ORIGINAL ARTICLE

Renal amyloidosis: validation of a proposed histological scoring system in an independent cohort

Joris J. Hoelbeek [ORCID] [1], Jesper Kers[1], Eric J. Steenbergen[2], Joris J.T.H. Roelofs[1] and Sandrine Florquin[1]

[1]Department of Pathology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands and [2]Department of Pathology, Radboud University Nijmegen Medical Center, Radboud University Nijmegen, Nijmegen, The Netherlands

Correspondence to: Joris J. Hoelbeek; E-mail: j.j.hoelbeek@amsterdamumc.nl

ABSTRACT

Background. In systemic amyloidosis, the kidney is frequently affected and renal involvement has a major impact on survival. Renal involvement is clinically characterized by decreased estimated glomerular filtration rate (eGFR) and proteinuria. The two most common renal amyloidosis types are light chain-related amyloidosis (AL) and serum amyloid A (AA) amyloidosis. Standardized histopathological scoring of amyloid deposits is crucial to assess disease progression. Therefore, we aimed to validate the proposed scoring system from Rubinstein *et al.* (Novel pathologic scoring tools predict end-stage kidney disease in light chain (AL) amyloidosis. Amyloid 2017; 24: 205–211) in an independent patient cohort.

Methods. We attempt to reproduce the scoring system, consisting of an amyloid score (AS) and a composite scarring injury score (CSIS), in a multicentre AL and AA case series. Additionally, we analysed all renal amyloidosis kidney biopsies performed in the Netherlands between 1993 and 2012.

Results. Similar to the original study, AS and CSIS correlated to eGFR ($r = -0.45$, $P = 0.0061$ and $r = -0.60$, $P < 0.0001$, respectively) but not to proteinuria at diagnosis. Furthermore, AS, but not CSIS, was associated with renal outcome. The scoring system was not reproducible in AA patients. The median incidence rate for renal amyloidosis in the Netherlands was 2.3 per million population per year, and increased during the study period.

Conclusions. In our AL case series and the original study, AS and CSIS were correlated to eGFR but not to proteinuria, and AS correlated with renal outcome. Overall, we regard this scoring system as competent for standardized histopathological assessment of amyloid deposits burden and thereby disease advancement in renal biopsies.

Keywords: amyloidosis, histological grading system, nation-wide incidence, renal biopsy, validation

INTRODUCTION

Amyloidosis refers to a spectrum of conditions in which misfolded proteins of various origins precipitate in a characteristic beta-sheet formation, forming fibrils. These extracellular depositions progressively impair organ functioning [1] and the estimated median survival for all systemic amyloidosis types is

32 months [2]. The heart and the kidney are the most affected organs and have the largest impact on survival [3–8]. Kidney involvement leads to proteinuria, impaired renal function and ultimately end-stage renal disease (ESRD).

In affected kidneys, amyloid deposits can be found in any renal compartment but are most often located in the glomeruli [9]. Currently, over 32 types of amyloid precursor proteins have been identified [1, 5]. The most prevalent renal amyloidosis types in the Western world are light chain-related amyloidosis (AL), where monotypic precipitates of kappa or lambda immunoglobulin light chains are found [incidence rate (IR) = 1–6 new cases per million of the population per year, pmp/y] [6–8, 10–13] and serum amyloid A (AA)-related amyloidosis, which is associated with chronic inflammation (IR = 1.6–2 pmp/y) [1, 12–15].

Despite the development of experimental non-invasive imaging techniques [5], for adequate comparison of (organ-specific) disease progression, both between patients and within patients at different points in time, the histopathological assessment remains essential. A standardized histopathological scoring system for the extent and localization of amyloid deposits is therefore crucial. Such a scoring system for renal AL was proposed by Rubinstein *et al.* [16]. We aimed to validate the described histopathological correlations in an independent, multicentre cohort study of biopsy-proven renal amyloidosis (both AL and AA).

This proposal was launched by the Immunonephrology Working Group of the ERA-EDTA.

MATERIALS AND METHODS

Incidence rate of renal amyloidosis in the Netherlands

Nation-wide IR data on renal amyloidosis are lacking in the Netherlands. To address this and assess the impact of renal amyloidosis nation-wide, we analysed renal amyloidosis biopsies included in the Dutch national pathology registry (PALGA) over a large period of time.

To obtain biopsy-proven renal amyloidosis IRs, we performed a search for renal amyloidosis biopsy reports in the 'Nationwide network and registry of histo- and cytopathology in the Netherlands' (PALGA Foundation). The PALGA registry gathers, manages and makes data available from all pathology reports issued by all pathology laboratories in the Netherlands since 1993 and is, therefore, a uniquely complete database. All reports coded with 'renal amyloidosis' between 1993 and 2012 were included. Repeat reports on the same biopsy (i.e. external revisions and consultations) and transplant biopsies were excluded. Population data from the Netherlands were retrieved from the website of 'Statistics Netherlands' (Central Bureau for Statistics) [17].

Validation of histopathological scoring system in a multicentre renal amyloidosis case series

For our retrospective renal amyloidosis case series, all consecutive cases of biopsy-proven renal AL and AA amyloidosis in native kidneys from the Amsterdam University Medical Centers (Amsterdam UMC) (between 1993 and 2012) and Radboud University Nijmegen Medical Center (RUNMC) (between 2004 and 2014), were reviewed. Biopsies with ≥7 glomeruli and available Jones Silver staining (or tissue to repeat staining) were included. Biopsies were assessed by light microscopy by an experienced renal pathologist (S.F.). We reassessed Congo Red staining, if available (at diagnosis, amyloidosis was confirmed by Congo Red positivity in all cases). Available

clinical data at the time of biopsy, including sex, age, serum creatinine, proteinuria per 24 h, amyloid type and the presence of nephrotic syndrome, were collected. Renal stage was computed as described by Palladini *et al.* [18]. We attempted to gather follow-up data on progression to ESRD (start of dialysis or renal transplantation) for Amsterdam UMC patients. In case proteinuria was only available as grams per litre, we multiplied this value by 1.5 to obtain grams per 24 h. Estimated glomerular filtration rate (eGFR) was computed using the Modification of Diet in Renal Disease (MDRD) formula. In case only descriptive information on renal function was available, we assigned the following: 'terminal renal insufficiency': eGFR = 14 mL/min/1.73 m^2 and 'normal renal function': eGFR = 61 mL/min/1.73 m^2 [19], and when creatinine clearance was available, this value was used instead of eGFR.

Chronic damage and amyloid deposits were scored using the system proposed by Rubinstein *et al.* [16]. The authors of this article kindly send us their raw data to allow statistical comparison of our patient cohorts.

The chronic damage score (composite scarring injury score, CSIS) is defined as the sum of percentage globally sclerotic glomeruli and percentage of tubulointerstitial fibrosis. Additionally, we scored tubulointerstitial fibrosis on a semi-quantitative scale ranging from 0 to 3 (0 = absent, 1 = <25%, 2 = 25–50% and 3 = >50% of affected interstitial area). The amyloid deposit score (amyloid score, AS) is defined as the sum of mesangial (M), capillary wall (CW), interstitial (I) and vascular (V) scores. Each of these individual scores was scored on a semi-quantitative scale ranging from 0 to 3 (0 = absent, 1 = <25%, 2 = 25–50% and 3 = >50% of glomerular compartment, vessel or interstitial area filled with amyloid deposits), averaged for all non-sclerotic glomeruli, interstitial areas and vessels present. Additionally, we scored interstitial amyloid deposits as percentage (to the nearest 10%).

We analysed the correlation between AS/CSIS and their components with eGFR and proteinuria at diagnosis.

For survival analysis, we used the cut-off values for CSIS (45) and AS (7.5) described by Rubinstein *et al.* [16].

Statistical analyses

For statistical analyses, Prism 5 for Windows was used. Quantitative variables are presented as median (interquartile range) since most were non-normally distributed. Mann–Whitney test was used to compare two independent continuous variables; Fisher's exact test was used to compare proportions. For comparison of more than two groups, the Kruskal–Wallis test was used, followed by *post hoc* Dunn's test when Kruskal–Wallis demonstrated a difference among groups. For correlation analysis, Spearman's rank test was used. For survival curve comparison, a log-rank (Mantel-Cox) test was used. A probability value (P-value) <0.05 was considered statistically significant.

RESULTS

Incidence rate of renal amyloidosis in the Netherlands

Between 1993 and 2012, 763 cases of biopsy-proven renal amyloidosis were identified through the PALGA database search, of which 432 (57%) were male patients. The mean (SD) age was 63 (13) years.

In the period 1993–2012, the population of the Netherlands was ∼16.0 million, with a male:female ratio of 1:1.02. Figure 1 shows the IR during the study period. The median IR for

renal amyloidosis was 2.3 pmp/y and increased (slope: 0.04 ± 0.016 pmp/y, $P = 0.01$) between 1993 and 2012. The IR differed between age groups ($P < 0.0001$) and peaked in the 65–79 years age group [10.3 (8.6–11.3) pmp/y, $P < 0.05$ compared with all other age groups] and males were more frequently affected than females [2.5 (2.3–3.2) pmp/y versus 1.9 (1.6–2.5) pmp/y, $P = 0.002$, respectively].

Multicentre, retrospective, observational renal amyloidosis cohort

Ninety-three cases of renal amyloidosis were identified (Amsterdam UMC, $n = 46$ and RUNMC, $n = 47$). Renal AL amyloidosis was diagnosed in 70 cases (75%), AA in 19 cases (21%) and 4 (4%) were 'Not Otherwise Specified'. Forty-four AL cases and 16 AA cases were included in the analysis. Figure 2 provides an overview of excluded cases.

Figure 3 shows representative AS examples. Concomitant renal diseases were not observed. RTx: renal transplant.

The authors of the article by Rubinstein *et al.* [16] kindly sent us their raw data. Table 1 summarizes the baseline
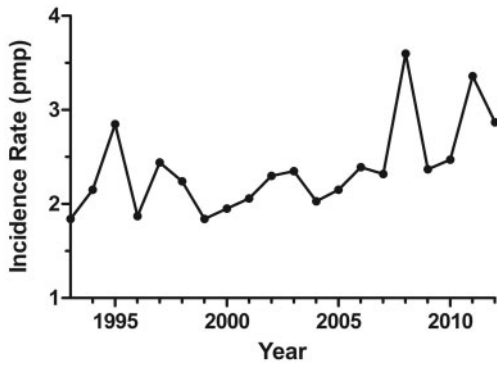

FIGURE 1: Incidence rate of renal amyloidosis in the Netherlands between 1993 and 2012; values are presented as per million population (pmp).
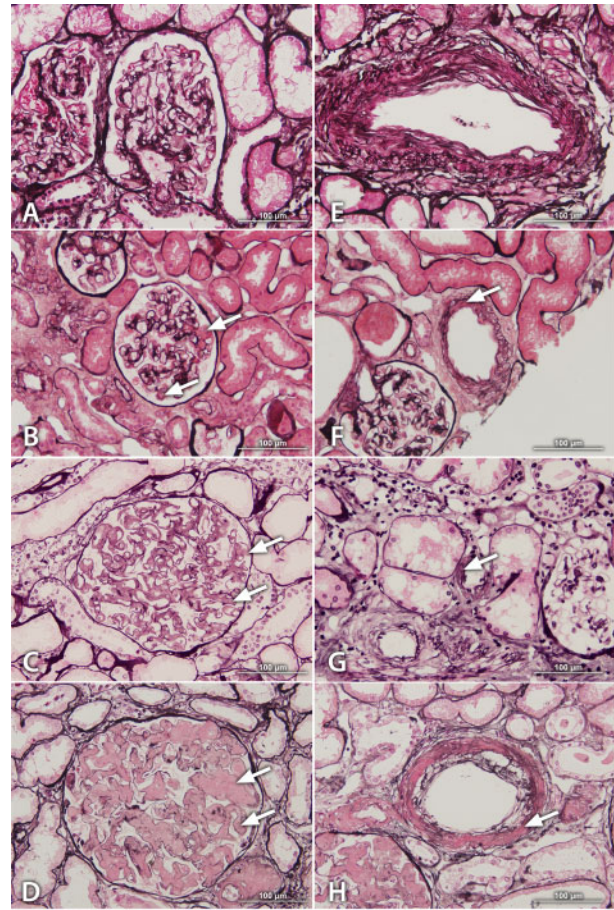
FIGURE 3: Examples of amyloid deposits score. (A–D) Representative slides showing the mesangial and capillary wall amyloid scores. $A = 0$, $B = 1$, $C = 2$ and $D = 3$ (arrows showing deposits, silver staining, $\times 20$). (E–H) Representative slides showing the vessel amyloid score. $E = 0$, $F = 1$, $G = 2$ and $H = 3$ (arrows showing deposits, silver staining, $\times 20$).

FIGURE 2: Flow chart showing the number of included and excluded renal amyloidosis cases in our patient cohort.

Table 1. Patient demographics

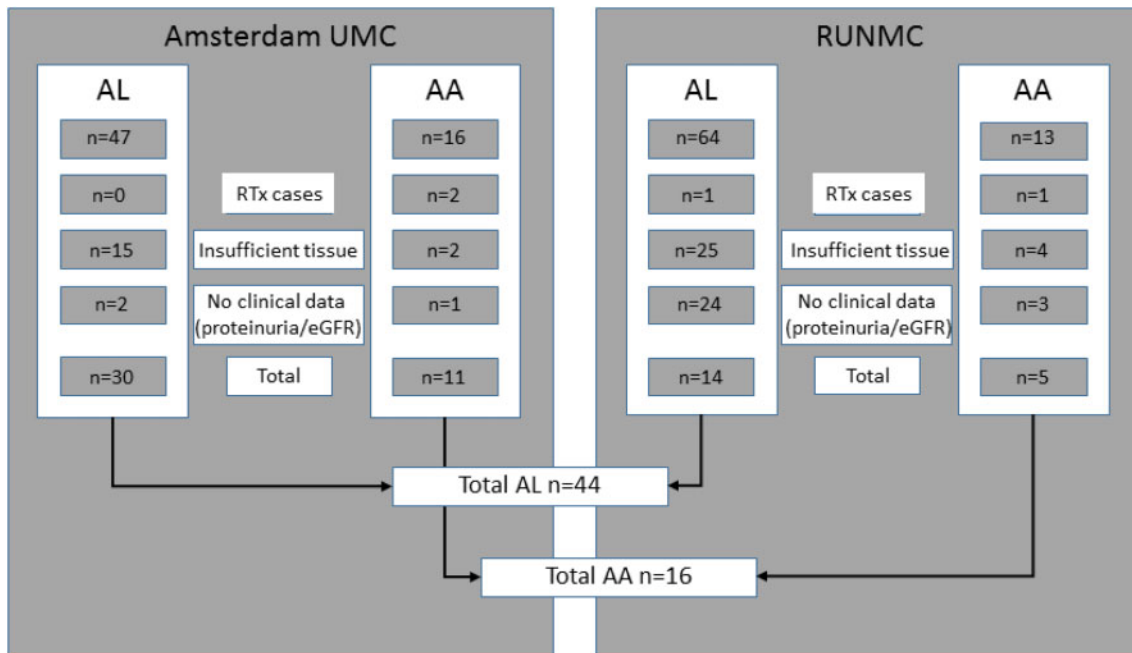| Patient subgroup	Variable (unit)	Amsterdam UMC	RUNMC	Total	Rubinstein *et al.* [16]	P-value[b]
AL		$n = 30$[a]	$n = 14$[a]	$n = 44$[a]	$n = 39$	
Clinical parameters	Male sex proportion (%)	21/30 (70%)	8/14 (57%)	29/44 (66%)	23/39 (59%)	0.6
	Age	65 (59–73)	61 (54–70)	64 (55–72)	56 (48–64)	0.0037
	eGFR (mL/min/1.73 m^2)	61 (41–84) ($n = 29$)	38 (21–66) ($n = 8$)	61 (36–82) ($n = 37$)	61 (23–79)	0.8
	Proteinuria (g/24 h)	6.5 (4.5–10.2) ($n = 29$)	8.5 (6.3–11.3) ($n = 12$)	7.0 (5.0–10.6) ($n = 41$)	7.1 (3.6–15.3)	0.9
	Nephrotic syndrome proportion (%)	18/25 (72%) ($n = 25$)	10/10 (100%) ($n = 10$)	28/35 (80%) ($n = 35$)		
	Palladini clinical stage [18]	2 (2–2) ($n = 28$)	2.5 (2–3) ($n = 6$)	2 (2–2.25) ($n = 34$)	2 (2–3)	0.96
	Stage 1, n (%)	4 (14)	0 (0)	4 (12)	7 (18)	
	Stage 2, n (%)	19 (68)	3 (50)	22 (65)	21 (54)	
	Stage 3, n (%)	5 (18)	3 (50)	8 (24)	11 (28)	
Histologic parameter	Number of glomeruli	14 (11–21)	12 (10–16)	13 (11–18)		
	CSIS	37 (13–70)	62 (18–84)	45 (18–79)	43 (20–70)	0.9
	AS	5 (3–9) ($n = 29$)	5 (4–7) ($n = 13$)	5 (3–7) ($n = 42$)	7 (5–10)	0.02
AA		$n = 11$[a]	$n = 5$[a]	$n = 16$[a]		
Clinical parameter	Male sex proportion (%)	7/11 (64)	2/5 (40)	9/16 (56)		
	Age	58 (54–63)	67 (29–74)	59 (42–67)		
	eGFR (mL/min/1.73 m^2)	49 (30–81)	40 (19–61) ($n = 2$)	49 (25–71) ($n = 13$)		
	Proteinuria (g/24 h)	7.1 (0.8–11.0)	2.4 (0.3–6.9) ($n = 4$)	6.5 (0.8–10.0) ($n = 15$)		
	Nephrotic syndrome proportion (%)	5/10 (50) ($n = 10$)	1/3 (33) ($n = 3$)	6/13 (46%) ($n = 13$)		
	Palladini clinical stage [18]	2 (2–2)	1 (1–1) ($n = 1$)	2 (2–2) ($n = 12$)		
	Stage 1, n (%)	0 (0)	1 (100)	1 (8)		
	Stage 2, n (%)	9 (82)	0 (0)	9 (75)		
	Stage 3, n (%)	2 (18)	0 (0)	2 (17)		
Histologic parameter	Number of glomeruli	11 (10–16)	20 (11–20)	11 (10–20)		
	CSIS	75 (40–119)	110 (80–163)	90 (46–120)		
	AS	5 (4–5) ($n = 10$)	7 (5–11)	5 (4–6) ($n = 15$)		
Total		$n = 41$[a]	$n = 19$[a]	$n = 60$[a]		
Clinical parameter	Male sex proportion (%)			38/60 (63)		
	Age			63 (55–71)		
	eGFR (mL/min/1.73 m^2)			55 (34–80) ($n = 50$)		
	Proteinuria (g/24 h)			7.0 (4.3–10.3) ($n = 56$)		
	Nephrotic syndrome proportion (%)			34/48 (71) ($n = 48$)		
	Palladini clinical stage [18]			2 (2–2) ($n = 46$)		
	Stage 1, n (%)			5 (11)		
	Stage 2, n (%)			31 (67)		
	Stage 3, n (%)			10 (22)		
Histologic parameter	Number of glomeruli			12 (10–18)		
	CSIS			56 (20–100)		
	AS			5 (4–7) ($n = 57$)		

Values presented as median (interquartile range) unless stated otherwise
[a]Only patients with available data were included in the analysis; in the case of missing data, the number of cases with available data is specified.
[b]Mann–Whitney tests were used to compare medians; Fisher's exact tests were used to compare proportions.
 AL: light chain-related amyloidosis; AA: serum amyloid A-related amyloidosis; eGFR: estimated glomerular filtration rate; AS: amyloid score; CSIS: composite scarring injury score; Amsterdam UMC: Amsterdam University Medical Centers; RUNMC: Radboud University Nijmegen Medical Center.

characteristics of both our and their patients. Our patient groups were similar regarding gender, baseline eGFR and proteinuria and renal stage. Our patients were, however, older; the median age was 8 years higher.

Amyloid and composite scarring injury scores

Since the scoring system proposed by Rubinstein *et al.* [16] was developed in a renal AL cohort, we primarily tried to reproduce their scoring system in our AL patients. CSIS was similar in our and their patients [45 (18–79) and 43 (20–70), respectively, P = 0.9], but AS was significantly lower in our cohort compared with their cohort [5 (3–7) and 7 (5–10), respectively, P = 0.02].

Correlation analysis of histological scores and clinical data at diagnosis is presented in Table 2. In AL patients, we found AS and CSIS to correlate to eGFR at diagnosis ($\rho = -0.44$, P = 0.009 and $\rho = -0.60$, P < 0.0001, respectively). We found no significant correlation between AS and CSIS and proteinuria at diagnosis ($\rho = -0.17$, P = 0.3 and $\rho = -0.01$, P = 0.9, respectively). AS and CSIS correlated with each other ($\rho = 0.58$, P < 0.0001).

Table 2. Correlations between amyloid and chronic damage scores (aggregated and individual) and clinical data at biopsy

AL (n = 44)

	eGFR ρ (CI); P-value (n = 37)[a]	Proteinuria ρ (CI); P-value (n = 41)[a]
AS	−0.44 (−0.68 to −0.11); 0.009 (n = 35)	−0.17 (−0.47 to 0.17); 0.3 (n = 39)
CW	−0.39 (−0.64 to −0.07); 0.02	0.16 (−0.17 to 0.45); 0.3
M	−0.52 (−0.72 to −0.22); 0.001	−0.15 (−0.44 to 0.18); 0.4
V	−0.23 (−0.53 to 0.12); 0.2 (n = 35)	−0.23 (−0.51 to 0.10); 0.2 (n = 39)
I	−0.23 (−0.53 to 0.12); 0.2 (n = 35)	−0.20 (−0.50 to 0.13); 0.2 (n = 38)
I%	−0.35 (−0.62 to −0.01); 0.04 (n = 35)	−0.23 (−0.52 to 0.10); 0.2 (n = 38)
CSIS	−0.60 (−0.78 to −0.33); <0.0001	−0.01 (−0.33 to 0.30); 0.9
IFTA %	−0.63 (−0.80 to −0.38); <0.0001	0.009 (−0.31 to 0.32); 0.96
IFTA 0–3	−0.69 (−0.83 to −0.47); <0.0001	−0.03 (−0.35 to 0.29); 0.8
Glom. Scler %	−0.34 (−0.61 to −0.01); 0.04	0.04 (−0.28 to 0.35); 0.8

AA (n=16)

	eGFR ρ (CI); P-value (n = 13)[a]	Proteinuria ρ (CI); P-value (n = 15)[a]
AS	−0.19 (−0.70 to 0.44); 0.5 (n = 12)	0.32 (−0.27 to 0.73); 0.3 (n = 14)
CW		0.62 (0.14 to 0.86); 0.01
CSIS	−0.32 (−0.75 to 0.29); 0.3	−0.02 (−0.54 to 0.51); 0.9

Total (n= 60)

	eGFR ρ (CI); P-value (n = 50)[a]	Proteinuria ρ (CI); P-value (n = 56)[a]
AS	−0.38 (−0.60 to −0.09); 0.009 (n = 47)	−0.05 (−0.33 to 0.23); 0.7 (n = 53)
CW		0.32 (0.05 to 0.54); 0.02
CSIS	−0.55 (−0.72 to −0.31); <0.0001	−0.08 (−0.35 to 0.19); 0.5

For correlation analysis, Spearman's rank test was used.

[a]Only patients with available data were included in the analysis; in the case of missing data, the number of cases with available data is specified.

Bold values indicate statistical significance (P < 0.05).

AL: light chain-related amyloidosis; AA: serum amyloid A-related amyloidosis; AS: amyloid score; CSIS: composite scarring injury score; eGFR: estimated glomerular filtration rate (mL/min/1.73 m^2); proteinuria: g/24 h; CW: capillary wall amyloid deposits (scored on a semi-quantitative scale ranging from 0 to 3); M: mesangial amyloid deposits (scored on a semi-quantitative scaleranging from 0 to 3); V: vascular amyloid deposits (scored on a semi-quantitative scale ranging from 0 to 3); I: interstitial amyloid deposits (scored on a semi-quantitative scale ranging from 0 to 3); I%: interstitial amyloid deposits (scored as percentage of affected renal interstitium); IFTA %: interstitial fibrosis and tubular atrophy (scored as percentage of affected renal interstitium); IFTA 0–3: interstitial fibrosis and tubular atrophy (scored on a semi-quantitative scale ranging from 0 to 3); Glom. Scler %: percentage of globally sclerotic glomeruli; ρ: rho correlation coefficient; CI: 95% confidence interval.

Percentage of globally sclerotic glomeruli and tubulointerstitial fibrosis (the two components of the CSIS score) correlated to eGFR ($\rho = -0.34$, P = 0.04 and $\rho = -0.63$, P < 0.0001, respectively). With tubulointerstitial fibrosis scored semi-quantitavely, the correlation with eGFR was stronger ($\rho = -0.69$, P < 0.0001). Of the AS components, M and CW scores correlated to eGFR at diagnosis ($\rho = -0.52$, P = 0.001 and $\rho = -0.39$, P = 0.02, respectively). We found no correlation between I or V scores and eGFR ($\rho = -0.23$, P = 0.02 and $\rho = -0.23$, P = 0.2, respectively). With I score as percentage, the score did correlate to eGFR at diagnosis ($\rho = -0.35$, P = 0.04).

We found no statistically significant correlations between AS/CSIS and eGFR/proteinuria at diagnosis in AA patients ($-0.32 \le \rho \le 0.32$, P $\ge$ 0.3); however, the CW score did correlate to proteinuria at diagnosis ($\rho = 0.62$, P = 0.01).

In our total cohort (AL + AA patients), AS and CSIS correlated to eGFR at diagnosis ($\rho = -0.38$, P = 0.009 and $\rho = -0.55$, P < 0.0001, respectively), but not to proteinuria at diagnosis ($\rho = -0.05$, P = 0.7 and $\rho = -0.08$, P = 0.5, respectively). However, CW score correlated to proteinuria at diagnosis ($\rho = 0.32$, P = 0.02).

Follow-up (progression to ESRD) data were available for 17 Amsterdam UMC patients (12 AL and 5 AA). Five of 12 AL patients progressed to ESRD, and time between diagnosis and ESRD was 776 (288–2324) days. Using the CSIS and AS cut-off values described by Rubinstein et al. [16], no significant difference in progression to ESRD was observed between patients with high (>45) or low (≤45) CSIS in the AL or the AL + AA subgroup (both P = 0.9). However, we did find significantly less progression to ESRD in AL patients with low (<7.5) compared with high (≥7.5) AS (P = 0.009). In the AL + AA subgroup, we found a trend towards less progression to ESRD for patients with AS <7.5 compared with AS ≥7.5 (P = 0.06).

DISCUSSION

Our results show a median IR of renal amyloidosis in the Netherlands of 2.3 pmp/y, with a peak incidence in the 65–79 years age group and a higher IR in males than in females. Unfortunately, around 40% of biopsy reports did not contain conclusive information about the amyloid type determination techniques. We are therefore unable to draw conclusions about the IRs of renal amyloidosis subtypes. Nation-wide studies performed elsewhere in Europe show IRs of 1.3 (Poland) [20] and 3.3 pmp/y (Spain) [21]. The IR in the Netherlands lies in between. The higher IR in males has been reported repeatedly in studies from Spain [21], Czech Republic [22] and Italy [23], but not in Poland [20]. Four of these studies [20–23], like us, describe an IR increase with age.

Several histological grading scores for renal amyloidosis have been proposed. Probably the most cited and used (mostly in adapted form) is the system proposed by Sen et al. [24]. Their system is based on the glomerular pattern of injury and does

not include non-glomerular deposits. Furthermore, their system was not related to clinical data and was developed in a cohort wherein 90% of patients had renal AA. This makes the applicability for AL (the most common type) uncertain.

Therefore, we decided to try to validate the grading system proposed by Rubinstein *et al.* [16] in our cohort. Their system, developed in an AL cohort, comprises glomerular, interstitial and vascular deposits that were predictive of ESRD. The baseline characteristics of the patients in both our groups were similar, except for a higher age and lower AS in our cohort. In our AL cohort, we could validate the association of AS and CSIS scores with renal function at diagnosis and of AS with progression to ESRD. These findings are supported by multiple studies reporting correlations between amyloid burden (both AL and AA) and renal function [25–30] and between chronic damage markers and renal function in AL and AA [25, 27, 31, 32]. Strikingly, the M score correlated stronger with baseline eGFR than AS in our AL cohort. In our opinion, and in line with previous reports [26–29, 32–34], this underscores the importance of glomerular deposits for renal function. Moreover, already 30 years ago, Shiiki *et al.* [25] described mesangial deposits specifically to correlate to renal function. Furthermore, corroborating on the results by Rubinstein *et al.* [16], we could not demonstrate a correlation between AS, CSIS or their components and proteinuria at diagnosis in AL patients either. In contrast, one Chinese study [29] reported glomerular AL deposits correlating to proteinuria both in univariate and in multivariate analysis. Moreover, multiple articles report similar correlations in mixed AL/AA cohorts [35].

In our AL cohort, AS and CSIS correlated well, and were both correlated to eGFR, which might indicate that they are both markers of nephron loss and chronic damage in renal amyloidosis.

Using the same cut-off values for AS and CSIS as Rubinstein *et al.* [16], we report similar results of progression to ESRD in AL. However, it should be mentioned that due to the limited sample size of our outcome analysis, our results should be interpreted with caution and drawing firm conclusions is difficult. It is indeed remarkable that we were able to reproduce this result despite the small number of patients in our survival analysis. Similarly, previous reports described correlations between amyloid load and renal outcome in AL + AA mixed cohorts [26, 36, 37]. Also, Yao *et al.* [29] reported a correlation between renal amyloid load and patient survival in 61 AL patients. On the other hand, a recent Japanese study [31] found no correlation between amyloid load and renal outcome.

In line with Rubinstein *et al.* [16], we found no difference in progression to ESRD for AL patients below or above the CSIS cut-off. This surprised us since CSIS correlated strongly with baseline eGFR and it has been repeatedly shown that renal function at diagnosis is related to both renal [18, 36] and patient survival in amyloidosis [37–40]. Rubinstein *et al.* [16] conclude that their study was underpowered to correlate CSIS to ESRD, and this might also be the case in our study.

We were unable to reproduce the scoring system in our AA cohort. We found no correlations between AS/CSIS and eGFR/proteinuria at diagnosis, in contrast to previous findings of clinicopathological correlations in AA [32, 34]. Interestingly, the CW score did correlate to baseline proteinuria in AA but not in AL patients. One study [34] could relate the pattern of glomerular AA deposits to baseline proteinuria, but not the deposit extent. It is unclear to us why CW deposits and proteinuria seem to be related in renal AA but not in AL. We deem amyloid distribution differences between AL and AA unlikely as

explanation, because we specifically analysed CW deposits in relation to proteinuria in both renal amyloidosis types.

All AA patients progressed to ESRD, impeding outcome analysis. Some studies could relate amyloid load to renal outcome in AA [32–34].

In our total (AL + AA) cohort, we found significant clinicopathological correlations at diagnosis and a trend in outcome analysis. We consider these correlations to overestimate the true association, since all of these correlations were only significant in either the AL or the AA patient group, and weaker in the total cohort.

The reason why this grading system seems to be applicable mainly to AL is unclear; however, we should emphasize the limited sample size of the AA subcohort. Alternatively, the difference in multicollinearity between the AL and AA amyloidosis subcohorts might be related to differences in deposit distribution as described in some [10, 25, 37] but not all studies [27, 28, 30, 31].

Limitations

Our findings and their interpretation are subject to uncertainties. The first factor introducing uncertainty is the retrospective nature of our data, implicating we had no control over the data collection and their accuracy. The second factor is the limited availability of clinical data in our multicentre cohort. Therefore, some analyses have been carried out with smaller sample subsets, potentially introducing selection bias. Also, this impeded adjustment for clinical variables in the outcome analysis, limiting the power of this analysis. Thirdly, the histological assessment of biopsy slides was performed by a single pathologist. Therefore, we were unable to analyse potential interobserver variation.

CONCLUSIONS

We aimed to validate the histopathological scoring system for renal amyloidosis proposed by Rubinstein *et al.* [16]. We obtained similar correlations between amyloid deposition scores and clinical parameters at diagnosis and progression to ESRD in our AL cohort. We could not reproduce the correlations of the scoring system in our renal AA amyloidosis patients. Overall, we regard this scoring system as competent for standardized histopathological assessment of renal AL amyloid deposits and thereby disease advancement in renal biopsies. Future studies ideally should focus on prospective validation and application of this histopathological scoring system in both renal amyloidosis subtypes.

FUNDING

CONFLICT OF INTEREST STATEMENT

None declared.

CLINICAL KIDNEY JOURNAL

ckj

REFERENCES

1. Khalighi MA, Dean Wallace W, Palma-Diaz MF. Amyloid nephropathy. *Clin Kidney J* 2014; 7: 97–106
2. Nienhuis HL, Bijzet J, Hazenberg BP. The prevalence and management of systemic amyloidosis in western countries. *Kidney Dis* 2016; 2: 10–19
3. Muchtar E, Gertz MA, Kyle RA *et al*. A modern primer on light chain amyloidosis in 592 patients with mass spectrometry-verified typing. *Mayo Clin Proc* 2019; 94: 472–483
4. Papa R, Lachmann HJ. Secondary, AA, amyloidosis. *Rheum Dis Clin North Am* 2018; 44: 585–603
5. Rysava R. AL amyloidosis: advances in diagnostics and treatment. *Nephrol Dial Transplant* 2019; 34: 1460–1466
6. Merlini G, Palladini G. Amyloidosis: is a cure possible? *Ann Oncol* 2008; 19 (Suppl 4): 63–66
7. Skinner M, Sanchorawala V, Seldin DC *et al*. High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: an 8-year study. *Ann Intern Med* 2004; 140: 85–93
8. Obici L, Perfetti V, Palladini G *et al*. Clinical aspects of systemic amyloid diseases. *Biochim Biophys Acta* 2005; 1753: 11–22
9. Said SM, Sethi S, Valeri AM *et al*. Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases. *Clin J Am Soc Nephrol* 2013; 8: 1515–1523
10. Kyle RA, Linos A, Beard CM *et al*. Incidence and natural history of primary systemic amyloidosis in Olmsted County, Minnesota, 1950 through 1989. *Blood* 1992; 79: 1817–1822
11. Satoskar AA, Burdge K, Cowden DJ *et al*. Typing of amyloidosis in renal biopsies: diagnostic pitfalls. *Arch Pathol Lab Med* 2007; 131: 917–922
12. von Hutten H, Mihatsch M, Lobeck H *et al*. Prevalence and origin of amyloid in kidney biopsies. *Am J Surg Pathol* 2009; 33: 1198–1205
13. Pinney JH, Smith CJ, Taube JB *et al*. Systemic amyloidosis in England: an epidemiological study. *Br J Haematol* 2013; 161: 525–532
14. Hemminki K, Li X, Försti A *et al*. Sundquist K; incidence and survival in non-hereditary amyloidosis in Sweden. *BMC Public Health* 2012; 12: 974
15. Larsen CP, Walker PD, Weiss DT *et al*. Prevalence and morphology of leukocyte chemotactic factor 2-associated amyloid in renal biopsies. *Kidney Int* 2010; 77: 816–819
16. Rubinstein S, Cornell RF, Du L *et al*. Novel pathologic scoring tools predict end-stage kidney disease in light chain (AL) amyloidosis. *Amyloid* 2017; 24: 205–211
17. Central Bureau for Statistics. Bevolking; kerncijfers. http://statline.cbs.nl/Statweb/publication/?DM=SLNL&PA=37296 NED&D1=0-2,8-18&D2=41-62&VW=T (12 July 2017, date last accessed)
18. Palladini G, Hegenbart U, Milani P *et al*. A staging system for renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. *Blood* 2014; 124: 2325–2332
19. Stevens PE, Levin A.; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013; 158: 825–830
20. Perkowska-Ptasinska A, Bartczak A, Wagrowska-Danilewicz M *et al*. Clinicopathologic correlations of renal pathology in the adult population of Poland. *Nephrol Dial Transplant* 2017; 32 (Suppl 2): 209–218
21. Rivera F, Lopez-Gomez JM, Perez-Garcia R. Spsnish registry of G; frequency of renal pathology in Spain 1994-1999. *Nephrol Dial Transplant* 2002; 17: 1594–1602
22. Maixnerova D, Jancova E, Skibova J *et al*. Nationwide biopsy survey of renal diseases in the Czech Republic during the years 1994-2011. *J Nephrol* 2015; 28: 39–49
23. Schena FP. Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. *Nephrol Dial Transplant* 1997; 12: 418–426
24. Sen S, Sarsik B. A proposed histopathologic classification, scoring, and grading system for renal amyloidosis: standardization of renal amyloid biopsy report. *Arch Pathol Lab Med* 2010; 134: 532–544
25. Shiiki H, Shimokama T, Yoshikawa Y *et al*. Renal amyloidosis. Correlations between morphology, chemical types of amyloid protein and clinical features. *Vichows Arch A Pathol Anat Histopathol* 1988; 412: 197–204
26. Castano E, Palmer MB, Vigneault C *et al*. Comparison of amyloid deposition in human kidney biopsies as predictor of poor patient outcome. *BMC Nephrol* 2015; 16: 1–7
27. Hopfer H, Wiech T, Mihatsch MJ. Renal amyloidosis revisited: amyloid distribution, dynamics and biochemical type. *Nephrol Dial Transplant* 2011; 26: 2877–2884
28. Min KW, Na W, Jang SM *et al*. Prognostic associations of clinical and histopathological features in renal amyloidosis. *Postgrad Med J* 2011; 87: 529–534
29. Yao Y, Wang SX, Zhang YK *et al*. A clinicopathological analysis in a large cohort of Chinese patients with renal amyloid light-chain amyloidosis. *Nephrol Dial Transplant* 2013; 28: 689–697
30. Kuroda T, Tanabe N, Hasegawa E *et al*. Significant association between renal function and area of amyloid deposition in kidney biopsy specimens in both AA amyloidosis associated with rheumatoid arthritis and AL amyloidosis. *Amyloid* 2017; 24: 123–130
31. Ozawa M, Komatsuda A, Ohtani H *et al*. Long-term prognosis of AL and AA renal amyloidosis: a Japanese single-center experience. *Clin Exp Nephrol* 2017; 21: 212–227
32. Kendi Celebi Z, Kiremitci S, Ozturk B *et al*. Kidney biopsy in AA amyloidosis: impact of histopathology on prognosis. *Amyloid* 2017; 24: 176–182
33. Uda H, Yokota A, Kobayashi K *et al*. Two distinct clinical courses of renal involvement in rheumatoid patients with AA amyloidosis. *J Rheumatol* 2006; 33: 1482–1487
34. Verine J, Mourad N, Desseaux K *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. *Hum Pathol* 2007; 38: 1798–1809
35. Luo CQ, Zhang YA, Li ZQ *et al*. Clinicopathological features of renal amyloidosis: a single-center study on 47 cases. *J Huazhong Univ Sci Technol Med Sci* 2015; 35: 48–53
36. Bohle A, Wehrmann M, Eissele R *et al*. The long-term prognosis of AA and AL renal amyloidosis and the pathogenesis of chronic renal failure in renal amyloidosis. *Pathol Res Pract* 1993; 189: 316–331
37. Sasatomi Y, Sato H, Chiba Y *et al*. Prognostic factors for renal amyloidosis: a clinicopathological study using cluster analysis. *Intern Med* 2007; 46: 213–219
38. Osawa Y, Kawamura K, Kondo D *et al*. Renal function at the time of renal biopsy as a predictor of prognosis in patients

with primary AL-type amyloidosis. *Clin Exp Nephrol* 2004; 8: 127–133

39. Gertz MA, Leung N, Lacy MQ *et al.* Clinical outcome of immunoglobulin light chain amyloidosis affecting the kidney. *Nephrol Dial Transplant* 2009; 24: 3132–3137

40. Bergesio F, Ciciani AM, Manganaro M *et al.*; on behalf of the Immunopathology Group of the Italian Society of Nephrology. Renal involvement in systemic amyloidosis: an Italian collaborative study on survival and renal outcome. *Nephrol Dial Transplant* 2007; 23: 941–951