journal homepage : https://acclmp.com/

Journal of Applied Biochemistry & Laboratory Medicine (2020) 01 (1):7-9

Review Article

Serum Free Light Chain Assay in Detection of Monoclonal Gammopathies : Relative Under-Detection of Lambda Dominant Kappa/Lambda Ratio

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ARTICLE INFO

Keywords :
Plasma cell dyscrasia,
free light chain,
protein electrophoresis,
immunofixation

How to cite this article
Banerjee D. Chances and Risk of Under-Detection of Lambda Dominant Kappa/Lambda Ratio Monoclonal Gammopathies using Serum Free Light Chain Assay predominantly. Journal of Applied Biochemistry & Laboratory Medicine 2020; 01 (1):7-9
Access link for this article
https://jablm.acclmp.com/2020/01/01_7-9.pdf

ABSTRACT

Plasma cell dyscrasias encompass a broad spectrum of diseases ranging from monoclonal gammopathy of undetermined significance (MGUS) to the potentially curable solitary plasmacytoma to the life-threatening conditions of multiple myeloma (MM) and light chain amyloidosis (AL). In the early 2000s an assay that measured serum free light chains (FLC) was developed. FLC assay is indicated in the evaluation and management of multiple myeloma and related plasma cell disorders (PCD). However, there are false positive and false negative test results due to several reasons. This study was performed to assess the frequency of false negative normal FLC ratio in presence of monoclonal gammopathy detected by serum protein and immunofixation electrophoresis. Serum FLC ratio was normal in 21.3% cases of monoclonal gammopathy cases that were detected by concomitant serum protein and/or immunofixation electrophoresis. Most of the cases with falsely normal FLC ratio were monoclonal gammopathies of lambda light chaintype.

Introduction:

Plasma cell dyscrasias include a wide range of diseases. It includes a wide range of hematological disorders. While monoclonal gammopathy of undetermined significance (MGUS) is on the lower spectrum of these disorders then the potentially curable solitary plasmacytoma, the life-threatening multiple myeloma (MM) and light chain amyloidosis (AL) form the most critical upper range. An estimation of circulating monoclonal immunoglobulins is one of the major diagnostic approach for a definitive diagnosis followed by an effective management and prognostic approach for each of these disorders. These diagnostic methods included mainly the serum and urine electrophoresis for estimation of the monoclonal immnunoglobulins until 1990. Some tests also included immunofixation electrophoresis (SIFE and UIFE) and nephelometric measurement of total immunoglobulins of serum. Although, these tests were able to diagnose a majority of MGUS and MM patients, they could not diagnose majority of patients with AL with sufficient accuracy and precision. Congruent to this more than the 3% of myeloma patients with non-secretory or oligo-secretory myeloma showed the similar trend. To complement these deficiencies, an assay for measuring the serum free light chains (FLC) was developed in the early 2000s [1] Typically characteristic polyclonal antibodies generated in this technique exhibited reaction with only those epitopes showing no association with the heavy chains. When associated with heavy chain, these epitopes become buried by the heavy chain and so they cannot be bound by these novel polyclonal antibodies. In normal conditions, renal glomeruli clear the serum FLCs and the proximal tubules metabolize them. So, the final serum concentrations of FLCs indicate the balance between the amount of production by plasma cells and their renal clearance thereafter[2] Under normal conditions the kidneys metabolize almost 1030g of FLC per day in comparison to normal plasma cell production of 0.51g per day in our body [3] Therefore, the serum FLC concentrations need to be elevated several times before the absorption limits are surpassed. This principle is the major contributing factor in imparting importance to this methodology for assay of FLCs recently. The methodology of serum FLC assay (FREELITE, The Binding Site Ltd., Birmingham, UK) is dependent on a reagent set of polyclonal antibodies available commercially. The major method used is the immunonephelometry that can be easily programmed and executed on a number of automated laboratory instruments [1] This assay has two major components: one to estimate kappa (k) FLC and the other to estimate lambda (l) FLC, both quantitatively. The reference interval with a 95% confidence interval for the kappa FLC was 3.319.4mg/l, while for the light FLC it was 5.726.3mg/l. The reference interval with 95% confidence limit was 0.31.2 for the k/l ratio. But, however, to include 100% of donors the normal diagnostic range for FLC k/l has been proposed to be 0.261.65.4 Reports from a multi-centric Indian study5 indicated the reference range concentrations slightly higher for the kappa and lambda FLCs. It was slightly higher in comparison to those published by Katzmann et al[4] Importantly, the concentration of the κ sFLCs was slightly higher than that of λ sFLCs, due to which the κ/λ sFLC ratio (0.36 2.33, 95% range) was found to be higher than that reported by the American study[1].The authors explained these findings by underscoring that the glomerular filtration rates of normal reference Indian subjects are lower than their Western counterparts. The important fact is that a severe renal impairment alters the dynamics of FLC clearance significantly. As GFR is reduced, the clearance of FLCs decreases. With advancement of the renal disorder, half-lives of κ FLCs and that of λ FLCs tend tobecome identical and as a result, their serum levels become more dependent on their underlying production rates. Consequently, the concentration of κ sFLCs increases greater than λ sFLCs in the serum. In a minority of patients the κ/λ ratio can exhibit higher concentration than the normal reference interval in the absence of monoclonal gammopathy[5].

Article submitted: 1st September, 2020
Revised article submitted : 10th October, 2020
Article accepted : 12th October, 2020.

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Hence, with an increase in stages of CKD, the median sFLC ratios showed elevation in a progressive manner. This led to the acceptance of the reference interval of 0.37 - 3.10 for a κ/λ sFLC, termed the "renal reference interval", in the patients with renal impairment[6] Although, sera from the patients having polyclonal hypergammaglobulinemia or renal impairment often show an elevated k FLC and l FLC due to increased synthesis or reduced renal clearance, the k/l FLC ratio (rFLC), however, usually remains within its normal reference range in these conditions [4].

FLC assay is supposed to be useful for evaluating and management of multiple myeloma and related plasma cell disorders (PCD). The baseline FLC value is given most weightage in virtually every PCD. Furthermore, it is also much useful for an initial screening for monoclonal gammopathy and quantitative monitoring of patients with oligo-secretory PCD. This includes AL, oligo-secretory myeloma and nearly two-thirds of patients supposed to have non-secretory myeloma. it is important to note that measurements of both monoclonal intact immunoglobulin and sFLC are essential for diagnosis and follow-up of IIMM as about 80% of all multiple myeloma (MM) patients produce monoclonal intact immunoglobulins, with 95% of these also producing monoclonal serum free light chains (sFLCs) forming the basis of a positiveresponse criteria.

Not only the presence of an abnormal FLC ratio, the extent to which the FLC ratio is abnormal also helps in predicting the risk of progression in the disorders like MGUS and other related disorders like smouldering multiple myeloma, amyloid light-chain (AL) amyloidosis, and solitary plasmacytoma.[7,8]. According to the reports of Dispenzieri and colleagues [8] patients with smouldering multiple myeloma and an altered to unaltered FLC ratio of 8 or more is an important marker as it heralds a 40% risk of poor prognostic outcome. Moreover, this worsening is evident within the first 2 years from diagnosis. Following this, Larsen and colleagues carried out their observations with 586 patients with smouldering multiple myeloma patients and made an effort to ascertain the threshold value of FLC ratio beyond which there is an 80% chance of progression to multiple myeloma or related malignant disease within 2 years. They observed this threshold value of FLC ratio as 100 in 90 (15%) patients of the total cohort. The FLC level in the affected ones was higher than the reference range in everyone. They described the progression as follows: the risk of progression to multiple myeloma was found to be 72% while the risk of progression to multiple myeloma or AL amyloidosis was reported to be 79%. Furthermore, when they carried out a joint assessment involving both the ratio and the quantitative FLC values they found that an FLC threshold ratio of 100 along with an involved FLC threshold value of 1000 mg/L increased the poor prognostic outcome to 82% at 2 years and 93% at 3 years for both multiple myeloma or AL amyloidosis. Most importantly, when an absolute FLC level threshold was considered in addition to the FLC ratio, it improved positive predictive value of the FLC assay, but lowered its sensitivity. It was revealed also that 27% of patients with an FLC threshold value of 100 had acute renal failure as the major myeloma-defining event. The importance of all these observations lie in the fact that they pointed out to the conclusion that an FLC threshold ratio of 100 performs as an important predictor of adverse outcome in smouldering multiple myeloma and hence a condition of multiple myeloma requiring therapy should be considered in such patients with due attention given accordingly. These findings have been strengthened by other studies world wide as described in the work by Kastritis and colleagues [9] from the Greek Myeloma Group. This study group reported that seven percent of patients out of ninety six patients of smouldering multiple myeloma showed an FLC ratio more than its threshold value of 100. Moreover, they also found that almost all of these patients showed significant poor outcome within the next 18 months. To reduce the possibility of error in all of these interpretations the new criteria has been modified to have a minimum involved FLC level of at least 100 mg/L. This is explained by the fact that a significantly altered k/l FLC ratio should clearly reflect a plasma cell neoplastic lymphoproliferative) disorder that secretes excess FLC leading to alteration in the normal balance between k and l secretion. However, as in all investigative procedures, there are some false positive and false negative outcomes due to several reasons. This study was performed to assess the frequency of false negative normal FLC ratio in presence of monoclonal gammopathy detected by serum protein and immunofixation electrophoresis.

Materialsand Methods

This study was conducted at a stand-alone clinical pathology laboratory in Kolkata, India from August 2019 to July 2020. Free light chain assays were requested for 535 patients (320 male and 215 female patients, M: F= 1.5:1) patients, either for screening because of clinical suspicion of plasma cell neoplasm or for monitoring known myeloma patients under therapy. Serum protein electrophoresis (SPEP) and/or serum immunofixation electrophoresis (SIFE) were advised in all cases. Urine protein or urine immunofixation electrophoresis were not advised in any of the cases. SPEP was carried out using a Hydrasis (Sebia, france) instrument and by using gels procured from Sebia (Hydragel 7). SIFE was also carried out using Hydrasis (Sebia, France) instrument and Hydragel 2 IF gels. Serum free light chains were assayed using Cobas 6000 analyzer, with reagent kits procured from the Binding Site (Birmingham, UK). The laboratory performing these and related tests is accredited bythe NABL.

Results :

Monoclonal gammopathy was detected in 127 (23.7%) cases using all the three techniques. Out of these, 100 (78.7%) cases showed altered FLC ratio and in 27 (21.3%) cases the monoclonal gammopathy was detected by SPEP and/or SIFE, while FLC ratio was normal. The ratio was greater than 100 in 22% cases and less than 0.01 in 14% cases with altered FLC ratio. The highest FLC ratio was 2695.25 and the lowest ratio was 0.012. Serum IFE was advised in 15 out of the 27 cases having normal FLC ratio. One case had bi-clonal (IgG kappa and IgALambda) gammopathy detected by IFE. Only 3 cases (21.4%) had kappa and 11 cases (78.6%) had lambda light chains out of the remaining 14 cases in which SIFE were done.

Discussion

Assay of free light chains for screening the suspected cases of plasma cell neoplasms and monitoring therapeutic response are becoming the mainstayinvestigations dayby day. However, this trend is found to be associated with a decrease in request for urine protein and immunofixation electrophoresis. To overcome any shortfall in diagnostic procedure, the International Myeloma Working Group (IMWG) has recommended an algorithm including SPEP/SIFE and UPEP/UIFE for the investigation of suspected monoclonal gammopathies [10-12]. However, among them UPEP/UIFE is not performed in many as they should be. This underutilization of UPEP/UIFE has been further complicated by an accompanying erroneous idea that SFLCA can replace UPEP/UIFE [13,14]. Monoclonal gammopathy was detected in 23.7% cases in this study by doing FLC assay and SPEP and/or SIFE. In 36% cases of monoclonal gammopathydetected with altered FLC ratio, a veryhigh (>100) or very low (<0.01) ratio was detected that served as a potent biomarker for multiple myeloma. However, the possibility of cases remaining undetected due to exclusion of UPEP and UIFE may be significantly high. Hence, we opine that although free light chain assay is a major advance, its limitations should be always kept in mind [15] First, there can be significant lot-to-lot variation (1920% CV) between different batches of polyclonal FLC antisera which may result in an incosistent immunoreactivity of individual monoclonal FLCs giving imprecise outputs. Second, some of the monoclonal light chains like particularly k FLC, do not exhibit a linear dilution and so may be underestimated if additional off line dilutions are not performed [15]. Third, excess antigen can result in a falsely lowered serum FLC values when measured using nephelometric techniques, which necessitates manual dilution for improving itsits detection limit for detection of

clinically suspicious samples. Furthermore, when light chains show variations in their amino acid sequence, certain light chain epitopes become non detectable to the FLC reagents, but can be detected using immunofixation or even electrophoresis. On the other hand, exxagerated polymerization can lead to an overestimation byas much as 10-fold. A significantly altered k/l rFLC strongly indicates to a plasma cell neoplasm(or lymphoproliferative disorder) that results in excessive secretion of FLC and thereby changes the balance between k and l chain secretion [16]. However, these results are associated with significant false positive and false negative results. The FLC ratio has been found to be normal in 5% cases of intact immunoglobulin monoclonal gammopathy. The engraftment/healing process is often accompanied by the development of oligoclonal pattern in patients treated with ASCT, which may be with or without a detectable neoplastic monoclonal protein. In those oligoclonal conditions, values of serum free kappa chains sometimes overwhelm the free light chains content in the serum due to over-production of the kappa variety leading to high κ/λ ratio in patients with lambda chain myeloma. About 15% of the lambda chain myelomas following ASCT exhibit such an aberrant κ/λ ratio, whereas an aberrant lambda dominant κ/λ ratio in patients with kappa chain myelomas was not detected in any [17].

In the present study we found that 21.3% cases detected with monoclonal gammopathy had normal FLC ratio. Among all, one had bi-clonal (Kappa and lambda) gammopathy that produced a normal FLC ratio. lambda was the dominant light chain in almost 80 percent of the cases in which SIFE were done. On the other hand, the medical necessity and clinical usefulness of SFLCA has been debatable as greater than 30% false positive and a more than 30% false negative rate for κ/λ ratio have been reported. The rate of false negative results from SFLCA is particularly worse for those patients who had lambda chain lesions. This is further underscored bythe observation that about 90% of the MGUS lesions with a lambda chain immunoglobulin type show normal κ/λ ratio [18]. The higher false negative rate for lambda chain lesions is also found for patients with SMM and MM and hence, it has been hypothesized that the high false negative rate for the lambda dominant κ/λ ratio in patients having monoclonal gammopathies with neoplastic lambda chain is most probably due to an under-detection of lambda light chains. It was suggested that due to the lambda chains show a greater tendency to dimerize than their kappa counterparts. It makes the target epitopes of the free dimerized lambda chains almost inaccessible that follows an under-detection of free lambda chains, compared to kappa chains. However, as an alternative explanation it has been proposed also that lambda chains are not produced in as much excess as the kappa chains which result in lower rates of lambda dominant κ/λ ratio in patient with a true lambda light chain neoplastic monoclonal gammopathies. Lastly, it has been also suggested that the polyclonal kappa light chains are overproduced in lambda chain monoclonal gammopathies, as commonly found in the case for tertiary care patients that leads to a false negative lambda dominant κ/λ ratio[18].

Conclusions

Although FLC assay plays a very significant role in the diagnosis and prognosis of plasma cell neoplasms of all types, it has some limitations. Asignificant number of cases may have normal FLC ratio in presence of monoclonal gammopathy. The falsely normal FLC ratio is mostly found in cases with lambda light chain containing monoclonal paraproteins. 24 hours urine protein and immunofixation electrophoresis have been mostly abandoned by clinicians when FLC assays are advised. Detection of monoclonal gammopathy may be missed in some cases as a result of this practice.

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