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Identification of Amyloidogenic Light Chains Requires the Combination of Serum-Free Light Chain Assay with Immunofixation of Serum and Urine

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BACKGROUND: The diagnosis of systemic immunoglobulin light-chain (AL) amyloidosis requires demonstration of amyloid deposits in a tissue biopsy and amyloidogenic monoclonal light chains. The optimal strategy to identify the amyloidogenic clone has not been established. We prospectively assessed the diagnostic sensitivity of the serum free light chain (FLC) κ/λ ratio, a commercial serum and urine agarose gel electrophoresis immunofixation (IFE), and the high-resolution agarose gel electrophoresis immunofixation (HR-IFE) developed at our referral center in patients with AL amyloidosis, in whom the amyloidogenic light chain was unequivocally identified in the amyloid deposits.

METHODS: The amyloidogenic light chain was identified in 121 consecutive patients with AL amyloidosis by immunoelectron microscopy analysis of abdominal fat aspirates and/or organ biopsies. We characterized the monoclonal light chain by using IFE and HR-IFE in serum and urine and the FLC κ/λ ratio in serum. We then compared the diagnostic sensitivities of the 3 assays.

RESULTS: The HR-IFE of serum and urine identified the amyloidogenic light chain in all 115 patients with a monoclonal gammopathy. Six patients with a biclonal gammopathy were omitted from the statistical analysis. The diagnostic sensitivity of commercial serum and urine IFE was greater than that of the FLC κ/λ ratio (96% vs 76%). The combination of serum IFE and the FLC assay detected the amyloidogenic light chain in 96% of patients. The combination of IFE of both serum and urine with the FLC κ/λ ratio had a 100% sensitivity.

CONCLUSIONS: The identification of amyloidogenic light chains cannot rely on a single test and requires the combination of a commercially available FLC assay with immunofixation of both serum and urine.

Systemic immunoglobulin light-chain (AL) amyloidosis[7] is a progressive disease caused by the deposition of insoluble fibrils formed by the aggregation of circulating monoclonal light chains produced by a usually small-sized bone-marrow plasma cell clone *(1)*. This process causes organ dysfunction and ultimately leads to death. However, effective chemotherapy, which suppresses the production of the amyloidogenic light chain before irreversible organ damage has occurred, can significantly extend survival *(2)*. Thus, early diagnosis is critical in the management of patients with AL amyloidosis. The diagnosis relies on both the demonstration of amyloid deposits in tissues and on the identification of the plasma cell clone producing a light chain, which should be proved amyloidogenic by direct biochemical or immunohistochemical typing of the amyloid deposits. The amyloidogenic clonal plasma cell population can be detected either by bone marrow analysis or by the identification of a monoclonal light chain in the patient's serum and/or urine *(3)*. The identification and quantification of the amyloidogenic light chain is also necessary in the follow-up of patients with AL amyloidosis to assess the hematologic response

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[7] Nonstandard abbreviations: AL amyloidosis, systemic immunoglobulin light-chain amyloidosis; FLC, circulating free light chains; IFE, agarose gel immunofixation electrophoresis; HR-IFE, high-resolution agarose gel immunofixation electrophoresis; IEH, immuno-electron histochemistry; NT-proBNP, N-terminal pronatriuretic peptide type B; cTnI, cardiac troponin I.

to therapy *(3)*. We investigated the optimal strategy to detect the amyloidogenic light chains, maximizing the diagnostic sensitivity. We performed a prospective study on 121 consecutive patients with AL amyloidosis, in whom the amyloidogenic light chain was detected in the amyloid deposits by use of immuno-electron microscopy *(4)* to assess the diagnostic sensitivity of the quantitative assay of circulating free light chains (FLC), of a commercial semiautomated agarose gel electrophoresis immunofixation (IFE) assay, and of the high-resolution agarose gel electrophoresis immunofixation (HR-IFE) technique developed at our referral center. We also assessed the contribution of urine analysis to the diagnosis.

Material and Methods

Study participants were 121 patients with biopsy-proven systemic AL amyloidosis consecutively enrolled between January 2004 and May 2005. None of the patients was previously treated. All the patients had been referred to our institution because of suspected systemic amyloidosis, and the diagnosis of AL amyloidosis was confirmed at our center. In that same period of time, a total of 179 such patients were referred to our center and the diagnosis of AL amyloidosis was not confirmed in 58 (32%) of them. All the patients gave written informed consent, and the study was approved by the review board of the *Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo*. Mutations associated with hereditary amyloidoses were excluded by DNA analysis. The amyloid deposits were characterized in all patients by immunoelectron microscopy analysis of abdominal fat aspirates and/or organ biopsies *(4)*. Six patients (5%) were excluded from the calculation of the diagnostic sensitivity of the tests because HR-IFE detected a biclonal gammopathy.

Serum and urine HR-IFE was performed with anti-IgG, -IgA, -IgM, -κ and -λ antibodies (Dako) on gels freshly prepared at our laboratory, following basically the technique proposed by the Malmö group *(5)*. Briefly, electrophoresis was performed in freshly prepared 1-mm thick agarose (SeaKem ME agarose, Cambrex Bio Science), 7.5 g/L in diethyl barbiturate buffer (75 mmol/L, pH 8.6, with calcium lactate 2 mmol/L). Custom dilution of the patient's serum, and 100-fold concentrated urine sample were placed in 5-mm–width slits, and 20 V/cm was applied until albumin had migrated 6.5 cm. After electrophoresis, filter paper strips soaked with 50 μL of appropriate antiserum were applied to each lane and incubated in a moist chamber for 1 h. The agarose plate was then washed 3 times with saline and once with deionized water, then dried and stained with Coomassie Blue R 250. With this method, the detection limit of a monoclonal component is 50–150 mg/L in serum and 30–100 mg/L in urine, depending on the intensity of the polyclonal background. Semiautomated serum and urine IFE was performed with a commercial Hydragel 2IF/BJ(HR) kit on a Hydrasys apparatus (Sebia). The detection limit reported by the manufacturer varies from 120–250 mg/L, according to the migration of the monoclonal component and to the intensity of the polyclonal background. Serum FLC concentration was measured by latex-enhanced immunoassay (The Binding Site) on a Behring BN II (Dade Behring) nephelometer. The reference intervals for κ and λ FLC are 3.3–19.4 mg/L and 5.7–26.3 mg/L, respectively, and the κ/λ ratio diagnostic range is 0.26–1.65 *(6)*. A patient was classified as producing monoclonal κ or λ light chains according to an abnormal κ/λ ratio *(6)*. Serum FLC immunoassays have a limit of quantification of <1 mg/L *(6)*.

Differences in diagnostic sensitivity were tested for statistical significance by Fisher exact test, and CIs were determined by the exact binomial distribution.

To assess disease severity, the number and type of organs affected by amyloidosis and the cardiac stage, based on serum N-terminal pronatriuretic peptide type B (NT-proBNP) and cardiac troponin I (cTnI), were recorded in patients in whom the amyloidogenic light chain was not identified by both serum IFE and HR-IF, and in those in whom it was missed by assay of the combination of FLC κ/λ ratio and serum IFE. Patients with both NT-proBNP ≤332 ng/L and cTnI <0.1 μg/L were listed as cardiac stage I, those with NT-proBNP >332 ng/L or cTnI ≥0.1 μg/L were defined as cardiac stage II, and patients with both NT-proBNP >332 ng/L and cTnI >0.1 μg/L were defined as cardiac stage III *(7)*.

Results

In 6 of the 121 patients with AL in whom the amyloidogenic light chain was identified by immuno-electron microscopy in the amyloid deposits, HR-IFE revealed a biclonal gammopathy (Table 1). In 4 of these patients, the FLC κ/λ ratio correctly identified the light chain type constituting the amyloid deposits, whereas high-resolution IFE did not identify monoclonal light chains in the urine of 2 patients (no. 117 and 118), and in 2 patients (no. 116 and 121) the opposite light chain was identified. In all the remaining 115 patients, HR-IFE detected the monoclonal amyloidogenic light chain in serum and/or urine and, in all patients, the type of light chain detected corresponded to that identified by immuno-electron microscopy as forming the amyloid fibrils. The amyloidogenic light-chain type was κ in 30 patients (26%) and λ in 85 patients (74%).

The diagnostic sensitivity of the tests is reported in Table 2. In 6 patients (5%) the HR-IFE detected the

Table 1. Results in 6 patients with a biclonal gammopathy.

ID no.	Serum HR-IFE	Urine HR-IFE	Serum IFE	Urine IFE	κ FLC, mg/L	λ FLC, mg/L	FLC κ/λ ratio	IEH typing
116	IgGκ	Free λ	IgGκ	Not detected	79.4	27.2	2.92 (κ)	κ
117	IgMκ/free λ	Not detected	IgMκ/free λ	Not detected	8.84	117	0.07 (λ)	λ
118	IgGκ/IgGλ	Not detected	IgGκ/IgGλ	Not detected	114	28.1	4.06 (κ)	κ
119	Free κ	Free κ/free λ	Not detected	Free κ/free λ	14.8	22.1	0.67 (Normal)	λ
120	IgGκ/free λ	Free λ	Free λ	Free λ	14.9	41.6	0.36 (Normal)	λ
121	IgGκ/IgGλ	Free λ	IgGκ	Not detected	152	27	5.63 (κ)	κ

amyloidogenic light chain only in urine, and in 6 cases (5%) only in serum. Five of the 6 amyloidogenic light chains detected only in urine were of the κ type, whereas 5 of the 6 light chains detected only in the serum were λ. The diagnostic sensitivity of both immunofixation techniques was significantly lower for κ than for λ light chains in serum (HR-IFE 83% vs 99%, $P = 0.004$; IFE 60% vs 87%, $P = 0.003$). Conversely, the FLC assay performed better with κ than with λ amyloidogenic light chains (97% vs 69%, $P = 0.002$). Notably, 5 patients with a FLC κ/λ ratio above the reference limit and κ Bence-Jones proteinuria did not show monoclonal κ light chains in serum with both immunofixation techniques (Table 3).

One patient with a serum IgAλ monoclonal component, λ Bence-Jones protein, and amyloid deposits typed as λ had a κ/λ ratio of 1.72 (κ FLC 146 mg/L and λ FLC 85 mg/L) and was incorrectly classified has having a κ clone by the FLC assay. This patient had renal failure (serum creatinine 221 μmol/L) and polyclonal hypergammaglobulinemia that could account for the increase of both κ and λ FLC. In the overall population the median serum creatinine concentration was 103 μmol/L (range: 44–619 μmol/L), and serum creatinine was within reference limits (males 115 μmol/L, females 97 μmol/L) in 65 patients (56%). In our patient population, renal failure did not significantly influence the diagnostic sensitivity of the κ/λ ratio, which was 78% (95% CI, 67%–87%) in patients with normal renal function and 73% (95% CI, 60%–84%) in patients with serum creatinine above the upper reference limit ($P = 0.58$). Serum creatinine was not significantly higher in patients in whom the FLC κ/λ ratio failed to identify the amyloidogenic light chain (median 100 vs 114 μmol/L, $P = 0.2$). Recently, a new reference interval for the FLC κ/λ ratio (0.37–3.1) has been proposed for patients with abnormal kidney function *(8)*. The diagnostic sensitivity of this reference interval in the patients with serum creatinine above the upper reference limit was 71% (95% CI, 58%–83%), very similar to that obtained with the commonly used reference range. As expected, most of our patients had hypogammaglobulinemia, and only 14 (12%) were found to have polyclonal hypergammaglobulinemia by serum

Table 2. Diagnostic sensitivity of HR-IFE, IFE, and FLC κ/λ ratio in 115 patients with systemic AL amyloidosis.

	Patients with κ clones (n = 30)		Patients with λ clones (n = 85)		Overall population (N = 115)	
	No. positive	% (95% CI)	No. positive	% (95% CI)	No. positive	% (95% CI)
Serum HR-IFE	25	83 (67–94)	84	99 (94–100)	109	95 (89–98)
Urine HR-IFE	29	97 (85–100)	80	94 (87–98)	109	95 (89–98)
Serum and urine HR-IFE	30	100 (90–100)	85	100 (96–100)	115	100 (97–100)
Semiautomated serum IFE	18	60 (42–76)	74	87 (79–93)	92	80 (72–87)
Semiautomated urine IFE	21	70 (52–84)	56	65 (55–75)	77	67 (58–75)
Semiautomated serum and urine IFE	27	90 (75–97)	83	98 (92–100)	110	96 (91–98)
FLC κ/λ ratio	29	97 (85–100)	59	69 (59–79)	88	76 (68–84)
Serum IFE + FLC κ/λ ratio	30	100 (90–100)	80	94 (87–98)	110	96 (91–98)
Urine IFE + FLC κ/λ ratio	29	97 (85–100)	77	91 (83–95)	106	92 (86–96)
Serum and urine IFE + FLC κ/λ ratio	30	100 (90–100)	85	100 (96–100)	115	100 (97–100)

Table 3. Results in 5 patients in whom serum immunofixation failed to identify the amyloidogenic κ light chain.

ID	Sex	Serum HR-IFE	Urine HR-IFE	Serum IFE	Urine IFE	κ FLC, mg/L	λ FLC, mg/L	FLC κ/λ ratio	IEH typing	Creatinine, μmol/L[a]	Organs involved	Cardiac stage[b]
8	F	Not detected	Free κ	Not detected	Free κ	329.0	21.2	15.52 (κ)	κ	94	Heart, liver	II
18	M	Not detected	Free κ	Not detected	Free κ	378.0	56.3	6.71 (κ)	κ	347	Kidney, heart, liver	II
22	M	Not detected	Free κ	Not detected	Free κ	505.0	13.8	36.59 (κ)	κ	138	Heart	III
74	F	Not detected	Free κ	Not detected	Not detected	395.0	27.8	14.21 (κ)	κ	322	Kidney, heart	II
108	F	Not detected	Free κ	Not detected	Free κ	158.0	53.1	2.98 (κ)	κ	394	Kidney, heart	II

[a] Upper reference limit: males 115 μmol/L, females 97 μmol/L.
[b] Stages of cardiac involvement according to Dispenzieri et al. (*7*).

electrophoresis (γ globulin concentrations of 18–24 g/L). The diagnostic sensitivity of the κ/λ ratio was 71% (95% CI, 46%–90%) in patients with hypergammaglobulinemia and 79% (95% CI, 70%–86%) in the other patients ($P = 0.52$).

The diagnostic sensitivity of commercial semiautomated serum and urine IFE was significantly higher than that of the serum FLC test (96% vs 76%, $P < 0.001$). The addition of serum IFE to the FLC κ/λ ratio enabled correct identification of the amyloidogenic light chain in all but 5 patients (4%), all of whom had a λ Bence-Jones protein detectable by urine IFE (Table 4). These 5 patients had symptomatic disease and were in need of prompt treatment. However, the combination of serum and urine IFE and of the FLC κ/λ ratio enabled correct identification of the amyloidogenic light chain in all the patients.

Discussion

The high-resolution immunofixation of serum and urine developed at our referral center enabled us to identify all amyloidogenic light chains in the study population. This result underlines the relevance of state-of-the-art techniques in the investigation of complex, rare diseases and the need for improving commercial immunofixation assays. However, this technique is not widely available. In our series, the diagnostic sensitivity of the FLC κ/λ ratio was 76%, that of commercial IFE of both serum and urine was 96%, and their combination correctly identified the amyloidogenic light chains in 100% of patients.

Previous studies on the diagnostic performance of the FLC assay in AL amyloidosis have led to different results. In the first 2 retrospective series by the United Kingdom National Amyloidosis Centre *(9)* and by the Mayo Clinic *(10)*, the quantitative FLC assay showed a greater diagnostic sensitivity than the association of serum and urine immunofixation electrophoresis (98% vs 79% and 91% vs 81%, respectively). These results were not confirmed in a subsequent retrospective study by the investigators of the Boston Amyloid Program, who reported a lower diagnostic sensitivity of the FLC assay (75%) compared to immunofixation (96%) *(11)*, or in a subsequent study by the Mayo Clinic group *(12)*, in which the FLC assay and the combination of serum and

Table 4. Results in 5 patients in whom the combination of serum immunofixation and κ/λ ratio failed to identify the amyloidogenic light chain.

ID	Sex	Serum IFE	Urine IFE	κ FLC, mg/L	λ FLC, mg/L	FLC κ/λ ratio[a]	IEH typing	Creatinine, μmol/L[b]	Organs involved	Cardiac stage[c]
2	F	Not detected	Free λ	29.0	60.3	0.48 (Normal)	λ	71	Kidney	I
5	M	Not detected	Free λ	11.2	11.7	0.96 (Normal)	λ	156	Kidney	I
39	F	Not detected	Free λ	22.3	37.8	0.59 (Normal)	λ	97	Heart	II
61	M	Not detected	Free λ	9.7	24.7	0.39 (Normal)	λ	94	GI[d]	I
91	F	Not detected	Free λ	53.5	71.3	0.75 (Normal)	λ	126	Kidney, heart, GI, PNS	III

[a] Also applying the recently published (*8*) new reference range for FLC ratio (0.37–3.1) for patients with abnormal renal function, the results do not change.
[b] Upper reference limit: males 115 μmol/L, females 97 μmol/L.
[c] Stages of cardiac involvement according to Dispenzieri et al. (*7*).
[d] GI, gastrointestinal system; PNS, peripheral nervous system.

urine immunofixation electrophoresis had a comparable diagnostic performance (diagnostic sensitivity 91% vs 95%, respectively). More recently, in a study from the Heidelberg group in which the study population included 11% of patients with multiple myeloma, the combination of serum and urine immunofixation had a slightly higher sensitivity than the FLC κ/λ ratio (92% vs 87%), and the 2 tests proved complementary *(13)*.

It is worth noting that none of the previous studies was based on the unequivocal typing of the amyloidogenic protein constituting the amyloid deposits *(1, 3)*. This result is achievable using various techniques, including immuno-electron microscopy *(4)*. In our series of consecutive patients the characterization of amyloidosis as AL type relied on immuno-electron microscopy results. Immuno-electron microscopy also confirmed that the light chain identified in the patients' serum and/or urine was actually amyloidogenic.

The observation of a higher diagnostic sensitivity of the FLC assay for κ than for λ light chains and of a lower resolution of serum immunofixation in patients with κ light chains in our series is in agreement with the results reported by the Boston and the Heidelberg groups *(11, 13)*. This difference may be due to the formation of aggregates of various size and electrophoretic mobility by κ light chains, resulting in the absence of a distinct electrophoretic band. This hypothesis is being tested at our center.

The introduction of the FLC assay also triggered a lively debate on the role of urine immunofixation in the diagnosis of AL amyloidosis. This issue was addressed in the study by Katzmann et al. on 110 patients with AL amyloidosis, in which the association of the FLC assay and serum immunofixation electrophoresis had a 99% diagnostic sensitivity and the addition of urine immunofixation electrophoresis did not improve this result *(12)*. In a subsequent retrospective study on 428 patients with plasma cell dyscrasia in whom a monoclonal light chain was detected in the urine by immunofixation, including 123 patients with AL amyloidosis, Katzmann and coworkers observed that the combination of the FLC assay and serum immunofixation identified a monoclonal light chain in 100% of the patients with amyloidosis *(14)*. However, this study included only patients with positive urine immunofixation, thus increasing the likelihood of an abnormal κ/λ ratio, because a high concentration of FLC is required to produce overflow proteinuria *(15)*. Studies performed on general hospital populations indicate that measurement of serum FLC may replace urine analysis in the initial screening of monoclonal gammopathies *(16, 17)*. The reported results of these studies showed that a false-positive κ/λ ratio was associated with polyclonal increases in immunoglobulin and with renal impairment. In our population, however, renal insufficiency and polyclonal hypergammaglobulinemia did not affect the diagnostic sensitivity of the κ/λ ratio. Also, the study by Bochtelr et al. *(13)*, performed in patients with AL amyloidosis, showed that renal insufficiency did not affect the diagnostic sensitivity of the FLC test. This discrepancy may be attributable to characteristics of the different patient populations. More recently, Dispenzieri et al. reported an unsatisfactory correlation between changes in FLC and urine monoclonal protein in multiple myeloma after chemotherapy, underlying the need for urine analysis in the follow-up of myeloma patients *(18)*. In the present series, the combination of serum IFE with the FLC assay detected the amyloid clone in 96% of the patients. Thus, our results indicate that, in patients with AL amyloidosis, urine immunofixation should also be performed to ensure optimal diagnostic sensitivity. Indeed, omitting urine immunofixation would have led to us missing 6% of patients with λ amyloidogenic clones, an important shortcoming given the severity of the disease and the need for early diagnosis. Only the combination of immunofixation electrophoresis of both serum and urine and the FLC assay was able to detect all the amyloidogenic clones. Semiautomated serum and urine IFE and the FLC test are widely available and should be combined for searching for a monoclonal component in patients with suspected AL amyloidosis.

Finally, it should be noted that in the present series 5% of the patients had a biclonal gammopathy and were not included in the assessment of the diagnostic sensitivity. In the 868 patients with AL amyloidosis evaluated at our center between 1986 and 2007 we observed a comparable proportion (6%) of cases with a biclonal gammopathy detectable with serum and urine HR-IFE. In the 6 patients with a biclonal gammopathy, the κ/λ ratio identified the amyloidogenic light chain in 4 cases (3 κ and 1 λ), whereas immunofixation produced mixed results. However, in patients with a biclonal gammopathy, although an altered FLC κ/λ ratio may suggest which light chain is involved, only the direct typing of the amyloid deposits allows the unequivocal identification of the amyloidogenic light chain, which should be considered the marker of hematologic response to therapy.

The availability of new reliable tools, such as the FLC assay, has dramatically improved the management of AL amyloidosis. Nevertheless, our findings underline the complexity of this disease, which still does not allow reliance on only a few diagnostic tests.

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References

1. Merlini G, Stone MJ. Dangerous small B-cell clones. Blood 2006;108:2520–30.
2. Merlini G, Palladini G. Amyloidosis: Is a cure possible? Ann Oncol 2008;19 Suppl 4:iv63–6.
3. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, 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, April 18–22, 2004. Am J Hematol 2005; 79:319–28.
4. Arbustini E, Verga L, Concardi M, Palladini G, Obici L, Merlini G. Electron and immuno-electron microscopy of abdominal fat identifies and characterizes amyloid fibrils in suspected cardiac amyloidosis. Amyloid 2002;9:108–14.
5. Jeppsson JO, Laurell CB, Franzén B. Agarose gel electrophoresis. Clin Chem 1979;25:629–38.
6. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, 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.
7. Dispenzieri A, Gertz MA, Kyle RA, Lacy MQ, Burrit MF, Therneau TM, et al. Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. J Clin Oncol 2004;22:3751–7.
8. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol 2008;9:11.
9. 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.
10. 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.
11. Akar H, Seldin DC, Magnani B, O'Hara C, Berk JL, Schoonmaker C, et al. Quantitative serum free light chain assay in the diagnostic evaluation of AL amyloidosis. Amyloid 2005;12:210–5.
12. Katzmann JA, Abraham RS, Dispenzieri A, Lust JA, Kyle RA. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. Clin Chem 2005;51:878–81.
13. Bochtler T, Hegenbart U, Heiss C, Benner A, Cremer F, Volkmann M, et al. Evaluation of the serum-free light chain test in untreated patients with AL amyloidosis. Haematologica 2008;93: 459–62.
14. Katzmann JA, Dispenzieri A, Kyle RA, Snyder MR, Plevak MF, Larson DR, et al. Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. Mayo Clin Proc 2006;81:1575–8.
15. Nowrousian MR, Brandhorst D, Sammet C, Kellert M, Daniels R, Schuett P, et al. Serum free light chain analysis and urine immunofixation electrophoresis in patients with multiple myeloma. Clin Cancer Res 2005;11:8706–14.
16. Hill PG, Forsyth JM, Rai B, Mayne S. Serum free light chains: an alternative to the urine Bence Jones proteins screening test for monoclonal gammopathies. Clin Chem 2006;52:1743–8.
17. Piehler AP, Gulbrandsen N, Kierulf P, Urdal P. Quantitation of serum free light chains in combination with protein electrophoresis and clinical information for diagnosing multiple myeloma in a general hospital population. Clin Chem 2008;54: 1823–30.
18. Dispenzieri A, Zhang L, Katzmann JA, Snyder M, Blood E, Degoey R, et al. Appraisal of immunoglobulin free light chain as a marker of response. Blood 2008;111:4908–15.