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Serum immunoglobulin free light chain measurement in AL amyloidosis: prognostic value and correlations with clinical features

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Short title: Immunoglobulin free light chain in amyloidosis

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ABSTRACT

Immunoglobulin free light chains (FLC) are the precursors of amyloid fibrils in primary amyloidosis (AL). We studied the relationship between FLC levels and clinical features in 730 patients with newly diagnosed AL. The plasma cell clone was λ in 72% patients, and κ in 28% patients. κ–AL had more GI tract and liver involvement, where as renal involvement was more with λ–AL. While the overall survival (OS) was similar for κ and λ–AL, the median OS for those without an identifiable serum heavy chain was significantly shorter (12.6 vs. 29.9 months; P=0.02). The OS was shorter among those with a higher dFLC (involved FLC - uninvolved FLC; κ>29.4 mg/dL or λ>18.2 mg/dL using median for cutoff); 10.9 vs. 37.1 months; P<0.001. In multivariate analysis, dFLC was independent of other prognostic factors. The type of light chain impacts the spectrum of organ involvement and the FLC burden correlates with survival in AL.

INTRODUCTION

Primary systemic or light-chain amyloidosis (AL) is characterized by multi-organ deposition of amyloid fibrils derived from immunoglobulin free light chains (FLC), either κ or λ.[1-5] The introduction of a nephelometric FLC assay (Freelite®) has enabled quantification of circulating FLC.[6-12] FLC assay, used along with serum and urine protein electrophoresis and immunofixation, significantly improves the detection of monoclonal proteins in AL.[13] The FLC assay by measuring the amyloid precursor protein provides us a unique opportunity to study disease biology. We undertook this study in a large cohort of patients with long follow-up, to better define the impact of the FLC measurements on clinical characteristics and survival.

PATIENTS, MATERIALS AND METHODS

The current study included patients with biopsy proven AL seen at Mayo Clinic between 1980-2006, who had FLC measurements within 90 days of diagnosis performed as part of clinical evaluation or subsequently on stored serum. Proof of a clonal plasma cell process, either by presence of monoclonal protein (on serum or urine protein electrophoresis or immunofixation or serum FLC assay) or presence of clonal marrow plasma cells, was required. Of the 1938 patients seen during this period, 730 (38%) satisfied the criteria. The study was conducted with approval from Mayo Clinic Institutional Review Board.

Major organ (cardiac, hepatic or renal) involvement was defined as previously described. Renal, cardiac or hepatic involvement required a positive biopsy of the

respective organ or 24-hour urine protein excretion >0.5 g/day, an interventricular septal thickness >12 mm, or an alkaline phosphatase >1.5 times normal, respectively. We used decreased serum carotene as a marker for intestinal involvement and resultant malabsorption.

Serum FLC quantitation was carried out as previously described using Freelite® FLC assay (The Binding Site Limited). The clonal light-chain is considered the "involved" FLC (iFLC) and the other is referred to as the "uninvolved" FLC (uFLC), with the numerical difference between the two denoted by dFLC.

The $\chi 2$ and Fisher exact tests were used to compare differences between nominal variables and the Mann-Whitney U test or Kruskal Wallis test for continuous variables. Kaplan Meier analysis was used for analyzing OS and survival curves were compared using log-rank test.[14]. Curves were generated with all patients surviving beyond 10 years censored at that time. Multivariate analysis was performed using the Cox Proportional Hazards model.[15]

RESULTS AND DISCUSSION

The median age was 63.3 years (range; 32 - 90) with 463 (63%) males; and the estimated median follow up from diagnosis was 58.4 months with 212 patients (29%) alive at the time of analysis. The baseline laboratory and clinical features are described in **Table-1**. The κ/λ FLC ratio was abnormal (<0.26 or >1.65) in 644 patients (88%), consistent with previous reports comparing the FLC assay to electrophoretic tests in serum and urine.[7,9-11] Based on immunofixation, marrow

immunohistochemistry or FLC assay, the clonal light-chain was determined to be λ in 528 (72.3%) patients, unlike in myeloma where κ is more often (60%) the clonal light-chain.[9,16] Also in contrast to myeloma, only 366 (51.3%) of the 714 patients with immunofixation results, had a detectable heavy chain. The median iFLC and dFLC was higher for κ–AL (31.4 and 29.4 mg/dL respectively) compared to 19.4 and 18.2 mg/dL respectively for the λ-AL. This is in contrast with myeloma where the median involved κ and λ concentrations were 37.1 and 71.3 mg/dl, respectively in one large study[16]. This is likely a reflection of the higher tumor burden and the higher prevalence of renal insufficiency in the κ–patients (**Table-1**).

Our study highlights interesting associations between the clinical features and the type and levels of FLC as shown in **Table-1**. Overall, cardiac, renal and liver involvement was seen in 65%, 55% and 20% of patients respectively. Patients with κ-AL were more likely to have liver and GI tract involvement. While the proportion of patients with nephrotic range proteinuria was higher among λ-patients, the proportion with serum creatinine > 1.5 mg/dL was higher among κ-patients. No relationship was found between frequency of cardiac involvement and type of light chain.

In order to assess the relationship between FLC burden and clinical features, patients were divided into a high (>19.6 mg/dL) and low (<=19.6 mg/dL) FLC group, using the median. Patients with high dFLC had more frequent and severe cardiac involvement with lower ejection fraction, and higher levels of cardiac

biomarkers troponin T and NT-ProBNP, consistent with previous reports (**Table**).[9] Similarly, higher dFLC was associated with more severe GI and renal involvement.

Previous studies have demonstrated prognostic value for FLC in different plasma cell disorders including MGUS, myeloma, and amyloidosis.[17,18] In AL, high baseline FLC level was associated with poor outcome in patients undergoing stem cell transplant and a reduction in FLC was associated with improved outcome.[19-21] One study did not find a prognostic value for baseline FLC levels, but was limited by small patient numbers.[11] In the current study, the median OS for the 86 patients with a normal κ/λ FLC-ratio (no clonal excess of light chain) was 63.6 months compared to 16.2 months for the remaining 644 patients, $P < 0.001$ (**Figure 1A**). In terms of the FLC burden, the median OS for patients with high dFLC was 10.1 months compared to 38.2 months for those with low dFLC, $P<0.001$. (**Figure 1B**). Given the significantly different median value for the dFLC between κ and λ-patients, we repeated the analyses using the respective medians for determining the high and low groups (29.4 mg/dL for κ–patients, 18.2 mg/dL for λ–patients). The results were similar; the median OS among patients with a high dFLC was 10.9 months compared to 37.1 months; $P < 0.001$ (**Figure 1C**). Given that treatments for AL have changed in recent years, we repeated the analysis using the more recent group of patients (1998-2006). The results were similar with a median OS for the high dFLC group of 11 months compared to 66 months for the rest; $P < 0.001$. In a multivariate model including NT-ProBNP, troponin T, number of organs involved, ventricular septal thickness, ejection fraction (EF), circulating plasma cells, and

serum uric acid level, dFLC was an independent predictor of survival. The impact of elevated free light chains on survival likely represents the increased availability of precursor light chain for amyloid fibril formation. Interestingly, this may also explain the poor prognosis seen with t(11;14) in AL as translocations involving the heavy chain locus are associated with higher FLC levels.[22,23]

We also examined if the type of light chain influenced outcome and found no relationship; median OS was 18.4 months for κ-patients compared with 19 months for λ-patients; P = 0.2. However, the fact that outcomes were similar despite lower levels of iFLC and dFLC for λ-patients suggest that λ–light chains might be more 'amyloidogenic'. Interestingly patients without an identifiable heavy chain had an inferior survival, 12.6 compared to 29.3 months for those with a heavy chain identified; P=0.02. It is important to note that patients without a heavy chain also had higher dFLC (25.5 vs. 15.3 mg/dL; P < 0.001), which likely impacted the extent of organ deposition and outcome. However, in a multivariate model including dFLC and the presence/absence of heavy chain, both were independently prognostic for survival. Unbound light chains may be inherently more prone to undergo misfolding into an amyloid configuration and might explain this finding.

In conclusion, the results of this study provide several valuable observations. The association between type of light chain and organ involvement provides unique insights and can potentially improve our understanding of biology. Finally, it provides an assessment of the prognostic value of FLC measurements in AL paving the way for its incorporation in future risk stratification models.

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AUTHOR CONTRIBUTIONS

SKK designed the study, analyzed the data and wrote the paper, AD, JAK, DL, CC, SRZ, MQL, SRH, FKB, NL, MRA, RAK, SVR and MAG were involved in the writing and reviewing of manuscript.

DISCLOSURES

AD has received honorarium from Binding Site.

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FIGURE LEGENDS

Figure 1

Panel A compares the OS between patients with an abnormal FLC (κ/λ) ratio to those with a normal ratio. The median OS for the 86 patients with a normal κ/λ FLC ratio (no clonal excess of light chain) was 63.6 months (95% CI; 39, 92) compared to 16.2 months (95% CI; 12, 19) for the remaining 644 patients, P < 0.001.

Panel B compares the overall survival from diagnosis between patients with a high dFLC using the same cutoff for κ and λ patients (19.6 mg/dl). The median OS for patients with a high dFLC was 10.1 months (95% CI; 7, 13) compared to 38.2 months (95% CI; 29, 51) for those with a low dFLC; P < 0.001.

Panel C compares the overall survival from diagnosis between patients with a high dFLC using different cutoffs (individual median values) for κ (29.4 mg/dl) and λ patients (18.2 mg/dl) and low dFLC. The median OS among patients with a high dFLC was 10.9 months (95% CI; 8, 13) compared to 37.1 months (95% CI; 27, 47); P < 0.001.

Table 1: Baseline characteristics and relationship between immunoglobulin light chain type/ levels and organ involvement

Variable	All patients	Light Chain type			dFLC		
		κ restricted patients	λ restricted patients	*P*	***High*** (>19.6 mg/dL)	***Low*** (<=19.6 mg/dL)	*P*
	Median (Range)	Median (Range)	Median (Range)		Median (Range)	Median (Range)	
κ FLC (mg/dL)	1.6 (0.032-1360)	31.4 (0.3-1360)	1.2 (0.032-13)	NA			
λ FLC (mg/dL)	11.4 (0.06-2480)	1.3 (0.06-24)	19.4 (0.9-2480)	NA			
κ:λ ratio abnormal	644 (88%)	191 (95)	453 (86)	<0.001			
dFLC (mg/dL)	19.6 (0.01-2478)	29.4 (0.01-1359)	18.2 (0.03-2478)	<0.001	56.2 (19.8-2478)	7.8 (0.01-19.6)	NA
Plasma cell %	8 (0-95)	9.5 (1-95)	8 (0-90)	0.04	10 (2-95)	6 (0-60)	< 0.001
	N (%)	***N (%)***	***N (%)***		***N (%)***	***N (%)***	***N (%)***
Heart	445 (65)	109 (60)	331 (67)	NS	244 (71)	196 (59)	<0.001
Septum > 12mm	423 (63)	104 (58)	316 (65)	0.09	233 (69)	187 (57)	0.0013
EF < 50%	151 (23)	34 (19)	117 (23)	NS	100 (30)	51 (16)	<0.001
cTnT > 0.035 ng/mL	265 (44)	60 (38)	205 (47)	0.05	154 (53)	111 (36)	<0.001
NT-ProBNP ≥332pg/mL	345 (70)	96 (71)	249 (70)	NS	244 (71)	196 (59)	<0.001

Liver	129 (20)	57 (31)	72 (16)	<0.0001	65 (20)	64 (20)	NS
Bilirubin >1.5 mg/dL	63 (10)	23 (13)	40 (9)	NS	40 (12.5)	23 (7)	0.03
Alk Phos > 1.5 x ULN	118 (18)	51 (28)	67 (14)	0.0002	59 (18)	59 (18)	NS
Serum carotene*	126 (12-662)	113 (12-370)	130 (23-662)	0.0006	107 (12-363)	141 (36-662)	<0.001
Kidney	385 (55)	80 (41)	35 (60)	<0.0001	158 (45)	227 (64)	<0.001
Creatinine > 1.5 mg/dL	160 (24)	62 (33)	98 (21)	0.0012	93 (28)	67 (20)	0.02
Urine Albumin > 3 gm/day	198 (29)	35 (19)	163 (33)	0.0003	79 (23)	119 (34)	0.007
Serum Alb < 3.5 g/dL	575 (80)	142 (74)	433 (83)	0.08	279 (78)	296 (83)	0.07

- *Median (Range)
- NA: not applicable
- The reference range for κFLC is 0.33 mg/dL to 1.94 mg/dL; for λFLC is 0.57 mg/dL to 2.63 mg/dL, and for the κ:λ ratio is 0.26 to 1.65.

Figure 1A

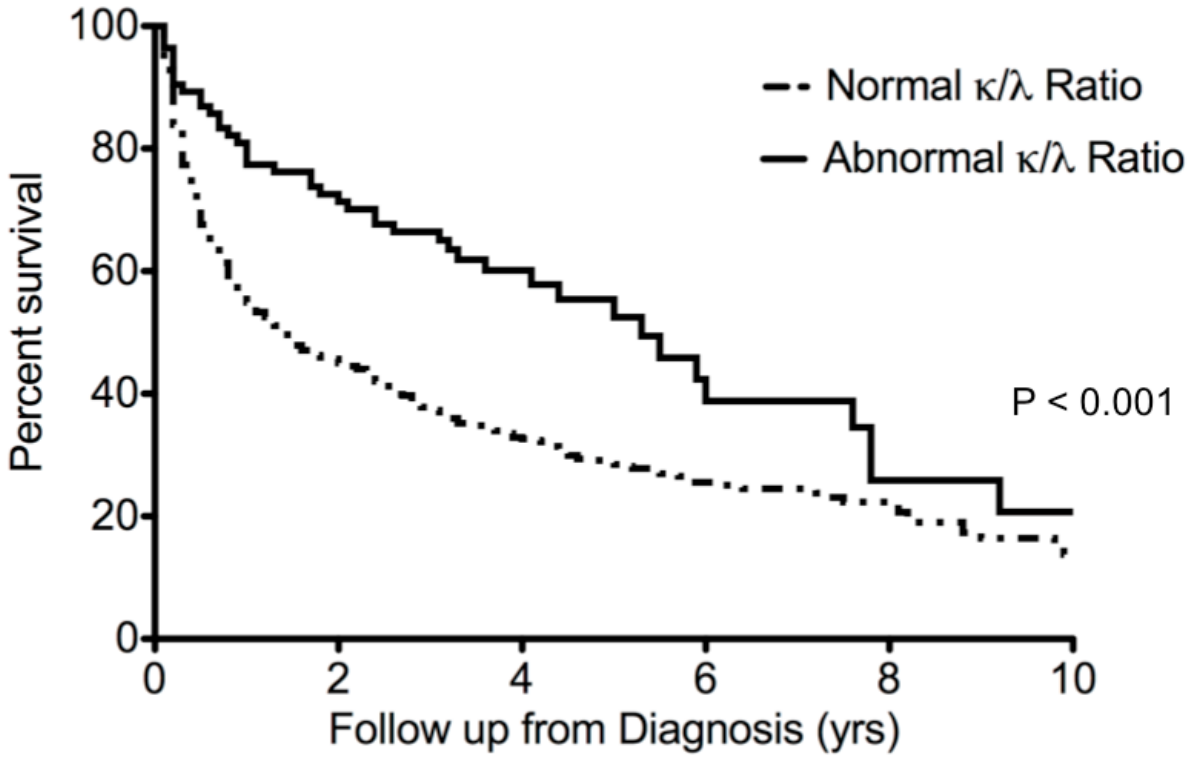

100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
Follow up from Diagnosis (yrs)
Normal κ/λ Ratio
Abnormal κ/λ Ratio
P < 0.001

Figure 1B

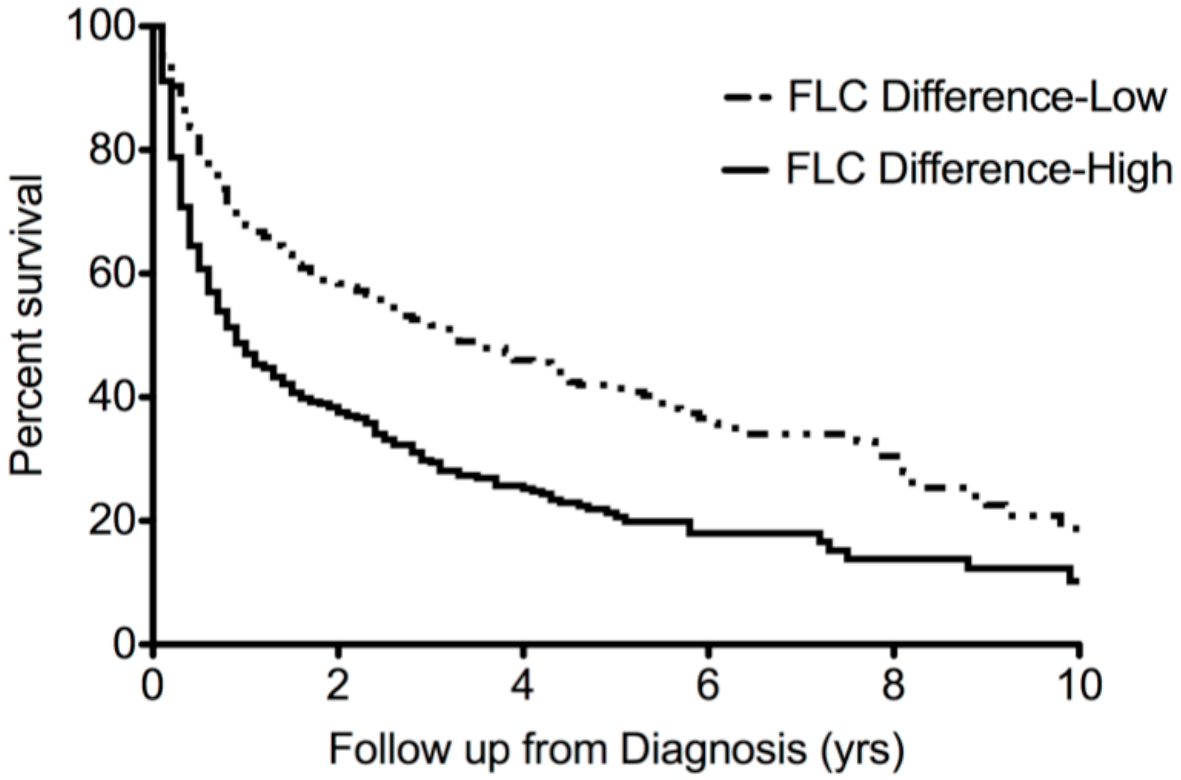
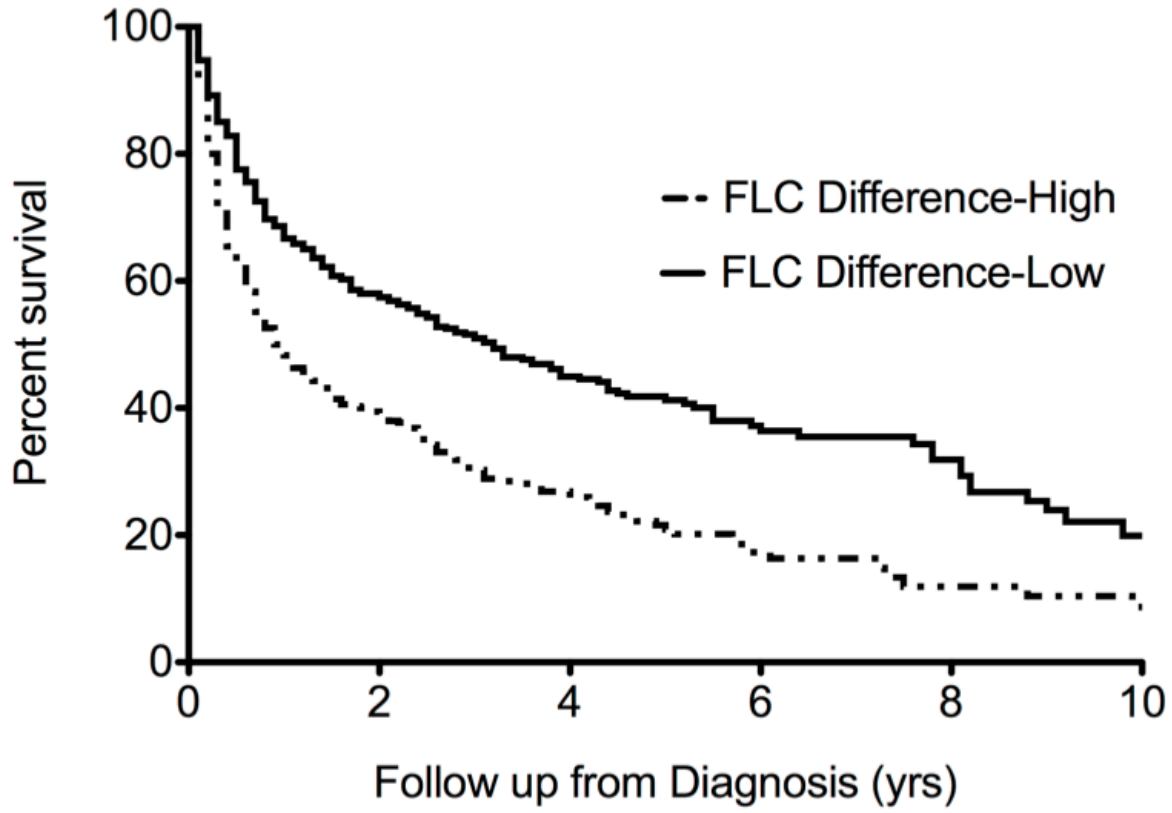

100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
Follow up from Diagnosis (yrs)
FLC Difference-Low
FLC Difference-High

Figure 1C


100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
Follow up from Diagnosis (yrs)
FLC Difference-High
FLC Difference-Low