

Nephelometric measurement of serum free light chains in nonsecretory myeloma

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Introduction

Nephelometric assays specific for immunoglobulin free light chains (flc) have recently been reported as useful for the diagnosis and monitoring of light chain monoclonal gammopathies. In one study (Blood. 2001; 97; 2900-2902), sera from patients classified as having nonsecretory myeloma (NSM) were found to contain abnormally high concentrations of immunoglobulin free light chains (flc). In some instances, the nephelometric assays utilised in this study measured flc concentrations >900mg/L although no monoclonal bands were visible by immunofixation.

	Free κ: mg/L	Free λ: mg/L	κ/λ ratio:	Bone marrow plasma cells, %	Other Results
Normal sera	8.4 (3.6-15.9)	14.5 (8.1-33)	0.60 (0.36-1.0)	-	-
Twelve: elevated free κ and increased κ/λ ratio					
1	1754	1.6	1096	85	IFE κ +/-
2	1201	3.6	333	82	BJP κ +/-
3	935	11	85	70	
4	487	6.6	74	20	
5	931	13.2	71	>90	IFE κ +/-
6	730	11.1	65	35	
7	978	19.4	50	65	
8	920	26.3	35	14*	
9	789	25.6	31	>50	BJP κ +/-
10	480	23.8	20	30	
11	151	11.5	13	66	Hist κ+ve
12	79.8	30.8	2.6	50	
Seven: elevated free λ with reduced κ/λ ratio					
13	11.2	196	0.057	20	IFE λ+
14	2.7	50.9	0.053	74	
15	2.6	61	0.043	6	
16	17.8	624	0.029	8	IFE λ
17	3.8	144	0.026	70	
18	7.7	389	0.019	60	
19	2.8	481	0.005	29	IFE λ+
Four: suppression of either κ, λ or both flc					
20	4.5	6	0.75	21	
21	1.2	1.6	0.75	55	
22	2.4	8.1	0.296	34	
23	3.6	13.1	0.274	70	
Five: κ or λ normal or borderline and normal κ/λ ratio					
24	16.2	23.4	0.692	67	
25	20.7	33	0.627	73	
26	77	142	0.543	18	IFE λ+
27	8.3	17.4	0.477	9	
28	8.6	25.2	0.341	80	

IFE +/- indicates immunofixation electrophoresis weak diffuse band; IFE +, weak narrow band; BJP +/-, low concentration Bence Jones protein in urine; Hist, immunohistochemical confirmation of multiple myeloma.

*Trepine biopsy positive for myeloma

Table: Reclassification of 28 patient sera, originally thought to have nonsecretory myeloma

The aim of this study was to ascertain whether the flc-specific nephelometric assays were over-reading the NSM sera or whether some property of the monoclonal light chain was affecting its immunofixation/electrophoretic detection. The presence of flc polymers has been noted in a number of studies and polymers could amplify the reading of a nephelometric assay whilst variably sized polymers could prevent the formation of clear bands by electrophoresis.

Methods & Results

Sera from two patients with NSM (with flc concentrations >900mg/L by nephelometry but no band / very weak band by immunofixation) were fractionated by gel filtration chromatography and nephelometric assays of the fractions revealed various flc polymers up to 200 kDa in size. However, a similar analysis of 2 light chain only myeloma sera (with clear monoclonal bands by immunofixation) indicated a similar distribution of polymers. Attempts to disrupt any potential polymers before electrophoresis by treatment with mercaptoethanol, sonication and sonication in the presence of detergent, failed to improve the resolution of bands by immunofixation. Similarly, sonication did not alter the results of the nephelometric assays.

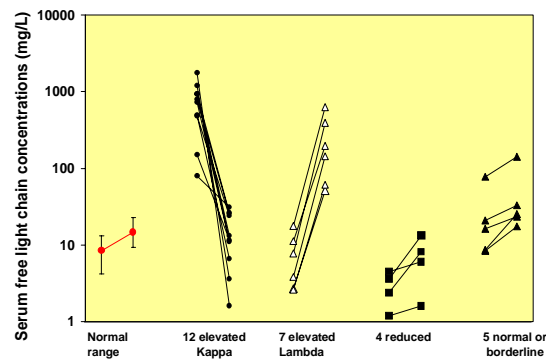


Figure 1. Graphical representation of the flc results for the 28 nonsecretory myeloma patients sera

Gel filtration chromatography was used to purify flc dimer and monomer from 2 urines, in sufficient quantities for them to be measured by flc nephelometry and total protein assay. The nephelometric assays of flc dimer did give higher protein estimates than the total protein assay but the difference between dimer and monomer was only 1.5 fold. In addition, by utilising an antibody affinity column made from flc-specific antibodies, flc were purified from one NSM serum and three other multiple myeloma sera. The flc concentration in the 4 sera was measured nephelometrically before purification and by total protein assay after purification (with corrections applied after gel electrophoresis assessment of purity). The results indicated a comparative (nephelometric) over-reading of the NSM serum of between 1.5 fold and 3.5 fold, relative to the other myeloma sera.

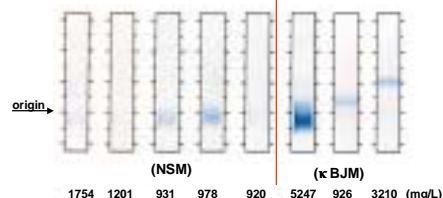


Figure 2. IFE of six nonsecretory myeloma (NSM) and two kappa Bence Jones myeloma (κBJM) sera fixed with a-total kappa antisera. Free kappa values using The Binding Site Ltd FREELITE™ assay are shown in mg/L. Clear monoclonal bands are present only in the κBJM sera.

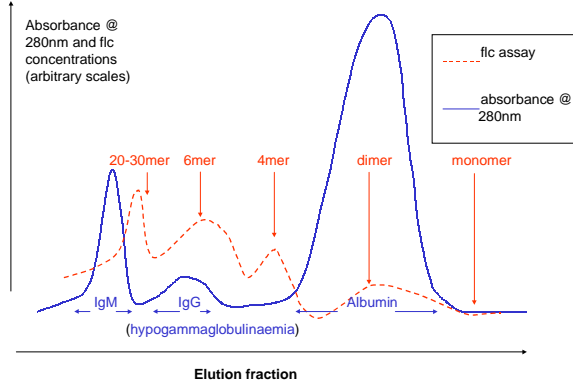


Figure 3. Gel filtration elution profile (S300 Pharmacia) of serum from nonsecretory myeloma patients. Total protein values (absorbance @ 280nm) and free light chain concentrations (nephelometric assay) of the fractions are plotted on arbitrary scales.

Conclusions

Although the gel-filtration analysis indicated similar degrees of flc polymerisation in both NSM and light chain multiple myeloma sera, the preliminary comparisons of nephelometric flc and total protein assays suggested there was some degree of over-reading by the nephelometric assays. However, an over-reading of approximately ten-fold would be necessary to explain the apparent discrepancy between the nephelometric and electrophoretic results for some NSM sera. Variable mutations or deletions in the light chain proteins could potentially give very diffuse electrophoresis/immunofixation bands while not affecting the nephelometric results. Investigation of this possibility is necessary.