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Quantitative serum free light chain assay in the diagnostic evaluation of AL amyloidosis

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Abbreviations: *SIFE = serum immunofixation electrophoresis; UIFE = urine immunoelectrophoresis; BM-IHC = bone marrow immunohistochemistry; FLC = free light chain; PPV = positive predictive value; NPV = negative predicitive value*

Abstract

We compared a new serum immunoassay for quantitation of serum free light chains (FLC) with the conventional tests for clonal immunoglobulin production: bone marrow immunohistochemistry, serum immunofixation electrophoresis, and urine immunofixation electrophoresis. Serum samples from 169 patients with AL amyloidosis and 20 controls were examined. Elevated levels of κ-FLC and λ-FLC were found in 94% and 93% of patients with the respective clonal disease. However, false positive elevations of κ-FLC and λ-FLC were found in 30% and 44% of patients with clonal disease of the other light chain subtype. We found that the FLC level was a reliable test for the diagnosis of clonal disease when the FLC κ:λ ratio was abnormal and was comparable to the conventional tests in patients with AL amyloidosis. After a histologic tissue diagnosis of amyloidosis, determining the type as AL amyloidosis relies on a panel of hematologic tests to determine light chain clonality and the exclusion other forms of amyloidosis.

Introduction

Primary amyloidosis (AL) is a plasma cell dyscrasia in which clonal plasma cells in the bone marrow produce an amyloidogenic monoclonal light chain [1–3]. A multisystem disease results due to the deposition of amyloid fibrils composed of monoclonal light chains in major organs including kidneys, heart, liver, and nervous system. AL amyloidosis progresses rapidly and eligibility for treatment protocols is based on organ involvement. An early diagnosis allows some patients to benefit from more aggressive treatment with high-dose melphalan and autologous stem cell transplantation [4–7].

A tissue biopsy, that shows green birefringence on polarization microscopy after Congo red staining, is the histologic proof required to make the diagnosis of amyloidosis. The AL type of amyloidosis is determined by the detection of a plasma cell dyscrasia by one of the following conventional tests: a monoclonal light chain in the serum by immunofixation electrophoresis (SIFE), a monoclonal light chain in the urine by immunofixation electrophoresis (UIFE), or monoclonal plasma cells in the bone marrow by immunohistochemistry (BM-IHC). Elevated levels of serum free light chains (FLC) may be an alternative test to identify a plasma cell dyscrasia, as in normal individuals most light chains circulate in association with heavy chains forming whole tetrameric antibody molecules [8,9]. Preliminary studies have suggested that elevated FLC levels correlate well with the presence of a plasma cell dyscrasia in myeloma and

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AL amyloidosis [10,11]. In this study, we compared the serum FLC immunoassay (FLC assay) to the conventional tests for plasma cell disease in patients with AL amyloidosis. Since FLC levels are elevated in renal insufficiency which is common in AL amyloidosis, the serum FLC κ:λ ratio was examined. FLC levels may also be elevated in polyclonal gammopathies and in the loss of renal function that occurs with aging.

Study design

The FLC assay was studied in serum samples from 169 patients with systemic AL amyloidosis and 20 controls. All patients with AL amyloidosis had a positive tissue biopsy by Congo red staining and evidence of a plasma cell dyscrasia by BM-IHC, SIFE, and/or UIFE. Samples from patients with multiple myeloma, other B cell lymphoproliferative diseases, and other forms of amyloidosis were excluded. Plasma cell percentages in the bone marrow were estimated by a single hematopathologist based on immunohistochemical staining of bone marrow core biopsy specimens for CD138, and clonality was determined by staining for κ and λ light chains [12]. Three patients had received a brief course of oral melphalan (84, 280, and 296 mg) prior to FLC sampling, but had persistent clonal disease. Control serum samples were obtained from 19 patients with a clinical diagnosis of familial amyloidosis confirmed by genetic testing (17 ATTR, 1 fibrinogen and 1 apolipoprotein A-II amyloidosis) and 1 patient with senile cardiac amyloidosis determined clinically by exclusion of other amyloid disease types. Serum samples were obtained from patients and controls at the time of initial evaluation in the Amyloid Clinic at Boston Medical Center between 1996 and 2003. All serum samples were stored at $-20\ ^{\circ}$C until used. Studies were approved by the Institutional Review Board of Boston University Medical Center.

Immunofixation electrophoresis

Serum and urine immunofixation electrophoresis (SIFE/UIFE) were performed using the Sebia HYRYS system (Sebia, Norcross, GA). SIFE (169 cases) and UIFE (167 cases) were both run on the Sebia HYDRAGEL 4 IF and visualized using acid violet staining. For SIFE, the detection limit is 12.5–25 mg/dl. UIFE was run using the Sebia Bence Jones protocol which detects both free and bound light chains and has a detection limit of 1–2 mg/dl. Results were interpreted as positive if there was a single restricted band on the gel in the κ or λ lanes, with or without an associated heavy chain, indicating a monoclonal protein.

Serum FLC assay

Serum FLCs were assayed using a latex-enhanced immunoassay on a nephelometric analyzer (Freelite™ the Binding Site, SanDiego, CA), with the Beckman Coulter Immage Immunochemistry System (Beckman Coulter, Fullerton CA). This FLC assay has a detection limit of 0.3 and 0.4 mg/dl for κ and λ light chains, respectively. For each sample, FLC levels and ratio were scored as normal if they were within the manufacturer's reference range: for κ and λ FLC those were 3.3–19.4 mg/L, 5.7–26.3 mg/L, respectively and for the κ:λ ratio was 0.26–1.65 [10].

Statistical analysis

All analyses were performed using SAS for Windows Release 8.02. Chi-square and Fisher's exact tests were used to compare categorical data. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined by comparing the FLC results to the conventional tests (SIFE, UIFE, BM-IHC) for the diagnosis of AL amyloidosis. The FLC κ was considered positive for disease if it was greater than 19.4, the FLC λ was considered positive if its value was greater than 26.3, and the κ:λ FLC ratio was positive if its value was less than 0.26 or greater than 1.65. Sensitivity, specificity, PVP, and NPV of the FLC measures were obtained separately for patients with κ and λ clonal disease.

Results

Samples from 169 patients with AL amyloidosis and 20 controls were assessed by conventional testing and the serum FLC assay (Table I). All controls had negative SIFE and UIFE, but BM-IHC was not performed since genetic testing had established the diagnosis of another form of amyloidosis. All patients with AL amyloidosis had at least one positive test for clonal disease among the conventional tests; 76% (128/169) had a monoclonal light chain on SIFE, 88% (148/169) had a monoclonal light chain on UIFE, and the BM-IHC was positive for clonal plasma cells in 89% (150/169) (Table II). Of the conventional tests, the UIFE was the most likely to identify a monoclonal protein in patients with AL amyloidosis and λ clonal disease (130/142 or 92% of patients), whereas the BM-IHC was the most likely test to identify a plasma cell dyscrasia in patients with κ clonal disease, (25/27 or 93% of patients). Either the SIFE or the UIFE was positive in 96% (163/169) of the patients with AL amyloidosis.

An elevated κ-FLC was found in 96% (26/27) of patients with known κ clonal disease and 89% (24/27) of patients had an abnormal κ:λ ratio (Table II). An elevated λ-FLC was found in 94%

Table I. Patient and control characteristics.

	Kappa Clonal ($n=27$)	Lambda Clonal ($n=142$)	Control group ($n=20$)
Age* (years)	62 (42, 79)	57 (32, 88)	60 (35, 83)
Gender, Female/Male	11/16	51/91	9/11
Bone Marrow Plasma Cells (%)	10 (5–20)	5 (2–20)	N.D.
Serum creatinine* (mg/dl)	1.2 (0.6–11.3)	1 (0.4–12.9)	0.9 (0.5–1.9)
κ-FLC* (mg/L)	130 (17, 1720)	15.6 (6.3, 144)	18 (7.9, 54.1)
λ-FLC* (mg/L)	26 (4.2, 172)	149 (8.3, 2800)	17.5 (8.2–27.8)
FLC κ:λ ratio*	4.8 (0.6, 277.4)	0.12 (0.01, 1.67)	1.18 (0.74, 2.25)

*Data are given as median values (minimum, maximum).
N.D. not determined.

Table II. Test results of patients and controls.

	AL ($N=169$)	AL Kappa clonal ($N=27$)	AL Lambda clonal ($N=142$)	Control ($N=20$)	Comparing Kappa to Lambda	Comparing AL to Control
	N (%)	*N* (%)	*N* (%)	*N* (%)	*p*-value	*p*-value
Abnormal Bone Marrow Clonality	150 (89%)	25 (93%)	125 (88%)	ND	0.74	ND
Abnormal SIFE	128 (76%)	16 (59%)	112 (79%)	0*	0.03	<0.0001
Abnormal UIFE	148 (88%)	18 (67%)	130 (92%)	0*	0.002	<0.0001
Abnormal SIFE or UIFE	163 (96%)	22 (81%)	141 (99%)	0	0.0004	<0.0001
Abnormal κ-FLC	69 (41%)	26 (96%)	43 (30%)	8 (40%)	NA	0.94
Abnormal λ-FLC	145 (86%)	12 (44%)	133 (94%)	1 (5%)	NA	<0.0001
Abnormal κ:λ ratio	127 (75%)	24 (89%)	103 (73%)	2 (10%)	0.07	<0.0001
Serum Creatinine $\leqslant 1.5$ mg/dl	128 (76%)	18 (67%)	110 (77%)	19 (95%)	0.23	0.051

*UIFE and SIFE testing were each not determined for one different control patient.
SIFE/UIFE: monoclonal light chain by serum/urine immunofixation electrophoresis.
Abnormal κ-FLC if quantitative κ-FLC was greater than 19.4, abnormal λ-FLC if quantitative λ-FLC was greater than 26.3, abnormal FLC κ:λ was less than 0.26 or greater than 1.65.
Chi-Square and Fisher's exact tests were used to compare categorical data among κ and λ clonal disease patients and among AL and control patients.
ND: Not determined.
NA: Not applicable.

(133/142) of patients with known λ clonal disease and 73% (103/142) of patients had an abnormal κ:λ ratio. An elevated FLC level was as likely to be found as a positive result of any of the conventional tests in patients within each respective clonal group, however abnormal levels of FLC of the other clonal type were also found. A false elevation of λ-FLC was found in 44% of patients (12 of 27) with κ clonal disease and false elevation of κ-FLC was found in 30% of patients (43 of 142) with λ light chain clonal disease. Abnormalities in renal function are reported to account for most false elevations of FLC [13]. In our study, approximately half (29/55) of the patients with a false positive elevation of FLC had a serum creatinine level higher than 1.5 mg/dl. In view of the abnormalities in FLC elevations associated with abnormalities in renal function, a finding that is common in AL amyloidosis, the κ:λ ratio was examined. An abnormal κ:λ ratio was found in 75% (127/169) of all patients with AL amyloidosis and was comparable to the overall finding of an abnormal SIFE at 76% (128/169).

The sensitivity, specificity, positive and negative predictive values of conventional tests and the FLC assay in all patients with each clonal disease are shown in Tables III and IV. The specificities of the conventional tests are 100% as that was the basis for the diagnosis of a plasma cell dyscrasia in this study. The sensitivity of the FLC test is also high and compares favorably to the conventional tests: the specificity of the κ-FLC in the diagnosis of AL among patients with κ clonal disease was 60% and the specificity of the λ-FLC in the diagnosis of AL among patients with λ clonal disease was 95% . The positive predictive value for the FLC κ:λ ratio was high in each clonal type: 92% for κ clonal disease and 98% for λ clonal disease.

Table III. Kappa clonal disease: validity of tests for the diagnosis of AL amyloidosis.

		AL Amyloidosis Conventional Tests*		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Yes	No				
Bone marrow clonality	Yes	25	0	93%	100%	100%	91%
	No	2	20	(83%–100%)	(100%–100%)	(100%–100%)	(79%–100%)
Abnormal SIFE	Yes	16	0	59%	100%	100%	65%
	No	11	20	(41%–78%)	(100%–100%)	(100%–100%)	(48%–81%)
Abnormal UIFE	Yes	18	0	67%	100%	100%	69%
	No	9	20	(49%–84%)	(100%–100%)	(100%–100%)	(52%–86%)
Abnormal SIFE or UIFE	Yes	22	0	81%	100%	100%	80%
	No	5	20	(67%–96%)	(100%–100%)	(100%–100%)	(64%–96%)
Abnormal SIFE and UIFE	Yes	12	0	44%	100%	100%	57%
	No	15	20	(26%–63%)	(100%–100%)	(100%–100%)	(41%–74%)
Abnormal κ-FLC	Yes	26	8	96%	60%	76%	92%
	No	1	12	(89%–100%)	(39%–81%)	(62%–91%)	(78%–100%)
Abnormal κ:λ ratio	Yes	24	2	89%	90%	92%	86%
	No	3	18	(77%–100%)	(77%–100%)	(82%–100%)	(71%–100%)

*Conventional Tests: One of the following positive: BM, SIFE or UIFE.

Table IV. Lambda clonal disease: validity of tests for the diagnosis of AL amyloidosis.

		AL Amyloidosis Conventional Tests*		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Yes	No				
Bone marrow clonality	Yes	125	0	88%	100%	100%	54%
	No	17	20	(83%–93%)	(100%–100%)	(100%–100%)	(83%–93%)
Abnormal SIFE	Yes	112	0	79%	100%	100%	40%
	No	30	20	(72%–86%)	(100%–100%)	(100%–100%)	(26%–54%)
Abnormal UIFE	Yes	130	0	92%	100%	100%	63%
	No	12	20	(87%–96%)	(100%–100%)	(100%–100%)	(46%–79%)
Abnormal SIFE or UIFE	Yes	141	0	99%	100%	100%	95%
	No	1	20	(98%–100%)	(100%–100%)	(100%–100%)	(86%–100%)
Abnormal SIFE and UIFE	Yes	101	0	71%	100%	100%	33%
	No	41	20	(64%–79%)	(100%–100%)	(100%–100%)	(21%–45%)
Abnormal λ-FLC	Yes	133	1	94%	95%	99%	68%
	No	9	19	(90%–98%)	(85%–100%)	(98%–100%)	(51%–85%)
Abnormal κ:λ ratio	Yes	103	2	73%	90%	98%	32%
	No	39	18	(65%–80%)	(77%–100%)	(95%–100%)	(20%–44%)

*Conventional tests: One of the following positive: BM, SIFE or UIFE.

Discussion

This is the first examination of the serum FLC assay in a large population of patients with AL amyloidosis and controls consisting of patients with other forms of systemic amyloidosis. It is the first study to describe the specificity and predictive value of the test in comparison to conventional tests for plasma cell dyscrasia in AL amyloidosis. We found the FLC assay to be highly sensitive for the diagnosis of a plasma cell dyscrasia and equal to or better than any one of the conventional tests. However, a falsely elevated result most often due to renal disease can be misleading. Performing both SIFE and UIFE appears to be the best screening test for a monoclonal protein; 96% of patients were positive for an abnormal test in either SIFE or UIFE.

Two recent studies of FLC in other large populations of patients with AL amyloidosis examined similar parameters. Both reports showed a high sensitivity for an abnormal FLC measurement, but in these studies there was a lower sensitivity for an abnormal value among the conventional tests [14,15]. Abraham, et al, found an elevated FLC in 91% of patients with AL amyloidosis, of whom 81% had an abnormal SIFE or UIFE. Lachmann, et al, reported 98% of patients with AL amyloidosis had an

elevated FLC, but only 79% had an abnormal SIFE or UIFE. These differences might be due to the IFE system used or related to the groups of patients studied. Neither study evaluated the κ:λ FLC ratio or reported the incidence of falsely elevated FLC levels in patients. The high frequency of falsely elevated FLC levels in our study lead us to examine the κ:λ ratio for sensitivity in detecting clonal disease. Our results show that the specificity and predictive value of an abnormal test for κ:λ ratio are superior to the FLC level in patients with κ clonal disease, although not quite as good for λ clonal disease when compared to the control population. It must be noted that 2 (10%) of the control patients had a kappa:lambda ratio that was just outside the normal range and based on this test could have been misdiagnosed as having clonal disease. Thus it is important for the clinician to diagnose clonal disease by at least one of the conventional ''gold standard'' tests: BM-IHC, SIFE, or UIFE.

The conventional tests that assess clonal light chain production and the serum FLC assay have major differences. The conventional tests are qualitative, with visual immunochemical evidence of clonality, while the FLC assay is quantitative, based on a raw number. The conventional tests may lead to an occasional error in interpretation, but are less likely to have false positives and are thus the most reliable for a diagnosis of clonal disease. The FLC assay, on the other hand, has the advantage of being quantitative which makes it a more useful test for monitoring patient response to treatment.

A shortcoming of our study and those of others is that data were obtained on samples from patients with known clonal disease. The FLC assay has yet to be tested for diagnostic sensitivity in patients in whom there is a clinical suspicion of amyloidosis and who have not had conventional tests already performed. In this study and in others, an elevated light chain inferred monoclonality when it was known that monoclonal light chains existed by other studies.

Currently the clinician has a broad array of tests that can be ordered for patients in whom the diagnosis of AL amyloidosis is suspected. We recommend beginning with a tissue biopsy or by testing for a plasma cell dyscrasia by either conventional (SIFE and UIFE) or FLC assay testing. More extensive testing is warranted for patients in whom AL amyloidosis is highly suspected because of multisystem involvement or macroglossia, and we recommend both tissue biopsy and tests for plasma cell dyscrasia including both conventional and FLC assay. If any of the studies are positive, the patient should be referred to a clinical center for additional testing and treatment. An early diagnosis is important and may allow the patient to have treatments found to provide greater benefit.

The FLC test was not superior to conventional testing in our study, but was complimentary. The diagnosis of AL amyloidosis continues to rest upon a panel of conventional hematologic tests, and in some cases, genetic testing to exclude other forms of amyloidosis. As a quantitative test, the FLC assay may have greater value in long-term monitoring of patients after treatment, and to compare with conventional testing for the monitoring of disease remission.

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