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Detection of κ and λ Light Chain Monoclonal Proteins in Human Serum: Automated Immunoassay versus Immunofixation Electrophoresis

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Recently, turbidimetric immunoassays for detecting and quantifying κ and λ free light chains (FLC) have become available and are promoted as being more sensitive than immunofixation electrophoresis (IFE) in detecting FLC monoclonal proteins. In this study, we assessed the ability of these turbidimetric assays to detect serum monoclonal proteins involving both free and heavy-chain-bound κ and λ light chains compared to standard immunofixation electrophoresis. Sera demonstrating a restricted band of protein migration (other than a definite M spike) by serum protein electrophoresis (SPE), which may represent early monoclonal proteins, were also examined. When compared to IFE, percent agreement, sensitivity, and specificity for the κ-FLC and λ-FLC were 94.6, 72.9, and 99.5% and 98.5, 91.4, and 99.7%, respectively, in detecting monoclonal proteins involving free and heavy-chain-bound light chains. The majority of sera (73.7%) demonstrating a restricted band of protein migration on SPE demonstrated abnormal IFE patterns suggestive of multiple myeloma or monoclonal gammopathy of unknown significance, but gave normal κ/λ FLC ratios using the turbidimetric immunoassays. In conclusion, the κ and λ FLC assays are significantly less sensitive (72.9 to 91.4%) than IFE, but specific in detecting serum monoclonal proteins. Moreover, the κ/λ ratio has little value in routine screening since the majority of sera with abnormal IFE patterns had normal κ/λ FLC ratios.

Multiple myeloma is a malignant clonal proliferative plasma cell disorder that is characterized by >10% plasma cells in the bone marrow, anemia, lytic bone lesions, and the presence of a serum and/or urine monoclonal protein. In contrast, monoclonal gammopathy of unknown significance is a more benign process associated with a monoclonal protein, but without an increase in bone marrow plasma cells.

Serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) are the essential assays used for identifying and immunotyping monoclonal proteins in patients with multiple myeloma, nonsecretory myeloma, amyloidosis, and monoclonal gammopathy of unknown significance. Turbidimetric and latex-enhanced nephelometric immunoassays to detect and quantify monoclonal κ and λ free light chains (FLC) using polyspecific antibodies have been described (14, 15). Recently, turbidimetric immunoassays for the detection and quantification of κ-FLC and λ-FLC using monospecific antibodies have become available and claim to be 50 to 100 times more sensitive than IFE or SPE in detecting FLC monoclonal proteins (2). It has been reported that these assays can detect the presence of FLC earlier than IFE in patients with nonsecretory myeloma and amyloidosis and are more useful when monitoring patients with free-light-chain disease (1, 3, 4, 10, 12).

In the current study, we sought to determine the sensitivity of the turbidimetric serum κ-FLC and λ-FLC assays compared to the standard IFE method for identifying definite monoclonal proteins involving both free and heavy-chain-bound κ or λ light chains, since the free light chains are typically produced in excess in heavy chain gammopathies. In addition, the IFE and FLC findings for sera demonstrating a restricted band of protein migration on SPE were also studied.

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MATERIALS AND METHODS

Clinical samples. Four hundred eighty-three consecutive sera sent to our laboratory for SPE and IFE testing were included in the study. All patient samples included in this study were processed according to the University of Utah Institutional Review Board-approved protocol 11409 to meet the Health Information Portability and Accountability Act patient confidentiality guidelines. No specific clinical information was available for these sera.

Serum protein electrophoresis. SPE was performed by capillary zone electrophoresis using equipment (Paragon capillary zone electrophoresis 2000) and reagents purchased from Beckman Coulter Inc. (Brea, CA). Serum protein electrophoresis by capillary zone electrophoresis is intended for the in vitro separation of human serum proteins through a fused silica capillary. The application of high voltage to the diluted serum sample causes the protein components to migrate through the capillary at differing rates. As the proteins pass the capillary window, detection is accomplished by monitoring the UV absorption at a wavelength of 214 nm. The absorbance is proportional to the peptide bonds present. An electropherogram of absorbance versus time is produced. Calculation of the relative percents of the protein fractions present in the sample is based on the absorbance recorded within a specified time period. Capillary zone electrophoresis has been shown to be a more sensitive method than agarose gel electrophoresis especially for monoclonal proteins of the immunoglobulin A isotype (5–8, 11).

Immunofixation electrophoresis. IFE testing was performed using equipment (HYDRASYS) and reagents (Hydragel IF; acid violet) purchased from Sebia (Norcross, GA). This assay was performed as stated in the manufacturer's product insert. Depending on the type of monoclonal protein, the lower limit of detection for this assay ranges from 12 to 25 mg/dl. Electropherograms were visually evaluated followed by final assessment and clinical interpretation.

Serum kappa and lambda free light chains by turbidimetry. Measurement of kappa and lambda free light chains by rate turbidimetry was performed using reagents (FREELITE) donated by the Binding Site (San Diego, CA) for use on the IMMAGE nephelometer (Beckman Coulter Inc., Brea, CA). These assays were performed as stated in the manufacturer's product insert. The lower limits of detection for the κ- and λ-FLC assays are 0.3 and 0.4 mg/dl, respectively. Normal reference intervals established by the manufacturer (9) for the FLC

TABLE 1. Correlation between κ-FLC turbidimetry and immunofixation electrophoresis in detecting free and heavy-chain-bound κ-MP in 463 sera[a]

κ-FLC turbidimetry result	IFE result	
	κ-MP (+)	κ-MP (−)
Abnormal	62	2
Normal	23	376

[a] Agreement, 94.6%; sensitivity, 72.9%; specificity, 99.5%.

TABLE 3. Correlation between λ-FLC turbidimetry and immunofixation electrophoresis in detecting free and heavy-chain-bound λ-MP in 463 sera[a]

λ-FLC turbidimetry result	IFE	
	λ-MP (+)	λ-MP (−)
Abnormal	64	1
Normal	6	392

[a] Agreement, 98.5%; sensitivity, 91.4%; specificity, 99.7%.

assays were utilized and are as follows: κ-FLC = 0.33 to 1.94 mg/dl; λ-FLC = 0.57 to 2.63 mg/dl; κ/λ ratio = 0.26 to 1.65. In order to rule out the possibility of antigen excess (prozone), sera possessing M proteins by SPE and IFE that produced low FLC values were repeated at a higher dilution.

Theses assays have been adapted for use on several analyzers and analytical/harmonization issues between two of them have been discussed (13). During our initial validations of the FREELITE assays using the Beckman IMMAGE nephelometer, we discovered that the latex particles in the reagent were adhering to the inner walls of the reaction cuvettes and producing an opaque film. The same analyzer (Beckman IMMAGE) was utilized by the developer, but this potential problem was not discussed in the original publication (2). If reagent cuvettes are not washed and replaced on a regular basis, assay precision is affected. We recommend performing wash cycles before and after each FLC run and replacing cuvettes every 2,000 tests. All sera in this study with discrepant FLC results by turbidimetry were repeated twice to ensure accurate results.

RESULTS

The serum κ-FLC assay showed an agreement of 94.6% but a sensitivity of only 72.9% and was 99.5% specific when compared to the standard serum IFE method for detecting definite kappa-type monoclonal proteins (Table 1). Of the 85 sera with κ-type monoclonal proteins on IFE, there were two light-chain- and 21 heavy-chain-bound (14 immunoglobulin G [IgG], four IgA, and three IgM) which demonstrated normal levels of κ-FLC (0.33 to 1.94 mg/dl) by turbidimetry (Table 2). Only 27 of these 85 (31.8%) sera produced abnormal κ/λ ratios (Table 2). In contrast, only 2 of 378 (0.5%) sera with normal patterns on IFE demonstrated abnormal concentrations of κ-FLC as well as abnormal κ/λ ratios (Table 1). One of these two sera demonstrated a restricted band of protein migration in the gamma region on SPE and produced a κ/λ ratio of 4.02. The second sample showed only a slight decrease in gamma on SPE and produced a κ/λ ratio of 10.27.

The serum λ-FLC assay showed an agreement of 98.5% and was 91.4% sensitive and 99.7% specific when compared to the standard serum IFE method in detecting definite λ-type monoclonal proteins (Table 3). Of the 70 sera with λ-type monoclonal proteins on IFE, there were six heavy-chain-bound (four IgG, one IgA and one IgM) that contained normal levels of λ-FLC by turbidimetry (Table 4). Only 22 of these 70 (31.4%) sera had abnormal κ/λ ratios (Table 4). In contrast, only 1 out of 393 (0.3%) sera with normal patterns on IFE had abnormal concentrations of λ-FLC as well as an abnormal κ/λ ratio (0.20). This sample also demonstrated a normal pattern on SPE.

There were 20 sera that demonstrated biclonal protein patterns on IFE (Table 5). Seventeen of 20 (85.0%) and 16 of 20 (80.0%) of these sera were found to have elevated levels of κ- and λ-FLC, respectively, but only 4 of 20 (20.0%) had abnormal κ/λ ratios (Table 5).

We next investigated the sensitivity of the κ- and λ-FLC assays using sera that had demonstrated a restricted band of protein migration (other than a definite M spike) in the gamma or beta region that are commonly noted on SPE. Ninety-five of the 483 (19.7%) sera tested had a restricted band of protein migration on SPE. Twenty-two of the 95 (23.2%) sera either demonstrated a normal pattern ($n = 9$) or had a polyclonal increase ($n = 13$) involving one or more immunoglobulins on IFE (Table 6). Only 1 of these 22 (4.5%) sera had an elevated FLC (κ) and an abnormal κ/λ ratio (this discrepant sample has been described previously in the above data for κ-FLC). The remaining 73 (76.8%) sera contained monoclonal (κ, $n = 30$; λ, $n = 30$) or biclonal ($n = 13$) proteins with free or heavy-chain-bound κ and/or λ light chains on IFE (Table 6). The κ-FLC and λ-FLC assays, therefore, detected 32 of 43 (74.4%) and 41 of 43 (95.3%), respectively, sera having monoclonal or biclonal proteins on IFE, but only 3 of these 73 (4.1%) had abnormal κ/λ ratios (Table 6).

DISCUSSION

In the current study, we have shown how new quantitative turbidimetric assays for detecting serum κ-FLC and λ-FLC compare to a standard serum IFE method in a laboratory setting for identifying monoclonal proteins involving both free

TABLE 2. κ-FLC turbidimetry results for 85 sera with free or heavy-chain-bound κ-MP by immunofixation electrophoresis

IFE	No. of κ-MP	No. of abnormal κ-FLC (%)	No. of abnormal κ/λ ratios (%)
IgG	55	41 (74.5)	17 (30.9)
IgA	10	6 (60.0)	5 (50.0)
IgM	10	7 (70.0)	2 (20.0)
IgG + IgM	2	2 (100.0)	0 (0.0)
κ-FLC	8	6 (75.0)	3 (37.5)
Total	85	62 (72.9)	27 (31.8)

TABLE 4. λ-FLC turbidimetry results for 70 sera with free or heavy-chain-bound λ-MP by immunofixation electrophoresis

IFE	No. of λ-MP	No. of abnormal λ-FLC (%)	No. of abnormal κ/λ ratios (%)
IgG	26	22 (84.6)	10 (38.5)
IgA	6	5 (83.3)	4 (66.7)
IgM	19	18 (94.7)	5 (26.3)
λ-FLC	19	19 (100.0)	3 (15.8)
Total	70	64 (91.4)	22 (31.4)

TABLE 5. FLC turbidimetry results for 20 sera with biclonal proteins by immunofixation electrophoresis

IFE	No. of biclonal proteins	No. with abnormal κ-FLC (%)	No. with abnormal λ-FLC (%)	No. with abnormal κ/λ ratios (%)
IgG(κ)/IgG(λ)	10	8 (80.0)	8 (80.0)	1 (10.0)
IgG(κ)/IgM(λ)	4	4 (100.0)	4 (100.0)	1 (25.0)
IgM(κ)/IgG(λ)	1	1 (100.0)	1 (100.0)	1 (100.0)
IgM(κ)/IgM(λ)	3	2 (66.7)	3 (100.0)	1 (33.3)
IgG + IgM(κ)/IgA(λ)	1	1 (100.0)	1 (100.0)	0 (0.0)
IgG + IgM(κ)/IgG + IgM(λ)	1	1 (100.0)	1 (100.0)	0 (0.0)
Total	20	17 (85.0)	16 (80.0)	4 (20.0)

and heavy-chain-bound κ and λ light chains. Since it is possible for samples to have an abnormal concentration of FLC with a normal κ/λ ratio, sensitivities for the serum FLC assays were based on their ability to demonstrate an abnormal concentration of the appropriate FLC as determined by IFE. For specificity purposes, sera having an abnormal FLC and κ/λ ratio with normal patterns on IFE were considered false positive, but patient sera with these results may represent true positives that were missed by IFE. The results for 20 sera with biclonal proteins were examined separately (Table 5) and were excluded from the monoclonal data.

The majority of discrepant sera between the two methods demonstrated abnormal IFE patterns indicative of multiple myeloma or monoclonal gammopathy of unknown significance, but had normal levels of FLC with normal κ/λ ratios by turbidimetry. Even though the FREELITE assays are intended for the detection and quantitation of κ and λ "free" light chains, one would also expect to detect the excess FLC often produced in patients with monoclonal proteins consisting of a heavy and a light chain. Discord between the two methods is most likely due to patient sera that are composed only of heavy-chain-bound light chains.

There were two sera with significantly abnormal concentrations of κ-FLC (20.00 and 15.40 mg/dl) as well as abnormal κ/λ ratios (4.02 and 10.27) that had normal IFE patterns. These sera may have been from patients with nonsecretory myeloma or primary amyloidosis that is sometimes missed by standard electrophoretic methods (1, 4). There was one similar discrepancy for λ-FLC, but this serum showed a moderate elevation (6.01 mg/dl) of λ-FLC and generated a less dramatic κ/λ ratio (0.20) that was slightly under the cutoff (0.26) established by the manufacturer (9).

TABLE 6. Immunofixation electrophoresis and FLC turbidimetry results for 95 sera having a restricted band of protein migration by serum protein electrophoresis

IFE	No. of sera	No. of abnormal κ-FLC (%)	No. of abnormal λ-FLC (%)	No. of abnormal κ/λ ratios (%)
IgG(κ)	19	12 (63.2)	12 (63.2)	0 (0.0)
IgA(κ)	2	2 (100.0)	2 (100.0)	1 (50.0)
IgM(κ)	4	2 (50.0)	3 (75.0)	1 (25.0)
IgG + IgM(κ)	2	2 (100.0)	2 (100.0)	0 (0.0)
κ-FLC	3	2 (66.7)	3 (100.0)	0 (0.0)
IgG(λ)	9	5 (55.6)	7 (77.8)	0 (0.0)
IgM(λ)	11	5 (45.5)	11 (100.0)	1 (9.1)
λ-FLC	10	8 (80.0)	10 (100.0)	0 (0.0)
Biclonal (κ + λ)	13	12 (92.3)	13 (100.0)	0 (0.0)
Polyclonal increase	13	9 (69.2)	11 (84.6)	0 (0.0)
Normal pattern	9	6 (66.7)	7 (77.8)	1 (11.1)

Patient sera often demonstrate a restricted band of protein migration in or near the gamma region on SPE which is not prominent enough to designate as an M protein or M spike. The majority (76.8%) of sera in this category actually contained monoclonal or biclonal proteins by IFE. It would be useful if the κ- and λ-FLC assays could be used to screen and identify M proteins in these sera by their κ/λ ratio, but the majority (95.9%) of these sera had a normal κ/λ ratio (Table 6).

Our data in this study show that the turbidimetric FLC assays are not suitable for routine screening of patients suspected of having multiple myeloma. Even though an excess of FLC was detected in the majority of sera with heavy-chain-bound light chains, less than half had an abnormal κ/λ ratio. Moreover, the majority of sera that demonstrated "free" light chains (not bound to heavy chain) by IFE had a normal κ/λ ratio (Tables 2 and 4). The κ- and λ-FLC assays seem to be most useful in identifying patients with nonsecretory myeloma and primary amyloidosis and for monitoring light chain disease (1, 3, 4, 10, 12), which limits their clinical utility.

In our opinion, IFE remains the primary method for detecting and characterizing monoclonal proteins. Visualization of the serum kappa or lambda light chain on immunofixation electrophoresis is essential for making the initial diagnosis rather than measuring the kappa/lambda ratio. The FLC turbidimetric assays are likely of real value for detecting patients with pure light chain disease (which represents about 25% of monoclonal proteins) and may also be utilized for monitoring disease activity in those patients with light chain disease confirmed and characterized by IFE.

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