ABSTRACT

Objectives: We aimed to evaluate the prevalence of lung hemorrhage, its determinants and its prognostic significance in adults patients with severe kidney involvement due to ANCA-associated vasculitis diagnosed and treated in a nephrology department.

Material and Methods: Seventy-five patients consecutively diagnosed by kidney biopsy with crescentic pauci-immune glomerulonephritis entered this cohort study and were grouped according to the presence of diffuse alveolar hemorrhage (DAH – diagnosed as diffuse alveolar pattern on chest radiographs and anemia without evidence of another external bleeding). ANCA’s were assessed by capture PR3-ANCA and MPO–ANCA ELISA or by indirect immunofluorescence.

Outcomes: Patients were followed for a median period of 38 (11.7; 65.8) months. The median age was 61.6 years. Median creatinine was 5.7 mg/dL and 17% of the patients needed temporary dialysis. Most of the patients (76%) had MPO-ANCA; 31% had pneuma-renal syndrome. DAH patients had more severe and active kidney disease, as reflected by higher serum creatinine (7.1 mg/dL vs 4.45 mg/dL; p=0.006) and higher hematuria (610/mm³ vs 230/mm³, p=0.003). The risk of DAH was not influenced by gender, age or ANCA specificity, but by smoking (smokers had a 4 (95%CI 1.18-14.2; p=0.002) times higher the risk of lung hemorrhage) and by season (patients diagnosed in winter and autumn had a 6 (95% CI 1.6-20.9; p=0.005) times higher the risk of lung hemorrhage). The proportion of responders and of patients with relapses, and time to maintenance dialysis or to death were similar irrespective to the occurrence of DAH at presentation.

Conclusions: In patients with ANCA associated pauci-immune glomerulonephritis, cold season, smoking and active kidney disease, but not ANCA specificity or inflammation were associated with lung hemorrhage. Although diffuse alveolar hemorrhage was the main cause of death, it was not related to short- or long-term outcome.
INTRODUCTION

Anti-neutrophil cytoplasmic antibody-associated small-vessel vasculitis (AAV) are rare but severe, systemic diseases.

More than 75% of the patients with AAV have kidney involvement (i.e. crescentic pauci-immune glomerulonephritis) and in around 30% of cases kidney is the only affected organ (1-3). Mortality is higher in patients with renal vasculitis as compared to those without renal involvement and the patients needing dialysis at presentation have the worse prognosis (2,4-6).

The most frequent causes of death are active vasculitis, infections and cardiovascular events in the first year after diagnosis, and infections, cardiovascular events and cancer, on long-term (4,7,8).

In contrast to kidney involvement, the reported incidence of diffuse alveolar hemorrhage (DAH) caused by pulmonary capillaritis varies between 8 and 36% but, in most of the cases, it is associated with pauci-immune glomerulonephritis and in half of these cases the patients are dialysis dependent at presentation (3,9-11). The AAV patients presenting with pneumo-renal involvement have the highest mortality (3,9-11). Nevertheless, why only some AAV patients suffer lung hemorrhage, it is unclear. A possible explanation of this association was the similar endothelial cells’ phenotype in glomeruli and alveoli (12).

If the severity of kidney injury was constantly associated with an adverse outcome, lung hemorrhage was only inconstantly found to predict death (11,13,14). One possible reason could be the heterogeneity of the investigated patients, with various associations of severe organ dysfunctions, as series originated from pulmonology, chest disease, rheumatology, and nephrology or internal medicine departments.

Accordingly, we aimed to evaluate the prevalence of lung hemorrhage at diagnosis and its prognostic significance in a cohort of patients with severe kidney involvement due to ANCA-associated vasculitis diagnosed and treated in a nephrology department.

MATERIAL AND METHODS

This is a cohort study on all adult patients consecutively admitted, diagnosed by kidney biopsy and followed in “Dr. Carol Davila” Teaching Hospital of Nephrology, Bucharest.

Patients

From January 2000 to January 2014, 104 patients were diagnosed with crescentic pauci-immune glomerulonephritis by kidney biopsy. Complete data could be retrieved in 75 patients, which were grouped according to the presence of DAH.

The study was approved by the local Ethic Committee and all patients provided written an informed consent.

Diagnosis and follow-up

The criteria for pauci-immune crescentic glomerulonephritis diagnosis were crescents in more than 50% of examined glomeruli and a direct immunofluorescence assay for complement and/or immunoglobulins of 0 to 1+ on a scale from 0 to 4+.

Markers of kidney damage were proteinuria measured in 24 hours urine collection, hematuria, proteinuria and serum creatinine. Dialysis therapy was considered temporary when needed for less than 3 months.

The standard for DAH diagnosis are bronchoalveolar lavage (BAL) and lung function tests with carbon monoxide transfer coefficient (TLCO) but these test were not available in our hospital (15). Therefore, in our cohort, DAH was diagnosed based on chest radiographs showing a diffuse alveolar pattern and on acute anemia without evidence of another external bleeding.

ANCAs were assessed by capture PR3-ANCA and MPO–ANCA ELISA (Euroimmun™, Lübeck, Germany) or by indirect immunofluorescence (IFI) with monoclonal mouse anti-human myeloperoxidase antibodies (Dako™, Glostrup, Denmark). The patients were grouped as MPO-ANCA patients (MPO-ANCA sero-positive or ANCAp pattern) and PR3-ANCA patients (PR3-ANCA sero-positive or ANCAc pattern).

Birmingham Vasculitis Activity Score version 3 (BVAS) was computed retrospectively by the same investigator and used to evaluate the severity of vasculitis.

Inflammation was assessed using erythrocytes sedimentation rate (ESR), serum fibrinogen, serum albumin, white blood cells and platelets number.

Response to therapy was defined as disappearance of hematuria, stable or improving serum creatinine and no signs of activity in other organs.
Relapse was defined in responders as reappearance of hematuria or hemoptysis accompanied by an increase in BVAS.

The measurements were performed with standard laboratory methods: biochemistry on an Olympus AU 400 auto-analyzer, and hematology on a MINDRAY BC 3000 auto-analyzer.

Follow-up protocol included monthly visits with clinical (BVAS) and laboratory assessments until remission and every three months thereafter for at least 2 years.

**Treatment**

Patients were treated by the same protocol over the whole study period. Induction therapy was done in the first 6-9 months with methylprednisolone, 0.5-1g, 3 daily intravenous pulses and cyclophosphamide 0.5-1g/m², one intravenous pulse each 2-4 weeks. Maintenance therapy consisted in prednisone, 0.5 mg/body weight per day, gradually tapered to 5-7.5 mg/day associated with azathioprine 1.5-2 mg/body weight per day, for 2 to 5 years. Plasmapheresis was performed in five patients; in all cases for severe hemorrhagic alveolitis.

**Statistical analysis**

Categorical variables are presented as percentages and comparison test were performed using Pearson $\chi^2$ test. Continuous variables are displayed as mean and 95% confidence interval (95% CI) or median and quartiles (1; 3), according to their distribution. Comparisons were done with Welch-ANOVA, Mann-Whitney and Kruskal-Wallis tests, as appropriate.

Determinants of DAH were examined in binary logistic regression models, where variables associated with DAH in univariate analyses ($p<0.3$) were introduced.

Patient and kidney survival was evaluated by Kaplan Meier analysis; the significance of differences in survival was tested with Log Rank test.

A $p\leq0.05$ was considered statistically significant.

Statistical analyses were performed with Analyse-it™ (Analyse-it Software, Ltd., Leeds, UK) or SPSS (SPSS Inc., Chicago, IL) packages.

**RESULTS**

Complete data were available for 75 patients who were followed for a median period of 38 (11.7; 65.8) months.
We present here a cohort of patients with ANCA-associated small arteries vasculitis with severe systemic and kidney disease. To the best of our knowledge, this is the first report in our country regarding pneumo-renal syndrome in ANCA-associated vasculitis.

The majority of patients with lung hemorrhage were diagnosed in cold seasons, suggesting a possible role of trigger by the upper respiratory tract infections, as reported by others (17,18). However, the long time to diagnosis (2 months) prevented us to ascertain an infectious trigger.

In our patients, smoking appeared to favor DAH, despite the lower prevalence of smoking in the entire cohort than in the general Romanian population, it was higher in patients with DAH. Indeed, smoking was very strongly associated not only with the risk of pulmonary hemorrhage in anti-glomerular basement membrane disease (formerly Goodpasture syndrome) (19), but also with other autoimmune diseases, like rheumatoid vasculitis (20). The underlying presumed pathogenic mechanism is the direct endothelial damage (21). On the other hand, nicotine was found to suppress the immune system and to be beneficial in addition to conventional maintenance treatment in ulcerative colitis (22,23). However, data from retrospective studies are controversial: smoking was reported in either higher or lower proportion of patients with AAV than in the general population (24,25).

Similar to other cohorts which included “nephrology” patients, we found a high incidence of DAH (31%) among patients with pauci-immune glomerulonephritis (3,7,26-28), which is much higher than reported in AAV with isolated DAH, where the incidence was lower than 10% (29). However, this clinically significant association was not yet underlined. It could be explained by the severity of vasculitis involving both organs - hypothesis sustained by the higher vasculitis activity (BVAS), higher serum creatinine, urea, and hematuria that we found in patients with hemorrhagic alveolitis. Severe uremia per se could also be involved by altering the capillary permeability, which favors alveolar bleeding. Nevertheless, the relationship uremia-DAH is biunivocal, because lung bleeding induces hypercatabolism and increases serum urea imposing more frequently dialysis, as noted in our patients.

In our cohort, 76% of patients were MPO-ANCA positive, and ANCA specificity was not related to DAH. Possible explanations are a selection bias (all patients had severe kidney disease) and the great predominance of MPO-
ANCA specificity, which could hinder statistical differences. However, the lack of correlation between ANCA specificity and lung hemorrhage observed in our patients is in line with other reports, which found a similar risk for DAH in patients with either MPO-ANCA or PR3-ANCA (2,30). Thus, at the time when organs lesions are severe, the trigger, i.e. ANCA specificity, seems less important than the mediators of tissue damage. However, neither inflammation markers did allow us to discern patients with DAH, which could be explained by the extreme severity of kidney damage, which concealed the additional inflammation resulting from lung lesions. The lack of a more specific test for systemic inflammation, like high-sensitive C-reactive protein could also be a contributor.

As in other similar cohorts, the mortality was high (32% vs 20-87%) as was the proportion of patients on maintenance dialysis (“kidney death”) at follow-up (29% vs. 23%) (4,11,13,14,31). However, in our data, DAH did not influence the outcome either of patient or of the kidney, and the therapeutic efficiency, i.e. the rate of response (60%) or of relapses (18%), was similar irrespective of lung hemorrhage, and in the range of literature reports (51% and 19-56%, respectively) (4,7,8,32). Although in our cohort the relapses were in the low range of reported data, probably related to our long-term maintenance immunosuppressive therapy, some of the relapses occurred in the lungs in patients without DAH at presentation and were the cause of death. Moreover, the site of relapse was not related to DAH at presentation (data not shown). Thus, DAH seemed to have little influence on outcome in patients with AAV and severe kidney disease, although it was a main cause of death.

There are some limitations of our study. The number of participants and the single center design could limit the extrapolation of the results to other population, but on the other hand, it is informative for the current practice in our country. As previously mentioned, a selection bias is also possible, since in the tertiary care nephrology setting, all patients had severe kidney disease. Additionally, the rather low numbers of patients and events impeded the statistical power of the study. As ANCAs were assessed by indirect immunofluorescence and/or by ELISA, and discrepancies between results obtained by these methods were described, data on ANCA specificity should be interpreted with caution (33).

CONCLUSION

In our patients with ANCA associated pauci-immune glomerulonephritis, cold season, smoking and active kidney disease, but not ANCA specificity or inflammation were associated with lung hemorrhage. Although diffuse alveolar hemorrhage was the main cause of death, it was not related to short- or long-term outcome.

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