Comparison of Clinico-pathologic features and outcomes of ANCA negative and ANCA positive pauci immune crescentic glomerulonephritis: A single centre study

Pallav Gupta  
Sir Ganga Ram Hospital

Vinant Bhargava  (vinant.bhargava.sgh@gmail.com)  
Sir Ganga Ram Hospital

Manish Malik  
Sir Ganga Ram Hospital

Anurag Gupta  
Sir Ganga Ram Hospital

AK Bhalla  
Sir Ganga Ram Hospital

Ashwini Gupta  
Sir Ganga Ram Hospital

Vaibhav Tiwari  
Sir Ganga Ram Hospital

D S Rana  
Sir Ganga Ram Hospital

R L Sapra  
Sir Ganga Ram Hospital

Research Article

Keywords: Pauci-immune, Crescentic, ANCA negative, Outcomes, histology

Posted Date: April 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1571226/v1

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Abstract

Introduction: Pauci-immune crescentic glomerulonephritis (PICN) is an important cause of rapidly progressive renal failure. 10-40% of PICN cases have ANCA negative serology. Present study compared clinico-pathologic features, Brix's renal risk score, Berden's histopathological classes and differences in outcome between ANCA-ve vs ANCA+ve PICN.

Methods: 61 patients of biopsy proven PICN were studied. Biochemical finding and ANCA serology were recorded. Renal biopsies slides were reviewed along with direct Immunofluorescence. Clinical and histological features were compared between ANCA negative and positive PICN using Man Whitney U test and chi square test. Patients were compared for distribution in Berden's histological classes and Brix's renal risk categories. Patient and renal survival were compared using Kaplan Meier survival analysis.

Results: ANCA negative PICN patients were younger (44.9±16.5 year vs 53.6±15.1 year, p=0.049). Nasal (0 vs 18%, p=0.035) and pulmonary involvement (9% vs 38%, p=0.014) were lower in ANCA negative group. Both ANCA groups had similar renal biochemical profile, percentage normal glomeruli, 16.27±18.25vs21.69±20.42 and percentage glomeruli with crescents, 64.45±28.12vs64.25±27.11. 27% ANCA negative cases fell in the sclerotic class in Berden's classification vs just 2.5% in ANCA positive group (p=0.037) without significant difference in Brix’s renal risk categories (p=0.329). 13% of ANCA –ve patients achieved complete remission on treatment compared to 33% in ANCA +ve patients. Patient survival and overall probability of progressing to ESRD were similar in the two groups.

Conclusion: ANCA negative PICN cases present at younger age. Nasal and pulmonary involvement is uncommon with fewer cases achieving remission on treatment. Patient survival and progression to ESRD is similar in both ANCA groups.

Introduction

Pauci-immune ANCA (anti neutrophil cytoplasmic antibody) associated crescentic glomerulonephritis is an important and common cause of patients presenting with rapidly progressive renal failure. These patients have rapid deterioration in renal function with severe clinical manifestations and untreated cases can progress to ESRD (end stage renal disease) or death. Morphologically patients have necrotizing crescentic glomerulonephritis with minimal to absent (0–1+) staining for immunoglobulin or complement on direct immune fluorescence performed on renal biopsies. [1, 2, 3] ANCA positivity has been reported in 75–90 percent in patients with pauci-immune crescentic glomerulonephritis (PICN). Remaining patients can have ANCA negative pauci-immune crescentic glomerulonephritis. [4, 5, 6] ANCA negative status has been documented in 10–39% patients with pauci-immune crescentic glomerulonephritis in various studies across the world with variation in the clinical, pathologic features and renal outcomes as compared to ANCA associated glomerulonephritis. [4, 5, 6, 7, 8] Present study is undertaken to study and compare the clinical, renal biopsy findings and prognostic systems (Berden's histological classification, [9, 10] and Brix's renal risk score, [11] among ANCA negative and ANCA positive patients.
Brix’s renal risk categories) in patients with ANCA negative and ANCA positive pauci-immune crescentic glomerulonephritis and to determine the patient and renal survival.

**Methods**

A retrospective single centre study was performed from January 2014 to October 2019. All methods were carried out in accordance with relevant guidelines and regulations (Declaration of Helsinki). A total of 61 cases of Pauci-immune crescentic glomerulonephritis were included in the study after excluding pediatric cases (age < 16 years), cases without complete clinical details, absent follow-up or inadequate renal biopsy (defined by less than 8 glomeruli in biopsy). All the patients had undergone serum ANCA (MPO/PR3) testing by serum IIF (indirect immune fluorescence) and ELISA on initial diagnosis or at time of biopsy and subsequent follow up. Biochemical profile of the patients including serum creatinine, 24 hour urinary protein, Urine protein: creatinine ratio (UP/CR), urine routine was obtained from hospital records. Renal biopsies from all the patients stained with Hematoxylin and eosin stain, periodic acid Schiff (PAS) stain, periodic acid silver methanamine (PSM) stain and Masson’s trichrome stain were evaluated by a renal pathologist for glomerular changes including crescents, glomerular tuft fibrinoid necrosis, Bowman’s capsule rupture, glomerulosclerosis, changes in the tubulo interstitial compartment including inflammation-fibrosis and changes in the vascular compartment for evidence of vasculitis. Direct Immunofluorescence findings of renal biopsies were also recorded in all the cases.

**Statistical Analysis**

The statistical data was analyzed using SPSS 17.0.0 software. Continuous variables were analyzed using Man Whitney U test and categorical variables were analyzed using chi-square test. Continuous variable were reported as mean ± standard deviation whereas categorical variables were reported as percentage. Kaplan Meier survival analysis was done to estimate renal and patient survival and compared using Log rank and Wilcoxon test. A P value of < 0.05 was considered to statistically significant.

**Results**

Sixty one patients were included in the study. The mean age of the patients was 50.48 ± 16.3 years. There were 32 males and 29 females. The mean serum creatinine at time of biopsy was 6.47 ± 4.4 mg/dl and mean e GFR was 16.25 ± 15.39 ml/min/1.73 m2. Mean 24 hour proteinuria at the time of biopsy was 2.88 ± 5.05 gm. All patients with C-ANCA showed activity against PR3 on ELISA and those with P-ANCA against MPO. There were 39 ANCA positive patients (21 C-ANCA/PR3, 18 P-ANCA/MPO) and 22 patients were ANCA negative. This accounted for 36% of all the cases of pauci-immune crescentic glomerulonephritis.

The mean age of ANCA negative patients was younger (44.9 ± 16.5 years) as compared to ANCA positive group (53.6 ± 15.1 years) and this was statistically significant, p = 0.049). The male to female ratio in
ANCA negative group was 0.8:1 as compared to 1.3:1 in ANCA positive group. The mean serum creatinine in the ANCA negative group was 6.35 ± 4.45 and mean e GFR was 14.7 ± 11.2. There was no statistically significant difference between the ANCA positive and negative group. All patients had microscopic hematuria at presentation. Proteinuria was present in all patients at time of biopsy with 5 of them having nephrotic range proteinuria. The 24 hour urinary protein was marginally higher in the ANCA negative group (3.36 ± 5.81 g/24hr) as compared to ANCA positive group (2.60 ± 4.62 g/24hr) and was not statistically significant (p = 0.620). Relatively higher number of patients in ANCA negative group(23%) had nephrotic range proteinuria as compared to ANCA positive group(15%). (Table 1)

Table 1
Clinical profile of patients with pauci-immune crescentic glomerulonephritis

<table>
<thead>
<tr>
<th>Feature</th>
<th>ANCA(-) 22</th>
<th>ANCA(+) 39</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>44.9 ± 16.5</td>
<td>53.6 ± 15.1</td>
<td>0.049</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>10M/12F</td>
<td>22M/17F</td>
<td>0.411</td>
</tr>
<tr>
<td>Fever</td>
<td>2/22 (9%)</td>
<td>17/39 (43%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Nasal Involvement</td>
<td>0/22 (0%)</td>
<td>7/39 (18%)</td>
<td>0.035</td>
</tr>
<tr>
<td>Pulmonary Involvement</td>
<td>2/22 (9%)</td>
<td>15/39 (38%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Serum Creatinine at Biopsy mg/dl</td>
<td>6.35 ± 4.45</td>
<td>6.53 ± 4.49</td>
<td>0.946</td>
</tr>
<tr>
<td>e-GFR at time of Biopsy ml/min/1.73 m2</td>
<td>14.7 ± 11.2</td>
<td>17 ± 17.39</td>
<td>0.892</td>
</tr>
<tr>
<td>24 hour urinary protein (g)</td>
<td>3.36 ± 5.81</td>
<td>2.60 ± 4.62</td>
<td>0.620</td>
</tr>
<tr>
<td>Nephrotic Range proteinuria</td>
<td>5/22 (23%)</td>
<td>6/39 (15%)</td>
<td></td>
</tr>
</tbody>
</table>

The ANCA negative group had lesser systemic involvement. Fever (p = 0.005), nasal involvement (p = 0.035) and pulmonary involvement (p = 0.014) were significantly more common in the ANCA positive patients as compared to ANCA negative patients (Table 1).Gastrointestinal involvement (10.2% ANCA + ve, 9% ANCA –ve), arthralgia (15.3% ANCA + ve ,13.6%) and skin rashes (17.9% ANCA + ve,18.1% ANCA –ve) were similar in both the groups.

Renal biopsy in both the groups showed nearly similar findings with respect to percentage normal glomeruli or percentage of glomeruli with total number of crescents. Breach of bowman's capsule (25.6% ANCA + ve, 9% ANCA –ve) and glomerular tuft fibrinoid necrosis (33% ANCA + ve, 18% ANCA –ve) were observed in higher number of ANCA positive patients suggesting more active disease but these differences with the ANCA negative group was not statistically significant.(Table 2) Tubular atrophy was observed in 51% patients with ANCA positive patients as compared to 59% patients with ANCA negative subjects. All the 61 cases of PICN including ANCA + ve and ANCA -ve showed absence of immunoglobulins and complements in direct Immunofluorescence studies performed on renal biopsies.
Table 2
Histological profile and risk score of patients with pauci-immune crescentic glomerulonephritis

<table>
<thead>
<tr>
<th>Feature</th>
<th>ANCA(-) 22</th>
<th>ANCA(+) 39</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% normal glomeruli</td>
<td>16.27 ± 18.25</td>
<td>21.69 ± 20.42</td>
<td>0.259</td>
</tr>
<tr>
<td>% glomeruli with crescent</td>
<td>64.45 ± 28.12</td>
<td>64.25 ± 27.11</td>
<td>0.982</td>
</tr>
<tr>
<td>Breach of Bowman’s capsule</td>
<td>2/22 (9%)</td>
<td>10/39 (25.6%)</td>
<td>0.118</td>
</tr>
<tr>
<td>Glomerular Fibrinoid necrosis</td>
<td>4/22 (18%)</td>
<td>13/39 (33%)</td>
<td>0.205</td>
</tr>
<tr>
<td>Berden’s Histologic classes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>2 (9%)</td>
<td>5 (12.8%)</td>
<td>0.037</td>
</tr>
<tr>
<td>Crescentic</td>
<td>10 (45%)</td>
<td>23 (59%)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>4 (18%)</td>
<td>10 (26%)</td>
<td></td>
</tr>
<tr>
<td>Sclerotic</td>
<td>6 (27%)</td>
<td>1 (2.5%)</td>
<td></td>
</tr>
<tr>
<td>Renal Risk Group (Brix et al.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk group Low</td>
<td>2 (9%)</td>
<td>9 (23%)</td>
<td>0.329</td>
</tr>
<tr>
<td>Risk group Medium</td>
<td>11 (50%)</td>
<td>19 (49%)</td>
<td></td>
</tr>
<tr>
<td>Risk Group High</td>
<td>9 (41%)</td>
<td>11 (28%)</td>
<td></td>
</tr>
</tbody>
</table>

Renal biopsies were also grouped according to Berden’s histological classification.\(^9\) ANCA negative group showed significantly higher number of patients in the sclerotic category (27%) as compared to just 2.5% in the ANCA positive group (\(p = 0.037\)). Similar number of cases were observed in the focal, and mixed categories in the two groups with only slightly lower percentage in crescentic category. The study subjects were also classified according to new renal risk score by Brix et al.\(^10\) It is a composite score based on eGFR, percentage normal glomeruli and degree of interstitial fibrosis and tubular atrophy and study subjects are characterized into low (score 0), medium (score 2–7) and high (score 8–9) renal risk groups. We observed maximum number of cases in the medium renal risk group in both ANCA negative and ANCA positive with no statistically significant difference in the distribution of cases in various risk categories between these two groups (\(p = 0.329\)), (Table 2).

Patients in the both the groups received similar treatment protocol. 39 patients received pulse intravenous methyl prednisolone and 8 patients received plasmapheresis. Cyclophosphamide was used as an inducing agent in 54 patients (15mg/kg body weight every 2 weeks, 3 doses and then every 3 weeks, 7 doses) with azathioprine in the maintenance phase. Oral steroids were given 1mg/Kg for 2 weeks and tapered thereafter.
Follow up period ranged from 3 months to 61 months with a mean of 19 months. During follow up, 50% cases of ANCA negative pauci immune crescentic patients progressed to ESRD as compared to 33% in the ANCA positive group and fewer patients achieved remission, 13% as compared to 33% patients in the ANCA positive group. Three patients died in the ANCA positive group as compared to 4 patients in the ANCA negative group on follow up. (Table 3)

<table>
<thead>
<tr>
<th></th>
<th>Deaths</th>
<th>ESRD</th>
<th>Refractory</th>
<th>Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANCA positive (39)</td>
<td>3</td>
<td>13</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>ANCA negative (22)</td>
<td>4</td>
<td>11</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Kaplan Meier survival analysis was done between the ANCA positive and ANCA negative groups to determine the renal and patient survival. Renal survival at 1 year and 3 year was 89% and 58% in the ANCA positive group as compared to 83% and 29% in the ANCA negative group, however overall renal survival was not significantly different in the two groups (wilcoxon, p = 0.063). (Fig. 1) All the deaths in both ANCA positive and ANCA negative group occurred during 1st year due to cardiorespiratory causes or sepsis. One year patient survival was 92% in the ANCA positive group as compared to 82% in the ANCA negative group. Overall patient survival was not significantly different. (wilcoxon, p = 0.169). (Fig. 2)

**Discussion**

Anti neutrophilic cytoplasmic antibodies directed against PR3 and MPO have been implicated in the pathogenesis of ANCA associated PICN.\[^{11}\] Other antibodies which have been implicated are anti hLAMP-2 anti-endothelial cell antibodies anti-moesin antibodies.\[^{12},^{13},^{14}\] Study by Peschel et al demonstrated anti-hLAMP-2 antibodies in 73% patients with ANCA negative PICN.\[^{15}\] ANCA negative pauci-immune crescentic glomerulonephritis is thought to be a result of antibodies directed against human lysosome-associated membrane protein-2 (hLAMP-2) and anti-endothelial cell antibodies although it is matter of further investigation.\[^{16}\] ANCA negative pauci-immune crescentic glomerulonephritis has also been reported in association with malignancies of lung and in association with chemotherapeutic agent Midostaurin with no clear cut pathogenesis.\[^{17},^{18}\] Some authors have also proposed the role of neutrophils and their activation by IL-6 and other cytokines in the pathogenesis of ANCA negative PICN.\[^{19}\] In our patients there was no association of ANCA negative PICN patients with any malignancy or chemotherapeutic agent.

In our study we found ANCA negative PICN patients had younger age of presentation as compared to ANCA positive patients. Similar observations have been made by earlier studies with a decade earlier presentation in ANCA negative patients as compared to ANCA positive counterparts.\[^{4},^{7},^{8},^{16}\] In our study as compared to ANCA positive patients lesser number of ANCA negative patients had systemic...
involvement in the form of fever, nasal and pulmonary involvement. Chen al have also observed lower prevalence of fever weight loss, arthralgia, upper respiratory tract and pulmonary involvement in ANCA negative PICN.\cite{7} Similar observations have also been recorded by Sharma et al, Hung et al and Hedger et al.\cite{8, 4, 5}

No signicant difference was observed in our study with respect to serum creatinine or e GFR at presentation and our observations were concordant to those made by Sharma et al.\cite{8} We found relative higher 24 hour urine protein excretion in the ANCA negative group but it was not statistically significant (0.620). However chen et al and Villacorta et al have found significant difference in the 24 hour urine protein excretion between the two groups higher being in ANCA negative PICN. \cite{7, 16} Study by Sharma et al didn't find any such difference in 24 hour urine protein excretion between the two groups.\cite{8}

On histology we found similar percentage of normal glomeruli, percentage of glomeruli with crescents and glomeruli with fibrinoid necrosis of the glomerular capillary tuft in both ANCA negative and ANCA positive groups. Similar observations by made by other studies.\cite{5, 16} In Berden's histological classification we found crescentic class was the most common class in both ANCA negative and ANCA positive groups similar to observations made by Villacorta et al.\cite{16} We noticed higher percentage of patients in the sclerotic class as compared to ANCA positive patients (27% vs 2.5%) whereas Sharma et al found more number of patients in the crescentic class.\cite{8} Hung et al observed more chronic glomerular lesion in the ANCA negative patients in their study. \cite{4} Chronic tubulo-interstitial disease in the form of tubular atrophy and interstitial fibrosis was similar both groups of PICN patients in our study and were concordant with study by Villacorta et al.\cite{16} Chen et al and Sharma et al observed higher prevalence and more severe interstitial fibrosis and tubular atrophy in the ANCA negative patients. \cite{7, 8} Our study is the first one to compare the ANCA negative and ANCA positive patients with respect to renal risk score (Brix et al.)\cite{10} We have found maximum number of cases in both the groups in Renal risk category medium with no statistically significant distribution of cases in all 3 renal risk categories, \( p = 0.329 \). This observation also confirms that renal survival is similar in two groups.

We observed similar patient survival in both groups of PICN. In our study renal survival at 3 years was lower in the ANCA negative group 29% vs 58% in the ANCA positive group however cumulative probability of reaching ESRD was similar in the two groups (\( p = 0.063 \)). We also observed that lesser number of patients in the ANCA negative group achieved remission on treatment. Villacorta et al observed similar patient and renal survival in both the groups\cite{16} whereas Chen et al observed poor renal outcome in the ANCA negative group but similar patient survival in both groups. \cite{7} Sharma et al showed that higher number of subjects showed deterioration in the ANCA negative group (\( p = 0.006 \)) and lesser number of subjects showed improvement on treatment (\( p = 0.02 \)) as compared to ANCA positive group. Villacorta et al observed similar patient and renal survival in both the groups\cite{16} whereas Chen et al observed poor renal outcome in the ANCA negative group but similar patient survival in both groups. \cite{7}
Conclusion

ANCA negative pauci-immune crescentic glomerulonephritis comprised about one third cases of pauci-immune crescentic glomerulonephritis in our study. These patients had younger age at disease onset and less evidence of systemic involvement in the form of fever, nasal and pulmonary involvement as compared to their ANCA positive counterparts. On histology more patients are likely to have a sclerotic class on Berden’s classification although they have similar involvement in terms of percentage normal glomeruli or percentage glomeruli with crescents, tubulo-interstitial changes and renal risk scores. Lesser number of ANCA negative patients achieves remission on treatment. Short term renal survival can be inferior as compared to ANCA + ve group although patient survival and probability of progressing to ESRD doesn’t seem to differ in them.

Declarations

Institutional Ethics committee approval was taken prior to initiation of study. Although the study was retrospective, informed consent was taken from the participants/legal guardian, wherever applicable. In terms of contribution to this manuscript, Pallav Gupta has contributed to drafting, Vinant Bhargava helped with editing and writing of the manuscript. All others authors have contributed in proof reading. RL Sapra has helped in statistical analysis of the data. No funding has been received for conduction of this study. No separate funding has been received for the study. Authors have no competing interests with any person, company or organization in conduction of this study.

The datasets generated and analyzed during the current study are not publicly available to protect patient privacy but shall be available from corresponding author on reasonable request.

Acknowledgments: We acknowledge the contribution of department of histopathology and nephrology towards this manuscript

Conflict of interest

None

Consent for Publication

NA

References


Figures

Figure 1

Kaplan Meier survival analysis depicting renal survival in ANCA positive and ANCA negative patients.
Figure 2

Kaplan Meier survival analysis depicting patient survival in ANCA positive and ANCA negative patients.