Prognostic Factors for Renal Amyloidosis: A Clinicopathological Study Using Cluster Analysis

Yoshie Sasatomi¹, Hiroshi Sato¹, Yoshiro Chiba³, Yasuhiro Abe¹, Seiji Takeda¹, Satoru Ogahara¹, Toshiaki Murata¹, Hidetoshi Kaneoka¹, Shigeo Takebayashi³, Hiroshi Iwasaki² and Takao Saito¹

Abstract

Objective There is no standardized therapy for renal amyloidosis, which shows rapid progression and poor prognosis. Here, we used cluster analysis to examine the correlation between amyloid-related renal damage and prognosis, and determined the clinicopathological prognostic factors for renal amyloidosis.

Methods and Patients We analyzed 125 patients with renal amyloidosis (men/women: 43/82; mean age at renal biopsy: 58.8±11.1 years, ±SD; range: 21-78 years). Cluster analysis was performed using clinical parameters, renal histological findings, type of renal amyloidosis, and follow-up data. We also analyzed survival data.

Results We divided 125 cases (prognosis was checked in 97 [77.6%] cases) into three groups by cluster analysis. In the cluster groups, accelerated progression correlated with serum creatinine (s-Cr) levels at renal biopsy and histological grade of renal damage by amyloid deposition (p<0.0001). The most important prognostic factors were glomerular, tubulointerstitial, and vascular lesions induced by amyloid deposition at biopsy (p<0.0001). We also found that amyloid-A (AA) type amyloidosis correlated more significantly with amyloid-mediated vascular (P=0.0010) and tubulointerstitial lesions (p=0.0705) than with amyloid-L (AL) type amyloidosis. Proteinuria and nephrotic syndrome were more severe in AL than AA amyloidosis (p=0.0836). The 10-year individual survival rate was about 20%, and most deaths were due to cardiovascular disease and infection.

Conclusion Our results indicate that the quantity of amyloid deposition in the kidney, and the extent of glomerular, tubulointerstitial, and vascular damage are significant renal prognostic factors in amyloidosis.

Key words: renal amyloidosis, AA and AL type, cluster analysis, renal prognosis, individual survival rate

(DOI: 10.2169/internalmedicine.46.1690)

Introduction

Renal amyloidosis is occasionally detected in patients with severe proteinuria and nephrotic syndrome as the first presenting symptom. Several outcome studies have discussed the onset and progressive mechanisms of renal amyloidosis; however, detailed analyses of a large number of cases have not been undertaken (1-4).

The therapeutic options for renal amyloidosis have been discussed in many studies (5-13), but standardized or effective therapeutic protocols have not yet been established. Because most cases of renal amyloidosis progress to renal failure, very few cases of remission of this disease are known so far. We previously reported our finding, based on cluster analysis, that severe tubulointerstitial and vascular damage was caused by amyloid deposition. This was rapidly progressive (4). In comparison with AL amyloidosis, amyloid deposition was more frequently detected in both tubulointerstitial tissue and vessels in AA amyloidosis. In this new
study we have examined more cases than in our previous study (4), with the goal of analyzing parameters related to possible cardiac involvement [cardio-thoracic ratio (CTR) and electrocardiogram (ECG)], since the commonest cause of death was cardiovascular disease. We used the clinical and renal pathological findings to classify cases into separate groups and performed cluster analysis to examine the correlation between renal pathological damage caused by amyloid deposition and prognosis. We also determined the clinico-pathological prognostic value of renal amyloidosis.

Patients and Methods

Patients

We analyzed 125 patients with renal amyloidosis. The diagnosis of amyloidosis was confirmed by renal biopsy. Clinically, these patients had proteinuria, nephrotic syndrome, and/or renal dysfunction. Biopsies were performed at Fukuoka University, Tohoku University, and associated hospitals from 1968 to 2001 (men/women: 43/82; mean age at biopsy: 58.8±11.1 years ±SD; range: 21-78 years). Prognosis was checked in 97 (77.6%) cases. The duration of follow-up after renal biopsy was 2.1±2.3 years (range: 0.014-15.6 years). The duration of follow-up for cases without end-stage renal disease (ESRD) reflected the time from biopsy to the date of the last prognosis check-up, while that of cases with ESRD represented the time from biopsy to commencement of hemodialysis.

Diagnosis of renal amyloidosis

Renal biopsy samples were embedded in paraffin and then stained with hematoxylin-eosin, periodic acid Schiff, periodic acid methenamine silver, and Congo red. At final diagnosis, all cases were confirmed to have amyloid fibrils by electron microscopy.

Differentiation of AA from AL amyloid types

After treatment with KMnO4, samples were examined for staining patterns with Congo red. Samples were also categorized immunohistochemically into AL and AA types using an antibody directed against the amyloid P component, amyloid A protein (Dako, Copenhagen, Denmark). We also determined monoclonal protein and urinary light chains by immunoelectrophoresis. We excluded any patients with transthyretin-associated hereditary amyloidosis (ATTR), determined by checking the family history for amyloidosis and polyneuropathy.

Clinical and laboratory data

The following clinical and laboratory variables were recorded and used for analysis: gender, age, serum creatinine levels (s-Cr), mean blood pressure (mBP) [diastolic BP + (systolic BP- diastolic BP)/3], nephrotic syndrome (proteinuria ≥ 3.5 g/day, persistent serum albumin: ≤ 3.0 g/dl for more than one month), chronic heart failure, CTR, ECG ab- normalities (low voltage, arrhythmia, etc), and medications received at biopsy.

Semiquantitative analysis of glomerular amyloid deposition

Each biopsy specimen contained at least seven glomeruli. We measured all cortical glomeruli in each visual field. The grade of pathological glomerular damage was evaluated semiquantitatively by the morphometric analyses of Rumpelt et al (14) and Ishitobi et al (15). The latter was determined based on staining of the sections with Congo red. The microscopic image was first recorded by a video camera and then digitized and displayed on the computer monitor using the “Nikon Cosmzone” software. All cortical glomeruli present in the visual field were examined, and amyloid deposits were encircled with a digital pen. We divided cases semiquantitatively into four grades, 0-3, according to the amount of glomerular amyloid deposition: 0, no deposition; 1+, <25% amyloid deposition in most glomeruli; 2+, <50% amyloid deposition in most glomeruli; and 3+, ≥ 50% amyloid deposition in ≥ 50% of glomeruli.

Semiquantitative analysis of tubulointerstitial damage

The point counting method (16) was used to evaluate amyloid deposition in the tubular basement membrane and/or interstitium. Under high magnification (×400) using an 81-point (100-square) eye piece micrometer, we analyzed at least 10 consecutive non-overlapping cortical fields in each biopsy section. The numbers of points overlying amyloid deposition in the tubular basement membranes and interstitial space were counted and expressed the result according to the following formula: (Number of grid intersections on amyloid deposition in the cortical interstitium / total number of grid intersections) ×100. The cases were divided semiquantitatively into four grades, 0-3, according to the degree of tubulointerstitial damage: 0, no amyloid deposition or tubulointerstitial damage; 1+, <25% tubulointerstitial damage with amyloid deposition; 2+, ≥ 25% to <50% tubulointerstitial damage with amyloid deposition; and 3+, ≥ 50% tubulointerstitial damage with amyloid deposition.

Semiquantitative analysis of vascular damage

Each biopsy specimen contained at least five vessels. Using the morphometric analyses of Rumpelt et al (14) and Ishitobi et al (15), we recorded the microscopic images using a video camera. The images were then digitized and displayed on the computer monitor. For analysis, amyloid deposits on arteriolar walls were encircled with a digital pen. The cases were divided semiquantitatively into four grades, 0-3, according to the amount of amyloid arterial deposits: 0, no amyloid deposition in all arterioles examined; 1+, <25% partial or total circumferential arteriole amyloid deposition; 2+, ≥ 25% to <75% partial or total circumferential arteriole amyloid deposition; and 3+, ≥ 75% partial or total circumferential arteriole amyloid deposition.
Table 1. Clinical and Histological Profiles of AL and AA Amyloidosis

<table>
<thead>
<tr>
<th>Factor</th>
<th>AL type</th>
<th>AA type</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>68</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Cause of amyloidosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary/MMA/RA/Tac/adult still/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchiolitis/Crohn/Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>men:woman</td>
<td>20:32</td>
<td>7:56</td>
</tr>
<tr>
<td>Mean age at biopsy (year)</td>
<td>59.6±9.7</td>
<td>57.8±12.6</td>
<td>0.5849</td>
</tr>
<tr>
<td>s-Cr (mg/dl)</td>
<td>1.3±0.8</td>
<td>2.3±1.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>92.7±17.3</td>
<td>96.9±14.1</td>
<td>0.1874</td>
</tr>
<tr>
<td>Chronic cardiac failure (%)</td>
<td>59.4±40.6</td>
<td>72.7±27.3</td>
<td>0.3127</td>
</tr>
<tr>
<td>&lt;50%/≥50%</td>
<td>73.7±26.3</td>
<td>23.1±78.9</td>
<td>0.00087</td>
</tr>
<tr>
<td>ECG abnormality (%)</td>
<td>47.6±52.4</td>
<td>41.7±58.3</td>
<td>0.6885</td>
</tr>
<tr>
<td>Nephrotic syndrome (%)</td>
<td>30.8±69.2</td>
<td>46.8±53.2</td>
<td>0.0836</td>
</tr>
</tbody>
</table>

Table 2-1. Clinical and Histopathological Background of Patients of the Three Cluster Groups

<table>
<thead>
<tr>
<th>Factors</th>
<th>Group (G)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>G-1</td>
<td>G-2</td>
</tr>
<tr>
<td>sex</td>
<td>men:woman</td>
<td>20:32</td>
</tr>
<tr>
<td>Mean age at biopsy (yrs)</td>
<td>58.2±9.0</td>
<td>57.0±12.3</td>
</tr>
<tr>
<td>GFR (mI/min/m²)</td>
<td>1.6±0.3</td>
<td>1.6±0.7</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>93.2±10.7</td>
<td>92.3±15.0</td>
</tr>
</tbody>
</table>

Nephrotic syndrome (%) 1.5 (1) GFR (mI/min/m²) 0.0297 0.0441 4.48 0.001

Mean of changes in GFR, GFR, and GFR significant difference in patients who received dialysis treatment vs. patients who did not receive dialysis treatment.

Management in cases of death. Cases of ESRD (including death due to hemodialysis or continuous ambulatory peritoneal dialysis) were categorized as “cases of outbreak.” Cases of patient death unrelated to renal disease, for example, from cardiac or gastrointestinal amyloidosis, were categorized as “cases of end of observation.”

Cluster analysis

The cluster analysis method allows for division of the sample into groups based on the relative quantitative value of certain parameters (17); each group then has characteristic parameters (18, 19). Using the total renal damage score (0-9 points) and s-Cr levels (mg/dl), we normalized the u-kidul distance. For cluster analysis, Ward’s method was used (15) (SAS International Computer Concepts, Inc.). In addi-

Total grade of renal pathological damage

The sum of glomerular, tubulointerstitial, and vascular damage associated with amyloid deposition was calculated, with 0-9 points indicating the total grade of renal pathological damage.

Rates of renal survival and death

Prognosis check. We performed a prognosis check every two to three years following renal biopsy. At each check, we recorded renal condition (remission, prolonged urinary abnormality, renal failure, and hemodialysis received).

Figure 1. Survival and life curves of patients with renal amyloidosis were poor. There was no difference between patients with amyloidosis type AL and AA.
tion, we regrouped the cluster groups in order to differentiate various types of prognosis.

**Method of analysis**

The following statistical analyses were performed: \(\chi^2\) test, Student’s t-test, Kaplan-Meier, Kruskal-Wallis, and generalized Wilcoxon tests, and multiple variant analyses including cluster analysis and Cox’s proportional hazards model. The p value was <0.05. Data are expressed as the mean ± SD. All statistical analyses were performed using SAS analysis software (SAS International Computer Concepts, Inc.).

**Results**

The amount of amyloid deposition varied from case to case. Significant amyloid deposits were noted in the glomeruli, blood vessels, and tubulointerstitium. In the blood vessels, a pattern of circumferential amyloid deposition was recognized, and most cases showed moderate dilatation of the vascular lumen.

**Renal survival curves and individual survival rates for AL and AA amyloidosis**

The renal survival curves and individual survival rates for AL (n=68) and AA (n=57) amyloidosis (prognosis was checked in only 97 cases) are shown in Fig. 1. There was no difference in these parameters between group AL and group AA. As indicated by these curves, the 5-year renal survival rate was about 30% and the 10-year individual survival rate was about 20%. The survival rates of both groups were poor.

**Comparison of AL and AA amyloidosis**

AA amyloidosis was frequent among female patients with high s-Cr levels, high CTR, high grades of tubulointerstitial damage, and significant renal vascular damage (Table 1-1). Of 68 patients with AL amyloidosis, 18 cases had been taking oral steroids and 3 had been taking melphalan. In 47 cases the treatment details were not available. Of 57 patients with AA amyloidosis, 32 patients had been taking oral steroids and 21 patients did not know what medications they were receiving.

**Groups by cluster analysis**

Performing cluster analysis using the total renal histological damage score and s-Cr levels enabled definition of three cluster groups (Table 2-1). The s-Cr level was higher in Group 3 than in Groups 1 or 2. When the renal histological damage grade was 3 to 4 points (average score, 3.6), cases were grouped into Group 2. Group 1 represented those patients with one or two points (average score, 1.4). The grade of histological damage differed significantly between Groups 1 and 2 (p<0.0001).

**Clinicopathological and cardiac functional background of each cluster group**

The clinical and histological backgrounds of each cluster group are described in Table 2-1. Severe vascular damage due to amyloid deposition was associated with high mBP in Group 3. Nephrotic syndrome was prominent in both Groups 1 and 2. The majority of cases in Groups 1 and 2 were AL cases, but AA predominated among patients in Group 3. The order of frequency of glomerular, tubulointerstitial, and vascular damage was Group 3 (1.8±0.5, 1.5±0.5, 1.8±0.4) > Group 2 (1.6±0.6, 0.6±0.6, 1.4±0.7) > Group 1 (0.9±0.6, 0.1±0.3, 0.4±0.5). Total renal histological damage scores were in the order Group 3 (5.1±0.8) > Group 2 (3.6±0.5) > Group 1 (1.4±0.5). There were significant differences between the three groups regarding renal function (s-Cr: Group 1, 1.0±0.3 mg/dl; Group 2, 1.4±0.7 mg/dl; Group 3, 2.4±1.9 mg/dl) and grade of pathological damage (p<0.0001). Furthermore, there were differences among the

---

**Table 2-2. Cardiac Functional Background of Patients of the Three Cluster Groups**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Group 1 (G-1)</th>
<th>Group 2 (G-2)</th>
<th>Group 3 (G-3)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic heart failure</td>
<td>81.8/18.2</td>
<td>61.1/38.9</td>
<td>60.0/40.0</td>
<td>0.4154</td>
</tr>
<tr>
<td>CTR</td>
<td>50.0/50.0 (&lt;50%:250%)</td>
<td>47.1/52.9 (N=17)</td>
<td>40.0/50.0 (N=20)</td>
<td>0.2377</td>
</tr>
<tr>
<td>ECG abnormality</td>
<td>66.7/33.3 (&lt;1%)</td>
<td>40.0/60.0 (N=15)</td>
<td>41.7/58.3 (N=24)</td>
<td>0.4978</td>
</tr>
</tbody>
</table>

| CTR, cardiothoracic ratio; ECG, electrocardiogram |

---

**Table 3. Multivariate Analysis for Groups Adjusted by Other Parameters**

<table>
<thead>
<tr>
<th>Factors</th>
<th>ESR</th>
<th>History of AL</th>
<th>CR</th>
<th>Chi-Square Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-0</td>
<td>0.200</td>
<td>1.000</td>
<td>0.270</td>
<td>0.034</td>
<td>0.0001</td>
</tr>
<tr>
<td>1-2</td>
<td>0.300</td>
<td>1.000</td>
<td>0.270</td>
<td>0.034</td>
<td>0.0001</td>
</tr>
<tr>
<td>2-3</td>
<td>0.400</td>
<td>1.000</td>
<td>0.270</td>
<td>0.034</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

| mean BP | 0.037 | 0.096 | 0.000 | 0.001 | 0.3919 |

Nephrotic syndrome

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

AL or AA type

<table>
<thead>
<tr>
<th>AL</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.042</td>
<td>1.000</td>
</tr>
</tbody>
</table>

---

Abbreviations: ESR, estimated serum creatinine; CR, standard error; CR, cardiothoracic ratio; AL, amyloid light chain; AA, amyloid associated.
three groups with regard to the mBP (p=0.0215), nephrotic syndrome (p=0.0110), AL and AA type (p=0.0219). There was no significant difference in cardiac function among the groups (Table 2-2).

**Renal survival curve for each cluster group**

The renal survival curve for each cluster group is shown in Fig. 2. The renal survival rates of Groups 2 and 3 were lower than that of Group 1 (p=0.0008). As indicated by these curves, the disease progressed slowly in Group 1 but rapidly in Groups 2 and 3.

**Multivariate analysis of poor prognostic factors in each cluster**

We entered various parameters into multivariate analysis to determine their prognostic significance (Table 3). Group 3 had a higher hazard ratio (1.000, p=0.0030) than Group 1. Mean BP was also correlated with a high hazard ratio (0.982, p=0.0572). Thus, progressive renal death was accelerated in Group 3.

**Individual survival rate at the end of the study**

The individual survival rate at the study endpoint is shown in Fig. 3. In both the ESRD and non-ESRD groups, the commonest cause of death was cardiac failure. In the ESRD group, other causes of death were infection, sepsis, and pneumonia, which were more frequent than in the non-ESRD group. In the non-ESRD group, amyloidosis, liver disease, gastrointestinal tract involvement, and sudden death were important causes. We also recognized three rare cases of remission of renal amyloidosis [a 51-year-old woman (AL type), a 52-year-old woman (AA type), and a 54-year-old woman (AL type)]. These cases were part of the cluster Group 1 with newly formed basement membrane between the podocyte and amyloid deposition.

**Discussion**

Some reports have claimed that renal failure or renal dysfunction in patients with AA amyloidosis is more frequent than in patients with AL amyloidosis (20, 21). In contrast, Bohle et al (3) reported that in the first five years (short-term) after diagnosis, patients with AL amyloidosis showed a more rapid deterioration of renal function than did those with AA. However, in our new study, there was no significant difference of clinical outcome between patients with AA amyloidosis and those with AL amyloidosis during the long-term patient follow-up. Our data (Table 3) were in agreement with the long-term results reported by Bohle et al (3) (generalized Wilcoxon test: AL type vs AA type, p=0.8946). We note the exception of one study which reported renal survival time reaching 10 years in a patient with AL amyloidosis (22). However, that patient progressed to ESRD after about 10 years and required one year for dialysis, and was then the successful recipient of a cadaveric renal transplant, which is still working excellently 10 years later, with little evidence of recurrent renal or systemic amyloidosis.

AL amyloidosis was also reported in combination with nephrotic syndrome. Shiiki et al (20) proposed that AL amyloidosis is occasionally characterized by deposition in the peribasement membrane. The amyloid deposition patterns of AA and AL amyloidosis in renal tissue were varied from patient to patient in the present study (Table 1). Specifically, AA amyloidosis was detected in tubulointerstitial tissue and vessels more frequently than was AL amyloidosis.

It is reasonable to expect a short renal survival time and poor individual survival rate with the progression of renal pathological damage due to amyloid deposition, as determined by renal biopsy (3). In the present study, patients in Groups 2 and 3 progressed rapidly, whereas those in Group 1 progressed slowly, to renal death (Fig. 2). The hazard ratio...
for Group 3 was 5.8 times higher than that of Group 1 (Table 3). In the present study, we found glomerular, tubulointerstitial, and vascular lesions as significant and poor prognostic factors for rapid deterioration to renal failure (Table 2-1). Bohle et al (3) reported that tubulointerstitial damage in renal amyloidosis, and especially in interstitial fibrosis, is an important determinant of renal prognosis. It was reported previously that amyloid deposition attracts mast cells to the site of renal amyloidosis, i.e., the interstitium (23). In the interstitium of renal amyloidosis, we found large populations of T cells, macrophages, fibroblasts, and tryptase-positive mast cells. We therefore suspect there is a strong relationship between the presence of interstitial fibrosis and cells such as mast cells (24).

Hypertension is one of the risk factors for impediment in many internal organs, for example, the brain, heart, kidney, blood vessels, and the retina (25-31). Several studies have discussed the role of hypertension as a renal prognostic factor (27-29), while others (32, 33) have reported that antihypertensive treatment improves renal function. The Japanese Society of Hypertension (JSH) recommended in 2004 that blood pressure levels of less than 130/80 mmHg are preferable to protect against renal dysfunction (34), but our results revealed no differences in the renal survival rate between patients with hypertension and those without it (mean BP, p=0.0572) (Table 3). On the other hand, hypotension is associated (3, 37). These studies have indicated that patients with nephrotic syndrome have a poor prognosis, but our results revealed no differences in the renal survival rate between patients with nephrotic syndrome and those without it (nephrotic syndrome (-) vs (+), p = 0.6893) (Table 3).

Only a few studies have investigated the individual survival rate (4, 38). Renal amyloidosis is associated with increased mortality in hemodialysis patients (36, 39) and in patients with rheumatoid arthritis (40). The present data showed that the 2-, 5-, and 10-year survival rates were about 55, 30, and 20 percent, respectively.

In our patients, the most frequent causes of death were cardiovascular disease and infection. Therefore, we investigated the differences in parameters related to cardiac function, namely CTR and ECG abnormalities (ultrasonography was not performed in all patients). The result showed higher CTR values in AA than in AL amyloidosis patients (p=0.0007) (Table 1), although the number of patients analyzed was relatively small. Further studies of larger samples are needed to confirm these findings.

In conclusion, our data indicate that the quantity of amyloid deposition in the kidney is a significant renal prognostic factor, that amyloid AA was evident in patients with severe vascular damage, and that the prognosis was especially poor in patients with renal lesions. The most common causes of death in the present patients were cardiovascular disease and infection. Therefore, taking appropriate measures against these complications in patients with amyloidosis is critical.

Special thanks to Miss Maho Watanabe for assistance.

This study was supported in part by a grant for the Progressive Renal Disease Research from the Ministry of Health, Labor and Welfare, Japan.

References


12. Komatsuda A, Morita K, Ohtani H, Yamaguchi A, Miura AB. Re-


© 2007 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imindex.html