A clinicopathological analysis in a large cohort of Chinese patients with renal amyloid light-chain amyloidosis

Ying Yao, Su-Xia Wang, You-Kang Zhang, Zhen Qu, Gang Liu and Wan-Zhong Zou

Correspondence and offprint requests to: Su-Xia Wang; E-mail: suxiawang@bjmu.edu.cn

ABSTRACT

Background. The aim of the study is to investigate the association between clinical and pathological features in a large cohort of Chinese patients with renal immunoglobulin light-chain amyloidosis (AL).

Methods. A series of 186 patients with renal AL amyloidosis diagnosed between 1990 and 2011 were retrospectively reviewed. The extent of amyloid deposition in glomeruli, blood vessels and tubulointerstitium were evaluated semiquantitatively. The renal amyloid load was defined by the sum of glomerular, vascular and interstitial deposits. The associations between the clinical manifestations and pathological features were analyzed.

Results. The extent of glomerular amyloid deposition was positively correlated with the level of proteinuria. Patients with codeposition of amyloid and immune complexes (ICs) in glomeruli had higher levels of proteinuria than those without ICs. Advanced glomerular amyloid deposition was an independent pathological factor associated with renal insufficiency at diagnosis. The degree of vascular amyloid (VA) deposition was positively correlated with cardiac involvement and hepatic involvement. Patients with AL-κ showed a higher prevalence of hepatic involvement and more severe VA deposition than patients with AL-λ. High renal amyloid load independently predicted the increased risk for overall death after adjusting for recognized confounders.

Conclusions. The degree and localization of amyloid deposits in the kidney of AL patients were associated with the degree of proteinuria and renal insufficiency, as well as extrarenal organs involvement. There were some differences between AL-κ and AL-λ in clinical and pathological characteristics. The renal amyloid load was an independent predictor for overall mortality.

INTRODUCTION

Immunoglobulin (Ig) light-chain amyloidosis (AL), which is usually associated with an underlying monoclonal plasma dyscrasia, is characterized by deposition of fibrillar proteins derived from Ig light chains or light-chain fragments produced by a clonal population of plasma cells in the bone marrow [1]. The kidney is the most frequently affected organ by AL, and renal involvement is often the major cause of morbidity in patients with AL [2]. It is reported that AL is the most common form of systemic amyloidosis in Western countries [1–3], but the prevalence and the clinical data of AL in Chinese patients are rarely reported.

Renal AL usually manifests to nephrotic range proteinuria and progressive worsening of renal dysfunction, and its pathological changes comprise a spectrum of glomerular, interstitial and vascular amyloid (VA) deposition, with the subsequent destruction of the tissue architecture [3]. It was reported that AL patients with vascular-limited deposition in the kidney showed less proteinuria and severe renal insufficiency [4], suggesting a strong correlation between clinical manifestations and the pattern of amyloid deposition in renal tissue. The clinical outcome of renal AL is related with urine protein excretion and serum creatinine at presentation [5].
Cardiac involvement is the most important prognostic de-
terminant of patients with AL amyloidosis [6]. However, the
pathological predictors of renal AL have not been clearly elu-
cidated. The aim of this study is to investigate the associ-
ations between the clinical characteristics and pathological
features in a large cohort of Chinese patients with renal AL.

MATERIALS AND METHODS

Patients

Of the 23,400 native renal biopsies performed in our insti-
tute between January 1990 and December 2011, 208 cases
(0.89%) were diagnosed as renal amyloidosis by the presence
of apple-green birefringence under a polarized light after
Congo red staining and the fibrils in a diameter of 8–12 nm
identified by electron microscopy. Among them, 186 cases
(89.4%) were classified as AL amyloidosis, 1 case as amyloid
A amyloidosis (AA) and 1 case as fibrinogen A α amyloidosis
(Afib). The remaining 20 cases were undetermined because of
the inconclusive result of immunohistochemistry (IHC)
staining and gene analysis. Therefore, 186 cases of renal AL
amyloidosis were collected for analysis.

Clinical and laboratory data

The following clinical and laboratory variables at the time
of renal biopsy were recorded and used for analysis: gender,
age, serum creatinine, serum albumin, serum alkaline phos-
phatase, immunofixation electrophoresis (IFE) of serum and
urine, 24-h urine protein excretion (UPE) and bone marrow
aspiration and/or biopsy examination.

Nephrotic syndrome was defined as a proteinuria higher
than 3.5 g/24 h and serum albumin lower than 30 g/L. Renal
insufficiency was defined as a serum creatinine level higher
than 1.5 mg/dL (133 μmol/L).

Extrarenal organs involvement was defined as previously
described [7, 8]. Cardiac involvement required a mean left
ventricular wall thickness >12 mm in the absence of hyper-
tension or other potential causes of left ventricular hypertro-
phy. Hepatic involvement was defined as a hepatomegaly
found by imaging in the absence of heart failure or the serum
alkaline phosphatase value was 1.5 times the upper limit of
the institutional normal value.

Informed consent was obtained for renal biopsy from each
patient. The design of this study was approved by the local
ethics committees.

Renal pathology

Renal biopsy specimens were examined by routine light
microscopy, immunofluorescence and electron microscopy.

For direct immunofluorescence examination, detection for
IgG, IgA, IgM, C3, C1q and fibrinogen were performed on
fresh frozen renal tissue immediately after the renal biopsy.
The results were graded on a scale of 0 to 4+ according to the
intensity of fluorescence. Deposition of immune complexes
(ICs) was defined as a score of 2+ or higher staining for any
kind of Ig, with or without complements.

Immunohistochemistry

IHC staining was performed on 4 μm formalin-fixed and
paraffin-embedded sections with a panel of antibodies, in-
cluded monoclonal antibodies directed against amyloid A
(1:200) and polyclonal antibodies directed against κ light
chain (1:10 000), λ light chains (1:10 000), transthyretin
(1:2000), lysozyme (1:2000), fibrinogen (1:10 000, all above
from DakoCytomation Denmark A/S) and apolipoprotein A-
I (1:2000, Merck, Calbiochem, Germany). DAKO EnVis-
ion™+ HRP kits, code Nos. K4008 was used as detection
system. As for staining with apolipoprotein A-I antibodies,
Polink-2 plus Polymer HRP Detection System for Goat
Primary Antibody (GBI, Inc.), was used. The diagnosis of AL
was confirmed by demonstration of selective staining for
light-chain κ (AL-κ) or λ (AL-λ) in the amyloid deposition
area, and with the exclusion of other forms of amyloidosis.

Quantification of renal amyloid deposition and scoring
of histological lesions

The extent of amyloid deposition in glomeruli, blood
vessels and interstitium and other additional histopathologic
lesions, such as global glomerular sclerosis, inflammatory in-
filtration, interstitial fibrosis and tubular atrophy, were scored
semiquantitatively according to the scoring system proposed
by Sen [9].

The extent of glomerular amyloid deposition (GA) was
divided to 5 scores (0–5) according to the percentage of
amyloid deposition to the total area of glomeruli: 1–10, 11–
25, 26–50, 51–75%, and >75%, respectively.

The extent of VA deposition was scored 0–4, according to
the percentage of amyloid deposition to the section area of
interlobular artery: absent, 1–10, 11–25, 26–50%, more than
50%, respectively.

The extent of interstitial amyloid (IA) deposition, inflam-
matory infiltration (Imm), interstitial fibrosis and tubular
atrophy (Ifib), and global glomerular sclerosis (GS) were
divided to 5 scores (0–4) according to the percentage of
lesion involvement: absent, 1–10, 11–25, 26–50%, and more
than 50%, respectively. The renal amyloid load (TA) was
defined by the sum of GA, VA and IA.

All histopathologic samples were reviewed and scored by
a single renal pathologist who was blinded to patients’ clinical
data. Twenty biopsies were re-reviewed by the study renal
pathologist to test intra-rater reliability. A second renal path-
ologist reviewed a random subset of 20 biopsies to assess
inter-rater reliability. In addition, quantitation of renal
amyloid deposition was measured by computerized image
analysis (Image–pro plus 6.0 software, Media Cybernetics,
MD) in a random subset of 20 biopsies. The results of quan-
titative computerized image analysis were compared with that
scored by the renal pathologist to assess inter-rater reliability.
(See Supplementary method 1).

Statistical analysis

Results were expressed as numbers (%) for qualitative vari-
ables and as mean ± SD or median (range) for quantitative
variables. One-way ANOVA, Student’s t-test, Kruskal–Wallis

Y. Yao et al.
test or the χ² test was used to compare the differences of variables between groups. A multivariate linear or logistic regression analysis was performed to investigate the independent variables associated with proteinuria and renal insufficiency at the time of renal biopsy. Kaplan–Meier analysis and Cox proportional hazards models were used for evaluation of overall survival in 66 patients who had follow-up data. Inter- and intra-rater reliabilities were measured using the Kappa statistics. Statistical analysis was performed using the SPSS software package (version 15.0; SPSS, Chicago, IL).

RESULTS

The ratio of light-chain types and clinical characteristics

One hundred and sixty (160/186, 86%) patients were confirmed as AL-λ by IHC staining, while only 26 patients (14.0%) were AL-κ. The ratio of the AL-λ to the AL-κ was 6.1:1. The clinical manifestations of renal AL amyloidosis at diagnosis are shown in Table 1. The mean age was 56.9 ± 10.8 years with 118 males (63.4%). Twelve patients (6.5%) with AL were younger than 40 years.

Multiple myeloma was diagnosed in 14 (7.5%) cases, all of which presented with light chain of λ type. A monoclonal protein was found in 65.1% (99/152) of patients by using immunofixation of serum or urine. Immunofixation of the serum showed a monoclonal protein in 54.6% (83/152) of the patients. A total of 39.5% of the patients had a monoclonal intact Ig (IgG 22.3%, IgA 13.2% and IgM 3.9%) and 15.1% of the patients had free monoclonal light chain (free λ 13.8% and free κ 1.3%) in the serum. Immunofixation of urine specimen was done in 87 patients, which showed a monoclonal protein in 72.4% (63/87) of the patients; 26.4% of those with intact Ig and 46% of those with free light chain (free λ 39.1% and free κ 6.9%) in the urine (see Supplementary tables). The types of light chains detected in renal tissue were all identical to those detected in serum and/or urine.

Only 15 (15/170, 8.8%) patients were found with overt anemia (hemoglobin lower than 100 g/L) at diagnosis, 10 of them with renal insufficiency.

All patients had different degrees of proteinuria, with a mean level of 6.4 ± 3.9 g/24 h (range, 0.44–18.7 g/24 h). Nephrotic syndrome was presented in 76.7% (132/172) of patients. The median of serum creatinine was 78 μmol/L (range: 31–1147 μmol/L). Thirty-one (31/167, 18.6%) patients presented with renal insufficiency at diagnosis, and only 10 (6%) patients presented with serum creatinine level higher than 265 μmol/L (3 mg/dL).

Forty (44%) of the 91 patients with echocardiographic examination results had cardiac involvement. Fourteen (15.6%) patients had an elevated serum alkaline phosphatase value and hepatomegaly was detected in 30 (29.4%) patients. Overall, 34 (33.3%) of 102 patients with hepatic function and/or imaging examination results had hepatic involvement. (Table 1).

Pathological findings

Of all 186 renal biopsy specimens, the number of glomeruli was in an average of 20 (5–81). We selected 10–15 glomeruli generally and at least 5 for scoring. We selected 10 vessels generally and at least 5 for scoring. There were 13 patients excluded because of the number of glomeruli was ≤5 or the number of vessels ≤5.

Glomerular amyloid deposition was detected in all cases. Twenty-three (13.3%), 39 (22.5%), 40 (23.1%), 50 (28.9%) and 21 (12.1%) were scored as GA = 1, 2, 3, 4 and 5, respectively. The extent of vascular amyloid deposition was varied. VA = 3 and VA = 4 were found in 44 (25.4%) and 51 (29.5%), respectively. Overt interstitial deposits (IA ≥2) were found only in 21 patients (12.1%) (Table 2) (Figure 1).

Codeposition of amyloid deposits and ICs in glomeruli were detected in 57 biopsies (32.9%). The types of Ig presented in the deposits were as follows: IgG in 25 cases, IgA predominant in 27 cases (IgA in 14 cases, IgA + IgG in 8 cases, IgA + IgM in 3 cases, IgA + IgG + IgM in 2 cases), IgM in 3 cases, IgG + IgM in 2 cases, respectively. By electron microscopy, electron dense deposits in mesangial matrix were found in 30 patients, 21 of them were IgA predominant, which were diagnosed with coexisted IgA nephropathy, other types of Ig included IgG in 8 cases and IgM in 1 case. These cases were diagnosed as IC-mediated glomerulonephritis (Figure 2).

Existence of casts in tubules were found in 20 biopsies, 11 of which exhibited fracture planes and surrounded by mononuclear cells that were consistent with light-chain cast nephropathy. The other cases were with a few hyaline casts in the tubules. By detailed examination of electron microscopy and immunofluorescent labeling for light chains, we did not find any evidence of light-chain deposition disease and light-chain proximal tubulopathy in patients of our cohort.

The results of amyloid deposition quantitation in glomeruli and blood vessels according to the scoring system had high intra-rater reliability (κ value = 0.801) and inter-rater reliability (κ value = 0.833). The evaluations of renal amyloid deposition by the renal pathologist were also highly correlated to computer image analysis (κ value = 0.767). The estimated scores by the renal pathologist tended to be higher than the computerized image analyzed results.

Associations between pathological parameters and urine protein excretion

The severity of proteinuria was clearly related to the extent of glomerular involvement in our study. In univariate analysis, the level of proteinuria was positively correlated with the grades of GA (spearman test, r = 0.278, P < 0.001). Patients with advanced glomerular deposition (GA = 5) had significantly higher proteinuria than other groups (GA = 1–4) (P < 0.05). It was also found that patients with codeposition of amyloid and ICs in glomeruli had higher level of proteinuria than those without ICs (7.85 ± 4.98 versus 5.74 ± 3.02, P = 0.006) (Table 2).

By multivariate linear regression analysis, codeposition of amyloid and ICs, GA and VA were three independent factors...
associated with proteinuria. Codeposition of amyloid and ICs ($\beta$ coefficient = 1.66; $P = 0.01$) and higher GA ($\beta$ coefficient = 0.986; $P < 0.001$) were associated with more severe proteinuria, while higher VA were associated with less proteinuria ($\beta$ coefficient = $-0.552$; $P = 0.015$) (Table 3). No relationships were found between proteinuria and other variables, including gender, age, IA and type of light chain involved.

**Associations between pathological parameters and renal function**

The incidence of renal insufficiency was significantly associated with GA and IA (linear-by-linear association, $P = 0.001$ and 0.003, respectively). No relationship was found between VA and renal insufficiency ($P = 0.934$) (Table 2).

Adjusted for proteinuria, advanced glomerular amyloid deposition (GA = 5), moderate or severe inflammatory infiltration (Iinf $\geq$ 3) and existence of casts in tubes were three independent variables associated with renal insufficiency by multivariate logistic regression analysis. Patients with GA = 5, Iinf $\geq$ 3 and tubular casts showed a significantly increased risk of renal insufficiency (OR 5.358, 95% CI 1.343–21.376, $P = 0.017$; OR 9.759, 95% CI 2.65–35.94, $P = 0.001$; OR 5.591, 95% CI 1.55–20.17, $P = 0.009$, respectively) (Table 4).

**Association between pathological parameters and the involvement of extrarenal organs**

Linear by linear association revealed that hepatic involvement was positively correlated with the extent of amyloid deposition in renal tissue, including GA ($P = 0.031$), IA ($P = 0.011$) and especially VA ($P < 0.001$). With GA, IA and especially VA increased, the incidence of hepatic involvement increased (Table 2).

Cardiac involvement was significantly correlated with the VA ($P = 0.016$). Patients with higher VA had higher incidence of cardiac involvement. No relationships between cardiac involvement and GA or IA were found.

**Comparison of the clinicopathological features of AL-κ and AL-λ.**

There were different clinicopathological features between AL-κ and AL-λ, as shown in Table 1. A monoclonal protein was found in serum in 39.1% (9/23) of patients with AL-κ, while in 57.4% (74/129) of patients with AL-λ ($P = 0.106$).
Table 2. Univariate analysis of the histopathological parameters and main clinical manifestations at renal biopsy in patients with renal AL

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Proteinuria g/24 h</th>
<th>P-value</th>
<th>Renal insufficiency n (%)</th>
<th>P-value</th>
<th>Cardiac involvement n (%)</th>
<th>P-value</th>
<th>Hepatic involvement n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular amyloid deposition, GA, score 1–5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23 (13.3)</td>
<td>5.37 ± 3.0</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (14.3)</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 (38.5)</td>
<td>0.856</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>2</td>
<td>39 (22.5)</td>
<td>5.36 ± 2.6</td>
<td></td>
<td>3 (8.8)</td>
<td>8 (47.1)</td>
<td></td>
<td></td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>3</td>
<td>40 (23.1)</td>
<td>5.60 ± 3.6</td>
<td></td>
<td>3 (8.6)</td>
<td>7 (43.8)</td>
<td></td>
<td></td>
<td>8 (40)</td>
</tr>
<tr>
<td>4</td>
<td>50 (28.9)</td>
<td>7.33 ± 4.7</td>
<td></td>
<td>6 (13)</td>
<td>12 (46.2)</td>
<td></td>
<td></td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>5</td>
<td>21 (12.1)</td>
<td>9.79 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>13 (61.9)</td>
<td>3 (27.3)</td>
<td></td>
<td></td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Vascular amyloid deposition, VA, score 0–4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13 (7.5)</td>
<td>5.27 ± 3.5</td>
<td>0.048&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2 (25)</td>
<td>0.934</td>
<td>0 (0)</td>
<td>0.016&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1</td>
<td>42 (24.3)</td>
<td>7.69 ± 3.6</td>
<td></td>
<td>7 (17.5)</td>
<td>10 (37)</td>
<td></td>
<td></td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>2</td>
<td>26 (15)</td>
<td>7.37 ± 4.3</td>
<td></td>
<td>3 (12)</td>
<td>2 (14.3)</td>
<td></td>
<td></td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>3</td>
<td>49 (28.3)</td>
<td>6.27 ± 4.4</td>
<td></td>
<td>8 (18.2)</td>
<td>12 (52.2)</td>
<td></td>
<td></td>
<td>11 (42.3)</td>
</tr>
<tr>
<td>4</td>
<td>43 (24.9)</td>
<td>5.35 ± 3.4</td>
<td></td>
<td>8 (20)</td>
<td>11 (64.7)</td>
<td></td>
<td></td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>Interstitial amyloid deposition, IA, score 0–4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>95 (54.9)</td>
<td>6.38 ± 3.9</td>
<td>0.227</td>
<td>8 (9.5)</td>
<td>0.003&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20 (43.5)</td>
<td>0.514</td>
<td>13 (25)</td>
</tr>
<tr>
<td>1</td>
<td>57 (32.9)</td>
<td>7.58 ± 4.1</td>
<td></td>
<td>12 (23.1)</td>
<td>13 (46.4)</td>
<td></td>
<td></td>
<td>9 (30)</td>
</tr>
<tr>
<td>2</td>
<td>13 (7.5)</td>
<td>5.38 (38.5)</td>
<td></td>
<td>5 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>4 (50)</td>
</tr>
<tr>
<td>3</td>
<td>5 (2.9)</td>
<td>2 (40)</td>
<td></td>
<td>2 (66.7)</td>
<td></td>
<td></td>
<td>3 (75)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3 (1.9)</td>
<td>1 (33.3)</td>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Codeposition of amyloid and immune complexes, 0 without, 1 with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>108 (67.1)</td>
<td>5.74 ± 3.0</td>
<td>0.006&lt;sup&gt;g&lt;/sup&gt;</td>
<td>16 (14.7)</td>
<td>0.086</td>
<td>25 (41)</td>
<td>0.797</td>
<td>19 (28.8)</td>
</tr>
<tr>
<td>1</td>
<td>53 (32.9)</td>
<td>7.85 ± 4.9</td>
<td></td>
<td>13 (26)</td>
<td>11 (44)</td>
<td></td>
<td></td>
<td>13 (39.4)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or numbers (%).
<sup>a</sup>compared between five groups according to GA.
<sup>b</sup>compared to patients with GA = 12,3,4; P < 0.05.
<sup>c</sup>linear by linear association of GA with clinical manifestations.
<sup>d</sup>compared between five groups according to VA.
<sup>e</sup>linear by linear association of VA with clinical manifestations.
<sup>f</sup>linear by linear association of IA with clinical manifestations.
<sup>g</sup>compared between patients with codeposition of amyloid and immune complexes and patients without immune complexes.
IgM was the major type (4/9) of monoclonal intact Ig in patients with AL-κ, whereas IgG was the major type (32/75) in patients with AL-λ (P < 0.001).

Compared with patients with AL-λ, patients with AL-κ showed a significantly higher frequency of hepatic involvement (75% versus 25.6%, P < 0.001). The incidence of cardiac involvement showed a trend to be higher in patients with AL-κ than in patients with AL-λ (72.7% versus 39.2%, P = 0.051). In patients with AL-κ, the proportions of VA = 3 (34.8%) and VA = 4 (47.8%) were higher than those in AL-λ (27.3 and 21.3%, respectively). Patients with AL-κ were more likely to have severe vascular amyloid deposition than those with AL-λ (χ² = 9.995, P = 0.001). No significant differences in age, proteinuria and renal insufficiency were observed between AL-κ and AL-λ.

**Follow-up data**

Because this was a retrospective study, only 66 patients in our cohort had follow-up data, with the minimum follow-up of 6 months if alive. Among them, 11 patients received high-dose melphalan and stem cell transplant, 31 patients received chemotherapy including melphalan plus prednisone, melphalan plus dexamethasone, bortezomib plus dexamethasone, and so forth. The other 24 patients did not receive any chemotherapy.

Median survival of the 66 patients after the diagnosis was 39 months (1–120 months). The estimated median survival was 39 months in patients with TA > 5 versus 46 months in patients with TA ≤ 5 (P = 0.047) (Figure 3). The Cox proportional hazards model was used to determine predictors of overall survival. After adjusting for hepatic involvement, cardiac involvement and therapy, renal amyloid load (TA > 5) independently predicted the increased risk for overall death (HR 3.337, 95% CI 1.310–8.502, P = 0.012).

**DISCUSSION**

Renal amyloidosis accounted for 0.89% of native renal biopsies from the data of our institution, which is lower than the incidence of the USA (1.3%) and Italy (2.9%) [10, 11]. Furthermore, correct typing of amyloidosis is very important for the treatment and predicting the prognosis of patients with amyloidosis. According to previous reports, amyloid typing
by IHC with antigen retrieval handling on paraffin sections is a specific and reliable method [12]. In the current study, typing of amyloidosis by IHC showed that AL was the major form of systemic amyloidosis in China, which accounted for 89.4% of renal amyloidosis, with a predominant light-chain type of λ. The ratio of the AL-λ to the AL-κ was as high as 6.1:1, consistent with previous reports [5].

Proteinuria was the main clinical manifestation of patients with renal AL in our cohort, and over three quarters of patients had nephrotic syndrome. At the time of renal biopsy, only 18.6% of patients had renal insufficiency that was lower than the incidence of renal insufficiency in patients with renal AL in the USA and Italy [11, 13]. The extent of amyloid deposition in glomeruli was positively associated

<table>
<thead>
<tr>
<th>Table 3. Multivariate linear regression analysis of the main relevant variables associated with proteinuria at renal biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariable analysis</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>β Coefficient (95% CI)</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Codeposition of amyloid and ICs</td>
</tr>
<tr>
<td>GA</td>
</tr>
<tr>
<td>VA</td>
</tr>
<tr>
<td>IA</td>
</tr>
<tr>
<td>CI, confidence interval; ICs, immune complexes; GA, the extent of glomerular amyloid deposition; VA, the extent of vascular amyloid deposition; IA, the extent of interstitial amyloid deposition.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4. Multivariate logistic regression analysis of the main relevant variables associated with renal insufficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariable analysis</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Proteinuria (per g/24 h)</td>
</tr>
<tr>
<td>With codeposition of amyloid and ICs</td>
</tr>
<tr>
<td>Advanced glomerular amyloid deposition, GA = 5</td>
</tr>
<tr>
<td>Overt interstitium amyloid deposition, IA ≥ 2</td>
</tr>
<tr>
<td>Moderate or severe inflammatory infiltration, Iinf ≥ 3</td>
</tr>
<tr>
<td>Moderate or severe interstitial fibrosis, Ifib ≥ 3</td>
</tr>
<tr>
<td>With casts in tubules</td>
</tr>
<tr>
<td>OR, odds ratio; CI, confidence interval; ICs, immune complexes; GA, the extent of glomerular amyloid deposition; VA, the extent of vascular amyloid deposition; IA, the extent of interstitial amyloid deposition; Iinf, the extent of inflammatory infiltration in interstitium; Ifib, the extent of interstitial fibrosis and tubular atrophy.</td>
</tr>
</tbody>
</table>
with the level of proteinuria and the prevalence of renal insufficiency by multivariate analysis in the present study. Our results are different from previous reports, which did not demonstrate any correlation between the extent of amyloid deposition and the severity of proteinuria [1, 14]. That may be attributed to the large sample size of our study. A few studies reported that vascular-limited deposits of amyloid exhibited more severe renal insufficiency and less proteinuria in both AA and AL patients [4, 15]. We also demonstrated that higher vascular deposition of amyloid was associated with lesser proteinuria, but not associated with renal insufficiency. Hence, the localization of amyloid deposits in different departments of renal tissue may affect clinical manifestations.

It was revealed that the extent of tubulointerstitial damage significantly correlated with the impairment of renal function in renal diseases [16]. In a study of 21 Japanese patients with AL renal amyloidosis, Osawa Y et al. [17] found that patients with interstitial damage in the kidney had significantly poorer prognosis of renal function than those without such damage. In our study, apart from advanced glomerular deposition, IA deposition, inflammatory infiltration and existence of casts in tubules were all associated with renal insufficiency. Moderate or severe inflammatory infiltration and existence of casts in tubules were independently associated with the increased risk of renal insufficiency.

Cardiac involvement is an independent predictor of overall survival in patients with amyloidosis. Based on an analysis of 11 cases of AL amyloidosis, Itabashi et al. [14] demonstrated that the vascular deposition pattern of amyloid in the kidney was associated with cardiac involvement and left ventricular thickening. However, Eirin et al. [4] did not find any differences in cardiac involvement between patients with vascular-limited deposition pattern and patients with the diffuse deposition pattern. In the current study, the extent of vascular amyloid deposition significantly correlated with the incidence of cardiac involvement. With the VA increased, the incidence of cardiac involvement increased. In addition, we demonstrated that hepatic involvement positively correlated with the degree of amyloid deposition in the kidney, especially with the degree of vascular amyloid deposition.

The high frequency of \( \lambda \) light chain involvement is a hallmark of AL amyloidosis [6], and our results confirmed that AL-\( \lambda \) was the predominant type of renal AL amyloidosis in Chinese patients. It was accepted that \( \lambda \) light chains might be more ‘amyloidogenic’ due to their physiologic properties [18], but the exact mechanisms have not been elucidated clearly. AL patients derived from different types of light chains showed different clinical and pathological characteristics and organ tropism. Compared with patients with AL-\( \lambda \), patients with AL-\( \kappa \) showed higher incidence of hepatic involvement and more severe vascular amyloid deposition in the kidney in the current study. Kumar et al. [8] also found that patients with AL-\( \kappa \) were more likely to have liver and GI tract involvement, and AL-\( \lambda \) mainly involved the kidney. Gertz et al. [5] pointed that \( \lambda \) light-chain amyloid not only showed a higher prevalence of renal involvement, but also greater urinary protein loss than \( \kappa \) type amyloid. The associations between the types of light chains and organs involvement provide unique insights and can potentially improve our understanding of biology of amyloidosis.

Another interesting finding in the current study was that patients with codeposition of amyloid and ICs in glomeruli had a higher level of proteinuria than those without ICs. In our cohort, above 30% of patients (57/186) with renal AL showed presence of Igs in renal tissue simultaneously, and electron dense deposits were identified in 30 patients, which were confirmed to be IC mediated glomerulonephritis superimposed on renal amyloidosis. The most common glomerulonephritis coexisted with AL is IgA nephropathy, which is very frequent in east Asia, especially in China. In addition, O’Nuallain et al. [19] recently showed that human sera contain antibodies specific for fibrils formed from different amyloidogenic precursor proteins, but did not react with these molecules in their native nonfibrillar forms. For patients with Ig deposition but without electron dense deposits, a specific antibody may have reacted with amyloid fibrils in situ, suggesting that humoral immunity could be involved in the pathogenesis of amyloidosis. Finally, the monoclonal Ig deposition in glomeruli suggests that the heavy chain of Ig might constitute the amyloid fibril. The identification of the heavy chain component of amyloid fibrils by the mass spectrometry method [20] is required.

We also found that as the renal amyloid load increased, the risk for overall death increased. Nevertheless, this is a retrospective study from a single center, and the number of patients with follow-up data was limited. The results should be proved by further study.

In summary, the present study revealed that the degree of amyloid deposition in glomeruli was closely associated with both proteinuria and renal function. The degree of vascular deposition positively associated with the incidences of heart and liver involvement. Patients with AL-\( \kappa \) were more likely to have liver involvement and severe vascular amyloid deposition. Patients with codeposition of amyloid and ICs in glomeruli had a higher level of proteinuria. We demonstrated, to our knowledge, for the first time that high renal amyloid load is an independent predictor of high overall mortality, which should be proved by further study.
CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


Received for publication: 14.4.2012; Accepted in revised form: 19.9.2012