Radboud Repository

Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
https://hdl.handle.net/2066/218890

Please be advised that this information was generated on 2021-05-13 and may be subject to change.

Clinical Chemistry 66:3
421–433 (2020)

Review

Mass Spectrometry for Identification, Monitoring, and Minimal Residual Disease Detection of M-Proteins

M. Zajec,[a,b,†] P. Langerhorst,[c,†] M.M. VanDuijn,[b] J. Gloerich,[c] H. Russcher,[a] A.J. van Gool,[c] T.M. Luider,[b] I. Joosten,[c] Y.B. de Rijke,[a,‡] and J.F.M. Jacobs[c,*,‡]

BACKGROUND: Monoclonal gammopathies (MGs) are plasma cell disorders defined by the clonal expansion of plasma cells, resulting in the characteristic excretion of a monoclonal immunoglobulin (M-protein). M-protein detection and quantification are integral parts of the diagnosis and monitoring of MGs. Novel treatment modalities impose new challenges on the traditional electrophoretic and immunochemical methods that are routinely used for M-protein diagnostics, such as interferences from therapeutic monoclonal antibodies and the need for increased analytical sensitivity to measure minimal residual disease.

CONTENT: Mass spectrometry (MS) is ideally suited to accurate mass measurements or targeted measurement of unique clonotypic peptide fragments. Based on these features, MS-based methods allow for the analytically sensitive measurement of the patient-specific M-protein.

SUMMARY: This review provides a comprehensive overview of the MS methods that have been developed recently to detect, characterize, and quantify M-proteins. The advantages and disadvantages of using these techniques in clinical practice and the impact they will have on the management of patients with MGs are discussed.

Introduction

Monoclonal gammopathies (MGs) are plasma cell disorders defined by the clonal expansion of plasma cells, resulting in characteristic excretion of a monoclonal immunoglobulin (Ig; M-protein). MGs encompass a broad spectrum of clinical disorders ranging from asymptomatic, benign MG of undetermined significance to life-threatening diseases such as multiple myeloma (MM) and amyloid light chain (AL) amyloidosis *(1)*.

M-protein detection and quantification are integral parts of diagnosis and monitoring of MG *(2)*. M-protein may consist of intact monoclonal Ig and/or monoclonal fragments such as free light chains (FLC) that can be detected in serum and/or urine. M-protein diagnostics is most commonly performed using electrophoretic methods, supplemented with additional assays for quantification and clonality testing *(3)*. Nonetheless, both traditional electrophoresis and immunochemical methods have analytical limitations that include standardization issues among different methods; poor analytical sensitivity, which hampers detection and/or accurate quantification of small M-proteins; and disease activity that remains unnoticed in patients with nonsecretory myeloma *(4)*.

Novel treatment modalities for MM have led to deeper responses, resulting in an increased percentage of patients that obtain stringent complete response (sCR), in which residual disease can no longer be detected using routine diagnostics in blood and/or urine *(5)*. Because many patients who obtain sCR will eventually relapse, analytically more sensitive assays capable of measuring minimal residual disease (MRD) are urgently needed. In addition, the introduction of therapeutic monoclonal antibodies (t-mAbs) can directly hamper traditional M-protein diagnostics because it can be challenging to distinguish the human(ized) t-mAbs from the endogenous M-protein.

Each M-protein is derived from recombination and somatic hypermutation events of both the heavy- and light-chain loci of the clonal B cell. Consequently, M-protein has both a unique amino acid sequence and unique molecular mass. Routine M-protein diagnostic methods, including electrophoretic and immunochemical methods, do not make use of these unique

[a] Department of Clinical Chemistry, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands; [b] Department of Neurology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands; [c] Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands.
*Address correspondence to this author at: Department of Laboratory Medicine, Radboud University Medical Center, Laboratory Medical Immunology (Route 469), Geert Grooteplein 10, 6525 GA Nijmegen, the Netherlands. Fax: +31-0-24-3619415; e-mail: h.jacobs@radboudumc.nl.
[†]Contributed equally.
[‡]Shared last authors.
Received July 23, 2019; accepted September 13, 2019.
DOI: 10.1093/clinchem/hvz041

©American Association for Clinical Chemistry 2020.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

M-protein features, beyond the general region of electrophoretic migration. Mass spectrometry (MS) is ideally suited to making accurate mass measurements or targeted measurements of unique M-protein peptides. Therefore, it is not surprising that new MS-based methods for the detection and quantification of M-proteins appeared in the literature beginning in 2014. Some of these novel methods have already been implemented in routine diagnostics. We anticipate that, in the near future, MS will play an increasingly important role in the field of M-protein diagnostics.

In this review we provide a comprehensive overview of current MS methods that can be applied to detect, characterize, and quantify M-proteins. The advantages and disadvantages to using these techniques to complement routine M-protein diagnostics and the impact they will have on the management of patients with MG are discussed.

Routine M-Protein Diagnostics

M-protein is a serum biomarker that relates directly to the clonal plasma cell burden in a patient with MG. The secreted M-protein can be used as a screening tool for the identification of MG and as a quantitative biomarker for disease prognostication to follow the course of disease and to monitor response to therapy. M-protein diagnostics are performed using high-resolution and semiautomated electrophoretic methods that are supplemented with additional assays for quantification and clonality testing *(3)*.

Serum protein electrophoresis (SPE) is performed using either agarose gel electrophoresis or capillary electrophoresis. These electrophoretic methods are commonly used for M-protein screening and quantification. Further characterization of the M-protein isotype is typically performed using immunofixation electrophoresis (IFE) or immunosubtraction–capillary electrophoresis. Turbidimetric and nephelometric analyses are performed to quantify total IgG, IgA, IgM, FLC, and heavy–light chain pairs *(2, 3)*. Katzmann et al. *(6)* have studied which panel of serologic tests is most cost-effective to screen for MG in a large cohort of patients with various plasma cell proliferative disorders. The heterogeneity of M-proteins and the limitation of each individual assay necessitates the use of multiple tests.

Numerous international guidelines provide recommendations for M-protein diagnostics of patients with a suspected MG and for patient follow-up *(3, 7–9)*. Despite these guidelines, test algorithms for M-protein diagnostics vary widely across laboratories *(10)*. M-protein quantification is further challenged by the analytical limitations and interferences observed both with electrophoretic methods and immunoassays applied within the field of M-protein diagnostics *(4, 11, 12)*. The actual spike of the M-protein as part of electrophoretic quantification remains a subjective procedure with suboptimal quantification of small M-proteins and those that comigrate with other abundant serum proteins, for example, in the β region *(4, 13)*. Recognition of the imprecision and inaccuracy of measurements of low-concentration monoclonal abnormalities is reflected in the International Myeloma Working Group (IMWG) guidelines that define a "measurable" M-protein as one that meets at least 1 of the following 3 criteria: serum M-protein ≥10 g/L; urine M-protein ≥200 mg/24 h; or serum involved FLC ≥100 mg/L, provided that the FLC ratio is abnormal *(14)*.

New treatment modalities have greatly improved the rates and depth of responses in patients with MM in the past decade *(15, 16)*. Because an increasing percentage of newly diagnosed MM patients obtain sCR, new assays need to be developed that can identify responses beyond conventionally defined sCR.

MRD Testing

Driven by the evolving framework of more effective multidrug treatment protocols, new methods have been developed to detect and quantify MRD. Current methodologies to assess MRD in MM patients focus on molecular and flow cytometric techniques performed on bone marrow aspirates *(5, 17)*. It is evident that among patients with MM who achieve sCR, MRD assessment by multicolor flow cytometry (MFC), allele-specific oligonucleotide–quantitative PCR (ASO-qPCR), or next-generation sequencing (NGS) can play an important role in patient management. MRD status is a major prognostic factor *(18)*. Moreover, MRD assessment can be applied to assess treatment effectiveness *(19)*. Consequently, new IMWG consensus criteria for MRD assessment have been defined that reach beyond the detection of the present therapy response criteria *(20)*. Generally, MRD negativity is defined by the absence of clonal plasma cells in bone marrow aspirates using methods with a minimum detection capability of 1 in $\geq 10^5$ nucleated cells.

The cellular method (MFC) and molecular methods (ASO-qPCR and NGS) to assess MRD allow the examination of millions of bone marrow cells or the corresponding amount of DNA (Fig. 1). Each technique has advantages and disadvantages that need to be considered (Table 1). The various MRD methods and their test characteristics have been extensively reviewed elsewhere *(5, 17, 19)*. Characteristics of an ideal MRD assay are high sensitivity, specificity, and reproducibility; feasibility for all MM patients; standardization among institutes; small sample volume; easy applicability; rapid turnaround time; and cost-effectiveness. None of the currently described methods to assess MRD meet all

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

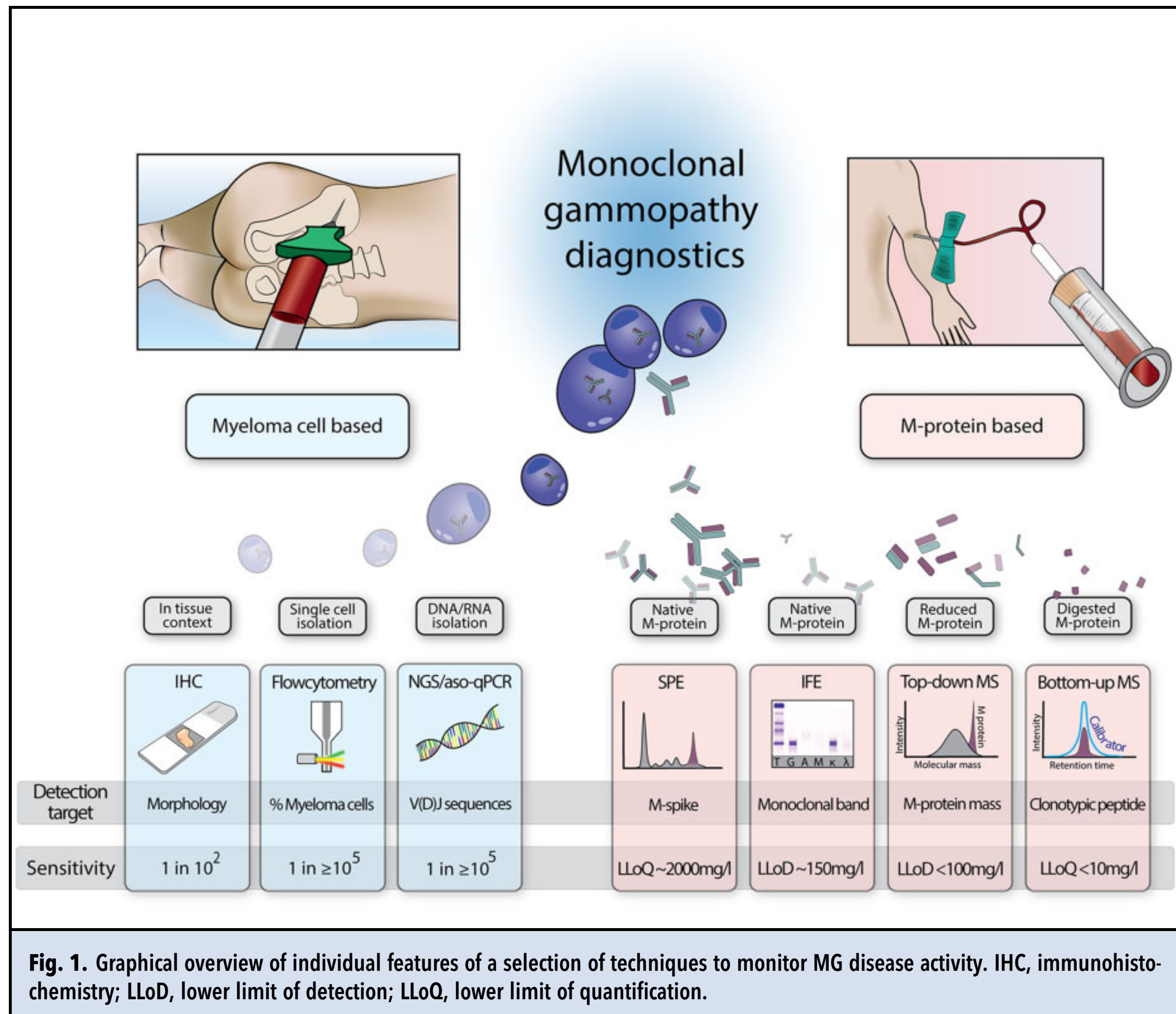

Fig. 1. Graphical overview of individual features of a selection of techniques to monitor MG disease activity. IHC, immunohistochemistry; LLoD, lower limit of detection; LLoQ, lower limit of quantification.

ideal test requirements. To assess differences in test characteristics in individual patients, the IMWG encourages inclusion of both MFC and NGS methods in prospective trials. This also allows direct comparison between the cellular methods that measure percentage of myeloma cells and the molecular methods that measure myeloma-specific gene sequences. It is further advised that MRD assessment should not be limited to a single time point because MRD kinetics over the disease course provide more robust evaluation of disease control in patients with MM after achieving sCR *(20)*.

The strongest limitation of the methods described is that disease monitoring must be performed on bone marrow aspirates, which introduces the risk of nonrepresentative sampling resulting from tumor heterogeneity *(21)*. The patchy nature of the disease has a direct negative impact on the reported results of these methods, and extramedullary MM outgrowth may give false-negative results even after repetitive bone marrow sampling. Another potential limitation is the complexity of these techniques, which makes them costly and difficult to standardize *(22)*. In addition, the need for repetitive bone marrow punctures for patient follow-up is a physical burden that reduces the quality of life for individual patients.

Evaluation of MRD in peripheral blood would represent an attractive minimally invasive alternative to circumvent the noted disadvantages of MRD assessment in bone marrow. Studies investigating the possibility of detecting MM disease activity in peripheral blood have emerged that use MFC on circulating myeloma cells and sequencing of tumor circulating DNA. Taken together, myeloma-specific targets in peripheral blood are available for evaluation of myeloma disease activity at diagnosis *(23)*. However, myeloma cells and tumor

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

Table 1. Characteristics of techniques to monitor multiple myeloma disease activity in bone marrow and serum.

	Bone marrow sampling			Serum sampling			
	MFC	ASO-qPCR	NGS	SPE/CE	IFE/IS-CE	Top-down MS	Bottom-up MS
Applicability, %	~100	~65	~90	~100	~100	~100	~100
Quantitative	Yes (clonal PCs)	Yes [V(D)J seq.]	Yes [V(D)J seq.]	Yes (M-spike)	No (visual M-peak)	No (arbitrary units)	Yes (internal standard)
Analytical sensitivity	1 in ≥ 10^5	1 in ≥ 10^5	1 in ≥ 10^5	LLoQ ~2 g/L	LLoD ~150 mg/L	LLoD ≤100 mg/L	LLoQ ≤10 mg/L
Baseline sample	Important but not mandatory	Mandatory	Mandatory	Not needed	Not needed	Not needed	Important but not mandatory
Fresh sample	Needed (<36 h)	Not needed[a]	Not needed[a]	Not needed	Not needed	Not needed	Not needed
Sample volume	≥5 x 10^6 cells[b]	≥1 x 10^6 cells[b]	≥1 x 10^6 cells[b]	500 µL	500 µL	≤ 100 µL	≤ 100 µL
Nonrepresentative sampling	Extramedullary and patchy disease			Non-secretory multiple myeloma (no M-protein biomarker)			
Turnaround time	2–3 h	BS: 3–4 weeks FU: ≤ 5 days	≤10 days	≤3 days	≤5 days	≤1 days	BS: 4–5 weeks[c] FU: ~5 days
Global availability	Intermediate	Limited	Limited	High	High	Few sites RUO[d]	Few sites RUO

BS, baseline sample (target identification); CE, capillary electrophoresis; FU, follow-up sample; IS, immunosubtraction; LLoD, lower limit of detection; LLoQ, lower limit of quantification; PC, plasma cell; RUO, research use only; M-spike, spike of the M-protein.

[a] DNA must be extracted within 36 h, analysis performed on archived DNA.

[b] More cells increase sensitivity.

[c] Without internal standard, the turnaround-time is equal to FU.

[d] Implemented in routine diagnostics at the Mayo Clinic in 2018.

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

circulating DNA are present at much lower levels in peripheral blood compared with the bone marrow. Consequently, disease activity measured at diagnosis becomes undetectable soon after effective therapy, even among electrophoretic-positive patients *(24)*. For that reason, these methods cannot be used for early detection of disease recurrence.

In summary, MFC, ASO-qPCR, and NGS are currently available molecular methods for MRD assessment in bone marrow that provide significantly improved sensitivity compared with conventional M-protein diagnostics. All 3 methods reach detection capabilities of at least 10^{-5}, defined as the detection of 1 MM cell among a background of at least 10^5 normal leukocytes. Because of the limited sensitivity of similar methods applied on peripheral blood, MRD investigation has been restricted to bone marrow. This may change in the near future with emerging novel methods for M-protein detection using MS.

Immunoglobulin Measurements Using MS

The impact of MS on laboratory diagnostics lies in both novel biomarker discovery and improved capacity to measure clinical analytes. MS has a long history, primarily for use in small-molecule quantification applied for confirmations of drugs of abuse, newborn screening, and screening for steroid hormones *(25)*.

MS methods that measure proteins were implemented much later in clinical laboratories because these assays are more complex to implement and require larger investments in terms of trained staff and equipment *(26)*. Increases in the linear dynamic range, as well as improved speed, resolution, and mass measurement accuracy, have made these instruments an attractive alternative for characterizing proteins. More user friendly and more robust, newer generation MS instruments have begun to play a role in clinical diagnostics *(26)*.

LC-MS is an analytical chemistry technique that combines the physical separation capacity of liquid chromatography with the mass analysis capacity of MS. This technique can be used to analyze complex samples. With the introduction of targeted LC-MS, quantification of protein biomarkers by measuring peptide surrogates has become feasible. As a result, different groups have pioneered methodology for Ig quantification using peptides derived from tryptic digestion of the constant Ig regions *(27, 28)*. In 2014, both groups published LC-MS/MS methods with stable isotope-labeled internal standards for quantification of total serum Ig and IgG subclasses *(27, 28)*. Our group demonstrated that accurate LC-MS/MS multiplex measurements of Ig heavy and light chains allowed complete Ig profiling including serum FLC quantification *(29)*. Van de Gugten et al. *(30)* used an optimized version of the IgG subclass LC-MS/MS method to demonstrate an apparent IgG4 cross-reactivity with immunonephelometric IgG subclass measurements. This cross-reactivity explains the discrepancies found between total IgG measurements and the total sum of the individual IgG subclasses observed in patients with IgG4-related disease. In addition to protein quantification, the rapid improvement in MS-based proteomics reveals structural Ig features that were previously unavailable with other techniques such as sequence information, polyclonal mass distributions, Ig glycosylation, and other posttranslational modifications *(31–33)*.

MS as a Novel Method for M-Protein Measurements in Peripheral Blood

Based on existing literature on analysis of t-mAbs *(34)*, a concept emerged that MS-based methods could be applied to measure patient-specific unique features of an M-protein. Proteomic methods are typically classified by preanalytical Ig processing into top-down, middle-down, and bottom-up (Fig. 2). The intact Ig is the starting analyte in top-down MS, and the fragmentation pattern further elucidates information on the primary structure. Conversely, bottom-up MS refers to the process in which the Ig is enzymatically digested into peptides. The Ig primary structure is inferred from the peptide sequences that are obtained by LC-MS/MS. These methods can be refined by reduction of the Ig into smaller fragments that can either be analyzed intact (middle-down) or after further digestion into peptides (middle-up) *(31, 35)*.

Important factors that contribute to optimal sensitivity and specificity of these MS methods are chemical reagents and methods used to isolate Ig and further cleave or digest these into fragments. Ig isolation decreases interference from other abundant proteins such as albumin in serum. Ig isolation can be achieved by physicochemical fractionation such as Ig precipitation, ion exchange chromatography (based on net charge), or size exclusion chromatography (based on size or molecular shape). Class-specific Ig purification can be achieved by protein A, protein G, or protein L affinity chromatography or immune capture directed against specific regions of the Ig of interest *(36)*. Cleaving Ig into smaller fragments through reduction of disulfide bonds, for example, with dithiothreitol or by enzymatic Ig cleavage will result in more manageable and more specific Ig fragments for further MS characterization. Peptides produced by further enzymatic digestion of these Ig fragments provide the input material for bottom-up MS profiling. Figure 1 provides a graphical overview of the MS methods to measure serum M-protein and their complementary value to other techniques that can be used to measure disease activity in blood and bone marrow of MG patients.

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

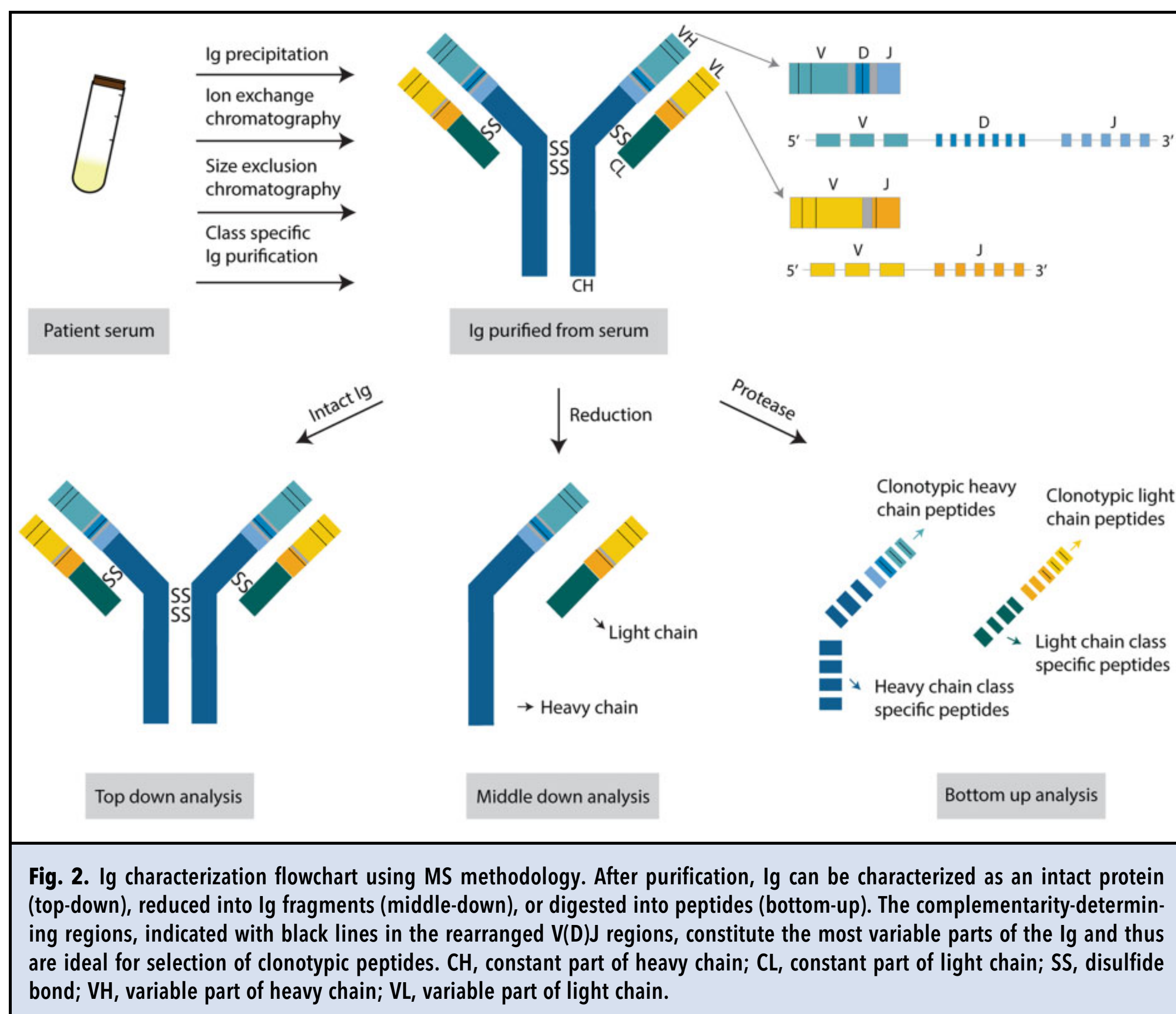

Fig. 2. Ig characterization flowchart using MS methodology. After purification, Ig can be characterized as an intact protein (top-down), reduced into Ig fragments (middle-down), or digested into peptides (bottom-up). The complementarity-determining regions, indicated with black lines in the rearranged V(D)J regions, constitute the most variable parts of the Ig and thus are ideal for selection of clonotypic peptides. CH, constant part of heavy chain; CL, constant part of light chain; SS, disulfide bond; VH, variable part of heavy chain; VL, variable part of light chain.

The chosen MS method and preanalytical process are dictated by the underlying clinical or research question. In the following paragraphs, we provide examples of how top-down MS can be used for high-throughput detection of an M-protein based on its unique mass; bottom-up MS can be used for highly sensitive quantification of an M-protein based on unique clonotypic peptides in the variable Ig region; and proteomics approaches can be used on tissue biopsy to detect monoclonal Ig deposits, for example, to diagnose AL amyloidosis.

M-Protein Quantification Using Intact Mass-Specific Methods (Top-down MS)

Intact protein analysis by MS (top-down MS) is based on monitoring the molecular mass of intact proteins or protein fragments (Fig. 3, A, upper panel). The application of intact protein analysis to M-protein diagnostics is based on the unique molecular mass and high abundance of the monoclonal Ig distinguishable from the otherwise polyclonal background. This unique mass can be determined by MS and serves as a personalized biomarker to monitor patients. Sample processing is simpler for intact analysis compared with peptide-based methods because protein digestion is not necessary. The only required sample processing step is Ig isolation and further cleavage into Ig fragments. Ig isolation increases the analytical sensitivity due to decreased interference from other highly abundant proteins such as albumin *(37)*.

The first evidence of feasibility of intact analysis to monitor M-proteins was described in 2014 by Barnidge et al. *(38)*. In their proof-of-principle study, they isolated the M-protein from sera from a single patient with MM and dissociated the Ig into light and heavy chains (middle-down MS), which were then subjected to liquid

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

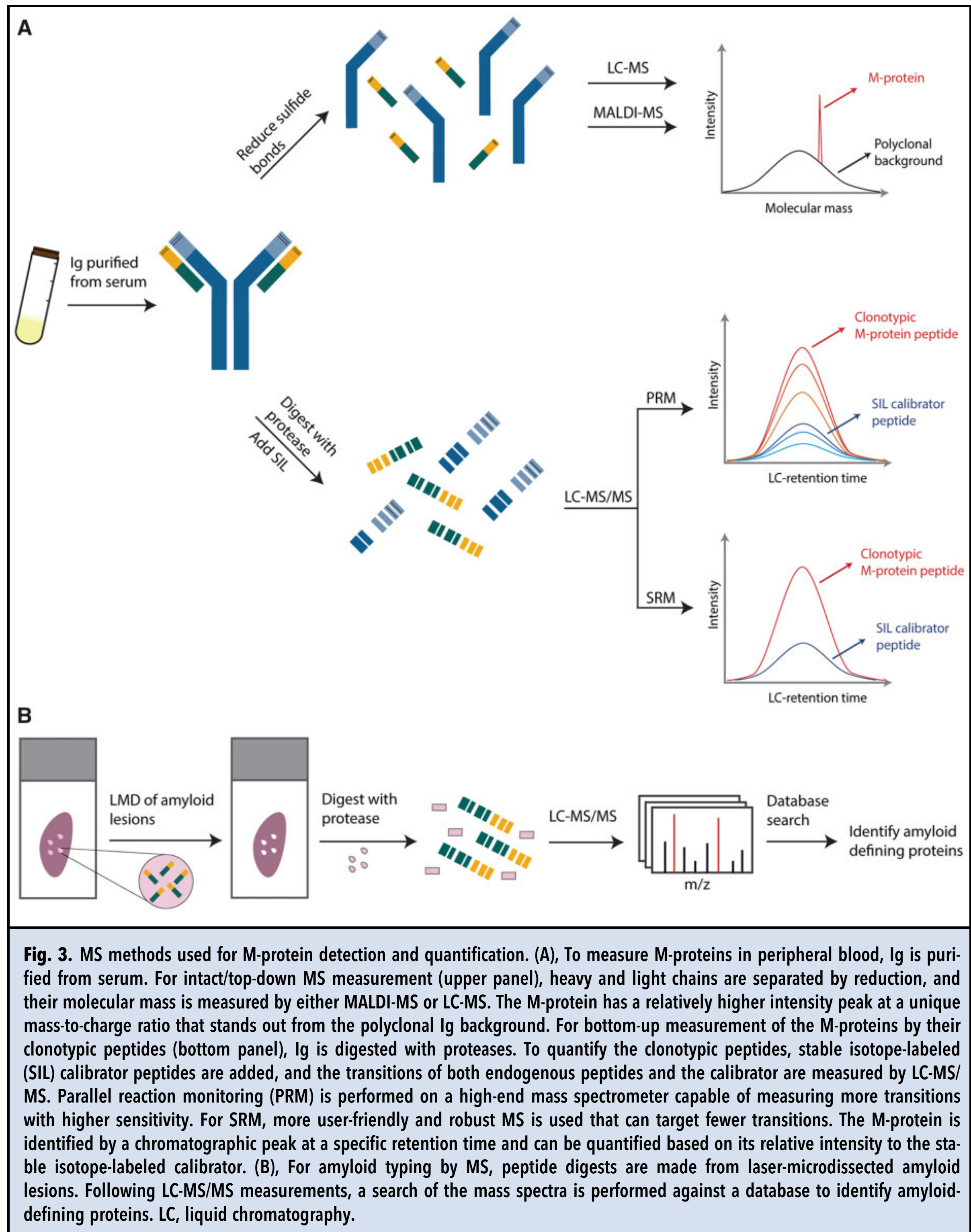

Fig. 3. MS methods used for M-protein detection and quantification. (A), To measure M-proteins in peripheral blood, Ig is purified from serum. For intact/top-down MS measurement (upper panel), heavy and light chains are separated by reduction, and their molecular mass is measured by either MALDI-MS or LC-MS. The M-protein has a relatively higher intensity peak at a unique mass-to-charge ratio that stands out from the polyclonal Ig background. For bottom-up measurement of the M-proteins by their clonotypic peptides (bottom panel), Ig is digested with proteases. To quantify the clonotypic peptides, stable isotope-labeled (SIL) calibrator peptides are added, and the transitions of both endogenous peptides and the calibrator are measured by LC-MS/MS. Parallel reaction monitoring (PRM) is performed on a high-end mass spectrometer capable of measuring more transitions with higher sensitivity. For SRM, more user-friendly and robust MS is used that can target fewer transitions. The M-protein is identified by a chromatographic peak at a specific retention time and can be quantified based on its relative intensity to the stable isotope-labeled calibrator. (B), For amyloid typing by MS, peptide digests are made from laser-microdissected amyloid lesions. Following LC-MS/MS measurements, a search of the mass spectra is performed against a database to identify amyloid-defining proteins. LC, liquid chromatography.

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

chromatography–quadrupole TOF analysis. Sequential monitoring of the unique mass of the M-protein light chain demonstrated improved analytical sensitivity compared with both gel-based techniques and FLC analysis. Furthermore, M-protein isotyping was possible because of the unique isotype-specific fragmentation pattern of the light chain. These proof-of-principle results led to further validation and development of the technique coined *miRAMM* (monoclonal Ig rapid accurate mass measurement) *(39)*. Subsequent studies revealed miRAMM to be a powerful tool in M-protein diagnostics and monitoring in matrices other than serum. A comparative study of light chain monitoring in urine by miRAMM and by IFE revealed equal analytical sensitivity of M-protein detection using miRAMM on neat urine and IFE on 200-fold concentrated urine (97.4% concordance) *(40)*. Further optimized versions of miRAMM demonstrated that this method can serve as a potential clinical assay that extends its analytical sensitivity for M-protein monitoring beyond that of conventional electrophoretic methods and the FLC assay. The superior analytical sensitivity of miRAMM was underscored in a study with 30 patients with MM reaching sCR after autologous stem cell transplantation *(41)*. At Day 100 after autologous stem cell transplantation, sCR samples had miRAMM identifiable M-proteins in 81% of patients, indicating increased analytical sensitivity compared with gel-based techniques. In this small data set, single time points of miRAMM M-protein status did not predict better progression-free survival. However, those patients whose miRAMM intensities decreased in 2 serial measurements had significantly longer progression-free survival compared with patients who did not experience decreased miRAMM intensities. The clinical relevance of miRAMM should be studied in a larger independent cohort with more frequent follow-up *(41)*. By analyzing nonreduced samples, miRAMM can also be applied for detection and quantification of monoclonal FLC, for example, for monitoring patients with light chain MM or AL amyloidosis *(42)*.

One drawback of miRAMM's limited use in high-volume routine diagnostics is the use of chromatography systems. Therefore, a chromatography-free system was developed by replacing micro Liquid Chromatography-Electrospray Ionization with MALDI *(43)*. This system allows higher sample throughput with measurement times of approximately 1 minute per patient sample. Moreover, the technique is simple in execution and has potential for automation. The combination of light chain immune enrichment and MALDI-TOF was termed *MASS-SCREEN* and provided a method to qualitatively screen for M-proteins in serum and urine. The clinical application of MALDI-TOF for M-protein monitoring in patient sera was demonstrated by Kohlhagen et al. *(44)*. In a comparative study performed in >500 patients, the authors demonstrated that MASS-SCREEN could be a cost-competitive screening method to detect M-proteins with a detection capability comparable to that of IFE. Because FLC ratios were found to be abnormal in 28% of MASS-SCREEN–negative samples, the method cannot replace FLC immunoassays. MALDI-TOF with minimal preanalysis was recently also applied for rapid screening of monoclonal FLC in urine *(45)*.

A drawback of MASS-SCREEN is the inability to distinguish Ig isotypes because the Ig isolation is performed with κ- and λ-directed nanobodies. To also account for Ig isotypes while retaining the benefits of MALDI-TOF analysis, MASS-FIX was introduced *(46)*. In the MASS-FIX workflow, 5 Ig isolations are performed with nanobodies directed against the constant domains of the heavy chains (IgG, IgA, and IgM) as and the light chains (κ and λ). This isolation strategy and subsequent MALDI-TOF analysis of the different fractions enables combined identification, isotyping, and quantification of M-proteins. In a comparative study between MASS-FIX and routine M-protein diagnostics, it was shown that M-proteins in 98% of sera and 95% of urine samples were similarly isotyped by IFE and MASS-FIX. In this study, the capability for detecting a serum M-protein by MASS-FIX was at least equal to that of IFE. MASS-FIX quantification, with interassay CVs of <20% in most samples, provided equivalent quantitative information to SPE *(46)*. A separate study that included clinical samples across the entire spectrum of plasma cell disorders confirmed that MASS-FIX had a comparable capacity to detect an M-protein compared with IFE *(47)*. Increased detection of abnormal FLC ratios was accomplished by performing an additional immunoenrichment using Sepharose-coupled antibodies against FLC followed by MALDI-TOF MS *(48)*. This approach allows direct FLC detection and provides added confidence for diagnosing MG based on monoclonal FLC.

Atypical mass spectra observed using MASS-FIX may provide additional information on posttranslational M-protein modifications *(47)*. An interesting observation was that a relatively large proportion (16%) of patients with AL amyloidosis had atypical spectra caused by glycosylated clonal light chains. It was further shown that these glycosylated light chains were present years before the diagnosis of AL amyloidosis *(49)*. This could be an interesting feature to screen for the risk of asymptomatic patients with MG to progress into AL amyloidosis. The pathologic and clinical impacts of these modifications warrant further research. Modified top- and middle-down MS can provide broad sequence coverage, which enables extensive mapping and glycoprofiling of M-proteins *(50)*.

Taken together, MASS-FIX is potentially a powerful alternative to gel-based techniques in M-protein

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

diagnostics, with competitive semiautomated sample throughput and some clear analytical advantages. In 2018, MASS-FIX replaced IFE in routine clinical practice at the Mayo Clinic.

M-Protein Quantification Using Peptide-Specific Methods (Bottom-up MS)

Bottom-up MS using targeted proteomics methods have been developed for ultrasensitive M-protein monitoring in peripheral blood that can potentially compete with MRD testing in bone marrow aspirates. The clonotypic (also called proteotypic) approach to measuring M-protein is based on peptide-targeted MS performed on serum digests from MM patients (Fig. 3, A, lower panel). Peptides unique for patient M-protein are selected and targeted with selected reaction monitoring (SRM) or parallel reaction monitoring *(28, 51, 52)*. Quantification of M-protein is possible by adding stable isotope-labeled peptides to serum or serum digest *(53)*. Stable isotope-labeled peptides are selected from the clonotypic candidates after assessing their performance for sensitivity and selectivity.

Clonotypic peptide candidates may be deduced from patient DNA or RNA sequencing information of the clonal plasma cells in the bone marrow. The Ig sequences of the clonal plasma cells are aligned to Ig germline sequences, and peptides with mutations relative to the germline sequence are selected. Because of the V(D)J clonal rearrangements and somatic hypermutations in the Ig complementarity-determining regions, these sites are considered to be of most interest for clonotypic peptide selection. There are 3 complementarity-determining regions on both heavy and light chains in the Ig antigen-binding part. For sequencing, 1 bone marrow aspirate taken during active disease is necessary. Efforts to develop methodology that no longer requires bone marrow are ongoing *(54)*. De novo sequencing on proteomics data may be feasible *(55)*. Computational de novo sequencing, in which a full amino acid M-protein sequence would be constructed from experimental, high-resolution, MS data, could eliminate the need for genome information and bone marrow sampling if adequate reliability can be achieved *(54, 55)*.

The detection capability of clonotypic targeted M-protein diagnostics is further improved by Ig purification during preanalysis to reduce the complexity of the patient serum. Digestion of the isolated Ig, including the M-protein, is most commonly performed with trypsin, and digested serum samples are measured on the mass spectrometer utilizing SRM (also called *multiple reaction monitoring*) *(28, 51)* and parallel reaction monitoring *(52)* technologies. SRM is usually performed with triple-quadrupole mass spectrometers to monitor targeted peptides and their selected fragments. Peptide and fragment ion pairs are called *transitions*, and in SRM, the transitions with the highest signal intensity have to be selected for every targeted peptide *(53)*. Conversely, parallel reaction monitoring is performed on high-resolution and high-accuracy mass spectrometers, and all fragments of targeted peptide can be detected in parallel, thus requiring less assay development than SRM (Fig. 3, A) *(56)*. Although clonotypic peptides have the potential to offer superior sensitivity, the process is more laborious and time consuming compared with MS methods performed on reduced Ig fragments such as miRAMM. It is important to note that effectiveness of the clonotypic MS assay can vary in individual patients because the number of suitable clonotypic peptides and their performance is patient-specific.

Murray et al. *(54)* showed that clonotypic targeting is >1.000 times more sensitive than SPE quantification and has the potential to be more sensitive than MRD analysis performed on bone marrow aspirates. MRD analysis in bone marrow and MRD analysis on M-protein in serum both have potential weaknesses. For bone marrow–based methods, as mentioned earlier, a significant portion of patients with MM present with focused lesions. Such solitary lesions, or extramedullary disease, would go unnoticed in a bone marrow aspirate unless performed at the exact site of the lesion. Nonrepresentative sampling can strongly bias MRD quantification in bone marrow aspirates. In contrast, disease activity would go unnoticed in serum-based assays when performed in the rare event of patients in whom the MM clone does not secrete an M-protein *(57)*. Furthermore, the M-protein is a surrogate marker of a cellular disease state. A confounding factor is the half-life of M-proteins in the blood: on average, 21 days for IgG and 10 days for IgA. This causes a delay between lysis of clonal plasma cells and the decrease in M-protein. It is challenging to compare the various MRD methods in terms of analytical performance because MFC measures myeloma cells, ASO-qPCR and NGS MRD techniques measure clonal DNA, and MS-based methods measure the M-protein. A good comparison between these methods for applicability, performance, and prognostic value is currently lacking.

MS Specifically Measures M-Protein without Interference from t-mAbs

The therapeutic landscape of MM has strongly evolved in the past decade. The first t-mAbs was approved for MM treatment, and a large list of biologics are being evaluated in clinical trials *(58)*. Such t-mAbs are all human(ized) mAbs that can appear on electrophoretic scans as small monoclonal bands *(59–61)*. In routine diagnostics, it may be challenging to differentiate the human(ized) t-mAbs from the endogenous M-protein.

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

Consequently, the IMWG response criteria have been modified to account for the presence of t-mAb interference *(62)*. However, comigration of t-mAbs and the endogenous M-protein can result in the inability to accurately assess therapeutic responses *(61, 63)*. Electrophoretic interference of t-mAbs can be overcome using a biologic-specific antibody that binds the t-mAbs and shifts SPE migration. For daratumumab, a so-called shift assay has been realized *(59)*. However, electrophoretic patterns will become increasingly difficult to interpret if multiple t-mAbs are combined for use in a single patient, and response assessment may not be possible.

MS methods can accurately quantify the M-protein without interference from multiple t-mAbs. Top-down MS makes use of the unique high-resolution mass of the t-mAbs *(37, 64, 65)*. In fact, initial proof-of-concept work for miRAMM technology was performed by serial dilutions of adalimumab in normal human serum *(38)*. In a recent study, miRAMM was able to correctly identify t-mAbs (daratumumab, elotuzumab, or isatuximab) and M-protein in 100% of the 192 samples tested *(66)*. The chance for an M-protein to have a mass so close to the mass of the t-mAbs to cause interference in top-down MS is estimated to be small. The problem of t-mAb interference is also solved in the targeted MS workflow by merely adding unique t-mAb peptides to the assay for targeting *(67)*. Our group has shown that M-protein can be detected in the presence of 3 additional t-mAbs without any cross-reactivity *(52)*. By adding reference stable isotope-labeled peptides for the t-mAbs and for the M-protein, all can be quantified in a single assay to allow additional therapeutic drug monitoring.

MS of Affected Tissue for Detection of AL Amyloidosis

Amyloidosis is a life-threatening disease caused by extracellular deposition of insoluble fibrils that can affect a wide variety of organs. Many different proteins have been identified that form these pathogenic fibrils. Misfolded monoclonal light chains are the most common and cause AL amyloidosis *(68)*. Accurate typing of amyloid is crucial for optimal treatment *(68)*.

Traditionally, histologic diagnosis of AL amyloidosis is based on Congo red staining to confirm the presence of amyloidosis. Congo red binds to amyloid fibrils, and green birefringence is seen under polarized light. Immunohistochemistry of tissue biopsies, staining the various potential amyloid proteins, is performed for further amyloid typing *(69)*. Immunohistochemistry using anti–light chain antisera can support confirmation of AL amyloidosis. However, immunohistochemistry interpretation is challenging because of the limited availability of type-specific antibodies, signal interference caused by tissue contamination from serum proteins, and false-negative results caused by the loss of epitopes in fixed tissue sections *(69)*. Moreover, the size of the plasma cell clone in patients with AL amyloidosis is often modest, which may further complicate and delay diagnosis *(70)*.

Proteomic typing of amyloid deposits using MS of affected tissue has not only improved amyloid diagnostics in terms of sensitivity and specificity but also has identified novel proteins as possible causes of amyloidosis *(70)*. Proteomic amyloid typing is performed on samples collected by laser microdissection; that way, affected lesions enriched for amyloid deposits can be analyzed using MS (Fig. 3, B). A complete proteome amyloid signature for diagnosis and typing of amyloidosis can be made by MS-based proteomics on subcutaneous fat aspirates, which could replace Congo red staining for confirmation of amyloidosis *(71, 72)*. The premise is that the protein causing amyloidosis represents a dominant protein in the deposits. Vrana et al. *(73)* have identified peptides from the constant regions of Ig in all cases of AL amyloidosis tested. Peptides from the variable regions can also be detected from patient MS data using an augmented database with light chain variable region sequence templates *(74)*.

MS-based proteomics have to be performed in specialized centers because the experimental methods and data processing require experienced personnel. New efforts are aimed at amyloid typing by targeted MS utilizing SRM technologies on more clinical laboratory–friendly, lower resolution mass spectrometers, without laser microdissection *(75)*. These methods target reference peptides for light chains to detect AL amyloidosis and heavy chains to control for serum contamination. The assays are multiplexed with other targets such as amyloid A and transthyretin amyloidosis to type amyloidosis with high sensitivity and specificity.

Conclusions

M-protein diagnostics can be challenging in individual patients because of patient-specific unique features. In addition, more sensitive assays are needed because improved treatment of patients with MG has resulted in deeper responses, with an increased number of patients who obtain sCR in which no disease activity is observed with routine M-protein diagnostics. Intact protein MS methods and clonotypic peptide MS methods have been developed that show promise for high-throughput M-protein detection and MRD measurements. These MS-based methods to measure M-proteins are applied on peripheral blood, which makes serial sampling possible to guide optimal personalized treatment. Consequently, they form an attractive alternative to the bone marrow–based methods currently applied for MRD detection (Table 1).

Although MS-based methods to measure M-proteins seem promising, several aspects have not been fully

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

addressed. First, current MS studies are based on relatively small sample sizes. Their feasibility and applicability in large cohorts have not yet been shown. Second, most studies have focused on MM and AL amyloidosis, with relatively little information on the applicability of MS-based methods in other MG, such as Waldenström's macroglobulinemia and plasmacytoma. Third, thresholds for sCR and MRD in peripheral blood using MS methods need to be defined. Finally, a direct comparison in which applicability, performance, prognostic value, and operational aspects such as cost and turnaround-time of MRD measured in bone marrow versus MS in peripheral blood has not yet been performed. MRD status obtained in bone marrow provides information that cannot be achieved by MS, such as clone evolution and bone marrow reconstitution. As such, we anticipate that, in the future, MS will not replace existing MRD tests in bone marrow but will have clinical value as a companion method, especially for monitoring of MRD in blood. This approach is in line with the recent IMWG recommendation *(20)* that the development of blood-based MRD monitoring should be the ultimate goal, as it would allow for serial sampling without the trauma of repeated bone marrow aspirations and ensures assessment of extramedullary disease, which is not evaluated by bone marrow biopsy.

Nonstandard abbreviations: MG, monoclonal gammopathy; Ig, immunoglobulin; M-protein, monoclonal immunoglobulin; MM, multiple myeloma; AL, amyloid light chain; FLC, free light chain; sCR, stringent complete response; MRD, minimal residual disease; t-mAb, therapeutic monoclonal antibody; MS, mass spectrometry; SPE, serum protein electrophoresis; IFE, immunofixation electrophoresis; IMWG, International Myeloma Working Group; MFC, multicolor flow cytometry; ASO-qPCR, allele-specific oligonucleotide–quantitative PCR; NGS, next-generation sequencing; miRAMM, monoclonal immunoglobulin rapid accurate mass measurement; SRM, selected reaction monitoring.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

A.J. van Gool, financial support.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: A.J. van Gool, Radboud University Medical Center.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: J.F.M. Jacobs, a grant from the Dutch Cancer Society (KWF Kankerbestrijding, No. 10817).
Expert Testimony: None declared.
Patents: None declared.

Acknowledgment: The authors thank Marilen Benner for the design of Fig. 1.

References

1. Glavey SV, Leung N. Monoclonal gammopathy: the good, the bad and the ugly. Blood Rev 2016;30: 223–31.
2. Willrich MAV, Murray DL, Kyle RA. Laboratory testing for monoclonal gammopathies: focus on monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Clin Biochem 2018;51:38–47.
3. Dimopoulos M, Kyle R, Fermand JP, Rajkumar SV, San Miguel J, Chanan-Khan A, et al. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood 2011;117:4701–5.
4. Keren DF, Schroeder L. Challenges of measuring monoclonal proteins in serum. Clin Chem Lab Med 2016;54: 947–61.
5. Mailankody S, Korde N, Lesokhin AM, Lendvai N, Hassoun H, Stetler-Stevenson M, et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. Nat Rev Clin Oncol 2015;12:286–95.
6. Katzmann JA, Kyle RA, Benson J, Larson DR, Snyder MR, Lust JA, et al. Screening panels for detection of monoclonal gammopathies. Clin Chem 2009;55: 1517–22.
7. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538–e548.
8. Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. Leukemia 2006;20:1467–73.
9. Kyle RA, Durie BG, Rajkumar SV, Landgren O, Blade J, Merlini G, et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. Leukemia 2010;24:1121–7.
10. Genzen JR, Murray DL, Abel G, Meng QH, Baltaro RJ, Rhoads DD, et al. Screening and diagnosis of monoclonal gammopathies: an international survey of laboratory practice. Arch Pathol Lab Med 2018;142: 507–15.
11. Jacobs JF, Tate JR, Merlini G. Is accuracy of serum free light chain measurement achievable? Clin Chem Lab Med 2016;54:1021–30.
12. Murray DL, Ryu E, Snyder MR, Katzmann JA. Quantitation of serum monoclonal proteins: relationship between agarose gel electrophoresis and immunonephelometry. Clin Chem 2009;55:1523–9.
13. Katzmann JA, Snyder MR, Rajkumar SV, Kyle RA, Therneau TM, Benson JT, et al. Long-term biological variation of serum protein electrophoresis M-spike, urine M-spike, and monoclonal serum free light chain quantification: implications for monitoring monoclonal gammopathies. Clin Chem 2011;57:1687–92.
14. Rajkumar SV, Harousseau JL, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood 2011;117:4691–5.
15. Chim CS, Kumar SK, Orlowski RZ, Cook G, Richardson PG, Gertz MA, et al. Management of relapsed and refractory multiple myeloma: novel agents, antibodies, immunotherapies and beyond. Leukemia 2018;32:252–62.
16. Barlogie B, Mitchell A, van Rhee F, Epstein J, Morgan GJ, Crowley J. Curing myeloma at last: defining criteria and providing the evidence. Blood 2014;124: 3043–51.
17. Bai Y, Orfao A, Chim CS. Molecular detection of minimal residual disease in multiple myeloma. Br J Haematol 2018;181:11–26.
18. Perrot A, Lauwers-Cances V, Corre J, Robillard N, Hulin C, Chretien ML, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood 2018;132:2456–64.
19. Paiva B, van Dongen JJ, Orfao A. New criteria for response assessment: role of minimal residual disease in multiple myeloma. Blood 2015;125:3059–68.
20. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

2016;17:e328–e346.
21. Rawstron AC, Orfao A, Beksac M, Bezdickova L, Brooimans RA, Bumbea H, et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica 2008;93:431–8.
22. Innao V, Allegra A, Russo S, Gerace D, Vaddinelli D, Alonci A, et al. Standardisation of minimal residual disease in multiple myeloma. Eur J Cancer Care 2017;26: e12732.
23. Kis O, Kaedbey R, Chow S, Danesh A, Dowar M, Li T, et al. Circulating tumour DNA sequence analysis as an alternative to multiple myeloma bone marrow aspirates. Nat Commun 2017;8:15086.
24. Pugh TJ. Circulating tumour DNA for detecting minimal residual disease in multiple myeloma. Semin Hematol 2018;55:38–40.
25. Jannetto PJ, Fitzgerald RL. Effective use of mass spectrometry in the clinical laboratory. Clin Chem 2016;62: 92–8.
26. Lehmann S, Brede C, Lescuyer P, Cocho JA, Vialaret J, Bros P, et al. Clinical mass spectrometry proteomics (cMSP) for medical laboratory: what does the future hold? Clin Chim Acta 2017;467:51–8.
27. Ladwig PM, Barnidge DR, Snyder MR, Katzmann JA, Murray DL. Quantification of serum IgG subclasses by use of subclass-specific tryptic peptides and liquid chromatography–tandem mass spectrometry. Clin Chem 2014;60:1080–8.
28. Remily-Wood ER, Benson K, Baz RC, Chen YA, Hussein M, Hartley-Brown MA, et al. Quantification of peptides from immunoglobulin constant and variable regions by LC-MRM MS for assessment of multiple myeloma patients. Prot Clin Appl 2014;8:783–95.
29. VanDuijn MM, Jacobs JF, Wevers RA, Engelke UF, Joosten I, Luider TM. Quantitative measurement of immunoglobulins and free light chains using mass spectrometry. Anal Chem 2015;87:8268–74.
30. van der Gugten G, DeMarco ML, Chen LYC, Chin A, Carruthers M, Holmes DT, et al. Resolution of spurious immunonephelometric IgG subclass measurement discrepancies by LC-MS/MS. Clin Chem 2018;64:735–42.
31. Murray D, Barnidge D. Characterization of immunoglobulin by mass spectrometry with applications for the clinical laboratory. Crit Rev Clin Lab Sci 2013;50:91–102.
32. Barnidge DR, Dasari S, Ramirez-Alvarado M, Fontan A, Willrich MA, Tschumper RC, et al. Phenotyping polyclonal kappa and lambda light chain molecular mass distributions in patient serum using mass spectrometry. J Proteome Res 2014;13:5198–205.
33. Jacobs JF, Wevers RA, Lefeber DJ, van Scherpenzeel M. Fast, robust and high-resolution glycosylation profiling of intact monoclonal IgG antibodies using nanoLC-chip-QTOF. Clin Chim Acta 2016;461:90–7.
34. Dekker LJM, Zeneyedpour L, Brouwer E, Duijn MM, Sillevis Smitt PAE, Luider TM. An antibody-based biomarker discovery method by mass spectrometry sequencing of complementarity determining regions. Anal Bioanal Chem 2011;399:1081–91.
35. Thoren KL. Mass spectrometry methods for detecting monoclonal immunoglobulins in multiple myeloma minimal residual disease. Semin Hematol 2018;55:41–3.
36. Gagnon P. Technology trends in antibody purification. J Chromatogr A 2012;1221:57–70.
37. Ladwig PM, Barnidge DR, Willrich M. Mass spectrometry approaches for identification and quantitation of therapeutic monoclonal antibodies in the clinical laboratory. Clin Vaccine Immunol 2017;24:e00545–16.
38. Barnidge DR, Dasari S, Botz CM, Murray DH, Snyder MR, Katzmann JA, et al. Using mass spectrometry to monitor monoclonal immunoglobulins in patients with a monoclonal gammopathy. J Proteome Res 2014;13: 1419–27.
39. Mills JR, Barnidge DR, Murray DL. Detecting monoclonal immunoglobulins in human serum using mass spectrometry. Methods 2015;81:56–65.
40. Botz CM, Barnidge DR, Murray DL, Katzmann JA. Detecting monoclonal light chains in urine: microLC-ESI-Q-TOF mass spectrometry compared to immunofixation electrophoresis. Br J Haematol 2014;167:437–8.
41. Mills JR, Barnidge DR, Dispenzieri A, Murray DL. High sensitivity blood-based M-protein detection in sCR patients with multiple myeloma. Blood Cancer J 2017; 7:e590.
42. Barnidge DR, Dispenzieri A, Merlini G, Katzmann JA, Murray DL. Monitoring free light chains in serum using mass spectrometry. Clin Chem Lab Med 2016;54: 1073–83.
43. Barnidge DR, Krick TP, Griffin TJ, Murray DL. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to detect monoclonal immunoglobulin light chains in serum and urine. Rapid Commun Mass Spectrom 2015;29:2057–60.
44. Kohlhagen MC, Barnidge DR, Mills JR, Stoner J, Gurtner KM, Liptac AM, et al. Screening method for M-proteins in serum using nanobody enrichment coupled to MALDI-TOF mass spectrometry. Clin Chem 2016;62: 1345–52.
45. Long S, Qin Q, Wang Y, Yang Y, Wang Y, Deng A, et al. Nanoporous silica coupled MALDI-TOF MS detection of Bence-Jones proteins in human urine for diagnosis of multiple myeloma. Talanta 2019;200:288–92.
46. Mills JR, Kohlhagen MC, Dasari S, Vanderboom PM, Kyle RA, Katzmann JA, et al. Comprehensive assessment of M-proteins using nanobody enrichment coupled to MALDI-TOF mass spectrometry. Clin Chem 2016;62: 1334–44.
47. Milani P, Murray DL, Barnidge DR, Kohlhagen MC, Mills JR, Merlini G, et al. The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic. Am J Hematol 2017;92:772–9.
48. Sepiashvili L, Kohlhagen MC, Snyder MR, Willrich MAV, Mills JR, Dispenzieri A, et al. Direct detection of monoclonal free light chains in serum by use of immunoenrichment-coupled MALDI-TOF mass spectrometry. Clin Chem 2019;65:1015–22.
49. Kourelis T, Murray DL, Dasari S, Kumar S, Barnidge D, Madden B, et al. MASS-FIX may allow identification of patients at risk for light chain amyloidosis before the onset of symptoms. Am J Hematol 2018;93: E368–E370.
50. He L, Anderson LC, Barnidge DR, Murray DL, Hendrickson CL, Marshall AG. Analysis of monoclonal antibodies in human serum as a model for clinical monoclonal gammopathy by use of 21 tesla FT-ICR top-down and middle-down MS/MS. J Am Soc Mass Spectrom 2017;28:827–38.
51. Barnidge DR, Tschumper RC, Theis JD, Snyder MR, Jelinek DF, Katzmann JA, et al. Monitoring M-proteins in patients with multiple myeloma using heavy-chain variable region clonotypic peptides and LC-MS/MS. J Proteome Res 2014;13:1905–10.
52. Zajec M, Jacobs JFM, Groenen P, de Kat Angelino CM, Stingl C, Luider TM, et al. Development of a targeted mass-spectrometry serum assay to quantify M-protein in the presence of therapeutic monoclonal antibodies. J Proteome Res 2018;17:1326–33.
53. Picotti P, Aebersold R. Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 2012;9:555–66.
54. Bergen HR 3rd, Dasari S, Dispenzieri A, Mills JR, Ramirez-Alvarado M, Tschumper RC, et al. Clonotypic light chain peptides identified for monitoring minimal residual disease in multiple myeloma without bone marrow aspiration. Clin Chem 2016;62:243–51.
55. He L, Anderson LC, Barnidge DR, Murray DL, Dasari S, Dispenzieri A, et al. Classification of plasma cell disorders by 21 tesla Fourier transform ion cyclotron resonance top-down and middle-down MS/MS analysis of monoclonal immunoglobulin light chains in human serum. Anal Chem 2019;91:3263–9.
56. Peterson AC, Russell JD, Bailey DJ, Westphall MS, Coon JJ. Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. Mol Cell Proteomics 2012;11:1475–88.
57. Patel UH, Drabick JJ, Malysz J, Talamo G. Nonsecretory and light chain escape in patients with multiple myeloma. Clin Lymphoma Myeloma Leuk 2018;18: e515–e519.
58. Touzeau C, Moreau P, Dumontet C. Monoclonal antibody therapy in multiple myeloma. Leukemia 2017;31: 1039–47.
59. McCudden C, Axel AE, Slaets D, Dejoie T, Clemens PL, Frans S, et al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. Clin Chem Lab Med 2016;54: 1095–104.
60. van de Donk NW, Otten HG, El Haddad O, Axel A, Sasser AK, Croockewit S, et al. Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflex assay (DIRA). Clin Chem Lab Med 2016;54:1105–9.
61. McCudden CR, Jacobs JFM, Keren D, Caillon H, Dejoie T, Andersen K. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. Clin Biochem 2018;51:72–9.
62. Durie BG, Miguel JF, Blade J, Rajkumar SV. Clarification of the definition of complete response in multiple myeloma. Leukemia 2015;29:2416–7.
63. Murata K, McCash SI, Carroll B, Lesokhin AM, Hassoun H, Lendvai N, et al. Treatment of multiple myeloma with monoclonal antibodies and the dilemma of false positive M-spikes in peripheral blood. Clin Biochem 2018;51: 66–71.
64. Willrich MA, Ladwig PM, Andreguetto BD, Barnidge DR, Murray DL, Katzmann JA, et al. Monoclonal antibody therapeutics as potential interferences on protein electrophoresis and immunofixation. Clin Chem Lab Med 2016;54:1085–93.
65. Moore LM, Cho S, Thoren KL. TOF mass spectrometry distinguishes daratumumab from M-proteins. Clin Chim Acta 2019;492:91–4.
66. Mills JR, Kohlhagen MC, Willrich MAV, Kourelis T, Dispenzieri A, Murray DL. A universal solution for eliminating false positives in myeloma due to therapeutic monoclonal antibody interference. Blood 2018;132:670–2.
67. Willrich M. Analysis of tryptic peptides from therapeutic monoclonal antibodies using LC-MS/MS. Methods Mol Biol 2019;1872:85–99.
68. Ramirez-Alvarado M. Amyloid formation in light chain amyloidosis. Curr Top Med Chem 2013;12:2523–33.
69. Picken MM. Proteomics and mass spectrometry in the diagnosis of renal amyloidosis. Clin Kidney J 2015;8: 665–72.
70. Palladini G, Merlini G. What is new in diagnosis and management of light chain amyloidosis? Blood 2016; 128:159–68.
71. Vrana JA, Theis JD, Dasari S, Mereuta OM, Dispenzieri A, Zeldenrust SR, et al. Clinical diagnosis and typing of

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020

systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics. Haematologica 2014;99:1239–47.

72. Mollee P, Boros S, Loo D, Ruelcke JE, Lakis VA, Cao KL, et al. Implementation and evaluation of amyloidosis subtyping by laser-capture microdissection and tandem mass spectrometry. Clin Proteom 2016;13:30.

73. Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR III, Dogan A. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. Blood 2009;114: 4957–9.

74. Dasari S, Theis JD, Vrana JA, Meureta OM, Quint PS, Muppa P, et al. Proteomic detection of immunoglobulin light chain variable region peptides from amyloidosis patient biopsies. J Proteome Res 2015;14:1957–67.

75. Conti M, Poppi I, Cavedagna TM, Zamagni E, Leone O, Corti B, et al. A targeted proteomics approach to amyloidosis typing. Clin Mass Spectrom 2018;7: 18–28.

Downloaded from https://academic.oup.com/clinchem/article-abstract/66/3/421/5729981 by Radboud University Nijmegen user on 15 July 2020