Editorial Commentary

How to quantify monoclonal free light chains in plasma cell disorders: which mass spectrometry technology?

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Plasma cell disorders are a cytogenetically heterogeneous group of hematologic diseases characterized by altered clonal plasma cell proliferation, the presence of a monoclonal protein (M-protein) in serum or urine, and/or the presence of monoclonal plasma cells in the bone marrow. Multiple myeloma (MM) is the malignant stage of these disorders, which is usually preceded by monoclonal gammopathy of undetermined significance (MGUS) which is an asymptomatic premalignant stage of MM. This stage has been noted in approximately 4% of the population over 50 years of age (1-3). The rate of progression from MGUS to MM is 0.5% to 1% per year. Smoldering MM (SMM) is an intermediary stage between MGUS and MM, with a risk of progression to MM of approximately 10% per year in the first 5 years after diagnosis (2).

The diagnosis of plasma cell disorders is based on clinical, imaging, and laboratory criteria. For laboratory testing of serum, the detection and quantification of a M-protein are essential to make diagnosis. Serum protein electrophoresis (PEL) is traditionally performed and allows relative quantification of the M-protein by integration of the area under the curve of the peak representing the monoclonal entity. Serum immunofixation electrophoresis (IFE) can then be used to determine the M-protein isotype. Finally, serum quantification of free light chains (sFLC) is necessary to diagnose and monitor patients with plasma cell disorders (1). The recommendations of the International Myeloma Working Group (IMWG) and most of the published studies have been based on sFLC measured using the first commercialized assay developed in 2001, namely the Freelite® assay (The Binding Site Group Ltd, UK) (4). This assay allows sFLC quantification using a nephelometric or turbidimetric analyzer, and it is based on sheep polyclonal anti-kappa and anti-lambda FLC antibodies. However, other commercialized assays have become available. The N Latex™ sFLC kappa and lambda assay (Siemens Healthineers Diagnostics GmbH, Germany) (5) is an immunonephelometry assay based on noncompeting mouse monoclonal antibodies. The Seralite™ assay (Sébia, Evry, France) (6) is a lateral flow immunoassay based on competitive inhibition. The Sébia Free Light Chain Assay™ (Sébia, Evry, France) is a sandwich enzyme-linked immunosorbent assay using rabbit polyclonal antibodies against free kappa or lambda light chains in the capture phase, and rabbit polyclonal horseradish peroxidase-conjugated antibodies directed against kappa or lambda light chains (7). The Diazyme Human kappa and lambda FLC assay™ (Diazyme Laboratories Inc., Poway CA, USA) is based on rabbit polyclonal antibodies, which is a latex particle-enhanced immunoturbidimetric assay. However, none of these assays directly quantifies monoclonal sFLC. Instead, the clonality

is indirectly assessed by the quantification of kappa and lambda sFLC and the interpretation of the sFLC kappa/lambda ratio. Indeed, the use of a kappa/lambda sFLC ratio appears to be a more sensitive marker of monoclonal FLC production than the absolute sFLC concentration, because the interpretation incorporates the suppression of the non-tumor sFLC. Moreover, the nephelometric/turbidimetric assays present numerous technical limitations: lot-to-lot variations, antigen excess, nonlinear responses especially at higher dilution, poor post-dilution linearity, detection and quantification of oligomerization of monoclonal sFLC, gap in quantification, under and overestimation with extreme values, a huge heterogeneity in FLC measurements illustrated in pairwise comparison with external quality controls, different results on the same samples according to the analytical platform used, and the reference ranges for the Freelite® test being dependent on the analytical platform (8,9). Regarding the Sebia FLC assay, technical experience reveals that several analytical improvements such as dilutions need to be made (10). No standard reference material is available to compare assays and analytical platforms, which is a major limitation of sFLC assays. And that is the reason why new commercialized assays are compared with the Freelite® assay, which is the first commercialized assay and the one used in establishment of guidelines (5,10-12). Numerous discrepancies between tests have been documented for patients especially with chronic kidney disease and AL amyloidosis. For example, while an extended kappa/lambda sFLC ratio has been proposed for patients with chronic kidney disease when the sFLC are evaluated with the Freelite® assay (13), this is not a requirement with the N Latex assay (8,14,15). But with a worsening of renal function, the Sebia Free Light Chain Assay detected an increase in both serum kappa and lambda FLC, and an increase in the kappa/lambda sFLC ratio (16). A number of studies have concluded that the various sFLC assays are safe but not commutable, as the numerical results can vary drastically for individual patients, while the qualitative results and the trends in monitoring are in agreement (8,10-12,17). Furthermore, all recommendations were established with Freelite® assay. So, in AL amyloidosis patients or when predicting of risk of progression from SMM to MM, the IMWG guidelines specify that the values refer to the Freelite® assay (1). Consequently, the other tests cannot be used for staging, especially in clinical trials, irrespective of the performance of these tests (11,18,19). Nevertheless, few studies to date have documented the performance of other sFLC assays in these conditions (10,17).

The use of mass spectrometry to detect M-proteins in serum and urine is a recent development, and its aim is to provide a greater degree of analytical sensitivity and specificity. Since 2014, scientists have sought to develop mass spectrometry for use in the diagnosis and management of plasma cell disorders and to demonstrate that mass spectrometry can advance the way that plasma cell disorders are screened, diagnosed, and monitored. Most of the publications to date have been based on MALDI-TOF methods with immunoenrichment (*Table 1*), and since the initial development of mass spectrometry development for use with plasma cell disorders, MALDI-TOF MS has consistently exhibited superior sensitivity and specificity compared to conventional methods. In mass spectrometry, the main advantage is that the compound of interest, in this case monoclonal immunoglobulins or monoclonal sFLC, is defined by both the retention time and the m/z ratio. Contrary to commercialized sFLC assays, mass spectrometry allows direct quantification of the monoclonal protein, without excess of antigen or nonlinearity issues, with high sensitivity to detect monoclonal protein over the polyclonal background (*Table 1*). However, previous studies have demonstrated abnormal FLC ratios in samples in which the FLC were measured with MALDI-TOF mass spectrometry, thus suggesting that the methods still need to be improved.

Indeed, in a recent study published in *Clinical Chemistry* titled "Direct Detection of Monoclonal Free Light Chains in serum by use of immunoenrichment-coupled MALDI-TOF Mass Spectrometry", Sepiashvili *et al.* sought to develop an analytically sensitive method for direct detection of monoclonal sFLC that was independent of the sFLC kappa/lambda ratio, thereby allowing conventional biochemistry techniques to be bypassed (29). The development of mass spectrometry to quantify sFLC has taken place in a series of steps. Initially, micro-LC-electrospray ionization-quadrupole-TOF-MS was shown to exhibit greater analytical sensitivity and specificity than PEL and IFE techniques (22,25). Methods were then developed for higher throughput approaches for routine clinical analyses that involved total immunoglobulin immunoenrichment with reduction and total light chain mass measurement (MASS-SCREEN and MASS-FIX) by MALDI-TOF. The analytical sensitivity of these methods was comparable to IFE. However, in patient's plasma samples, there was a high abundance of light chains from intact immunoglobulins, and the challenge was to

Table 1 Published mass spectrometry methods for the detection and quantification of monoclonal proteins in plasma cell disorders

Reference	Mass spectrometry method	Enrichment/ purification	Detected protein	Matrix	Effective	Method	Results
Milani 2017 (20)	MASS-FIX	Ig-enrichment	M-protein	Serum and urine	257	PEL, IFE, FLC	• MASS-FIX has a sensitivity comparable to PEL/IFE/FLC when both the serum and the urine determination are combined • Most of the AL amyloidosis patients had atypical spectra • The assay works across the spectrum of plasma cell disorders and in patients with renal failure
Kohlhagen 2016 (21)	miRAMM with nanobody Ig immunoenrichment and MALDI-TOF MS	Nanobody Ig immunopurification	M-protein	serum	556	PEL, IFE	• MASS-SCREEN could be used to detect serum M-proteins with good overall sensitivity (92%) and specificity (80%)
Barnidge 2016 (22)	miRAMM with microLC-ESI-Q-TOF	No	Free light chains	serum	27	FLC Freelite	• Detection of monoclonal FLC in AL patients • High resolution and accurate mass measurements
Mills 2016 (23)	MASS-FIX (MALDI-TOF MS)	Nanobody Ig immunoenrichment	M-protein	Serum and urine	152 sera; 55 urines	PEL, IFE, Hevylite assay	• MASS-FIX detected all M-proteins in serum and urine, provided good isotype information, and accurately quantified the M-proteins • MASS-FIX could simultaneously measure FLC κ/λ ratios for IgG, IgA, and IgM • MASS-FIX can be used for screening, diagnosis, and monitoring plasma cell disorders
Mills 2015 (24)	miRAMM with microflow LC-ESI-Q-TOF MS	Ig enrichment using Melon gel	M-protein	serum	NA*	PEL, IFE	• The sensitivity to detect a monoclonal Ig above the polyclonal Ig background is significantly improved compared to PEL and IFE • Mass spectrometry is a tool to monitor M-proteins in patients with monoclonal gammopathy
Barnidge 2015 (25)	MALDI-TOF MS	Ig enrichment using Melon gel	M-protein and monoclonal free light chain	Serum and urine	NA*	PEL	• MADLI-TOF MS can be used to screen serum and urine samples for monoclonal Ig
VanDuijn 2015 (26)	LC-MS	Selected Reaction Monitoring	sFLC and M-immunoglobulin	Serum	NA	Nephelometry	• Interpretation with MALDI-TOF MS is more straightforward than ESI
Botz 2014 (27)	microLC-ESI-Q-TOF (miRAMM)	Ig enrichment using Melon gel then DTT reduction	Monoclonal light chains	Urine	257	PEL IFE	• The sensitivity of MALDI-TOF MS is similar to PEL

Table 1 (*continued*)

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Reference	Mass spectrometry method	Enrichment/ purification	Detected protein	Matrix	Effective	Method	Results
Barnidge 2014 (28)	LC-MS then microLC-ESI-QTOFMS (miRAMM)	Ig enrichment using Melon gel then DTT reduction	M-immunoglobulin	Serum and urine	23	PEL	• The light chain of M-protein can be monitored based on its molecular mass • Quantification and isotype identification could be performed in the same run • MicroLC-ESI-QTOFMS provides superior sensitivity and specificity compared to conventional methods

*, methodology article. sFLC, serum free light chain; M-protein, monoclonal protein; MASS-FIX, matrix-assisted laser desorption ionization time-of-flight mass-spectrometry; MicroLC-ESI-Q-TOF, electrospray ionization time-of-flight mass spectrometry; miRAMM, monoclonal immunoglobulin rapid accurate molecular mass; NA, not available.

distinguish monoclonal sFLC against the background from light chains associated with intact monoclonal immunoglobulin and/or polyclonal immunoglobulins. Moreover, an abnormal sFLC ratio in MASS-SCREEN-negative samples was noted in previous studies (20), suggesting that specific considerations are required for sFLC measurements. To address this issue, the authors developed sFLC enrichment coupled with MALDI-TOF MS methods (FLC MALDI-TOF MS). One hundred sixty-seven sera were selected based on the presence of M-protein as determined by IFE and the sFLC ratio and these were then used to quantify sFLC with FLC MALDI-TOF MS. The results were compared with IFE, MASS-FIX, and the sFLC ratio determined with Freelite® FLC.

With specific immunoenrichment, the overall agreement of FLC MALDI-TOF MS with the sFLC ratio was 98% for sera with a low (lambda-positive) kappa/lambda sFLC ratio and for sera with normal (negative) kappa/lambda sFLC ratio. For sera with high kappa/lambda sFLC ratio (kappa-positive), the overall agreement was 79%. One hundred percent of cases with abnormal sFLC ratios with detectable M-protein on IFE were confirmed with FLC MALDI-TOF MS and 76% of cases with abnormal sFLC ratios undetectable by IFE. FLC MALDI-TOF MS allows better identification of sera with lower sFLC ratios, which enhanced the confirmation of the monoclonal sFLC by 43% compared with IFE. However, 24% of the M-proteins were identified by the sFLC ratio rather than by FLC MALDI-TOF MS. The authors suggest the increased of the binding capacity or the analytical sensitivity and resolution for optimization of the preanalytical processing. Furthermore, the authors demonstrated that FLC MALDI-TOF MS can detect monoclonal sFLC at lower concentrations of monoclonal sFLC than IFE and other mass spectrometry techniques. FLC immunoenrichment eliminated a significant proportion of the polyclonal background than could have masked the presence of monoclonal sFLC.

Finally, mass spectrometry is presently the only methodology that allows detection and quantification of monoclonal sFLC independently of the κ/λ sFLC ratio. Although the FLC immunoenrichment method eliminates the polyclonal background, the sensitivity of the method needs to be increased in order to identify all monoclonal sFLC. Preanalytical optimization is still needed to improve the performance of sFLC extraction.

FCL immunoenrichment coupled to MALDI-TOF MS is an attractive approach, although a number of considerations should be taken into account. These

comprise its feasibility in routine laboratories, optimization of the manual preanalytical process, cost, and technical and clinical limitations. The clinical performance of the method should now be evaluated in patients with monoclonal gammopathy with or without renal impairment, in AL amyloidosis patients, while the pertinence of the test to assess treatment response, disease recurrence, and residual disease should also be evaluated. And one question remains unanswered: what is the place of this technique in clinical trials whose IMWG recommendations are based on the use of the Freelite assay?

Despite the quantitative results of sFLC, the authors state that the methodology is essentially based on qualitative detection of monoclonal sFLC and that quantification approaches should be developed for monoclonal sFLC.

Finally, the method published by Sepiashvili *et al.* appears to be the culmination of years of study (*Table 1*) that ultimately resulted in the development of what is now generally considered to be the best mass spectrometry method to detect sFLC in plasma cell disorders.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm.2020.03.200). OD reports grants from Sébia, grants from Siemens, grants from Binding site, outside the submitted work; CM reports grants from Sebia, grants from Binding Site, grants from Siemens, outside the submitted work. These grants were used in independent clinical trials to buy assays to tests all assays commercialized to measure free light chains. CRL has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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