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Review Article

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Free Monoclonal Immunoglobulin Light Chains in Serum and Urine

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Received: 21 March 2022; **Revised:** 16 April 2023; **Accepted:** 17 April 2023; **Published:** 21 April 2023

Abstract

Plasma cells, normal, reactive, and malignant, generally produce more light chains than heavy chains of immunoglobulins. The excess light chains, polyclonal and monoclonal, can be detected in serum and urine. Qualitative and quantitative ascertainments of light chains in serum and urine are instrumental in diagnosis and monitoring of plasma cell disorders in general, and multiple myeloma in particular. There are differences in biological behaviors of kappa and lambda light chains, and there is a preference for kappa light chains. Antisera/antibodies to light chains fall into two groups: (a) reacting with free light chains as well as those part of an intact immunoglobulin, or (b) specific to free light chains only. The commercially available antisera/antibodies to free light chains are not equivalent and differ in their reactivity with light chains.

Measurement of serum free light chains has been promoted for screening for monoclonal gammopathies and inaccurately touted as replacing the need for urine examination. Other purported uses for serum free light chain measurement have been for determination of stringent complete response to treatment in multiple myeloma, and for diagnosing myeloma defining conditions, however, the validity of these uses has been challenged. Use of serum free light chain measurement in other disorders with monoclonal gammopathy, e.g., amyloidosis has not been fully explored.

Quantification of serum free light chains is broadly accepted for monitoring of light chain multiple myelomas and for diagnosis of light chain predominant multiple myelomas. Ascertainment of monoclonal light chains in serum and urine has been proposed to serve as marker for residual disease. A gel-based assay for detection of monoclonal light chains in serum, using antisera specific to free light chains, has been shown to be more sensitive than a mass spectrometry-based assay. Similarly, urine immunofixation electrophoresis using antisera specific to free light chain has demonstrated superior sensitivity to monoclonal light chains in urine. The usefulness of antisera to combination of heavy and light chains, "Hevylite®" in detecting residual disease remains to be examined.

Keywords: *Multiple myeloma; Free immunoglobulin light chains; Serum protein electrophoresis; Urine protein electrophoresis; Light chain predominant multiple myeloma; Free light chain modified serum protein immunofixation electrophoresis; Free light chain urine immunofixation electrophoresis; Monoclonal light chains*

Abbreviations: *SPEP, and UPEP: Serum and urine protein electrophoresis, SIFE and UIFE: Serum and urine immunofixation electrophoresis, MGUS: Monoclonal gammopathy of undermined significance, SMM: Smoldering multiple myeloma, PC/MM: Plasma cell/multiple myeloma, LCMM: Light chain MM, LCPMM: Light chain predominant multiple myeloma, SFLC: Serum free light chains, FLC-UIFE: UIFE with antibodies to free light chains, ASCT: Autologous stem cell transplantation, FLC-Modified SIFE: SIFE conducted on undiluted serum using antisera to free light chains, ISUB: Capillary electrophoresis and antibody mediated subtraction*

Introduction

Immunoglobulins are the dominant component of adaptive humoral immune response to infections. Immunoglobulins generally consist of, covalently linked, two heavy chains and two light chains. Each molecule has only one type of heavy chain and one type of light chain. The five different classes of heavy chains are designated, gamma, alpha, mu, delta and epsilon. The two types of light chains are kappa and lambda. Thus, there are ten different types of immunoglobulins, though only five isotypes are recognized based on the heavy chain type. IgG has four subclasses and IgA has two subclasses [1,2]. IgG, IgA, IgM, IgD and IgE each present with either kappa or lambda light chain. IgA exists in secretions in dimeric form with attached J chain and secretory piece. IgM is generally pentameric. Through a combination of DNA rearrangement, diversity region DNA and somatic hypermutation, humans can generate more than 10^{16} different types of immunoglobulins with distinct antigen binding sites [3].

Usual laboratory tests for immunoglobulins include quantification of different classes of immunoglobulins and in the case of IgG, quantification of its four subtypes [1]. Additional assessment is generally carried out by electrophoretic analysis of serum and urine by serum or urine protein electrophoresis (SPEP, and UPEP) and immunofixation analysis of these fluids (SIFE and UIFE). Assays for functional activity towards microorganisms and autoantigens comprise a large area of laboratory testing but are not addressed in this communication.

The process of maturation of lymphocytes to immunoglobulins producing plasma cells involves rearrangement of DNA to generate diversity of immunoglobulins types [4]. A similar process generates the diversity of T-lymphocyte receptors and olfactory receptors [5,6]. In the process of DNA rearrangement, kappa chain components are rearranged first and only if that is unsuccessful does the lymphocyte proceed to rearrangement of lambda chain DNA. There seems to a natural preference for kappa chain associated immunoglobulins. In humans the ratio of kappa chain associated immunoglobulins to lambda chain associated immunoglobulins is about 2:1 [7]. In mice the ratio is 19:1 [8]. Plasma cells are the mature form of lymphocytes that secrete the immunoglobulins found in blood and other body fluids. As an aside, immunoglobulins in camels, llamas and some species of sharks consist of heavy chains only and may be referred to as nanobodies [9]. These antibodies have been used in some assays, e.g., MASS-FIX MALDI TOF for identification of monoclonal immunoglobulins and monoclonal light chains in serum [10]. However, these antibodies, not infrequently, fail to react with free light chains, especially free monoclonal light chains [11,12].

Immunoglobulin light chains are generally synthesized in greater abundance than heavy chains. Excess free light chains are detectable in blood, though it is serum that is generally tested [13-16]. Monomeric light chains are about 25 kilo Dalton in size; are filtered freely by the renal glomerulus and are detectable in urine. Free lambda light chains tend to form dimers and have longer half-life in serum [13,16]. This advantage of longer half-life for lambda chains is lost in renal failure and patients with renal insufficiency usually have a kappa dominant abnormal kappa/lambda ratio, in addition to abnormal elevation of both types of free light chains in serum [17]. The "normal" ratio of kappa to lambda light chains in serum is not applicable to patients in renal failure and different ratio has been proposed for such patients, despite the limited usefulness of this measurement [18-22].

Antisera/antibodies reactive with free light chains

The antibodies to light chains in common use react with both the light chains complexed with heavy chains and free light chains. Figure 1 illustrates the differences in accessible epitopes in light chains that are part of an intact immunoglobulin vs. free light chains. The figure schematically illustrates the accessible epitopes in free light chains that are "hidden" in the intact immunoglobulin molecule. Bradwell brilliantly exploited this difference and raised polyclonal antibodies that reacted specifically and exclusively with free light chains [13].

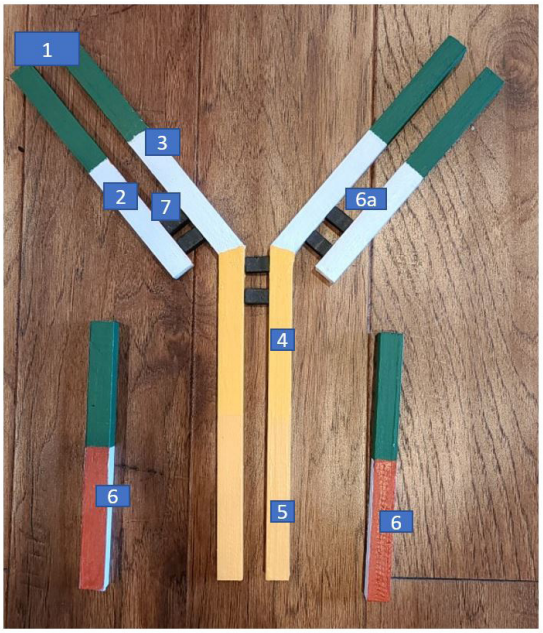

Figure 1: Schematic representation of Immunoglobulin structure: The generic structure of an immunoglobulin G molecule is depicted showing a tetrameric molecule consisting of two heavy chains and two light chains. Two free light chains are shown in the lower part, one on either side of the Fc region of the heavy chains. (A). The green shaded areas, marked 1, represent the variable regions in heavy and light chains and the antigen binding sites. (B). Site marked 2 represents the constant region of light chain. (C). Sites labelled 3, 4, 5 mark the constant domains of heavy chain. The regions constituting domains 4 and 5 are the Fc region of the molecule and are involved in secondary actions, e.g., complement activation, and binding to cells. (D). The region of light free light chain marked 6, in red paint, represents the epitopes that are hidden in the intact immunoglobulin. Antisera/antibodies to this region constitute free light chain specific antibodies. The site marked 6a, in the intact immunoglobulin molecule, is meant to illustrate the hidden epitope of light chains shown in red paint in the free light chain. (E). The black blocks labelled 7 indicate the intermolecular disulfide bonds. (F). Carbohydrate molecules are usually attached to the constant regions of heavy chain marked 4 and 5.

The antisera generated by The Binding Site Company were employed in latex turbidimetric assays for quantification of free light chains in serum. The Binding Site assay evolved through the stages of radial immunodiffusion and nephelometric measurement though the current assays is based on turbidimetry [13]. Other parties have raised similar antibodies in other animals, e.g., rabbits by Agilent and monoclonal mouse antibodies by Siemens [19,23] The assay for serum free light chains by The Binding Site, called Freelite®, and by other vendors generally generate comparable results [24-28]. The Freelite® being the first to market has been used the longest and most of the diagnostic criteria using concentration of serum free light chains are based on Freelite® results. One measure of abnormality in serum free light chain (SFLC) concentrations has been the ratio of concentration of kappa SFLC to the concentration of lambda SFLC, i.,e., kappa/lambda ratio [16].

Other suppliers of reagents for SFLC measurement include Siemens -N Latex assay using monoclonal antibodies; Sebia – Sebia FLC; Abingdon Health – Seralite; and Diazyme. The results from the assays from different vendors are more or less comparable but are not interchangeable. Siemens assay has been recommended as not being affected by renal failure, however a head-to-head comparison of the Freelite® (Binding Site assay) and N Latex FLC (Siemens assay) did not reveal meaningful difference in the performance of the two assays [29]. An additional shortcoming of the N Latex FLC was the limited reactivity of monoclonal antibodies with the wide variety of epitopes on light chains thus missing identification of a limited number of light chain molecules, particularly monoclonal light chains [30].

Neither Freelite® nor subsequent assays have been adapted, commercially, for quantification of free light chains in urine, cerebrospinal fluid or other body fluids. Individual laboratories, including ours, have developed methods to quantitate free light chains in urine. However, the assay has not been proven to have clinical utility. In particular, the assay did not help in identifying urine samples for which UIFE may be omitted. [unpublished observation]. A drift has been observed in the performance of Freelite® assay and it may result in erroneous diagnoses [31-33].

There has also been limited utilization of these antisera in gel electrophoresis analysis of serum or urine, despite demonstrated potential of modified electrophoretic assays in enhancing the sensitivity for detecting monoclonal light chains [11,23,34,35]. In the modified SIFE assay, undiluted serum was used to inoculate electrophoresis gels. This change increased the concentration of serum proteins in gel such that usual washing process did not remove some of the proteins that were not part of the immunocomplexes with antisera to free light chains. This limitation was overcome by adding three wash steps following incubation with free light chain specific antisera. Using undiluted serum improved the sensitivity of the assay in detecting free monoclonal light chains in serum. In parallel testing, the modified SIFE method was documented to be more sensitive than MASS-FIX MALDI for detection of free monoclonal light chains in serum. The greater sensitivity of the modified SIFE method rendered it suitable for detecting minimal residual disease [11]. It warrants reiteration UIFE for detection of monoclonal light chains is underutilized and SFLC assay does not obviate the need for UIFE, statements to the contrary notwithstanding [7,34-37]. The greater sensitivity of the UIFE by using antisera to free light chains makes is useful for detecting residual disease [23].

Greater abundance of light chains than heavy chains: As noted for normal plasma cells, malignant plasma cells also generally synthesize light chains in greater quantity than heavy chains [7,38]. In most cases of monoclonal immunoglobulin production, free monoclonal light chains are detectable in serum and urine [11,23,35-40]. In neoplastic monoclonal gammopathies, i.e., monoclonal gammopathy of undermined significance (MGUS), smoldering multiple myeloma (SMM) and plasma cell/multiple myeloma (MM) free monoclonal light chains may be detectable in serum and other body fluids [35]. In general kappa chain associated monoclonal gammopathies produce higher levels of free monoclonal light chains than lambda chain associated lesions [7,15,41-46]. For example, lambda chain associated MGUS lesions have normal kappa/lambda ratio in about 90% of the lesions compared to a normal ratio in 60% in kappa associated MGUS lesions when FLC is measured by Freelite® [7,22]. The same phenomenon extends to SMM and MM as well. A limited but not infrequent cases of lambda light chain associated multiple myelomas, i.,e., IgG, and IgA lambda monoclonal gammopathies, an excess amount of lambda light chains is not detectable [22]. The kappa/lambda ratio in such patients remains normal throughout the course of illness and free light chain assays are ineffective in diagnosis and monitoring. There is controversy about this observation, and it has not been settled if the lack of detection of excess free lambda light chain in some of these lesions is truly due to lack of excess production of free light chains or if it is due to lack of reactivity of available antisera to a sub-type of free lambda light chains. In support to the latter notion is the observation of variability of reactivity of antisera from different vendors [11,23,29,30]. This variability is not limited to lambda light chains and has also been observed for kappa free light chains. This observation along with the observed variation in the commercial assays for quantification of free immunoglobulin light chains warrants an effort at harmonization of reagents and methods.

About 15% of MM lesions produce only monoclonal light chain, without synthesizing intact immunoglobulins, generally referred to as light chain MM (LCMM) [7,44-46]. About 18% of intact immunoglobulin producing MM secrete a larger quantity of free monoclonal light chains. This subset of MM designated light chain predominant MM (LCPMM) has higher prevalence of renal

damage and about 2-year shorter survival [15,45,46].

Monoclonal free light chains are also detectable in other lesions associated with production of monoclonal immunoglobulins, e.g., lymphoma, amyloidosis, light chain deposition disease and monoclonal gammopathies associated with auto-immune disorders etc. [7]. Measurement of free light chains in general and, monoclonal light chains in particular, are useful in the diagnosis and monitoring of non-myeloma lesions, e.g., amyloidosis [47]. Abnormal carbohydrates associated with monoclonal light chains have been implicated in the pathogenesis of amyloidosis [48,49].

Polyclonal increase in serum free light chains (SFLC)

The reference ranges for SFLC levels for kappa and lambda light chains were based on the findings in blood donors. Though the "normal" kappa/lambda ratio was expanded from 0.3-1.2 to 0.26 to 1.65 to improve sensitivity for detection of MM in screening assays using SPEP and SFLC assay [13,16]. While it is the usual practice to use "healthy" subjects to establish reference ranges, it may have been more appropriate to base the reference ranges on data from patients in the same age group and with similar co-morbidities as seen in patients with MM. When patients presenting to a tertiary care healthcare setting were examined for SFLC, more than 36% displayed an abnormal kappa/lambda ratio, without any evidence of monoclonal gammopathy. Such patients may be put through additional investigation based on this high false positive rate [21].

In patients with renal failure the serum levels of free light chains are generally higher than in healthy persons. The ratio of kappa to lambda light chains is also altered. When using Binding Site assay for SFLC quantification the "normal" kappa/lambda ratio has been revised to be 0.37-3.1 for patients with renal failure [21]. Different methods for measuring SFLC have more discordant results in the presence of renal failure. The monoclonal antibody-based assay by Siemens was reported to be not affected as much as the Binding Site assay, however direct comparison of the two methods did not reveal advantage with one method over the other [29].

The excess production of free light chains is higher for kappa than lambda light chains in non-malignant gammopathies as well as in neoplastic monoclonal gammopathies, including MM. This is reflected in relatively higher production of kappa free light chains than lambda free light chains in patients with increase in immunoglobulin production due to (a) physiological responses to stimuli, such as infections, (b) chronic inflammation due to infections or (c) non-infectious causes of inflammation such as rheumatoid arthritis and other autoimmune disorders, and (d) disorders of immunoglobulin metabolism as seen in liver cirrhosis [7,20]. Patients in the age group in which MM is prevalent have a 36% incidence of abnormal kappa/lambda ratio and in nearly 90% of these circumstances the abnormal ratio is kappa dominant, i.e., >1.65, without any evidence of a monoclonal gammopathy. More than half of patients with polyclonal increase in gamma globulins, due to any cause, have abnormal kappa/lambda ratio and in nearly 95% of the instances the abnormal ratio is kappa dominant. The kappa dominance is likely due to an unexplained preference for kappa light chains [21].

Thus, it is recommended that UIFE be conducted in patients with abnormal kappa/lambda ratio before assigning a label of monoclonal gammopathy. As presented later, for initial investigation of monoclonal gammopathy, it would be prudent to conduct UIFE with antisera specific to free light chains to detect monoclonal light chains because a normal kappa/lambda ratio does not exclude monoclonal gammopathy and an abnormal kappa/lambda ratio is not diagnostic of monoclonality [7,22,39,42,43,50].

Initial evaluation of suspected monoclonal gammopathy

Initial investigation of monoclonal gammopathy usually consists of SPEP, SIFE, UPEP, UIFE and SFLC measurement or a combination of these tests. Gel electrophoresis and capillary electrophoresis are in use in different laboratories [51]. Different forms of mass-spectrometry have been proposed for screening but are not on common use [10,52]. For diagnostic evaluation of monoclonal gammopathies, SFLC measurement is recommended by the International Myeloma Working Group (IMWG) and an expert panel of the College of American Pathologists (CAP) [40,53,54]. Initial study by Mayo Clinic recommended performing serum protein electrophoresis (SPEP) and serum immunofixation electrophoresis (SIFE) and SFLC assay, based on the observation that this testing would detect abnormal results in virtually all cases of MM. The investigators argued against the need for examination of urine. However, it appears that the recommendation has been misapplied because an abnormal kappa/lambda ratio is not diagnostic of monoclonal gammopathy and a normal kappa/lambda ratio does not exclude monoclonal gammopathy [7,13,16,21,22,50]. Anecdotal observations, not uncommonly, have revealed patients with an abnormal kappa/lambda ratio being labeled as having MGUS, without any evidence of monoclonality. On the other hand, detection of monoclonal light chains by urine immunofixation electrophoresis (UIFE) is diagnostic of monoclonality [23]. It was also argued that urine examination is not needed in patients with light chain MM (LCMM), However, all patients with LCMM had monoclonal light chains in urine, even though the authors used a less sensitive method that omitted the usual step of concentrating the specimen before electrophoresis. The authors suggested that post-treatment, kappa/lambda ratio was abnormal more often than the detection of monoclonal light chains in urine. However, no independent verification of the abnormal kappa/lambda ratio being so due to

monoclonal light chains was presented [34,36,50].

Examination of urine by immunofixation electrophoresis using antisera specific to free light chains revealed the presence of free monoclonal light chains in urine in 18% more specimens than was detectable by conventional UIFE [23]. Neither the total urine protein nor serum levels of free light chains were predictive for the detection of monoclonal light chains in urine. Therefore, it has been suggested that the optimum initial screen for monoclonal gammopathy would be SPEP, SIFE and UIFE using antisera specific to free light chains [23]. More pointedly, SFLC assay has been over- and mis - used. SFLC assay is needed to identify LCPMM and high levels of light chains in LCMM as this has prognostic significance [15,45,46,54]. The other valid use for SFLC assay is in monitoring light chain MM [44]. An additional observation of a multi-institution analysis of conventional UIFE was the lack of usefulness of testing for heavy chains in urine. A heavy chain was not detected in any of the urine specimens that was not noted in serum by conventional SIFE [23,34].

International myeloma working group (IMWG) recommended 24-hour urine examination for evaluation of MM, however, this is not in common use. The collection of 24-hour urine is prone to errors and there is little evidence that it adds any value to the information gathered from examination of concentrated random urine specimens by UIFE for detection of monoclonal light chains [23]. The area where there may use for 24-hour urine examination would be for quantification of monoclonal light chains in urine, however, the lack of clinical evidence for usefulness of this measurement is the likely reason for poor utilization of this test.

Both the IMWG and an expert panel of College of American Pathologists (CAP) recommend using SFLC despite the fact that a normal kappa/lambda ratio does not exclude monoclonal gammopathy and an abnormal ratio is not diagnostic monoclonal gammopathy [7,40,50,52,54]. The recommendation that SFLC assay can replace UIFE is eminently debatable. Detection of a monoclonal light chain in UIFE is diagnostic of monoclonal gammopathy. As cited earlier, an example is the publication by Dejoie T, et al. (2016) showing that SFLC ratio was abnormal in all cases of light chain myeloma at the same time the authors demonstrated that UIFE displayed monoclonal light chain in urine, even though the authors used unconcentrated urine. Their assertion that SFLC ratio was more frequently abnormal in LCMM following treatment than the presence of monoclonal light chains in UIFE, ignored the fact that they never demonstrated that the abnormal SFLC ratio was due to monoclonal light chains in urine [36]. Katzmann JA, et al. (2006) recommended using SFLC assay in preference to UIFE with part of the argument being that SFLC assay is less expensive than UIFE [37]. However, as noted above, SFLC measurement is neither diagnostic of monoclonal gammopathy nor does it exclude monoclonal gammopathy, while UIFE is diagnostic and FLC UIFE has greater sensitivity [7,23].

Diagnosis of LCPMM

About 80% of the MM lesions produce intact monoclonal immunoglobulins, about 15% produce only light chains, i.e., LCMM. A retrospective review of SFLC levels in patients with MM producing intact immunoglobulins, revealed that patients with higher levels of SFLC had higher incidence of renal failure, dialysis, and death. About 18% of lesions producing intact monoclonal immunoglobulins meet the criteria of excessive free monoclonal light chain production and have been designated light chain predominant MM, i.e., LCPMM [45,46]. The criteria take into account the concentration of the intact monoclonal immunoglobulin and concentration of free involved light chain. In lesions associated with kappa light chain a light chain concentration of 67 mg/g of involved monoclonal Ig met the requirement for designation of the lesion as LCPMM. The corresponding figure for lambda chain associated lesions is 43.5 mg/g [15,44,45]. Survival in the high SFLC level group, i.e., LCPMM group, was 22 months shorter than that in the group with lower levels. A similar review of LCMM revealed the change point for high levels of SFLC concentration to be 455 mg/L [46]. There were insufficient cases to derive light chain specific criteria for LCMM. The higher SFLC level group exhibited a more than 2-year shorter survival [15,45,46,55]. At this time there are no effective therapies specifically addressing light chains, even though the nephrotoxicity of monoclonal light chains has been known for nearly a century [55,56]. Attempts have been made to reduce the concentration of monoclonal light chains with large pore membrane dialysis and plasmapheresis, however, controlled trials have not documented benefit of such procedures. It has been suggested that future controlled trials of treatment of MM, the sub-class of LCPMM be identified and treatments evaluated to address the need and effectiveness of selectively targeting light chains [55]. The same issues would be applicable to sub-class of LCMM with high levels of free monoclonal light chains. The initial investigation pegged the dividing line at 455 mg/L of free light chains, however, there were insufficient number of cases to develop light chain specific criteria that this remains a gap in our understanding of the behavior of LCMM and effectiveness of treatment for the subclass [46].

It bears noting that virtually all IgD multiple myelomas are light chain predominant lesions. The excess of monoclonal light chains, producing a separate band in addition to IgD, in IgD myelomas has erroneously been called biclonal lesions, without evidence of differences in the epitopes/clonotypes of light chains associated with monoclonal delta heavy chains and free monoclonal light chains. As an aside, IgD myelomas are more commonly associated with lambda light chains than other MM lesions. Taking note of these peculiarities of IgD myelomas criteria have been developed for presumptive diagnosis of IgD myeloma, pending confirmation by SIFE using antisera to delta heavy chains or by quantification

of serum IgD levels [57]. For the sake of completion, it is noted that IgE myelomas are extremely rare with only about 50 cases reported in the literature [58].

An additional contribution of the recognition of the lesion LCPMM is in clarification of light chain escape. Light chain escape refers to detection of monoclonal light chain in SIFE following treatment of patients with intact immunoglobulin MM. This entity, if it exists, must be exceedingly rare as no case of light chain escape was observed at this institution over a 20-year period and more than 600 MM cases. The so-called light chain escape generally reflects the earlier detection of free monoclonal light chains in patients with LCPMM, following treatment [7]. The entity of light chain escape was described before the discovery of light chain predominant multiple myeloma subtype thus the difference in opinion about the validity of the entity of light chain escape.

Monitoring of monoclonal gammopathy

As about 85% of the MM lesions are associated with secretion of intact immunoglobulins, estimation of the monoclonal immunoglobulin in serum provides a non-invasive/minimally invasive method for measuring the disease burden. It is imperative that the location of monoclonal peak in SPEP be documented at the initial evaluation. For the monoclonal immunoglobulins migrating in the gamma region, densitometric scanning of the protein peak provides a reasonable estimate of the concentration of the monoclonal immunoglobulin. Vertical drop and tangent skimming methods provide different results due to interference by the background level of polyclonal immunoglobulins. Similarly, manual demarcation of the peak and automated detection of the peak yield different results. However, change in the concentration of the protein in the peak is more important than the absolute accurate concentration. Thus, any method, applied consistently, in a given laboratory is usually adequate to monitor the progress of disease [7,40,54].

The monoclonal immunoglobulins migrating in the beta region are affected by the background proteins to a greater extent than the proteins migrating in the gamma region. In practice, change in the concentration of the peak, that may include C3 component of complement, or transferrin or both may suffice to monitor the progress of disease. It has been proposed that measuring Hevylite® concentration of IgA monoclonal immunoglobulins would provide a more accurate measure of the monoclonal IgA proteins migrating in the beta region, however, measuring Hevylite® did not offer any advantage over monitoring the total concentration of IgA [13,59]. Subtraction of C3 by heat inactivation and subtraction of transferrin concentration by immunochemical measurement of the protein have been proposed but neither the Hevylite® nor the subtraction methods are in common use for accurate quantification of monoclonal immunoglobulins migrating in the beta region. Accurate measurement of monoclonal immunoglobulins migrating in beta region may be important in appropriate identification of LCPMM lesions as the monoclonal intact immunoglobulin concentration is used as the denominator to normalize the concentration of free light chains [15,45,60].

In about 15% of MM lesions the tumors secrete light chains only. Serum free light chain concentration of the involved light chain in LCMM is one of the compelling reasons for the uses of SFLC assay. The results allow one to monitor the disease burden as no other non-invasive quantitative measure of tumor mass is available [44]. UIFE with antisera specific to free light chains (FLC-UIFE) should also be used along with SFLC assay as the FLC-UIFE can detect monoclonal light chains in the face of normal serum levels of free light chains. FLC-UIFE is especially important in lambda light chain associated lesions [23]. Following treatment, monoclonal lambda light chains were detectable in urine in a quarter of patients with normal kappa/lambda ratio of SFLCs [42,43]. It has been proposed that FLC-UIFE on concentrated urine specimens could serve as a marker of minimal residual disease [23].

Urine examination is especially relevant in patients' post-treatment with stem cell transplantation. Even though a controlled trial did not document any improvement in overall survival, autologous stem cell transplantation has been adopted as a standard treatment and is applied in about 70% of the patients [60]. An oligoclonal pattern is seen in about 70% of patient status post autologous stem cell transplantation [7,39,61] The oligo-clones tend to be kappa predominant and alter the SFLC kappa/lambda ratio in favor of kappa dominant finding. In patients treated with ASCT, who had a lambda light chain associated MM, i.,e., IgG lambda or IgA lambda MM, about 11% had the expected lambda dominant kappa/lambda ratio while 15% had kappa dominant kappa/lambda ratio. If the same conservative rate of false positive kappa dominant abnormal kappa/lambda were applied to kappa light chain associated MM, about 40% of the abnormal kappa/lambda ratios could be interpreted to be false positives [39,42,43]. These results challenge the use of a normal kappa/lambda ratio as criterion for stringent complete response. Examination of serum and urine for monoclonal light chains, using antisera specific to free light chains, would provide a more sensitive marker of residual disease [11,23].

The reagents and techniques described for monitoring the monoclonal immunoglobulins in MM lesions are also applicable to MGUS and SMM. Non-secretory MM lesions constitute less than 1% of MM cases and are not suitable for monitoring by these techniques [7]. While the monoclonal immunoglobulins in other lesions, e.g., amyloidosis, chronic auto-immune demyelinating

polyneuropathy, and lymphomas also could be monitored by the methods used in MM, these disorders often have other complicating issues [7].

Higher average levels of kappa light chains than lambda light chains in monoclonal gammopathies

Greater production of light chains than heavy chains extend to monoclonal gammopathies and as noted for non-monoclonal gammopathies, there a greater abundance of excess free kappa light chains than lambda light chains. As noted earlier, in MGUS lesions nearly 90% of lambda light chain associated lesion have a normal kappa/lambda ratio compared to 60% in kappa associated MGUS lesions. A similar 30% higher prevalence of normal kappa/lambda ratio is noted in global lesions of monoclonal gammopathies, associated with lambda light chains than kappa light chains [43].

A similar disparity in the abundance of free light chains in favor of kappa light chains is noted in patients with MM. On average SFLC level of kappa light chains are present in >4.0-fold greater abundance in kappa chain associate MM lesions than in lambda chain associated lesions [41,43]. Using median levels, a five-fold greater abundance of kappa light chains as compared to lambda monoclonal light chains has been reported [41]. Conversely, levels of uninvolved light chain are twice as high in lambda chain associated monoclonal gammopathies than in kappa chain associated gammopathies. This kappa predominance alters the ratio of kappa/lambda light chain concentrations and often renders the use of this ratio untenable. As noted earlier the oligoclonal pattern usually seen after ASCT has more prominent kappa chain associated clones and further interferes with the validity of the kappa/lambda ratio in monitoring response or ascertainment of stringent complete response [7,39,43].

The usually higher levels of kappa free light chains in kappa chain associated monoclonal gammopathies were not considered in the description of light chain concentration-based criterion for myeloma defining condition. The criterion, as stated, requires concentration of involved light chain greater than 100.0 mg/L and ratio of involved to uninvolved light chains of greater than 100. If the predominance of kappa light chains had been taken into consideration, the criterion could have been kappa light chain concentration >100.0 mg/L and lambda chain concentration >25.0 mg/L. Similarly, the ratio of involved to uninvolved light chains could have been 100 for kappa chain lesions and 12.5 for lambda light chain lesions. This may be a moot issue as treatment of myeloma defining conditions is not recommended, without the presence of end-organ damage [7].

Stem cell transplantation

An oligoclonal and polyclonal gammopathy is often noted in patients' status-post stem cell transplantation. Allogeneic stem cell transplantation is complicated by graft vs. host disease and varying abnormalities in immunoglobulin synthesis. Allogeneic stem cell transplantation is uncommonly used in the treatment of MM. However, autologous stem cell transplantation is done as a standard treatment for MM patients. Even in ASCT, there is a detectable oligoclonal response in about 70% of the patients. The oligoclonal response is generally dominated by kappa clones than lambda clones. This kappa dominance results in patients with lambda chain associated MM having abnormal kappa/lambda ratio that is kappa dominant more often than being lambda dominant, contrary to the expected lambda dominance. This phenomenon of kappa predominant oligoclonal response renders the use of normal kappa/lambda ratio as a determining factor in the diagnosis of stringent complete response to be invalid [39,42,43,61]. The background increase in polyclonal kappa light chains following ASCT and other treatments for MM may account, in part, for the normal kappa/lambda ratio despite the detection of monoclonal lambda light chains in urine [39,62].

Variability in antibody reactivities and methods

The various methods and reagents used in the investigation of monoclonal immunoglobulins are generally comparable but not equivalent [9,11,19,28] This applies to the screening methods of SPEP and SIFE versus Capillary electrophoresis and antibody mediated subtraction (ISUB) to guide demarcation for identification and quantification of monoclonal immunoglobulins [7,51]. As noted earlier, these variations are generally not meaningful in affecting diagnosis and monitoring of MM as long as the methods are applied consistently at each site. Part of the lack of standardization of quantification of monoclonal immunoglobulins is due to unavailability of purified preparations of monoclonal immunoglobulins that could be used as standards. Quantification of total immunoglobulins is more standardized, and performance is monitored by external proficiency testing, e.g., by the College of American Pathologists.

There is greater variability in the results of quantification of free light chains. Even using reagents from one vendor, different testing platforms provide different results [18,19,28,35]. As in the case of monoclonal intact immunoglobulins, lack of pure standard preparations of free light chains is a stumbling block in harmonization of the methods. An additional source of variability in the results of measurement of free light chains is the uneven reactivity of antibodies/antisera to the epitopes that are essential for detecting free light chains selectively. The genetic diversity of light chain amino acid sequences and the epitopes determining the hidden sites makes it impractical to have reagents that would cover all of the variations. This is illustrated by the observation by Bradwell of lack of reactivity of the antiserum to free kappa light chains of a patient [13]. This was corrected by supplementing the antigens used for raising antibodies/antisera

to free kappa light chains. A similar lack of reactivity of rabbit antiserum to free lambda light chains from one vendor while reagent from a different vendor having the requisite reactivity has been documented [11,23]. In investigating the usefulness of FLC-UIFE, lack of rabbit antiserum to free kappa light chains from a patient with Waldenstrom's macroglobulinemia was noted. Antiserum to light chains that reacted with free kappa light chains and kappa light chains bound to IgM validated the presence of free kappa light chains in urine [23]. The lack of reactivity of the antiserum to mu heavy chains in the urine supported the contention that the monoclonal band of kappa light chains in urine detected by the conventional antiserum documented free kappa monoclonal light chains in urine. On the other hand, FLC-UIFE detected 10% more monoclonal kappa lights and 30% more lambda monoclonal light chains in urine than was detectable by conventional antiserum on parallel testing of concentrated urine. Overall FLC-UIFE detected 18% more monoclonal light chains in urine than conventional UIFE [23]. Part of the greater sensitivity of FLC-UIFE was determined to be due to detection of monoclonal free light chains co-migrating with intact monoclonal immunoglobulin. Free monoclonal light chains co-migrating with the cognate intact immunoglobulins could not be identified by the conventional antisera to intact monoclonal immunoglobulins, due to obscuration by the intact immunoglobulin. The more marked increased detection of free monoclonal lambda light chains in urine, by FLC-UIFE may be due to lack of reactivity of conventional antisera to dimeric/polymeric lambda chains in urine.

A similar vagary in the reactivity of nanobodies used in the concentration step of MASS-FIX MALDI likely caused the markedly lower level of detection of monoclonal light chain in serum. In direct comparison of FLC-Modified SIFE using undiluted serum and MASS-FIX MALDI, the latter missed a significant number of monoclonal light chains [11]. As an instance in a parallel study of FLC-Modified SIFE and MASS-FIX MALDI, 43 specimens from patients with monoclonal gammopathy were examined. FLC-Modified SIFE identified monoclonal light chains in 42 of 43 specimens whereas MASS-FIX MALDI identified monoclonal light chain in only 19 of the specimens. The results of FLC-Modified SIFE were in consonance with the expected finding based on clinical history and other immunodiagnostic assays [11]. A likely explanation of this deficit in the performance of MASS-FIX MALDI is the failure of nanobodies to bind to free monoclonal light chains in serum thus preventing their detection in the MALDI-TOF phase of the assay [11]. A different method for concentration of immunoglobulins with a broader spectrum of reactivity to monoclonal light chains may be needed to address this shortcoming of MASS-FIX MALDI. A MALFI-TOF-MS method described for screening analysis of monoclonal proteins in serum did not use the concentration step, however, the method did not specifically address the detection of free monoclonal light chains in serum [10,11].

Conclusion

In summary, SFLC assay is important in (a) diagnosis of light chain predominant intact immunoglobulin MM lesions, and high light chain burden LCMM lesions as these have a significantly worse prognosis; and (b) monitoring the progress of LCMM. SIFE and UIFE performed with antisera specific to free light chains enhance the sensitivity of the assays for detection of monoclonal immunoglobulin light chains in serum and urine and in assessing minimal residual disease. Whether using Hevylite® in modified SIFE assays, as used in FLC Modified SIFE, would improve the sensitivity of detection of residual monoclonal intact immunoglobulins remains to be addressed. It is proposed that the initial evaluation for suspected monoclonal gammopathy should utilize SPEP, SIFE and UIFE only, and UIFE could be limited to assessing light chains only using FLC-UIFE. Usefulness, if any, of quantification of free light chains in urine remains to be addressed.

Conflict of Interest

Dr. Singh serves a member of the medical advisory board of HealthTap.

Disclosure

This study was conducted at Medical College of Georgia at Augusta University and the protocol was approved by the Institutional Review Board.

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