Clinical Chemistry 51:5
878–881 (2005)

Hematology

Diagnostic Performance of Quantitative κ and λ Free Light Chain Assays in Clinical Practice

Jerry A. Katzmann,* Roshini S. Abraham, Angela Dispenzieri, John A. Lust, and Robert A. Kyle

Background: **The quantitative assay for free light chains (FLCs) is a recently introduced commercial test reported to be sensitive and specific for detecting FLC diseases such as primary systemic amyloidosis (AL), light chain deposition disease (LCDD), nonsecretory multiple myeloma (NSMM), and light chain multiple myeloma. We evaluated its diagnostic performance in clinical practice.**
Methods: **All FLC clinical test results generated in 2003 were abstracted from the Laboratory Information System. Diagnoses were obtained from the Dysproteinemia database and the patient medical history.**
Results: **In 2003, we received samples for FLC assays from 1020 Mayo Clinic patients. The majority of these patients (88%) had bone marrow-derived monoclonal plasma cell disorders (PCDs). The 121 patients who did not have monoclonal gammopathy all had FLC κ/λ ratios within the range of values obtained for a reference population in our laboratory. Among the patients with monoclonal gammopathies were patients with multiple myeloma (330), AL (269), monoclonal gammopathy of undetermined significance (114), smoldering multiple myeloma (72), plasmacytoma (22), NSMM (20), macroglobulinemia (9), LCDD (7), and a variety of other PCDs. Among the 110 AL patients who had not been previously treated and who had a FLC assay performed within 120 days of diagnosis, the FLC κ/λ ratio was positive in 91% compared with 69% for serum immunofixation electrophoresis (IFE) and 83% for urine IFE. The combination of serum IFE and serum FLC assay detected an abnormal result in 99% (109 of 110) of patients with AL.**
Conclusion: **The performance of the FLC assay in this analysis of clinical laboratory data is consistent with results from published retrospective validation studies.**

A quantitative nephelometric assay for free light chains (FLCs)[1] has recently been introduced as a commercial test. The assay measures κ and λ light chains that circulate as light chain monomers or dimers and are not bound to immunoglobulin heavy chain. Quantification of the κ and λ FLCs and calculation of the FLC κ/λ ratio have been reported to be sensitive and specific for detection of excess monoclonal FLCs. We have recommended a diagnostic range for the FLC κ/λ ratio that included 100% of a 282-sample reference population to maximize the diagnostic specificity and minimize false-positive results *(1)*. Retrospective studies using stored serum from populations of patients with nonsecretory multiple myeloma (NSMM) *(2)*, primary systemic amyloidosis (AL) *(3, 4)*, light chain deposition disease (LCDD) *(1)*, and light chain multiple myeloma (LCMM) *(5)* have documented the sensitivity of these assays and established their use as a complement to immunofixation electrophoresis (IFE). In addition to its diagnostic use in the FLC diseases, the assay is used for monitoring disease course in AL, LCDD, NSMM, and LCMM, in which there may be a band detected on IFE that cannot be quantified by protein electrophoresis.

Our clinical laboratory implemented FLC testing in late 2002. In 2003, we performed FLC assays on 1020 samples from Mayo Clinic patients. These patients were seen predominantly by clinicians in the Division of Hematology. To assess the performance of the FLC assay in our

Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology, and Division of Hematology, Department of Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN.
*Address correspondence to this author at: Department of Laboratory Medicine and Pathology, Hilton 210, Mayo Clinic, Rochester, MN 55905. Fax 507-266-4088; e-mail katzmann@mayo.edu.
Received December 15, 2004; accepted February 23, 2005.
Previously published online at DOI: 10.1373/clinchem.2004.046870

[1] Nonstandard abbreviations: FLC, free light chain; NSMM, nonsecretory multiple myeloma; AL, primary systemic amyloidosis; LCDD, light chain deposition disease; LCMM, light chain multiple myeloma; IFE, immunofixation electrophoresis; PCD, plasma cell disorder; MGUS, monoclonal gammopathy of undetermined significance; and SMM, smoldering multiple myeloma.

routine clinical laboratory practice, we reviewed the diagnoses and FLC results for these 1020 patients.

Materials and Methods

We queried the Laboratory Information System for the FLC results of all Mayo Clinic patients who were tested for serum κ and λ FLCs from January 1, 2003, to December 31, 2003. The list of 1020 patients was merged with data from the Dysproteinemia database, which contained each patient's diagnosis, date of diagnosis, and serum and urine IFE results. Individual patient histories were reviewed for any patients not contained in the database. If a patient had more than one sample tested, only the initial 2003 sample was included in this study. The samples were obtained at the patient's initial presentation, during disease monitoring, or depending on the diagnosis, post treatment. The treatment status of the AL and NSMM patients was determined from the patient history. All queries to the Laboratory Information System, Dysproteinemia database, or patient histories followed a protocol approved by the Mayo Institutional Review Board.

The serum FLC assay was performed on the same day as the venipuncture and was reported to the patient's medical record. The FLC assay (FREELITE™; The Binding Site Ltd.) *(6)* was performed on a Dade Behring BNII automated nephelometer. This assay consists of two separate measurements: one to quantify κ FLCs and the other to quantify λ FLCs. In addition to reporting the κ and λ FLCs, the assay report also contains the FLC κ/λ ratio. Patients with ratios >1.65 have excess κ FLCs and are presumed to be producing clonal κ FLCs. Patients with ratios <0.26 have excess λ FLCs and are presumed to be producing clonal λ FLCs.

Results

Among the 1020 patients with FLC assays performed, 899 (88%) had monoclonal plasma cell disorders (PCDs; Table 1). Among the PCD patients, the most common diagnoses were multiple myeloma (37%), AL (30%), monoclonal gammopathy of undetermined significance (MGUS; 13%), and smoldering multiple myeloma (SMM; 8%). There were 121 patients with FLC results who did not have a monoclonal gammopathy. These patients included 52 non-AL amyloidosis patients who were diagnosed with localized amyloid (n = 23), hereditary amyloid (n = 16), senile amyloid (n = 6), secondary amyloid (n = 3), or amyloid of unknown type (n = 4). The 69 remaining patients with nonmonoclonal gammopathy had peripheral neuropathy, anemia, proteinuria, lymphoproliferative disease, possible AL, and several other miscellaneous conditions.

In all 121 patients with nonmonoclonal gammopathy, the FLC κ/λ ratio was within the values obtained for our reference population (Table 2). Among the PCD patient groups, the FLC results were comparable to published retrospective data for patients with NSMM, AL, LCDD, and MGUS. The 5 untreated NSMM patients all had an abnormal FLC κ/λ ratio. The 6 NSMM patients with normal FLC κ/λ ratios had all received a stem cell transplant, and 5 of the 6 had achieved a complete bone marrow response. Among the 269 AL patients, 110 had not yet received treatment, and the FLC assay had been performed within 120 days of diagnosis. The FLC κ/λ ratio was abnormal in 100 (91%) of these patients. Among the LCDD patients, all 7 patients had abnormal FLC κ/λ ratios. Among the 114 patients with MGUS, 44% had an abnormal FLC ratio, a percentage almost identical to that in a retrospective cohort study of MGUS patients that was balanced regarding whether the patients had progressed to malignant disease *(7)*. Among the 72 untreated SMM patients, 88% had abnormal FLC ratios. The performance of the FLC assay in patients with SMM has not been reported previously.

Table 1. Distribution of PCDs (n = 899).

Diagnosis	No. of cases
Multiple myeloma	330
NSMM	20
Osteosclerotic myeloma	15
SMM	72
Indolent/evolving myeloma	8
Plasmacytoma (solitary)	22
Extramedullary myeloma	5
Multiple solitary plasmacytoma	3
Macroglobulinemia	9
IgM lymphoproliferative disease	2
IgM lymphoma	5
Smoldering macroglobulinemia	2
Primary systemic amyloidosis	269
LCDD	7
MGUS	114
Idiopathic Bence Jones proteinuria	4
Heavy chain disease	2
Cryoglobulinemia	4
Acquired Fanconi syndrome	3
Scleromyxedema	2
Plasma cell leukemia	1

The assay results of the serum FLC κ/λ ratio, serum IFE, and urine IFE for the 110 untreated AL patients are shown in Table 3. As we have seen in retrospective studies, the FLC assay is more sensitive (91%) than the serum or urine IFE assay (69% and 83%, respectively). In addition, the 3 assays are complementary for the detection of monoclonal FLCs in AL patients. If serum and urine IFE assays are evaluated, 95% of AL patient had an abnormal result in at least 1 of the 2 assays. If serum IFE and FLC assays are evaluated, 109 of the 110 AL patients (99%) had an abnormal result in at least 1 of the 2 tests, and use of the urine IFE did not add any information.

Discussion

The diagnostic performance of the FLC assay during the first year of use in our clinical practice closely matched the published retrospective data. These assays were per-

Table 2. FLC results.

	Current study		Retrospective published data		
Diagnosis	n	Abnormal FLC ratio, %	n	Abnormal FLC ratio, %	Reference
Normal			282	0	Katzmann et al. *(1)*
Polyclonal			25	0	Katzmann et al. *(1)*
Nonmonoclonal gammopathy	121	0			
NSMM, untreated	5	100	28	68	Drayson et al. *(2)*
NSMM, treated	15	60			
AL, untreated	110	91	262	98	Lachmann et al. *(3)*
			34	88	Abraham et al. *(4)*
LCDD	7	100	19	89	Katzmann et al. *(1)*
MGUS	114	44	97	43	Rajkumar et al. *(7)*
SMM	72	88			

formed by the clinical laboratory as samples were received, and during this 1-year time frame, we used 2 different reagent lots. Earlier, we chose to define the reference interval for the FLC κ/λ ratio as the interval that included all of the reference population to ensure high specificity *(1)*. The absence of abnormal results in the 121 patients with no monoclonal gammopathy in this study, however, was unexpected. We assume that 2 factors may have contributed to the absence of false-positive results. The first is that most of the requests were from the Division of Hematology and not from general medical practice. As the assay becomes more widely requested, we expect to see false-positive FLC results. The second, and perhaps more important, factor is that in this study some of the clinical diagnoses may have been influenced by the FLC results. (It should be remembered, however, that in AL the diagnosis requires histopathologically confirmed AL amyloid.) With these 2 caveats in mind, we are reassured regarding the diagnostic use of the FLC assay as a tool that is complementary to other laboratory tests.

The current gold standard for detection of a monoclonal FLC is IFE. IFE assays are qualitative, and although their sensitivities may vary among laboratories and among antiserum lots, nonlaboratorians tend to think of them as black or white with no ambiguity in results. The use of a quantitative assay with defined normal cutoffs relies on low assay variability and long-term reagent stability that will yield consistent results as new reagents are prepared. The FLC assay is currently produced by a single manufacturer, and there is no defined international standard. Although we are reassured by the performance of the FLC assay during this 1-year study period, a verifiable standard needs to be developed.

Table 3. Diagnostic performance in AL (n = 110)

Assay	% Positive (CI)[a]
FLC κ/λ ratio	91 (84–96)
Serum IFE	69 (60–78)
Urine IFE	83 (74–89)
Serum IFE + urine IFE	95 (90–99)
FLC κ/λ ratio + urine IFE	91 (84–96)
FLC κ/λ ratio + serum IFE	99 (95–100)
All 3 assays	99 (95–100)

[a] CI, confidence interval determined by the exact binomial distribution.

In addition to the absence of false-positive results, this study has also validated use of the FLC κ/λ ratio as a diagnostic tool in the light chain diseases. The identification of abnormal results in various disease groups closely matches the published retrospective data. The diagnostic results in AL are similar to those in 2 published studies *(3, 4)*. In this study and in our previous retrospective study *(4)*, the sensitivity of the FLC assay in AL was lower than the sensitivity of 98% reported by Lachmann et al. *(3)*. That study used a 95% reference interval for the FLC κ/λ ratio (0.3–1.2) vs our use of a range that encompassed 100% of the reference population (0.26–1.65). None of the 110 AL patients, however, had borderline FLC κ/λ ratios that would have been categorized differently by the 2 criteria. Any differences in the diagnostic sensitivity therefore cannot be explained by the different criteria used to define normal FLC κ/λ ratios and may reflect differences in the patient populations. The slightly higher sensitivity in the group of 5 untreated NSMM patients compared with the sensitivity reported by Drayson et al. *(2)* is most likely an artifact attributable to the small number of patients in this study. In addition, the FLC and IFE data validate our retrospective studies that indicated that the FLC assays are more sensitive than individual serum or urine IFE assays for detection of FLCs in AL and LCDD *(1)*.

Multiple myeloma patients were the largest group of patients in which FLC assays were ordered. We are not aware of any data that suggest that the FLC assay is useful for the diagnosis or monitoring of most myeloma patients. Some observations suggest that measurement of serum FLC may be better than urine protein electrophoresis for monitoring monoclonal light chains in LCMM patients, but no published studies have validated this approach. We assume that during the first year the FLC assay was introduced into routine practice, our hematologists were ordering the assay in myeloma patients simply to get a better understanding of the assay performance.

The third and fourth largest patients groups were MGUS and SMM. We have recently published evidence that results of the FLC assay provide a prognostic indicator for progression of MGUS to malignant disease *(7)*. Those data, however, were not available until late in 2003. The use of FLC assays as part of risk stratification of patients with MGUS may become part of the clinical management of these patients. In addition, the collection of FLC data on the 72 patients with SMM will allow us to evaluate the use of FLC assays as a prognostic marker for progression in SMM.

We conclude that the FLC assay is a reliable clinical laboratory test and is performing as predicted by the retrospective validation studies. In current practice, it is reasonable to perform serum and urine protein electrophoresis and IFE in patients suspected of having multiple myeloma or AL and in patients with unexplained renal disease, cardiac failure, bone fractures, osteolytic lesions, or immune deficiency. If these assays are negative and clinical suspicion remains high, the FLC assay may be performed as an additional diagnostic test. In addition to these diagnostic uses, the FLC assay provides prognostic information in patients with newly diagnosed MGUS and can be performed at diagnosis to identify patients at low risk for transformation to malignant disease. Lastly, the FLC assay is useful for monitoring disease activity in patients with NSMM, LCMM, AL, and LCDD in whom there is no measurable M-spike on serum or urine protein electrophoresis.

This work was supported in part by a research grant (CA62242) from the National Cancer Institute.

References

1. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, et al. Serum reference intervals and diagnostic ranges for free κ and free λ immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437–44.
2. Drayson MT, Tang LX, Drew R, Mead GP, Carr-Smith HD, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood 2001;97:2900–2.
3. Lachmann HJ, Gallimore R, Gillmore JD, Carr-Smith HD, Bradwell AR, Pepys MB, 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.
4. Abraham RS, Katzmann JA, Clark RJ, Bradwell AR, Kyle RA, Gertz MA. 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.
5. Bradwell AR, Mead GP, Carr-Smith HD, Drayson MT. Serum test for assessment of patients with Bence Jones myeloma. Lancet 2003;361:489–91.
6. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, et al. Highly sensitive automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem 2001;47:673–80.
7. Rajkumar SV, Kyle RA, Therneau TM, Clark RJ, Bradwell AR, Melton LJ III, et al. Presence of monoclonal free light chains in the serum predicts risk of progression in monoclonal gammopathy of undetermined significance. Br J Haematol 2004;127:308–10.