Evaluation of the serum-free light chain test in untreated patients with AL amyloidosis

Tilmann Bochtler,[1] Ute Hegenbart,[1] Christiane Heiss,[2,4] Axel Benner,[2] Friedrich Cremer,[1] Martin Volkmann,[3] Jochen Ludwig,[3] Jolanta B. Perz,[1] Anthony D. Ho,[1] Hartmut Goldschmidt,[1] and Stefan O. Schonland[1]

[1]Department of Internal Medicine Division of Hematology/Oncology, Amyloidosis Clinic, University of Heidelberg; [2]Division of Biostatistics (C060), German Cancer Research Center, Heidelberg; [3]Department of Internal Medicine I and Clinical Chemistry, University of Heidelberg; [4]Department of Internal Medicine Division of Hematology/Oncology, University of Heidelberg, Germany[4]

ABSTRACT

We evaluated the Serum Free Light Chain (FLC) test in a series of 133 untreated patients with systemic AL amyloidosis. The FLC test detected the monoclonal gammopathy in 87% compared with 92% for immunofixation of serum and urine in combination. However, both tests proved complementary. The FLC test was also a valuable tool in patients with advanced renal failure in spite of uninvolved light chain retention. Higher FLC levels were associated with higher bone marrow plasmocytosis, poorer Karnofsky index and heart involvement, and therefore reflected disease severity.

Key words: serum-free light chain test, AL amyloidosis, Karnofsky index, bone marrow plasmocytosis, heart disease.

Citation: Bochtler T, Hegenbart U, Heiss C, Benner A, Cremer F, Volkmann M, Ludwig J, Perz JB, Ho AD, Goldschmidt H and Schonland SO. Evaluation of the serum-free light chain test in untreated patients with AL amyloidosis. Haematologica 2008 Mar; 93(3):459-462. doi: 10.3324/haematol.11687

Introduction

AL amyloidosis (AL) is a plasma cell dyscrasia characterized by the deposition of immunoglobulin light chains in different tissues. The amyloidogenic light chains are produced by monoclonal plasma cells in the bone marrow. The standard techniques to detect the underlying plasma cell disorder are electrophoresis of serum and urine with subsequent immunofixation, the quantitative determination of light chain excretion in the urine and bone marrow examination. However, immunofixation techniques are not quantitative and depend on subjective visual detection of the electrophoresis bands. In AL, the diagnostic value of serum electrophoresis and urine light chain excretion is poor due to the usually low amount of monoclonal protein. A new quantitative nephelometric test has been developed to detect immunoglobulin light chains in the serum.[1] Since this assay specifically detects the free light chains in the serum, but not the bound forms, it is also referred to as the Free Light Chain (FLC) test. Previous reports have studied the role of the FLC test in AL.[2-7] Recently, the test has been included in the consensus guidelines for patients with AL to evaluate and quantify the monoclonal gammopathy.[8]

Design and Methods

Patients

This study included a series of 133 consecutive untreated patients from a single institution with a histologically confirmed diagnosis of systemic AL. Patient characteristics are summarized in Table 1. Thirteen of the AL patients met the diagnostic criteria for a concomitant multiple myeloma (MM) stage I as defined by a bone marrow plasmocytosis > 30%, a light chain excretion > 1 g/day in the urine, IgG > 35 g/L or IgA > 20 g/L without bone lesions.[9,10] Patients with MM requiring therapy[11] had been excluded from the analysis. Heart, kidney and GI tract involvement were the most frequent clinical manifestations, affecting 80%, 66% and 37% patients respectively. The study was approved by the ethics committee of the University of Heidelberg and patients gave written informed consent.

Methods

The concentrations of serum free light chains were measured by a latex enhanced immunoassay (Freelite TM Human Kappa Free Kit kindly provided by The Binding Site GmbH, Schwetzingen, Germany) on a Behring 2 nephelometer. The

TB and UH contributed equally to this study. Manuscript received May 5, 2007. Manuscript accepted November 28, 2007.
Correspondence: Stefan Schönland MD, Medizinische Klinik V, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.
E-mail: stefan.schoenland@med.uni-heidelberg.de
The online version of this article contains a supplemental appendix.

reference ranges were used according to the manufacturer's instructions as follows: 3.3-19.4 mg/L for kappa light chain, 5.7-26.3 mg/L for lambda light chain and 0.3-1.6 for kappa/lambda ratio.[12] The FLC test was considered positive when the criteria of both an abnormal FLC ratio and an elevation of the involved light chain above the respective upper range were met. Immunofixation of serum and urine was performed using the Paragon Electrophoresis System kit by Beckman Coulter. In case of an ambiguous result, the immunofixation was repeated after prior denaturation with 2-mercaptoethanol and kits by Dako, Binding Site or Technoclon were applied additionally. For the quantification of urine light chain excretion antisera by Dade Behring (N Antisera to Human Immunglobulin/L-chains) were used. For renal function assessment we used the creatinine clearance as determined by a 24 hr. urine collection. In 15 patients with no available creatinine clearance, the value was calculated by the MDRD formula.[13] Cytological smears were used to compare FLC values with the bone marrow plasma cell content. Eligibility for high dose chemotherapy was only analyzed in patients under 70 years of age. Organ involvement was determined according to consensus criteria.[8]

Statistical Analysis

For linear regression analyses, the log transformation was performed for dependent variables, namely involved and uninvolved light chain concentrations as well as FLC ratio. Univariate and multivariate regression analyses were used to relate transformed FLC values to clinical variables. The output shown in the *Online Supplementary Table* provides the estimates of the regression coefficients and their confidence intervals resulting from univariate and multivariate linear regression analyses. To account for multiple testing by univariate linear regression, p-value adjustment was made according to Bonferroni-Holm.[14] In the multivariate linear regression model the Karnofsky index, the number of affected organs, heart involvement, plasma cell content and creatinine clearance were included.

The McNemar test was used to test the agreement of the sensitivity of FLC test with the sensitivity of immunofixation. The distribution of log-transformed FLC ratios depending on the detection of monoclonal protein in electrophoresis was analyzed using the exact Wilcoxon rank sum test. The correlation between quantitative light chain excretion in the urine and the serum free light chain test was estimated using Pearson's correlation coefficient r including the 95% confidence interval.

All statistical tests were two-sided. All p values < 0.05 were considered to be statistically significant. All statistical analyses were performed using R, version 2.4.1.[15]

Results and Discussion

The sensitivity of the FLC test is comparable to that of combined immunofixation in serum and urine

A positive FLC test was observed in 87% (116/133) of patients. In comparison, the diagnostic sensitivities of immunofixation were 69% (92/133) in serum and 86% (115/133) in urine. A sensitivity of 92% (123/133) was achieved when serum and urine immunofixation were assessed in combination. Details are shown in Figure 1 and Table 1. The FLC test proved statistically superior to serum immunofixation alone ($p<0.001$), but had a sensitivity comparable to immunofixation of the urine alone ($p=1.00$) and immunofixation of serum and urine in combination ($p=0.21$). Previous studies in AL have reported a wide variation in frequencies for a positive FLC test ranging from 75% to 98%.[2-7] Compa-

Table 1. Baseline characteristics of 133 untreated patients with systemic AL amyloidosis.

General characteristics	
Age, median (range)	63 (40–81), years
Gender (male/female)	76/57
Karnofsky index, median (range)	80 (40–100), %
Eligibility for high dose chemotherapy	57 patients (43%)
AL amyloid type κ/λ	37/96
Hematologic parameters	
Median FLC Ratio/median conc	
κ restricted patients	21.5/322 mg/L
λ restricted patients	0.064/195 mg/L
Elevated involved FLC conc	130 patients (98%)
κ restricted patients	36/37 patients
λ restricted patients	94/96 patients
Abnormal FLC ratio	118 patients (89%)
κ restricted patients	36/37 patients
λ restricted patients	82/96 patients
Positive FLC test *	116 patients (87%)
κ restricted patients	36/37 patients
λ restricted patients	80/96 patients
Positive Immunofixation in serum or urine	123 patients (92%)
Intact immunoglobulines	61 patients (46%)
Bone marrow plasmocytosis, median (range)	10% (2 – 42)
Concomitant multiple myeloma stage I **	13 patients (11%)
Organ involvement pattern	
Median number of affected organs (range)	2 (1–5)
Heart	106 patients (80%)
Kidney	88 patients (66%)
Gastrointestinal tract	49 patients (37%)
Soft tissue	36 patients (27%)
Peripheral polyneuropathy	33 patients (25%)
Liver	32 patients (24%)
Lung	2 patients (2%)

**The FLC test was considered positive when the criteria of both an abnormal FLC ratio and an elevation of the involved light chain above the respective upper range were met. **The diagnosis of MM I was based on a plasma cell content > 30% in 3 patients , on a light chain excretion in the urine above 1g/day in 6 patients , on both these criteria in another 3 patients and on an IgG > 35g/L in 1 patient. conc: concentration.*

risons with the sensitivity of immunofixation either showed superiority of the FLC test over immunofixation with 98% *vs.* 79%[2] or inferiority with 75% *vs.* 96%.[5] The sensitivities of 87% for the FLC test and 92% for immunofixation in our series were within the range of the frequencies reported so far. In our study, the FLC test and immunofixation were complementary. Among the 10 patients with a negative immunofixation in both serum and urine, the FLC test was diagnostic for the detection of monoclonal gammopathy in 8. Vice versa, among the 17 patients with a negative FLC test, combined immunofixation was diagnostic in 15. Thus, the FLC test and the combined immunofixation of serum and urine allowed the monoclonal gammopathy to be detected in 131 out of 133 patients. In the two remaining patients with a negative FLC test and a negative immunofixation, the diagnosis of systemic AL was based on the clinical picture, immunohistochemical assessment of organ biopsy material,[16,17] and exclusion of hereditary amyloidosis by DNA analysis.[18]

In our series, the FLC assay seemed to be particularly effective in patients with clonality for kappa light chain with 97% (36/37) positive test results *vs.* 83% (80/96) in λ restricted patients (p=0.04). These differences were attributable to a higher rate of abnormal FLC ratios in the kappa group whereas the absolute concentrations of the involved light chain were elevated at a very high frequency in both groups. This might be due to an over strict lower reference range of the FLC ratio.

The FLC test is a diagnostic tool even in patients with renal insufficiency

Though renal insufficiency is a known confounding factor for the serum free light chain test,[12,5] the FLC test was also a valuable diagnostic tool in patients with impaired kidney function. Worsening of renal function was associated with an increase in uninvolved serum free light chain concentrations (p=0.03 for the kappa group and p<0.001 for the lambda group). No statistical significance was reached for the involved serum free light chain (p=0.24 for kappa, p=0.07 for lambda). Nevertheless, the increase in the uninvolved light chains did not translate into a statistically significant reduction in the FLC test's sensitivity. In particular, among patients with a creatinine clearance < 30 mL/min, 21 out of 24 (88%) patients still showed a positive FLC test. Since determination of the light chains in urine is not reliable in patients with renal insufficiency, the FLC test is of particular value to quantify the amount of the monoclonal gammopathy in these patients.

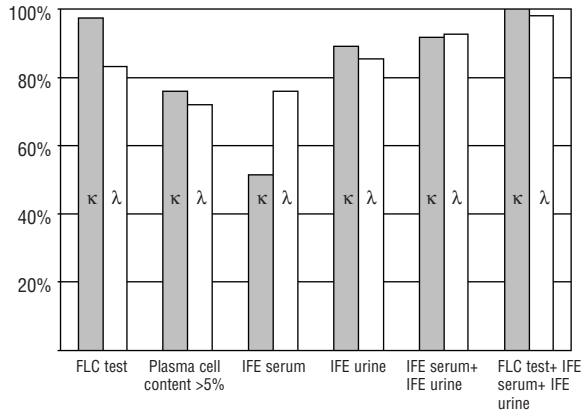

Figure 1. Sensitivities of the FLC test compared with bone marrow plasmocytosis and immunofixation specified according to light chain restriction: the FLC test has a higher sensitivity than serum immunofixation (p<0.001). IFE: immunofixation

Higher plasma cell content, concomitant multiple myeloma stage I and positive serum immunofixation were associated with more pronounced FLC ratios

A higher plasma cell content in the bone marrow was positively associated with the FLC ratio in univariate linear regression analysis (p=0.002 for kappa, p<0.001 for lambda). Concomitant MM stage I was also associated with more pronounced FLC ratios (p=0.009 for kappa and p<0.001 for lambda). Correspondingly, the FLC ratio was diagnostic in all 13 patients with a concomitant MM stage I. A positive immunofixation in serum was also found more frequently among patients with a more pronounced FLC ratio (p=0.04 for kappa and p<0.001 for lambda). However, this was not the case for immunofixation positivity in the urine. The detection of a monoclonal protein in the serum electrophoresis had no impact on the FLC ratio either (p=0.37 for kappa, p=0.93 for lambda). The FLC test´s diagnostic failures were equally distributed among patients who had a monoclonal intact immunoglobulin versus those without (p=0.8). The heavy chain subtype also had no impact on the FLC test´s diagnostic sensitivity. The quantitative light chain excretion in the urine was also not associated with the serum free light chain ratio (r=0.25 [-0.08, 0.54] for kappa, r=0.29 [0.09, 0.47] for lambda).

Cardiac involvement and a poorer Karnofsky index were associated with higher concentrations of the involved serum free light chain

Patients with cardiac involvement had higher involved FLC concentrations (p=0.002, see *Online Supplementary Table*) as previously reported.[6] Univariate linear regression analysis also showed a statistically significant effect on FLC concentrations for the cardiac biomarkers cTNT (p=0.007) and NT-proBNP (p<0.001). We conclude that patients with high concentrations of circulating free light chains seem therefore to be at high risk for amyloid deposition in the heart. Apart from heart involvement, neither other organ involvement or the number of involved organs were statistically significantly related to light chain concentrations.

Remarkably, patients with higher involved light chain concentrations were in worse clinical condition as reflected by a poorer Karnofsky index ($p<0.001$). Although it has been suggested that high FLC values reflect advanced amyloid disease,[6] this is the first report to show an association between the FLC test and performance status.

To determine the most important factors for the concentration of the involved free light chain we developed a multivariate model including bone marrow plasmocytosis, creatinine clearance, Karnofsky index, number of affected organs and heart involvement. In our view, these represent very well the biology of the underlying plasma cell disorder and the severity of the amyloid disease. Bone marrow plasmocytosis and the Karnofsky index were identified to be statistically independently associated with higher serum light chain concentrations ($p<0.001$ and $p=0.03$ respectively), and heart involvement retained borderline significance ($p=0.07$). Since heart involvement has a major impact on Karnofsky index, both factors were certainly competing with each other in the multivariate analysis, which may explain why cardiac involvement lost the significance observed in univariate analysis.

In conclusion, our results demonstrate that the FLC test is a very useful tool for the initial assessment of the plasma cell dyscrasia in patients with AL. The FLC concentration also reflects the expansion of the aberrant clone and, to some extent, the severity of organ involvement.

Authorship and Disclosures

TB, UH, SOS designed the research, analyzed the data and wrote the paper.; JBP, HG, MV, JL, FC, ADH contributed to the data analysis; CH, AB performed the statistical analysis. The FLC test was provided free of charge by The Binding Site GmbH, Schwetzingen, Germany. The authors reported no potential conflicts of interest.

References

1. Bradwell A, Carr-Smith H, Mead G, Tang L, Showell P, Drayson M, et al. Highly sensitive, automated immunoassay for the immunoglobulin free light chains in serum and urine. Clin Chem 2001;47:673-80.
2. Lachmann H, Gallimore R, Gillmore J, Carr-Smith H, Bradwell A, Pepys M, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. Br J Haematol 2003;122:78-84.
3. Abraham R, Katzmann J, Clark R, Bradwell A, Kyle R, Gertz M. Quantitative analysis of serum free light chains. A new marker for the diagnostic evaluation of primary systemic amyloidosis. Am J Clin Pathol 2003;119:274-8.
4. Katzmann J, Abraham R, Dispenzieri A, Lust J, Kyle R. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. Clin Chem 2005;51: 878-1.
5. Akar H, Seldin D, Magnani B, O'Hara C, Berk J, Schoonmaker C, et al. Quantitative serum free light chain assay in the diagnostic evaluation of AL amyloidosis. Amyloid 2005;12:210-5.
6. Dispenzieri A, Lacy M, Katzmann J, Rajkumar S, Abraham R, Hayman S, et al. Absolute values of immunoglobulin free light chains are prognostic in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. Blood 2006;107: 3378-83.
7. Morris K, Tate J, Gill D, Kennedy G, Wellwood J, Marlton P, et al. Diagnostic and prognostic utility of the serum free light chain assay in patients with AL amyloidosis. Int Med J 2007;37:456-63.
8. Gertz M, Comenzo R, Falk R, Fermand J, Hazenberg B, Hawkins P, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France. Am J Hematol 2005; 79:319-28.
9. Skinner M, Sanchorawala V, Seldin D, Dember L, Falk R, Berk J, et al. High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: an 8-year study. Ann Int Med 2004; 140: 85-94.
10. Durie B. Staging and kinetics of multiple myeloma. Semin Oncol 1986; 13:300-9.
11. Kyle R, Child J, Anderson K, Barlogie B, Bataille R, Bensinger W, et al. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Hematol 2003;121:749-57.
12. Katzmann J, Clark R, Abraham R, Bryant S, Lymp J, Bradwell A, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437-44.
13. Levey A, Bosch J, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461-70.
14. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979;6:65-70.
15. R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org. 2007.
16. Linke R, Oos R, Wiegel N, Nathrath W. Classification of amyloidosis: misdiagnosing by way of incomplete immunohistochemistry and how to prevent it. Acta Histochem 2006;108:197-208.
17. Kebbel A, Röcken C. Immunohistochemical classification of amyloid in surgical pathology revisited. Am J Surg Pathol 2006;30: 673-83.
18. Lachmann H, Booth D, Booth S, Bybee A, Gilbertson J, Gillmore J, et al. Misdiagnosis of hereditary amyloidosis as AL (primary) amyloidosis. N Engl J Med 2002;346:1786-91.