Pathological, Clinical Characteristics and Prognostic Analysis of Amyloid Nephropathy

Xue Zhao
Department of Nephrology, Shandong Provincial Hospital affiliated to Shandong First Medical University
Rong Wang
Department of Nephrology, Shandong Provincial Hospital affiliated to Shandong First Medical University
Che Yu (yuchesdu@hotmail.com)
Department of Nephrology, Shandong Provincial Hospital affiliated to Shandong First Medical University

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Abstract

Background: The number of patients diagnosed with renal amyloidosis is increasing year by year, and the pathological grade and prognostic factors are not clear. This study attempts to investigate the pathological features of renal amyloidosis and the prognostic factors in patients with amyloidosis.

Method To evaluate the pathological sections of 184 patients with renal amyloidosis in the Department of Pathology, Shandong University, and to retrospectively analyze the clinical data of 175 patients with amyloidosis in our center and to follow up them.

Results: There was a positive correlation between different pathological grades and creatinine levels (rs = 0.275, P = 0.38), the higher the pathological grade, the higher the incidence of renal insufficiency (χ2 = 8.44, P = 0.015); the more severe the tubulointerstitial damage, the higher the incidence of renal insufficiency (χ2 = 14.15, P = 0.007); different degrees of tubulointerstitial damage and glomerulosclerosis affected the urinary protein quantification, and the difference was statistically significant (P < 0.05). The treatment effect of older is worse (P=0.030，OR=1.040，95%CI=1.004，1.078) and the effect of selecting an effective treatment is better (P = 0.002, OR = 0.618, 95% CI = 0.456, 0.839). Hypotension, cardiac involvement, decreased serum albumin, and increased TnT were independent risk factors for survival; while receiving effective treatment modalities was an independent protective factor.

Conclusion: The pathological grade of renal amyloidosis is associated with the incidence of renal insufficiency, in which the degree of tubulointerstitial chronic lesions significantly affects renal function.

Background

Amyloidosis is a systemic disease, mostly caused by extracellular deposition of amyloid from β-structured fibrin, which can be deposited in multiple organs such as kidney, heart, and skin causing damage(1), and can be secondary to a variety of diseases, such as plasma cell disease, chronic inflammatory diseases, and gene mutations, with an incidence of about 10 people/million per year in the population. There are different proteins in different types of amyloidosis. At present, 36 human proteins have been associated with amyloidosis, including immunoglobulin light chain (AL), serum amyloid A (AA), transthyretin (ATFR), etc.(2), and the mechanism is relatively complex. AL and AA are currently the two most common forms, the former being more common in developed countries, and as a plasma cell disease, monoclonal proteins can be detected in urine or serum in more than 95% of patients(3); the latter is common in developing countries and is secondary to active or recurrent inflammatory diseases(4). The clinical manifestations are complex and diverse due to different organs involved, such as heart failure, massive proteinuria, edema, skin ecchymotic papules, hepatosplenomegaly, and tongue hypertrophy, which are not specific, have a high rate of clinical misdiagnosis, and have a poor prognosis. Among them, renal involvement can be 74%(5). Compared with Western countries, the proportion of renal involvement in Chinese patients(6).

Studies have shown that its clinical manifestations may be related to the location and mode of amyloid deposition, glomerular deposition is mainly manifested as severe nephrotic syndrome with volume overload, while patients with tubulointerstitial or vascular deposition usually show lower proteinuria and progressive renal damage(7). Amyloid is deposited in glomeruli, renal interstitium, renal vessels, and tubules in different ways. Pathologists such as Watanabe and Saniter(8), Dikman(9), and Shiiki(10) continuously defined and updated the deposition pattern of amyloid, and Verine et al defined the distribution of glomerular amyloid deposits as six categories: nodular, diffuse intimal, capillary or mixed, mainly extramembranous or perimembranous, and pulmonary(11). However, a clear relationship between the degree of amyloid deposition and the severity of the clinical presentation remains unconfirmed, renal amyloid biopsy reports are not standardized, and amyloidosis progresses rapidly without a specific treatment regimen and has a poor prognosis. Based on the above reasons, Sait et al proposed a histopathological classification, scoring, and grading system for renal amyloidosis to provide ideas for pathological grading as well as prognosis(12).

In addition to the extent of amyloid deposition and other pathological changes, recent guidelines have shown that urinary protein quantification, serum creatinine levels and glomerular filtration rate(13), free light chains(14), cardiac involvement(15) and other markers (e.g. cTNT(16), BNP(17), albumin(18), etc.) all affect the prognosis of amyloidosis. To further investigate the relationship between pathological changes and clinical characteristics and prognosis of amyloidosis nephropathy, risk factors that may affect prognosis, and different clinical and prognostic characteristics of renal amyloidosis and amyloidosis in other organs, we evaluated the pathological sections of amyloidosis nephropathy and also retrospectively analyzed the clinical data of amyloidosis in our center.

Method

1. Patients 184 patients with renal amyloidosis who underwent pathological analysis of renal biopsy in the Department of Pathology, Shandong University School of Medicine, 17 prefectural tertiary hospitals and 15 secondary hospitals in Shandong Province from March 2000 to March 2020 were selected. And 175 patients were confirmed to have amyloidosis by pathological biopsy in Shandong Provincial Hospital were selected as the study subjects.

2. Inclusion and exclusion criteria *Inclusion criteria:* (1) diagnosed with renal or other organ amyloidosis by pathological biopsy; (2) patients with case data in our hospital or other hospitals; *Exclusion criteria*: (1) patients with severe cardiovascular and cerebrovascular diseases, malignant tumors, end-stage renal disease, etc.; (2) do not cooperate with the follow-up.

3. Study methods (1) 184 cases of renal amyloidosis pathological sections and reports were repeatedly read and evaluated, the histopathological classification, scoring and grading of renal amyloidosis were performed, and the correlation between pathological changes and clinical characteristics of amyloidosis nephropathy was analyzed; (2) The clinical and pathological data of patients in our center were collected to determine the prognosis of patients (cure, remission, no improvement, death), and factors that may

affect the prognosis. (3) follow-up was performed from definite diagnosis to patient death or the most recent clinical follow-up, with the endpoint of patient death.

4. Diagnostic and Scoring Criteria

(1) Diagnostic criteria for renal amyloidosis: Congo red staining of renal tissue was positive and yellow-green birefringence was observed under a polarized microscope. Electron microscopy showed a distribution of fine fibrillar structures 8–12 nm in diameter, unbranched, and disorganized. The diagnostic criteria for other organ involvement are shown in Table 1.

Table 1
The diagnostic criteria for organ involvement

Organ involvement	Diagnostic criteria
Kidney	24-hour urinary protein > 0.5 g/day (mainly albumin); or renal biopsy for amyloidosis
Liver	Volume > 15 cm, excluding heart failure; alkaline phosphatase > 1.5 times the upper limit of normal
Nerve	Peripheral neuropathy (symmetrical bilateral lower extremity sensorimotor neuropathy); autonomic Lesions (impaired gastric emptying, pseudo-obstruction, micturition disorder)
Gastrointestinal Tract	Biopsy Confirmation
Lungs	Biopsy confirms diagnosis; interstitial X lug
Soft tissue	Macroglossia, arthropathy, lameness, lymph node biopsy, skin biopsy, muscle biopsy, Carpal tunnel syndrome

(2) Histopathological classification and scoring criteria of renal histopathological changes: The scores included 7 aspects— Class of glomerular amyloid deposition (GAP), Percentage of glomerular amyloid deposition (GA%), renal vascular amyloid deposition (VA), renal interstitial amyloid deposition (IA), tubulointerstitial chronic lesions (Ifib), renal interstitial inflammatory cell infiltration (Iinf) and degree of glomerular sclerosis (GS). The degree of amyloid deposition in different tissues of the kidney was semiquantitatively scored based on the percentage of Congo red-positive material to the area of the corresponding site. The histopathological classification of renal amyloidosis is shown in Table 2,and specific scoring criteria are shown in Table 3.

Table 2
The histopathological classification of renal amyloidosis

Classifications	Definition
Class I	Minimal renal deposition
Class II	Mesangial minimal amyloid deposition
Class III	Focal mesangiocapillary amyloid deposition
Class IV	Diffuse mesangiocapillary amyloid deposition
Class V	Membranous amyloid deposition
Class VI	Advanced amyloid deposition

Table 3
Scoring of Histopathologic Findings

Item	Method	Result	Score
GAP	Pattern of amyloid deposition in glomeruli	Absent, hilar, minimal mesangial, focal mesangial, mesangiocapillar, membranous, global sclerotic	0–6
GA%	The percent of amyloid in the entire glomerular area	0, 1%-10%, 11%-25%, 26%-50%, 51%-75%, 76%-100%	0–5
VA	Different degrees of amyloid deposition in blood vessels	Absent, minimal, focal, moderate, severe amyloid deposition	0–4
IA	Different degrees of amyloid deposition in renal interstitium	Absent, minimal, focal, moderate, severe amyloid deposition	0–4
Ifib	Degree of tubular atrophy and renal interstitial fibrosis	0, 1%-10%, 11%-25%, 26%-50%, 51%-100%	0–4
Iinf	inflammatory cell infiltration as a percent of tubulointerstitial area	0, 1%-10%, 11%-25%, 26%-50%, 51%-100%	0–4
GS	Percent of glomeruli with glomerulosclerosis	0, 1%-10%, 11%-25%, 26%-50%, 51%-100%	0–4

(3) *Renal amyloid prognostic score (RAPS) and grade*: RAPS is the total score of GAP + GA% + VA + IA + Ifib + Iinf + GS, and renal amyloidosis is further classified into three levels: grade I, early renal amyloidosis, 1–7 points; grade II, late renal amyloidosis, 8–15 points; and grade III, advanced renal amyloidosis, 16–31 points.

5. Statistical analysis SPSS 23.0 statistical software was used for data analysis. The enumeration data were expressed as n and percentage (%), the measurement data of normal distribution were expressed as $x \pm s$, and the measurement data of skewed distribution were expressed as M (P25, P75). The means

between the two groups were compared using the t-test, and the means of multiple groups were compared using one-way analysis of variance; the non-normal distribution data were analyzed using the non-parametric test Kruskal-Wallis H test, and the rates were compared using the χ2 test or Fisher's exact test (sample size < 40 or frequency 0); the Kapln-Meir method was used to compare the survival time between different levels of each index. Multivariate Cox regression analysis was performed using indicators with statistical significance on univariate analysis as independent variables to explore independent factors of survival. P < 0.05 was considered statistically significant.

Results

1. Clinical characteristics

175 patients with amyloidosis in our center, 99 males and 76 females, male *vs* female was 1.3:1. The mean age was 56.23 ± 12.61 years, with the highest proportion (34.85%) between 50 and 59 years. It mainly involved the kidney in 75 patients (42.86%), heart in 38 patients (21.71%), skin in 26 patients (14.86%), and 19 patients (10.86%) had multiple organ involvement. The clinical manifestations of renal involvement were proteinuria (34.86%) and edema (34.26%), accompanied by decreased albumin; patients with cardiac involvement mainly presented with chest tightness, wheezing (20.00%), and fatigue (12.57%), and 9.14% of patients presented with hypotension. Other organ involvement was characterized by skin itching, papules and pigmentation (13.14%), abdominal pain (10.29%), hepatomegaly (12.77%), splenomegaly (9.57%), ascites (26.60%), cough, sputum (9.14%), pleural effusion (52.08%), memory loss (2.86%), limb disorders (1.14%), hoarseness (3.43%), and tongue hypertrophy (1.14%). Immunofixation electrophoresis was measured in 61 patients and 27 showed positivity (44.26%). However, free light chains were measured in 54 patients and the ratio was abnormal in 36 patients (66.67%); 52 patients underwent bone marrow puncture, MGUS in 23 patients and MM in 18 patients. 24 with hormone/immunosuppressive therapy, 8 with hemodialysis, 30 with chemotherapy, and 10 with surgery. During the follow-up, 40 patients died, 55% had cardiac involvement, and most of them died due to heart failure and multiple organ failure. Cumulatively, only 58 patients (33.14%) were diagnosed before 2014, and 117 patients (66.86%) were diagnosed from 2014 to 2019.

Among the 184 patients with renal amyloidosis in Shandong Province, 113 were male and 71 were female, with a male to female ratio of approximately 1.6:1; the mean age was 58.71 ± 9.02 years, the proportion was as high as 75% at 50–69 years of age, and only 3.8% (7/184) were under 40 years of age. A total of only 20 patients (10.87%) were diagnosed before 2014 and 164 patients (89.13%) were diagnosed after 2014, of which 32 patients were diagnosed in 2019.

2. Renal pathological changes

Glomerular damage was predominant in 181 biopsy specimens, and three showed only interstitial or perirenal tissue damage, specific renal pathological findings are shown in Table 4. We found that light chain κ/λ staining was performed in 141 patients, of which 51.06% (72/141) were λ positive and 26.95% (38/141) were κ positive, with a ratio of approximately 1.89:1, and 35 were both κ and λ positive. Twenty-

six patients underwent immunohistochemical staining, and only four patients underwent kappa and Lambda staining, which showed varying degrees of positivity; nine patients considering membranous nephropathy had negative PLA2R staining; and 11 patients with hepatitis B virus infection had negative HbsAg and HBeAg staining.

Table 4.
Pathological characteristics of renal biopsy (n = 184)

Pathological items	Pathological features	Number（%）
Amyloid deposition	glomerulus	181（98.37）
	mesangial area	169（91.85）
	capillary wall	19（10.33）
	subendothelial	4（2.17）
	renal tubule	6（3.26）
	renal interstitium	9（4.89）
	interstitial small vessel wall	99（53.80）
	peribulbar tissue	2（1.09）
Masson	haemophilin deposition	20（10.87）
	no haemophilin deposition	164（89.13）
PASM	"Eyelash" change	99（53.80）
	deposition of red substance	9（4.89）
	negative	76（41.30）
PAS	deposition of PAS positive material negative	18（9.78）
Immunofluorescence staining	IgG	166（90.22）
	IgA	31（17.03）
	IgM	44（24.18）
	C3	82（45.05）
	C1q	37（20.33）
	Fn	60（32.97）
	K	22（12.09）
	λ	38（26.95） n=141）
Congo red staining	positive	72（51.06） n=141）
	weak positive	6（3.35）
	negative	5（2.72）
Electron microscope	Patchy irregular filamentous material with a diameter of	143（77.72）

8 – 12 nm

3. Correlation analysis between renal pathological changes and clinical features

Fifty-seven patients with amyloidosis nephropathy who underwent semi-quantitative scoring and grading in our center were selected and their pathological changes were correlated with urinary protein quantification, renal impairment and prognosis.

3.1 Correlation between renal pathological changes and proteinuria quantification

The proteinuria levels in patients with RAPS scores grade I, II, III respectively were 4.65 (2.81, 7.68) g/d, 4.63 (3.63, 5.81) g/d, 4.18 (2.67, 7.71) g/d, and there was no significant difference. Spearman correlation analysis showed that different pathological grades were not correlated with the amount of urinary protein. The urinary protein quantification of patients with GS = 0, 1, 2, 3, 4 respectively was 4.75 (3.33, 8.13) g/d, 4.04 (3.37, 4.89) g/d, 4.88 (3.99, 8.67) g/d, 5.46 (4.78, 9.82) g/d, 1.24 (1.16, 1.61) g/d, and the difference was statistically significant ($P < 0.05$); GS = 4 was significantly lower than the other groups ($P < 0.05$). However, the urinary protein quantification in patients with Ifib = 0, 1, 2, 3, 4 respectively was 5.24 (2.81, 7.68) g/d, 4.42 (2.22, 4.89) g/d, 4.17 (3.40, 7.11) g/d, 6.07 (5.05, 12.99) g/d, 1.32 (1.16, 2.26) g/d, and the difference was statistically significant ($P < 0.05$). Pairwise comparison showed that Ifib = 3 was significantly higher than other groups ($P < 0.05$). But the results showed that there was no significant difference in the quantification of urinary protein among different score groups of GAP, GA%, VA, and IA.

3.2 Correlation between renal pathological changes and renal function impairment

The rates of renal insufficiency in patients with RAPS grade I, II, III respectively were 25%, 18.75%, 58.8%, and the difference was statistically significant ($\chi2 = 8.44$, $P = 0.015$); Spearman analysis showed that different pathological grades were positively correlated with creatinine levels ($rs = 0.275$, $P = 0.38$). Further analysis showed that the rates of renal insufficiency in patients with Ifib = 0, 1, 2, 3, 4 respectively were 12.5%, 0%, 34.6%, 50%, 100%, with statistically significant difference ($\chi2 = 8.44$, $P = 0.015$). Ifib = 4 was significantly higher than other groups ($P < 0.05$). However, there was no significant difference in the incidence of renal insufficiency among patients with different scores in each group of GAP, GA%, VA, IA, GS, and Iinf.

3.3 Correlation between renal pathological changes and prognosis

The mortality rates of patients with grade I, II, III were 66.7%, 27.3%, 36.4%. The difference was not statistically significant. And correlation analysis showed that RAPS was not associated with mortality.

(Table 5)

Table 5
Comparison of clinical features of renal amyloidosis with different pathological grades(n = 57)

Clinical features	I	II	III	*P*
Age (years)	57.63 ± 11.0	56.63 ± 10.52	55.88 ± 8.59	0.919
Serum albumin (g/L)	23.58 ± 6.76	22.26 ± 7.16	24.87 ± 7.93	0.494
Urine protein (g/24 h)	4.65(2.81, 7.68)	4.63(3.63, 5.81)	4.18(2.67, 7.71)	0.867
Renal insufficiency(n)	2(25.0)	6(18.8)	10(58.8)	0.015
Death (n)	4(66.7)	6(27.3)	4(36.4)	0.204

4. Analysis of different clinical characteristics

4.1 Comparative analysis of amyloidosis patients with different organ involvement

The clinical characteristics and prognosis were compared between the renal involvement and no-renal involvement groups. And the results showed that the length of hospital stay, serum albumin, serum calcium, glomerular filtration rate, urinary protein and creatinine were higher in the renal involvement group, and the differences had statistical significance ($P < 0.05$). While the differences in other indicators had no statistical significance. Therefore, patients with renal amyloidosis have a higher incidence of renal impairment than those with other organ involvement. (Table 6)

Table 6
Comparison of clinical features of amyloidosis in different organs (n = 175)

Clinical features	Renal amyloidosis	Other organ amyloidosis	*P*
Age (years)	56.6 ± 11.1	55.9 ± 13.7	0.710
Length of stay (days)	16.1 ± 11.7	12.8 ± 6.9	0.033
Hypertension (n)	32(50.0)	32(50.0)	0.147
Hemoglobin (g/L)	128.7 ± 28.9	128.2 ± 21.6	0.905
Serum albumin (g/L)	25.9 ± 15.5	36.7 ± 6.2	< 0.001
Urine protein quantitation (g/24 h)	5.1 ± 3.1	1.0 ± 1.4	< 0.001
eGFR	87.2 ± 41.1	105.4 ± 37.4	0.003
Serum creatinine(umol/L)	126.9 ± 149.3	77.4 ± 48.3	0.007
cTnT(pg/ml)	103.0 ± 116.7	113.3 ± 143.9	0.817
BNP(pg/ml)	5510.1 ± 7628.1	5283.7 ± 5023.2	0.905
Blood calcium (mmol/L)	2.0 ± 0.2	2.3 ± 0.2	< 0.001
Recovered or improved (n)	29(42.6)	39(57.4)	0.539
Death (n)	18(45.0)	22(55.0)	0.450

4.2 Analysis of clinical characteristics of amyloidosis patients in different years

In our study, 98 patients diagnosed in the past 5 years and 77 patients in the first 15 years. Compared clinical characteristics and prognosis of patients in the two groups, the results showed that the age of diagnosis of amyloidosis patients in recent 5 years was higher than before, and the glomerular filtration rate was lower. The differences were statistically significant ($P < 0.05$). However, there was no significant difference in other laboratory parameters between two groups. But we can found that the mortality rate of this disease decreased and the cure rate increased in the past 5 years. (Table 7)

4.3 Analysis of clinical characteristics in amyloidosis patients with different prognosis

The comparison of different prognostic groups showed that the serum albumin in the death group was significantly lower than that in the non-death group, and the BNP was higher in death group ($P < 0.05$). The difference in other indicators was not statistically significant. Therefore, decreased serum albumin and increased BNP may increase mortality. (Table 8)

Table 7
Comparison of clinical features of amyloidosis patients in different years (n = 175)

Clinical characteristics	Nearly 5 years	First 15 years	*P*
Age (years)	58.6 ± 11.2	53.2 ± 13.7	0.004
Gender (M/F)	58/40	41/36	0.432
Length of stay (days)	13.1 ± 7.9	15.6 ± 10.9	0.083
Hypertension (n)	40(40.8)	24(31.2)	0.210
Hemoglobin (g/L)	130.9 ± 24.0	125.2 ± 26.0	0.135
Serum albumin (g/L)	32.2 ± 14.0	31.9 ± 9.9	0.880
Urine protein quantitation (g/24 h)	4.5 ± 3.7	4.2 ± 2.7	0.659
eGFR	88.6 ± 28.5	109.1 ± 48.8	0.001
Serum creatinine(umol/L)	83.9 ± 51.1	117.3 ± 148.9	0.063
cTnT(pg/ml)	110.0 ± 136.1	110.5 ± 141.7	0.996
BNP(pg/ml)	5238.1 ± 5849.0	7065.0 ± 7901.5	0.556
Blood calcium (mmol/L)	2.2 ± 0.2	2.2 ± 0.2	0.999
Recovered or improved (n)	48(57.8)	20(45.5)	0.183
Death (n)	23(27.7)	17(38.6)	0.207

Table 8
Clinical characteristics of amyloidosis patients with different prognosis (n = 127)

Metrics	Death Group	Non-death group	*P*
Age (n = 127)	61.20 ± 8.01	55.31 ± 11.87	0.969
Hospital stay (n = 127)	13.55 ± 8.18	12.43 ± 6.42	0.399
Hypertension (n = 127)	14 (35.00)	48 (35.56)	0.949
Hemoglobin (n = 126)	124.03 ± 24.11	129.87 ± 23.27	0.200
Serum albumin (n = 127)	28.46 ± 8.27	33.31 ± 14.36	0.049
Serum creatinine (n = 127)	93.67 ± 60.66	83.38 ± 56.68	0.354
Blood calcium (n = 126)	2.16 ± 0.24	2.18 ± 0.20	0.590
EGFR (n = 127)	90.50 ± 36.99	97.03 ± 34.25	0.332
cTnT (n = 39)	147.72 ± 107.19	78.11 ± 166.74	0.132
BNP (n = 54)	7722.57 ± 6355.15	3141.24 ± 4483.77	0.003
ALP (n = 127)	139.13 ± 143.31	114.64 ± 119.60	0.317
Urine protein quantitation (n = 58)	4.10 ± 3.11	4.05 ± 3.72	0.965
Immunofixation electrophoresis positive (n = 61)	8 (40.00)	19 (46.34)	0.640

5. The results of survival time analysis

Results displayed that the average survival time of patients was 93.36 months, while the survival time results were not obtained. The follow-up time was 20.00 months. The 1-year, 2-year, 3-year and 5-year survival rates were 71.7%, 69.3%, 67.7% and 64.9%, respectively. And the mean survival time of patients with renal involvement was 79.96 months, but the survival time was not obtained; the 1-year, 2-year, 3-year, and 5-year survival rates respectively were 69.4%, 65.7%, 61.6%, and 51.3%. Univariate analysis indicated that the survival time of groups with cardiac involvement, multiple organ involvement, hypotension, decreased serum albumin, increased BNP, increased cTNT, decreased eGFR and only symptomatic treatment were lower than the control group ($P < 0.05$). Other factors had no significant effect on the survival rate. Further multivariate Cox regression analysis combined with regression coefficient results showed that hypotension, cardiac involvement, and serum albumin and cTnT at the time of diagnosis were independent risk factors for mortality ($P < 0.05$), and receiving treatment was an independent protective factor for mortality ($P < 0.05$). (Table 9)

Table 9
Cox regression analysis of factors influencing survival rate

Metrics	B	S.E	Wald	Df	P	Exp(B)
Hypertension	-0.064	0.366	0.031	1	0.860	0.938
Hypotension	-0.961	0.418	5.300	1	0.021	0.382
ALB	-0.036	0.017	4.610	1	0.032	0.965
BNP	-1.305	0.551	5.601	1	0.018	0.271
cTNT	-1.086	0.522	4.319	1	0.038	0.338
eGFR	0.622	0.322	3.723	1	0.054	1.862
Cardiac involvement	-2.170	0.435	24.936	1	0.000	0.114
Multiple organ involvement	0.131	0.526	0.062	1	0.803	1.140
Treatment	0.959	0.366	6.864	1	0.009	2.608

Discussion

As the number of confirmed cases of renal amyloidosis gradually increases, the disease is no longer rare and unfamiliar. Renal amyloidosis is mainly due to deposition of amyloid in the kidney, non-specifically manifested as edema, proteinuria and renal impairment. In our study, the number of amyloid patients diagnosed after 2014 was significantly higher than before, especially in patients with renal amyloidosis, with up to 89.13% of patients diagnosed in the past 5 years. The reasons are mainly related to the improvement of test methods and the continuous improvement of the understanding of the disease. After 2014, most hospitals in China carried out free light chain detection, combined with immunofixation electrophoresis and bone marrow aspiration results, for multi-level differential diagnosis, significantly improving the diagnosis rate. At the same time, the addition of light chain κ and λ staining items in immunofluorescence staining of pathological biopsy is also conducive to improve the diagnosis rate.

Sammer et al. collected 22,330 cases of renal biopsy in the United States, of which 474 cases were diagnosed as amyloidosis nephropathy, accounting for 2.1%(19); in relevant Spanish studies, renal amyloidosis accounted for 3.7% of renal biopsy(20), while the incidence in Italy was 2.5%(21). Compared with foreign countries, relevant studies in China reported that amyloidosis nephropathy accounted for 0.89% ~ 1.27% of renal biopsy cases in the same period(22); in this study, renal amyloidosis patients accounted for 1.30% of renal biopsy cases in the same period in Shandong Province, which was lower than the incidence in foreign countries and consistent with domestic studies. Some patients did not undergo renal biopsy due to personal reasons, some patients visited other departments with extrarenal manifestations and didn't confirm by renal biopsy because of the insufficient communication between nephrology department and non-nephrology room doctors. What's more, amyloidosis nephropathy was

rare before 2010, only few case reports in China. There were lack of large-sample studies about amyloidosis and deep recognition of this disease, resulting in a high rate of clinical misdiagnosis.

With renal involvement in our study, amyloid was mainly deposited in the glomeruli, mostly in the interstitial small vessel wall, and only a small part was deposited in the renal tubules and renal interstitium; there were varying degrees of tubular atrophy and interstitial fibrosis, with inflammatory cell infiltration, which was consistent with most studies. Six patients showed a weak positive Congo red staining, possibly due to less amyloid deposition in the early stages of the disease, or inadequate staining due to inappropriate section thickness. Eight cases which showed minimal glomerular lesions were finally diagnosed with early amyloidosis nephropathy combined with the results of special staining. Hetzel and Sayed also showed that patients with amyloidosis nephropathy may present with minimal glomerular lesions in the early stage(23, 24), and the final diagnosis of amyloidosis is confirmed after electron microscopy, multiple stains, or repeated renal biopsy. The possible reasons include: patient has both of these diseases; the morphological variations of amyloid that are indistinguishable from minor glomerular lesions on light microscopy; or amyloid deposition is too small to detect in early stages. Therefore, the initial diagnosis of amyloidosis nephropathy needs to be combined with light microscopy, special staining, electron microscopy and clinical manifestations. Moreover, the time interval between the diagnosis of minimal glomerulopathy and renal amyloidosis was up to 241 days(24). Therefore, in clinical practice, for patients with pathological results of minimal glomerulopathy, accompanied by plasmacytosis, ineffective hormone therapy, or other organ involvement, and abnormal relevant clinical indicators (light chain, immunofixation electrophoresis, etc.), it is necessary to attach great importance to whether it is amyloidosis disease and timely make a correct diagnosis.

In this study, the light microscopic results of one patient who underwent renal biopsy at an outside hospital showed membranoproliferative glomerulonephritis, but electron microscopy revealed patchy irregular filamentous fibrous material deposited in the renal tissue, suggesting renal amyloidosis. The other case showed glomerular mesangial ganglion hyperplasia lesions, poor therapeutic effect after several months of hormone combined with immunosuppressive agents, and lower extremity venous thrombosis, and pathological sections were consulted in the pathology department of our hospital, and Congo red staining was performed again to show positivity, confirming the diagnosis of renal amyloidosis. Therefore, not only minimal glomerular lesions, but also other pathological types can present the same situation. So electron microscopy, Congo red counterstaining, and secondary renal biopsy are all important ways to reduce the misdiagnosis rate.

Amyloid nephropathy is easily confused with other renal diseases like as fibroid glomerulonephritis(25), immune vibrissae glomerulopathy(26), collagen type III glomerular disease(27), and light chain deposition disease(28), all of which present with proteinuria and edema, and there is fibroid or granular material deposition under electron microscopy, which has been poorly recognized by doctors in the early years, leading to easy misdiagnosis in clinical practice. These diseases can be differentiated by Congo red staining and electron microscopy. Recent studies have shown the presence of DNAJB9 protein in the glomeruli of patients with fibroid glomerulonephritis but not in patients with several other diseases, and

immunohistochemical detection of glomerular DNAJB9 has 98% sensitivity and > 99% specificity(29, 30).In our study, one patient was not the age of high incidence of amyloidosis because of his young age, and there were pathological reports of fibroid glomerulonephritis with Congo red positivity(31), and to rule out the disease, DNAJB9 immunohistochemical detection was performed with negative results, combined with electron microscopic results to finally diagnose amyloidosis nephropathy. Therefore, the diagnosis rate can be improved by Congo red counterstaining, special immunofluorescence staining, and high magnification observation under electron microscope.

Sait et al classified and scored 305 cases of renal amyloidosis, 90% of which were AA type amyloidosis, and found that pathological grade was correlated with glomerular histopathological classification, and the severity of glomerular amyloid deposition was positively correlated with interstitial fibrosis and inflammation(12). Sasatomi's study showed that serum creatinine level and pathological grade at renal biopsy were associated with accelerated progression of the disease, of which the most important prognostic factors were glomerular, tubulointerstitial and vascular lesions due to amyloid deposition(32). Yao Ying et al summarized the pathological changes of 199 patients with AL renal amyloidosis by semi-integral quantitative method and found that the degree of glomerular amyloidosis was positively correlated with the amount of urinary protein, and the incidence of renal insufficiency was higher in patients with GA and severe renal interstitial damage(22). This study found that different pathological grades were positively correlated with creatinine levels, and the higher the pathological grade, the higher the incidence of renal insufficiency; the more severe the tubulointerstitial damage, the incidence of renal insufficiency, and the relatively higher the urinary protein quantification; at the same time, the degree of glomerulosclerosis would also affect the urinary protein quantification. However, there were no significant correlations between the degree of amyloidosis in different renal tissues and proteinuria quantification or the incidence of renal insufficiency. There was also no correlation between pathological grade and mortality in patients with different amyloid nephropathy. Since the number of patients with complete renal pathology, clinical and follow-up data in our center is small, and the sample size is smaller in patients with severe pathological lesions, the relationship between the degree of amyloid deposition in different renal tissues and clinical indicators still needs to be further verified by large-sample study; the actual prognosis and clinical applicability of RAPS and renal amyloidosis patients need to be further validated.

AL amyloidosis is the most common type of amyloidosis nephropathy, accounting for 81.0–92.7%(19)]. In this study, the specific classification was not performed, but it is worth noting that the addition of light chain κ and λ staining in the immunofluorescence staining program after 2014. In this study, 141 patients underwent light chain κ/λ staining, of which 51.06% were λ positive and 26.95% were κ positive. For one of κ or λ staining, it showed positive (+ + or + + +), while the other showed negative, then the pathological report considered AL amyloidosis; for both κ and λ, it was considered very likely to be AL; if both κ and λ were negative or weakly positive (+/- or +), further immunohistochemical staining was required to determine their classification. Therefore, according to the results of light chain κ/λ immunofluorescence staining, in the absence of immunohistochemistry and genetic testing, whether the clinical rough classification can be made in combination with the results of free light chain and immunofixation

electrophoresis will provide our clinical and pathologists with great preliminary diagnosis and treatment direction.

Correlation analysis of risk factors in our study showed that blood pressure, serum albumin, eGFR, cTnT and BNP, cardiac and multiple organ involvement, and treatment methods all affected mortality. And hypotension, cardiac involvement, decreased albumin, and increased TnT were independent risk factors for survival, while receiving effective treatment was an independent protective factor for survival. For renal amyloidosis, domestic studies have shown that massive proteinuria and creatinine level at the time of diagnosis will affect the prognosis of patients(15, 32). Mayo Group study indicated that creatinine level did not affect the survival time of patients, but the decrease of urinary protein quantification greater than 95% within 1 year may prolong the survival time of patients(33). However, in this study, it was found that decreased serum albumin was an independent risk factor for patient survival, which was related to massive proteinuria and renal impairment. So, it is crucial for doctor to pay close attention to the level of albumin, proteinuria, creatinine and eGFR.

The key to the treatment of this disease lies in stopping or reducing the production of amyloid sources and promoting clearance. At present, the removal of free light chains and small molecules mainly uses high cut-off dialysis, but inhibition of amyloid production is determined by its typing. Localized amyloidosis had a good effect of surgical treatment and a good prognosis in our study. The Mayo team followed up 413 patients with localized amyloidosis and showed that 61% of patients chose first-line surgery, with a 10-year survival rate as high as 78% and no systemic progression(34). Patients with multiple organ involvement are usually complicated to treat and have a significantly higher mortality rate than single organ involvement. Chemotherapy is the main treatment for AL amyloidosis. Studies have shown that the optimal regimen is high-dose melphalan combined with autologous stem cell transplantation (ASCT), which can achieve hematological remission in about 40% of cases and control the treatment-related mortality at 10%(35). However, this surgery requires strict requirements for patients and professional centers. In our center, only 6 patients underwent ASCT, with a mortality rate of 16.67%. The mortality rate is higher than other studies possibly because of small sample. Patients not eligible for ASCT receive standard-dose chemotherapy based on alkylating agents, steroids, proteasome inhibitors, and immunomodulatory drugs(3). The most commonly regimen is bortezomib combined with dexamethasone, and studies have shown that 65% of patients on this regimen can achieve complete or partial remission, with a 5-year survival rate of 80% in intermediate-risk patients(36). 18 patients chose this regimen in our study, with the 1-year survival rate was 70.6% which was lower than that in foreign studies. Recent studies have shown that monoclonal antibodies can not only provide treatment against malignant plasma cells, but also target and remove amyloid from organs, with the advantages of high selectivity and low toxicity, becoming one of the expected clinical treatments in the future(37, 38).AA amyloidosis is mainly associated with long-term inflammatory conditions(39, 40), and treatment is based on the control of inflammation, clinically mainly using factors, antibiotics, colchicine and other treatments(41, 42). For patients with poor efficacy or severe organ failure, organ transplantation was performed. Patients with hereditary amyloidosis are treated by liver transplantation or gene knockout. Although treatment modalities continue to be updated, organ failure is irreversible for patients with

advanced disease. Therefore, early diagnosis, classification and treatment are particularly important, and clinicians should continuously improve their understanding and attention to the disease.

This study is a retrospective analysis. The time span of admission for the included patients is large, the pathological and clinical data and data of some patients are missing, the conditions for medical examination in the early years are limited, and only a small part of clinical indicators can be determined. The majority of patients diagnosed in our unit are not clearly classified, so the number of patients with AL, AA or other types of amyloidosis cannot be determined, with the pathological, clinical characteristics and prognosis analysis among different types cannot be performed. During the follow-up, some patients are lost to follow-up due to the change of contact information. For these reasons, the survival time of some clinical indicators cannot be obtained, which has impact on the later analysis and prediction, with some limitations. However, with the continuous refinement of diagnosis and treatment techniques, as well as the increase of clinician communication and cooperation in various departments, early and correct diagnosis and effective treatment will effectively improve the prognosis of amyloidosis patients.

Conclusion

The pathological grade of renal amyloidosis is associated with the incidence of renal insufficiency, in which the degree of tubulointerstitial chronic lesions significantly affects renal function. The relationship between the degree of starch deposition in different renal tissues and the levels of proteinuria and renal function still needs to be explored in large sample studies. At the same time, clinicians should pay more attention to the relevant risk factors to improve the early diagnosis rate and improve the prognosis of patients.

Declarations

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Availability of data and materials

The data sets in this study are available from the corresponding author on reasonable request.

Authors' contribution

Study concept and design, drafting of manuscript: ZX. Acquisition of data, or analysis of data: ZX. Revision of manuscript for important intellectual content: YC, WR. All authors have read and approve of the final version of the manuscript.

Competing interests

The authors report no conflict of interest.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Provincial Hospital of Shandong University. Written informed consent was obtained from all patients participating in the study.

Consent for publication

Not applicable.

References

1. Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, et al. Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis. 2014;21(4):221-4.
2. Benson MD, Buxbaum JN, Eisenberg DS, Merlini G, Saraiva MJM, Sekijima Y, et al. Amyloid nomenclature 2018: recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis. 2018;25(4):215-9.
3. Gertz MA. Immunoglobulin light chain amyloidosis: 2018 Update on diagnosis, prognosis, and treatment. American journal of hematology. 2018;93(9):1169-80.
4. Papa R, Lachmann HJ. Secondary, AA, Amyloidosis. Rheumatic diseases clinics of North America. 2018;44(4):585-603.
5. Rysava R. AL amyloidosis: advances in diagnostics and treatment. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2019;34(9):1460-6.
6. Nienhuis HL, Bijzet J, Hazenberg BP. The Prevalence and Management of Systemic Amyloidosis in Western Countries. Kidney diseases (Basel, Switzerland). 2016;2(1):10-9.
7. Beimler J, Zeier M. [Renal involvement in amyloidosis and sarcoidosis]. Deutsche medizinische Wochenschrift (1946). 2018;143(2):101-9.
8. Watanabe T, Saniter T. Morphological and clinical features of renal amyloidosis. Virchows Archiv A, Pathological anatomy and histology. 1975;366(2):125-35.
9. Dikman SH, Churg J, Kahn T. Morphologic and clinical correlates in renal amyloidosis. Human pathology. 1981;12(2):160-9.
10. Shiiki H, Shimokama T, Yoshikawa Y, Toyoshima H, Kitamoto T, Watanabe T. Renal amyloidosis. Correlations between morphology, chemical types of amyloid protein and clinical features. Virchows

Archiv A, Pathological anatomy and histopathology. 1988;412(3):197-204.

11. Verine J, Mourad N, Desseaux K, Vanhille P, Noël LH, Beaufils H, et al. Clinical and histological characteristics of renal AA amyloidosis: a retrospective study of 68 cases with a special interest to amyloid-associated inflammatory response. Human pathology. 2007;38(12):1798-809.
12. Şen S, Sarsık B. A Proposed Histopathologic Classification, Scoring, and Grading System for Renal Amyloidosis: Standardization of Renal Amyloid Biopsy Report. 2010;134(4):532-44.
13. Dittrich T, Benner A, Kimmich C, Siepen FAD, Veelken K, Kristen AV, et al. Performance analysis of AL amyloidosis cardiac biomarker staging systems with special focus on renal failure and atrial arrhythmia. Haematologica. 2019;104(7):1451-9.
14. Sidana S, Tandon N, Gertz MA, Dispenzieri A, Ramirez-Alvarado M, Murray DL, et al. Clinical features, laboratory characteristics and outcomes of patients with renal versus cardiac light chain amyloidosis. British journal of haematology. 2019;185(4):701-7.
15. Dittrich T, Kimmich C, Hegenbart U, Schönland SO. Prognosis and Staging of AL Amyloidosis. Acta haematologica. 2020;143(4):388-400.
16. Palladini G, Barassi A, Klersy C, Pacciolla R, Milani P, Sarais G, et al. The combination of high-sensitivity cardiac troponin T (hs-cTnT) at presentation and changes in N-terminal natriuretic peptide type B (NT-proBNP) after chemotherapy best predicts survival in AL amyloidosis. Blood. 2010;116(18):3426-30.
17. Hwa YL, Gertz MA, Kumar SK, Lacy MQ, Buadi FK, Dingli D, et al. Prognostic restaging at the time of second-line therapy in patients with AL amyloidosis. Leukemia. 2019;33(5):1268-72.
18. Palladini G, Hegenbart U, Milani P, Kimmich C, Foli A, Ho AD, et al. A staging system for renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. Blood. 2014;124(15):2325-32.
19. Said SM, Sethi S, Valeri AM, Leung N, Cornell LD, Fidler ME, et al. Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases. Clinical journal of the American Society of Nephrology : CJASN. 2013;8(9):1515-23.
20. Panizo N, Rivera F, Lopez-Gomez JM. Decreasing incidence of AA amyloidosis in Spain. European journal of clinical investigation. 2013;43(12):1371.
21. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Seminars in hematology. 1995;32(1):45-59.
22. Yao Y, Wang SX, Zhang YK, Qu Z, Liu G, Zou WZ. A clinicopathological analysis in a large cohort of Chinese patients with renal amyloid light-chain amyloidosis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2013;28(3):689-97.
23. Hetzel GR, Uhlig K, Mondry A, Helmchen U, Grabensee B. AL-amyloidosis of the kidney initially presenting as minimal change glomerulonephritis. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2000;36(3):630-5.

24. Sayed RH, Gilbertson JA, Hutt DF, Lachmann HJ, Hawkins PN, Bass P, et al. Misdiagnosing renal amyloidosis as minimal change disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2014;29(11):2120-6.
25. Rosenstock JL, Markowitz GS. Fibrillary Glomerulonephritis: An Update. Kidney international reports. 2019;4(7):917-22.
26. Schwartz MM, Korbet SM, Lewis EJ. Immunotactoid glomerulopathy. Journal of the American Society of Nephrology : JASN. 2002;13(5):1390-7.
27. Chen T, Achan A, Li K, Harris D. Collagenofibrotic Glomerulopathy. Nephrology (Carlton, Vic). 2018;23(6):601-2.
28. Wang Q, Jiang F, Xu G. The pathogenesis of renal injury and treatment in light chain deposition disease. Journal of translational medicine. 2019;17(1):387.
29. Nasr SH, Fogo AB. New developments in the diagnosis of fibrillary glomerulonephritis. Kidney international. 2019;96(3):581-92.
30. Nasr SH, Vrana JA, Dasari S, Bridoux F, Fidler ME, Kaaki S, et al. DNAJB9 Is a Specific Immunohistochemical Marker for Fibrillary Glomerulonephritis. Kidney international reports. 2018;3(1):56-64.
31. Andeen NK, Troxell ML, Riazy M, Avasare RS, Lapasia J, Jefferson JA, et al. Fibrillary Glomerulonephritis: Clinicopathologic Features and Atypical Cases from a Multi-Institutional Cohort. Clinical journal of the American Society of Nephrology : CJASN. 2019;14(12):1741-50.
32. Sasatomi Y, Sato H, Chiba Y, Abe Y, Takeda S, Ogahara S, et al. Prognostic factors for renal amyloidosis: a clinicopathological study using cluster analysis. Internal medicine (Tokyo, Japan). 2007;46(5):213-9.
33. Palladini G, Dispenzieri A, Gertz MA, Kumar S, Wechalekar A, Hawkins PN, et al. New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(36):4541-9.
34. Kourelis TV, Kyle RA, Dingli D, Buadi FK, Kumar SK, Gertz MA, et al. Presentation and Outcomes of Localized Immunoglobulin Light Chain Amyloidosis: The Mayo Clinic Experience. Mayo Clinic proceedings. 2017;92(6):908-17.
35. D'Souza A, Dispenzieri A, Wirk B, Zhang MJ, Huang J, Gertz MA, et al. Improved Outcomes After Autologous Hematopoietic Cell Transplantation for Light Chain Amyloidosis: A Center for International Blood and Marrow Transplant Research Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(32):3741-9.
36. Palladini G, Milani P, Foli A, Vidus Rosin M, Basset M, Lavatelli F, et al. Melphalan and dexamethasone with or without bortezomib in newly diagnosed AL amyloidosis: a matched case-control study on 174 patients. Leukemia. 2014;28(12):2311-6.

37. Popkova T, Hajek R, Jelinek T. Monoclonal antibodies in the treatment of AL amyloidosis: co-targetting the plasma cell clone and amyloid deposits. British journal of haematology. 2020;189(2):228-38.
38. Godara A, Siddiqui NS, Lee LX, Toskic D, Fogaren T, Varga C, et al. Dual Monoclonal Antibody Therapy in Patients With Systemic AL Amyloidosis and Cardiac Involvement. Clinical lymphoma, myeloma & leukemia. 2020;20(3):184-9.
39. Lane T, Pinney JH, Gilbertson JA, Hutt DF, Rowczenio DM, Mahmood S, et al. Changing epidemiology of AA amyloidosis: clinical observations over 25 years at a single national referral centre. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis. 2017;24(3):162-6.
40. Lachmann HJ, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, et al. Natural history and outcome in systemic AA amyloidosis. The New England journal of medicine. 2007;356(23):2361-71.
41. Obici L, Merlini G. AA amyloidosis: basic knowledge, unmet needs and future treatments. Swiss medical weekly. 2012;142:w13580.
42. Westermark GT, Fandrich M, Westermark P. AA amyloidosis: pathogenesis and targeted therapy. Annual review of pathology. 2015;10:321-44.