impossible. A chance for achieving remission also is lower when the treatment is longer.
REFERENCES


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