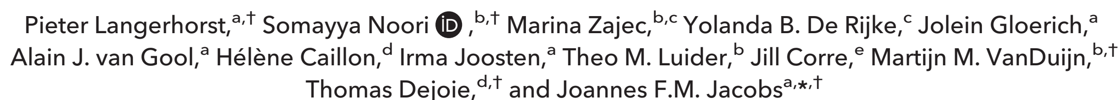
Clinical Chemistry 67:12
1689–1698 (2021)

Multiple Myeloma Minimal Residual Disease Detection: Targeted Mass Spectrometry in Blood vs Next-Generation Sequencing in Bone Marrow



Pieter Langerhorst,[a,†] Somayya Noori ,[b,†] Marina Zajec,[b,c] Yolanda B. De Rijke,[c] Jolein Gloerich,[a] Alain J. van Gool,[a] Hélène Caillon,[d] Irma Joosten,[a] Theo M. Luider,[b] Jill Corre,[e] Martijn M. VanDuijn,[b,†] Thomas Dejoie,[d,†] and Joannes F.M. Jacobs[a,*,†]

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

BACKGROUND: Minimal residual disease (MRD) status assessed on bone marrow aspirates is a major prognostic biomarker in multiple myeloma (MM). In this study we evaluated blood-based targeted mass spectrometry (MS-MRD) as a sensitive, minimally invasive alternative to measure MM disease activity.

METHODS: Therapy response of 41 MM patients in the IFM-2009 clinical trial (NCT01191060) was assessed with MS-MRD on frozen sera and compared to routine state-of-the-art monoclonal protein (M-protein) diagnostics and next-generation sequencing (NGS-MRD) at 2 time points.

RESULTS: In all 41 patients we were able to identify clonotypic M-protein-specific peptides and perform serum-based MS-MRD measurements. MS-MRD is significantly more sensitive to detect M-protein compared to either electrophoretic M-protein diagnostics or serum free light chain analysis. The concordance between NGS-MRD and MS-MRD status in 81 paired bone marrow/sera samples was 79%. The 50% progression-free survival (PFS) was identical (49 months) for patients who were either NGS-positive or MS-positive directly after maintenance treatment. The 50% PFS was 69 and 89 months for NGS-negative and MS-negative patients, respectively. The longest 50% PFS (96 months) was observed in patients who were MRD-negative for both methods. MS-MRD relapse during maintenance treatment was significantly correlated to poor PFS ($P < 0.0001$).

CONCLUSIONS: Our data indicate proof-of-principle that MS-MRD evaluation in blood is a feasible, patient friendly alternative to NGS-MRD assessed on bone marrow. Clinical validation of the prognostic value of MS-MRD and its complementary value in MRD-evaluation of patients with MM is warranted in an independent larger cohort.

Introduction

Multiple myeloma (MM) is the second most common hematological malignancy, characterized by the accumulation of clonal plasma cells, usually within the bone marrow (1). MM is an incurable disease, however the introduction of novel therapies has led to major improvements in clinical outcome (2). Routine monoclonal protein (M-protein) diagnostics play an important role in the screening and monitoring of MM patients (3). M-protein diagnostics are reliable, fast, and inexpensive, but not sensitive enough to detect low M-protein concentrations (4, 5). More than 50% of newly diagnosed patients reach a stringent complete response (6). However, most patients relapse despite achieving such deep responses, which stresses the need for new assays that can identify responses beyond conventionally defined stringent complete response.

Current methods to assess minimal residual disease (MRD) in MM patients focus on molecular and flow cytometric techniques performed on bone marrow aspirates (7, 8). Generally, MRD-negativity is defined by the absence of clonal plasma cells on bone marrow aspirates with a minimum sensitivity of 1 myeloma cell in $\geq 10^5$ nucleated cells. MRD assessment by multiparameter flow cytometry (MFC), allele-specific oligonucleotide (ASO)-quantitative PCR, or next-generation sequencing (NGS), can play an important role in the

[a]Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, the Netherlands; [b]Department of Neurology, Erasmus University Medical Center, Rotterdam, the Netherlands; [c]Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, the Netherlands; [d]Laboratoire de Biochimie, Centre Hospitalier Universitaire (CHU), Nantes, France; [e]Unité de Génomique du Myélome, Institute Universitaire du Cancer de Toulouse Oncopole, Toulouse, France.
*Address correspondence to this author at: Department of Laboratory Medicine, Laboratory Medical Immunology (route 469), Radboud University Medical Center, Geert Grooteplein 10, 6525 GA Nijmegen, the Netherlands. E-mail: H.Jacobs@Radboudumc.nl
[†]These authors contributed equally to this work.
Received April 26, 2021; accepted August 18, 2021.
DOI: 10.1093/clinchem/hvab187

©American Association for Clinical Chemistry 2021.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

management of patients in complete remission. MRD status has evolved into a major prognostic factor (9–11). Moreover, MRD assessment can be applied to assess treatment effectiveness in clinical trials (12). Consequently, new International Myeloma Working Group (IMWG) consensus criteria for MRD assessment have been defined that reach beyond the detection of the present therapy response criteria (6). A disadvantage of MRD evaluation on bone marrow aspirates is the risk of non-representative sampling, resulting from heterogeneous dispersion of MM cells and possibly hemodilution (13). Extramedullary MM outgrowth may give negative results even after repetitive sampling. In addition, repetitive bone marrow biopsies are invasive to patients.

Blood-based mass spectrometry (MS) that targets M-protein-specific peptides has recently been introduced as a sensitive and minimally invasive alternative for MRD-evaluation performed on bone marrow (14–16). In this study we evaluated the feasibility of the MS-MRD assay in a cohort of 123 sera obtained from 41 MM patients in the IFM 2009 clinical study. For all patients, serum protein electrophoresis, immunofixation electrophoresis, free light chain analysis, NGS-MRD results, and a median of 51 months clinical follow-up were available (17). Blood-based MS-MRD performance was compared to conventional M-protein diagnostics and NGS-MRD evaluation performed on bone marrow.

Materials and Methods

PATIENT SAMPLES

All samples were collected from the IFM 2009 clinical study (ClinicalTrials.gov identifier NCT01191060) (17), after written informed consent, and clinical and genomic data were de-identified in accordance with the Declaration of Helsinki and approval for this study was provided by our Institutional Review Board (2018-4140). The IFM 2009 trial was a phase 3 study started in 2012 enrolling 700 patients under 66 years who were eligible for autologous stem cell transplantation (ASCT). Treatment started with 3 cycles of lenalidomide, bortezomib, and dexamethasone (RVD) followed by either ASCT or additional 5 RVD cycles. The disease evaluation was done before and after 1 year of lenalidomide maintenance. At these time points, the disease evaluation was assessed on both blood and bone marrow aspiration. Among the 700 patients included in the IFM 2009 trial, we selected patients for this study based on the following inclusion criteria: (a) availability of NGS-MRD data performed pre-maintenance and post-maintenance; (b) availability of RNA sequencing data; and (c) availability of serum samples at baseline, pre-maintenance and post-maintenance. In total 41 patients fulfilled all 3 criteria.

ROUTINE M-PROTEIN DIAGNOSTICS

M-protein diagnostics were performed in a centralized laboratory as routine diagnostic workup of the IFM 2009 clinical study. Serum protein electrophoresis (SPEP) was performed by capillary zone electrophoresis (Capillarys, Sebia) and immunofixation electrophoresis (IFE) was performed on the Hydrasys 2 system using the Hydragel 4 IF kit (Sebia). Serum free light chain (sFLC) measurements were performed using Freelite reagents (The Binding Site) on a BNII nephelometer (Siemens Healthcare), according to the manufacturer's protocol. Reference intervals for the sFLC ratio are 0.26 to 1.65.

MS-MRD

The M-protein sequences targeted in the MS-MRD approach were selected based on bioinformatics analysis of a 100,000-reads sample extracted from RNA sequencing data produced for the NCT01191060 trial (18). A contig was constructed based on targeted assembly of reads from highly expressed mRNA related to the M-protein. Assembly was performed with the TASR software tool, directed by short seeding sequences from either conserved immunoglobulin regions, or from M-protein-specific complementary-determining region (CDR)3 sequences that could be revealed by the MiXCR software package (19, 20). Tryptic peptides from the M-protein sequence were predicted in silico and assessed for containing mutations compared to an immunoglobulin germline reference (IMGT). Qualifying peptide targets were evaluated experimentally by targeted mass spectrometry analysis on the matching diagnostic serum sample in the cohort, as well as other serum samples as controls. For peptides yielding the strongest signal in the diagnostic serum sample while remaining negative in the controls, stable isotope-labelled peptides were synthesized (Pepscan). Samples were reduced with 10 mmol/L dithiotreitol, alkylated with iodoacetamide, and digested overnight with trypsin in 150 mL/L acetonitrile, 50 mmol/L ammonium bicarbonate. During LC-MS data acquisition, a synthetic peptide mixture was sampled at every 10th injection to validate performance of chromatography and mass spectrometry systems. All targeted LC-MS data were obtained after separation on a Pepmap Acclaim C18 column (75 µm x15 cm, 30 min gradient, 300 nL/min) and a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific) at 120 000 resolution, $1x10^6$ AGC target and 1.4 *m/z* isolation. The collision energy was optimized by analyzing a mixture of all stable isotope-labelled (SIL) peptides at different collision energies, ranging from 18

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

to 36 normalized collision energy. The collision energy resulting in the highest signal intensity was determined for each peptide and subsequently used for the measurements. The targeted masses, monitored transitions, and optimized collision energy of each peptide can be found in online Supplemental Table 1. All targeted LC-MS data were analyzed with Skyline; an rdotp score of >0.89 was used as the threshold for positivity (21). All MS-MRD negative test-results were confirmed by re-injecting these samples. Examples of MS-MRD positive and MS-MRD negative samples are shown in online Supplemental Fig. 1. Online Supplemental Fig. 2A shows a replicate analysis from 3 patients in whom all 3 sample time points were digested and analyzed in 3 independent experiments. Sample digest stability was tested in 9 samples in which sample digests were stored at 8 °C for 12 weeks and re-analyzed on the same instrument. A linear fit of log-transformed data had a slope of 1.0091 and an R^2 of 0.9969 between fresh and 12-week-old samples (Supplemental Fig. 2, B). M-protein quantification was based on the average of the heavy and light chain clonotypic peptide, if applicable. For additional MS-MRD sensitivity of disease monitoring, all samples were analyzed a second time after 10 µL of serum was enriched for immunoglobulins using 100 µL of Melon Gel (Thermo Fisher Scientific) following the manufacturer's instructions. All MS-MRD analyses were performed blinded with respect to NGS-MRD results and survival data of the patients.

NGS-MRD

Collected bone marrow samples were frozen as dry pellets and stored at −80 °C until analysis. MRD was performed as previously described (9). Briefly, the clonal rearrangements were identified using the NGS commercial Clonoseq kit for MRD assay (Adaptive Biotechnologies®). For MRD quantification, DNA was extracted from the bone marrow samples and amplified by polymerase chain reaction using immunoglobulin gene-specific primers; the amplified products were then sequenced. The sensitivity was 10^{-6}, i.e., one malignant plasma cell within 1 000 000 bone marrow cells.

STATISTICAL ANALYSIS

GraphPad Prism 5.03 was used to perform statistical analyses and make the figures. The Kaplan–Meier method was used to estimate time-to-progression distributions, and statistical comparisons were done using log-rank (Mantel–Cox) tests.

Results

IDENTIFICATION OF CLONOTYPIC PEPTIDES

Patient -specific clonotypic peptides were selected based on the assembled clone sequences derived from RNAseq datasets (Fig. 1). For each heavy and light chain clone sequence in all 41 MM patients, multiple candidate clonotypic peptides could be identified from the immunoglobulin (Ig)-variable region. For the heavy chain on average 5.4 clonotypic peptides (range 2 to 7 peptides) and for the light chain on average 3.3 clonotypic peptides (range 2 to 5) were identified. Based on proteomics data, per M-protein 2 optimal clonotypic peptides were selected in terms of specificity and sensitivity in the mass spectrometer (Table 1). Clonotypic peptides were identified in sequences located at both the variable CDR and the more conserved framework regions, covering the full variable gene sequence (Table 1).

METHOD COMPARISON MS-MRD WITH ROUTINE M-PROTEIN DIAGNOSTICS

In all 41 patients a baseline sample and 2 follow-up samples were measured using routine M-protein diagnostics (SPEP and IFE), free light chain analysis and MS-MRD. Of these 123 samples, 52 sera were negative using IFE (online Supplemental Table 2), sFLC ratio was normal in 69 sera (online Supplemental Table 3), and MS-MRD measured 17 MRD negative sera in total (Supplemental Tables 2 and 3). In 39 sera the results were MS positive whereas IFE results were negative. In 52 sera the MS result was positive whereas the sFLC ratio was normal. In all 17 MS-MRD negative samples, the IFE result was also negative and the sFLC ratio was normal. This indicates that routine M-protein diagnostics were never more sensitive than MS-MRD in any of the sera measured in this cohort.

METHOD COMPARISON MS-MRD WITH NGS-MRD

NGS-MRD was performed on 81 bone marrow aspirates in this cohort taken at both pre-maintenance and post-maintenance treatment. MS-MRD was performed on serum acquired at the same time points. Nineteen percent (15/81) of the samples were NGS-MRD negative and 21% (17/82) of the samples were MS-MRD negative. Overall concordance between both methods was 79% (Table 2).

Progression-free survival (PFS) analysis suggests that with both MRD methods MRD negativity is associated with longer PFS, however the longer PFS was not found statistically significant ($P = 0.14$) which could be due to the relatively small cohort size (Fig. 2). The 50% PFS was 49 months for patients who were NGS-positive directly after maintenance treatment (Table 3). The 50% PFS of MS-positive patients was identical (49 months). The 50% PFS was 69 and 89 months for NGS-negative and MS-negative patients, respectively. The longest 50% PFS (96 months) was observed in patients who were MRD-negative for both methods.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

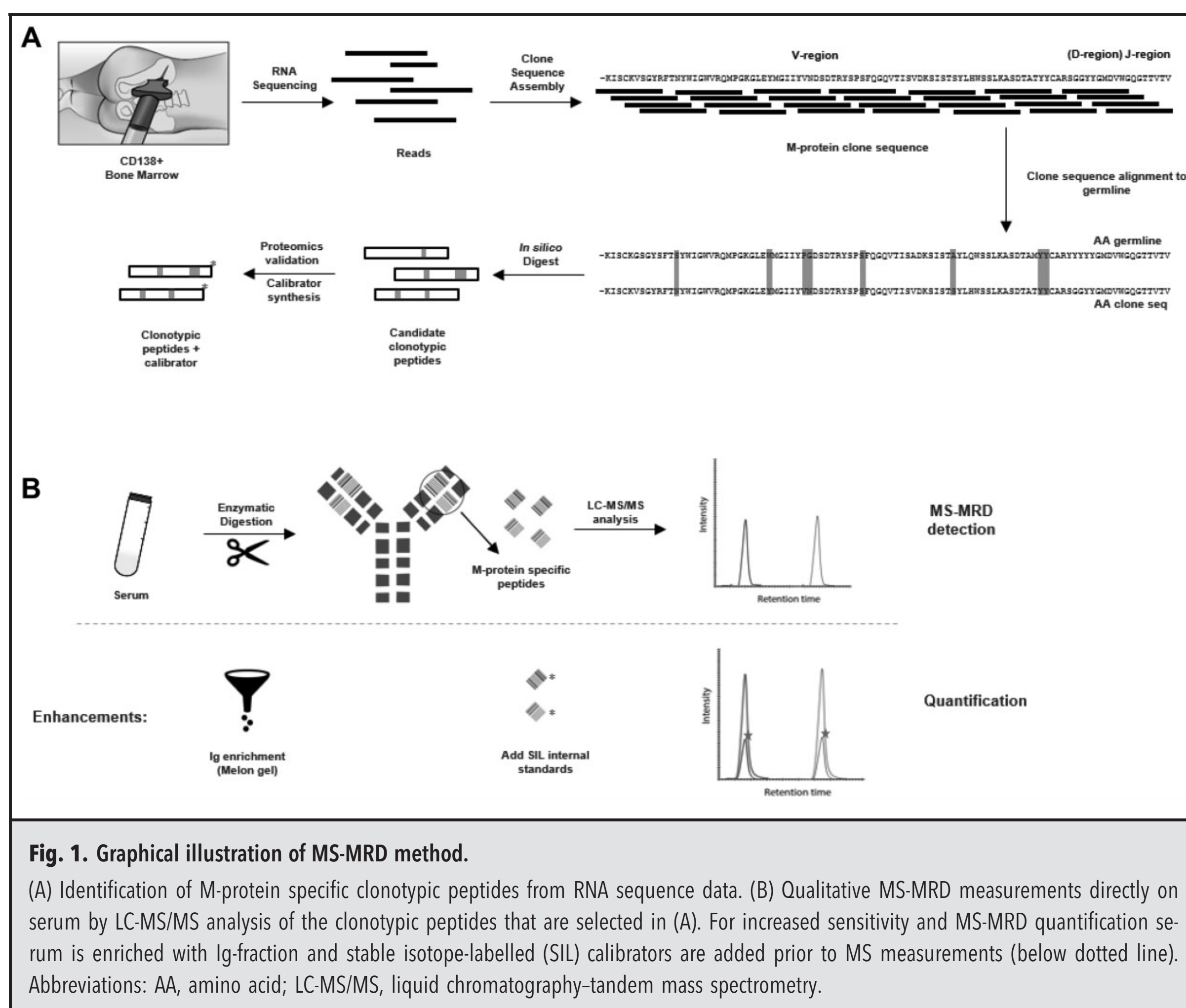


Fig. 1. Graphical illustration of MS-MRD method.

(A) Identification of M-protein specific clonotypic peptides from RNA sequence data. (B) Qualitative MS-MRD measurements directly on serum by LC-MS/MS analysis of the clonotypic peptides that are selected in (A). For increased sensitivity and MS-MRD quantification serum is enriched with Ig-fraction and stable isotope-labelled (SIL) calibrators are added prior to MS measurements (below dotted line). Abbreviations: AA, amino acid; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

OPTIMIZING MS-MRD

For quantitative MS-MRD measurements, SIL clonotypic peptides with a known concentration were synthesized for each patient. To quantify the M-protein, the quantifications of the heavy and light chain clonotypic peptides were averaged. Small differences between heavy and light chain clonotypic peptides were observed, but they showed similar trends between the 3 measured time points (online Supplemental Fig. 3). To further increase the sensitivity of the MS-MRD assay, a pre-analytical antibody purification step using Melon Gel was introduced (Fig. 1, B). The M-protein could be quantified down to approximately1 mg/L with a sufficient signal-to-noise ratio (online Supplemental Fig. 4). Re-analysis of all 82 MRD samples with SIL calibrators and Ig-enrichment reduced the number of MS-MRD-negative samples from 21% to 11% (9/82 samples).

DISEASE MONITORING USING MS-MRD

MS-MRD quantification by addition of the SIL calibrators allows for the first time monitoring of M-protein concentrations in patients who achieve stringent complete remission. We observed that the M-protein concentration in 35 out of 41 patients (85%) decreased during maintenance treatment; in the remaining 6 patients (15%) we observed an increased M-protein concentration during maintenance treatment (Fig. 3, A). MS-MRD relapse during maintenance treatment was significantly correlated to poor PFS (Fig. 3, B, $P < 0.0001$).

Discussion

Driven by the evolving framework of more effective multidrug treatment regimes, new methods have been developed to detect and quantify MRD in MM patients

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

Table 1. Overview of clonotypic peptides selected for each patient.[a]

Patient ID	M-protein	Heavy chain clonotypic peptide	Gene-location (begin-end)	Light chain clonotypic peptide	Gene-location (begin-end)
001 002PE	IgA-L[b]	YWGQGTLVTVSSASPTSPK	CDR3 - FR4[b]	VEDGDEADYYCQVWDSSSAHVVFGGGTR	FR3 - FR4
001 004SR	IgG-K[b]	SAGYYWSWIR	CDR1 - FR2	LEPEDFAVYFCQHYNTSPLFTFGPGTK	FR3 - FR4
007 003RF	IgG-K	AGTFDYWGQGTLVTVSSASTK	CDR3 - FR4	SSHSLYSSHNK	CDR1 - FR2
015 001GY	IgG-K	GLEWVTVIWHDGSK	FR2 - CDR2	WLAWYQQKPGK	FR3 - CDR3
015 002DP	IgG-L	GLEWVASISSK	FR1 - FR2	SEDEAEYYCASWDVSR	FR3 - CDR3
015 005GC	IgG-K	GSGYTFIDYYIHWVK	FR2 - CDR2	LEPEDFAVYFCQQYGSSPR	CDR1 - FR2
017 007VA	IgA-L	GLEWVSLITWDAGSTYYADSVK	FR2 -FR3	FSGSNSGSMATLTISR	FR3 - FR3
019 004BM	IgG-L	ALEWLAHIFSNDEK	FR2 - CDR2	QSVLTQPPSVSAAPGQR	FR1 - FR1
019 005BP	IgG-L	GLEWIGEIYHNGNANYNPTLK	FR2 - FR3	LMIYEVSGRPSGVSNR	FR2 - FR3
019 007SM	IgG-K	SDGGTTDYSAPVK	CDR2 - FR3	SSQSLLHSNGNNYLDWYLQ KPGQSPQLLIYLGSNR	FR1 - FR3
020 001DJ	IgA-K	EVQLVESGGALVEPGGSLR	FR1 - FR1	LLIFAASNLQSGVPSR	FR2 - FR3
020 004HC	IgG-K	ASGGIFTNSIITWVR	FR1 - FR2	ASSLEGGVPSR	CDR2 - FR3
022 004DJ	IgA-L	GLEWIGYIYHSGSTLYNPSLK	FR2 - FR3	DNTANQLVFGGGTK	CDR3 - FR4
022 006ML	IgG-K	YNWFDEWGQGTLVIVSSASTK	CDR3 - FR4	LLIHDASR	FR2 - FR3
024 004GM	IgG-L	GLEWVSSIGTVAADVYFPVSVR	FR2 - FR3	VEAGDEADYYCQVWDTTTN QGVFGGGTK	FR3 - FR4
025 002LD	IgG-K	GLEWVSYISSSGTYTNYADSVK	FR2 - FR3	LLIYDTSNLETGVPSR	FR2 - FR3
025 007MM	IgG-K	QAPGQGLEWMGAHIPIF GTPNYAQNFQGR	FR2 - FR3	ASQSVSGSYFAWYQQKPGQAPR	FR2 - FR2
034 005MA	IgG-K	NEFSLNLR	FR3 - FR3	APQLLIYAASNLK	FR2 - FR3
034 008DD	IgA-K	SLPIGELTSWGPGTLVTVSSASPTSPK	CDR3 - FR4	EIELTQSPGTLSLSPGEGATLSCR	FR1 - FR1
034 017AR	FLC-K[b]	Heavy chain not expressed		LLIYTASSLQNGVPSR	FR2 - FR3
036 001PR	IgG-L	GSSLSYYGMDVWGQGTTVTVSSASTK	CDR3 - FR4	QKPGQSPVLLIYQDNK	FR2 - FR3
038 008MG	IgG-K	DGGSSSTWYR	CDR3 - CDR3	AIQLTQSPSSLSASVEDR	FR1 - FR1
042 006BJ	IgA-L	EGQLVESGGALVEPGGSLR	FR1 - FR1	LLTQPPSASGTPGLR	FR1 - FR1
044 012MM	IgG-K	SQGGLIPLDSWGQGTLVAVSSASTK	CDR3 - FR4	LLIYDASTLEDGVPSR	FR2 - FR3
044 018CH	FLC-K	Heavy chain not expressed		LLIHATSNLQSGVPSR	FR2 - FR3
047 001HM	IgA-K	GLEFIGTMYYTGSTYYNPSLK	FR2 - FR3	LEPEDFAMYYCQQYGDSLWTFGQGTK	FR3 - FR4

Continued

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

Table 1. (continued)

Patient ID	M-protein	Heavy chain clonotypic peptide	Gene-location (begin-end)	Light chain clonotypic peptide	Gene-location (begin-end)
052 003LN	IgA-K	GLEWIGYVYYSGSTSYNPSLK	FR2 - FR3	APQLLIHTASNLQSGVPSR	FR2 - FR3
052 005CF	FLC-L	Heavy chain not expressed		VTISCSGSSSNIGR	FR1 - CDR1
052 007GC	IgG-K	DDSTNTVFLQMNNVR	FR3 - FR3	STLQGGVPSR	CDR2 - FR3
054 002LM	IgG-K	GLEWMGVIYPGDSDSR	FR2 - FR3	ASQNINFWLAWYQQKPGK	FR1 - FR2
054 006VN	IgG-K	QAPGTGLEWIAYISTGSTAIHYADSVK	FR2 - FR3	SVSSDLAWYQQK	CDR1 - FR2
067 005BJ	IgA-K	GMCVSWIR	CDR1 - FR2	APILLIYDASNLETGVPSR	FR2 - FR3
091 004SH	IgG-K	GLEWVSYISSSGSTIYHAASVQGR	FR2 - FR3	VTITCQASQDISNYLNWYQQK	FR1 - FR2
091 006GJ	IgG-K	DVDYHGMDVWGQGTTVTVSSASTK	CDR3 - FR4	SQGVTTWMAWYR	FR1 - FR2
091 010SM	IgG-L	LSCAAPGFTLNSYAMHWVR	FR1 - FR2	YVSWFQQHPGK	CDR1 - FR2
092 002 MJ	IgA-L	TVSGFSLSNPR	FR1 - CDR1	SVSGSPGQSVTISCTGTNSDVG GYDYVSWYQQHPGK	FR1 - FR2
099 006BE	IgG-K	GSGYTFSENAIHWVR	FR1 - FR2	ASEGVSSYYLAWYQQK	FR1 - FR2
104 002 PS	IgG-K	LSCAASGFTFSNYGMHWVR	FR1 - FR2	TNWLWTFGQGTK	CDR3 - FR4
152 002CL	IgG-L	NQVVLTMTNMDPMDTATYYCAHR	FR3 - CDR3	QPVLTQPPSSSASPGESAR	FR1 - FR1
152 005FM	IgG-K	GLEWLAVISGDETTK	Fr2 - CDR2	LLIYAASTLENGVPSR	FR2 - FR3
152 018MM	IgG-K	LDHGGNPFMYWGPGTLVAVSSASTK	CDR3 - FR4	FSGSGSVTDFTLEISR	CDR3 - FR3

[a]Per patient in the cohort, the selected clonotypic peptides and their respective location in the gene.
[b]Abbreviations: L, lambda; CDR, complementarity-determining region; FR, framework; K, kappa; FLC, free light chain.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

Table 2. Method comparison NGS-MRD and MS-MRD.[a]

	NGS-MRD positive	NGS-MRD negative
MS-MRD positive	57	8
MS-MRD negative	9	7

[a]MS-MRD method without Ig-purification and without stable isotope-labelled calibrators.

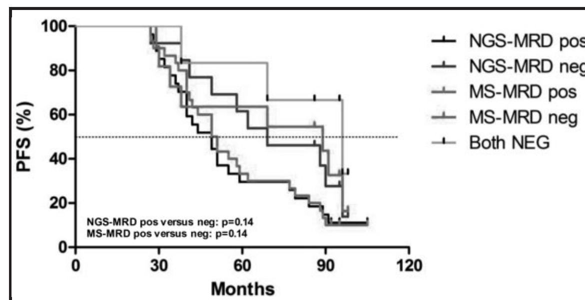


Fig. 2. **Kaplan–Meier curves for progression-free survival (PFS).**

PFS among patients who were tested for MRD-status directly post-maintenance treatment. The 50% PFS of patients who tested MRD-positive with either NGS-MRD (illustrated with black line) or MS-MRD (red) was 49 months. The 50% PFS increased to 69 months in the group of patients that tested NGS-MRD negative (blue) and 89 months in patients who tested MS-MRD negative (green). The longest PFS was observed in the group of patients who tested negative for both NGS-MRD and MS-MRD (grey).

(22). Consequently, new IMWG consensus criteria for MRD assessment have been defined that reach beyond the detection of the present therapy response criteria (6). In this study we evaluated MS-MRD performance in a cohort of 41 MM patients from the IFM 2009 clinical study. Blood-based MS-MRD performance was compared to conventional M-protein diagnostics and NGS-MRD evaluation performed on matching bone marrow samples.

MS-MRD testing necessitates identification of M-protein-specific clonotypic peptides in each patient. In our recent in silico study performed on a cohort of 609 patients, we demonstrated that multiple clonotypic peptides within the variable part of the M-protein could be identified from RNA sequence data in all MM patients (23). In this study we confirmed that on average 8 MS-suitable clonotypic peptides could be identified in each

Table 3. Progression-free survival (PFS) and correlation to MRD-status.

MRD status[a] measured post-maintenance	Number of patients	50% PFS (months)
NGS-MRD positive	27	49
MS-MRD positive	30	49
NGS-MRD negative	13	69
MS-MRD negative	11	89
NGS-MRD negative and MS-MRD positive	4	34
NGS-MRD positive and MS-MRD negative	7	58
NGS- and MS-MRD positive	23	49
NGS- and MS-MRD negative	6	96

[a]MS-MRD method without Ig-purification and without stable isotope-labelled calibrators.

patient. For a robust and confident M-protein quantitation by the MS-MRD assay, we selected a clonotypic M-protein peptide from both the heavy and light chain based on highest MS intensity for optimal sensitivity and specificity. When both clonotypic peptides are measured, it becomes feasible to also monitor disease activity in patients with free light chain escape (24). In practice, we did not observe this phenomenon in the current cohort. Three out of 41 patients had a light chain-only MM at study entry and for these patients we selected both peptides from the clonal light chain. The 150-bp paired-end sequencing on HiSeqX10 genome analyzers provided full coverage of the Ig-clone. That is an advantage over other sequencing approaches that limit information to a segment containing the CDR3 due to primer choice in a PCR amplification step. It allowed us to include clonotypic peptides from the entire variable region of the M-protein.

For ease of use, we initially performed the MS-MRD analyses directly on serum without enrichment of immunoglobulins and without addition of SIL calibrators to obtain qualitative MS-MRD data. MS-MRD performed on 123 sera was compared to routine electrophoretic M-protein diagnostics and FLC analyses that were performed on the same sera. In total 52 sera tested IFE negative, 69 sera had a normal sFLC ratio, and 17 sera were MS-MRD negative. All sera that were either IFE positive ($n = 64$) or had an abnormal sFLC ratio (54) were also found MS-MRD positive. This indicates that MS-MRD is more sensitive than IFE and sFLC analysis in all tested samples.

In 2 recent method comparisons between NGS and MFC MRD-evaluation performed on the same bone

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

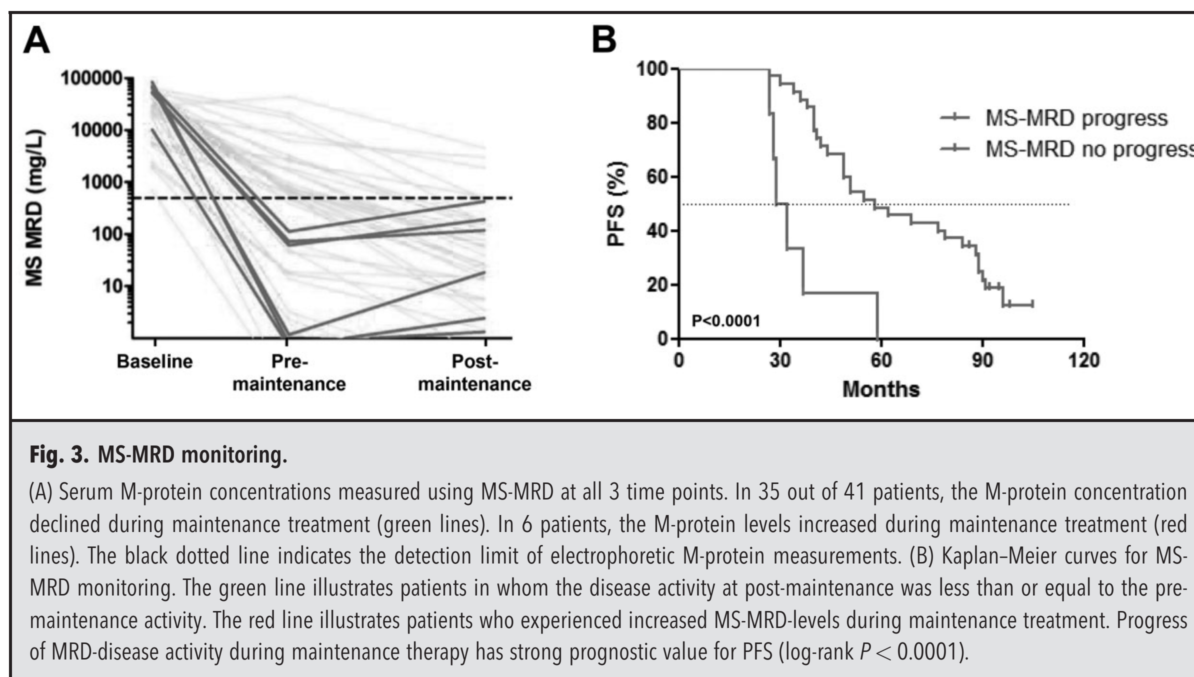


Fig. 3. MS-MRD monitoring.

(A) Serum M-protein concentrations measured using MS-MRD at all 3 time points. In 35 out of 41 patients, the M-protein concentration declined during maintenance treatment (green lines). In 6 patients, the M-protein levels increased during maintenance treatment (red lines). The black dotted line indicates the detection limit of electrophoretic M-protein measurements. (B) Kaplan–Meier curves for MS-MRD monitoring. The green line illustrates patients in whom the disease activity at post-maintenance was less than or equal to the pre-maintenance activity. The red line illustrates patients who experienced increased MS-MRD-levels during maintenance treatment. Progress of MRD-disease activity during maintenance therapy has strong prognostic value for PFS (log-rank $P < 0.0001$).

marrow aspirates, concordance rates of 68% (25) and 93% (26) were described. The overall concordance rate between NGS-MRD and MS-MRD in this study was 79%. That is high, considering that the two methods measure different biomarkers, as NGS measures DNA reads from myeloma cells in the bone marrow and MS measures M-protein in the blood. Our data are in line with the 63% concordance between NGS-MRD and clonotypic peptide MS analysis on serum that was recently reported by Derman et al. (15). Compared to NGS-MRD, their blood-based MRD assay was at least as sensitive to detect MRD and appeared to be a superior predictor of PFS (15). Also Martins et al. recently showed that targeted proteomics to detect clonotypic peptides in the serum of patients with MM is possible, even in patients who were tested negative with MFC MRD evaluation performed on bone marrow (16).

For patients with extramedullary myeloma disease spreading, blood-based MS-MRD will likely be more reliable. In contrast, in patients with non-secreting MM NGS performed on bone marrow will likely be more reliable. Another confounding factor for the evaluation of MS-MRD results, is the half-life of M-proteins in the blood which is 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. In individual patients it is difficult to prove the exact reason for a discordant result between NGS and MS. The complementary nature of both MRD analyses was further stressed by the fact that longest PFS was observed in patients who were MRD negative for both NGS and MS.

In this study we showed that the sensitivity of the MS-MRD analysis can be further increased using pre-analytical Ig-enrichment procedures and the introduction of SIL calibrators. Pre-analytical Ig-enrichment and addition of SIL calibrators increases the confidence with which low-concentration M-proteins are measured. As a consequence, an increasing number of patients test MS-MRD positive. In this cohort, only 9 out of 82 MRD sera were evaluated as MRD positive. In order to operate as a biomarker with the same prognostic value as NGS-MRD, this would necessitate the introduction of an MS-MRD cutoff value. The MS-MRD cutoff value that corresponds to the current MRD cutoff (1 myeloma cell in $\geq 10^5$ nucleated cells), must be assessed in a future study in a large independent cohort. The lower limit of our MS-MRD quantification method is estimated to be 1 mg/L M-protein (27).

While an MRD-negative treatment response is favorable, sustained MRD negativity is likely to be associated with even better prognosis. Sequential MRD analysis further has the potential to detect relapses early, before overt biochemical or clinical relapse (28). MS-MRD quantification was possible by addition of SIL calibrators, which allowed for the first time monitoring of M-protein concentrations in patients who achieve stringent complete remission. In this study we showed that MS-MRD relapse during maintenance treatment was significantly correlated to poor PFS. It is important

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

to note that it takes 4 to 5 weeks to develop and validate a patient-specific MS-MRD assay. The MS-MRD measurements for subsequent monitoring of a patient can be processed within 2 days, which makes it very suitable for dynamic MRD monitoring. For clinical implementation of MS-MRD this long turnaround time for the first MS-MRD measurement is likely not problematic since MRD evaluation is not relevant within the first half year after the start of therapy. Additionally, Martins et al. have shown that stable isotope-labelled control-Ig might be able to replace the most time-consuming element, namely the synthesis and validation of the SIL calibrators (16).

Conclusion

Our data indicate proof-of-principle that MS-MRD evaluation in blood is a feasible, patient friendly alternative to NGS-MRD assessed on bone marrow. 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 on bone marrow, but will have clinical value as a companion method especially for minimally invasive, longitudinal monitoring of MRD in blood. Further research is warranted to specify the exact role of MS-MRD analyses in the management of MM patients in larger cohorts.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: MRD, minimal residual disease; MM multiple myeloma; MS-MRD, blood-based targeted mass spectrometry; M-protein, monoclonal protein; NGS, next-generation sequencing, PFS, progression-free survival; MS, mass spectrometry; IFE, immunofixation electrophoresis; sFLC, serum free light chains; SIL, stable isotope-labelled; Ig, immunoglobulin.

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.*

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: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding : J.F.M. Jacobs received a research grant from the Dutch Cancer Society (#10817). This study was further sponsored by Sebia and an e-Infrastructure grant of the Dutch Research Counsel (#35856).
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: The authors thank Charles Guidet for his help with sample logistics.

References

1. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011;364:1046–60.
2. Moreau P, Attal M, Facon T. Frontline therapy of multiple myeloma. Blood 2015;125:3076–84.
3. Dimopoulos M, Kyle R, Fermand JP, Rajkumar SV, San Miguel J, Chanan-Khan A, et al.; International Myeloma Workshop Consensus Panel 3. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood 2011;117:4701–5.
4. Jacobs JFM, Turner KA, Graziani MS, Frinack JL, Ettore MW, Tate JR, et al. An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part II: limit of detection and follow-up of patients with small M-proteins. Clin Chem Lab Med 2020;58: 547–59.
5. Turner KA, Frinack JL, Ettore MW, Tate JR, Graziani MS, Jacobs JFM, et al. An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part I: factors impacting limit of quantitation of serum protein electrophoresis. Clin Chem Lab Med 2020; 58:533–46.
6. 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 2016;17:e328–e346.
7. Bai Y, Orfao A, Chim CS. Molecular detection of minimal residual disease in multiple myeloma. Br J Haematol 2018;181:11–26.
8. 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.
9. 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.
10. Tschautscher MA, Jevremovic D, Rajkumar V, Dispenzieri A, Lacy MQ, Gertz MA, et al. Prognostic value of minimal residual disease and polyclonal plasma cells in myeloma patients achieving a complete response to therapy. Am J Hematol 2019;94:751–6.
11. Munshi NC, Avet-Loiseau H, Anderson KC, Neri P, Paiva B, Samur M, et al. A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma. Blood Adv 2020;4: 5988–99.
12. 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.
13. Rawstron AC, Orfao A, Beksac M, Bezdickova L, Brooimans RA, Bumbea H, et al.; European Myeloma Network. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica 2008;93:431–8.
14. Zajec M, Langerhorst P, VanDuijn MM, Gloerich J, Russcher H, van Gool AJ, et al. Mass spectrometry for identification, monitoring, and minimal residual disease detection of M-proteins. Clin Chem 2020;66:421–33.
15. Derman BA, Stefka AT, Jiang K, McIver A, Kubicki T, Jasielec JK, et al. Measurable residual disease assessed by mass spectrometry in peripheral blood in multiple myeloma in a phase II trial of carfilzomib, lenalidomide, dexamethasone and

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022

autologous stem cell transplantation. Blood Cancer J 2021;11:19.

16. Martins CO, Huet S, Yi SS, Ritorto MS, Landgren O, Dogan A, et al. Mass spectrometry-based method targeting Ig variable regions for assessment of minimal residual disease in multiple myeloma. J Mol Diagn 2020;22: 901–11.
17. Attal M, Lauwers-Cances V, Hulin C, Leleu X, Caillot D, Escoffre M, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. N Engl J Med 2017;376:1311–20.
18. Cleynen A, Szalat R, Kemal Samur M, Robiou Du Pont S, Buisson L, Boyle E, et al. Expressed fusion gene landscape and its impact in multiple myeloma. Nat Commun 2017;8:1893.
19. Warren RL, Holt RA. Targeted assembly of short sequence reads. PLoS One 2011;6:e19816.
20. Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nat Methods 2015;12:380–1.
21. Pino LK, Searle BC, Bollinger JG, Nunn B, MacLean B, MacCoss MJ. The Skyline ecosystem: informatics for quantitative mass spectrometry proteomics. Mass Spectrom Rev 2020;39:229–44.
22. Diamond BT, Rustad E, Maclachlan K, Thoren K, Ho C, Roshal M, et al. Defining the undetectable: The current landscape of minimal residual disease assessment in multiple myeloma and goals for future clarity. Blood Rev 2021;46:100732.
23. Langerhorst P, Brinkman AB, VanDuijn MM, Wessels H, Groenen P, Joosten I, et al. Clonotypic features of rearranged immunoglobulin genes yield personalized biomarkers for minimal residual disease monitoring in multiple myeloma. Clin Chem 2021;67:867–75.
24. Brioli A, Giles H, Pawlyn C, Campbell JP, Kaiser MF, Melchor L, et al. Serum free immunoglobulin light chain evaluation as a marker of impact from intraclonal heterogeneity on myeloma outcome. Blood 2014;123: 3414–9.
25. Kriegsmann K, Hundemer M, Hofmeister-Mielke N, Reichert P, Manta CP, Awwad MHS, et al.; for the German-speaking Myeloma Multicenter Group (GMMG). Comparison of NGS and MFC Methods: key metrics in multiple myeloma MRD assessment. Cancers (Basel) 2020;12:2322.
26. Ho C, Syed M, Roshal M, Petrova-Drus K, Moung C, Yao J, et al. Routine evaluation of minimal residual disease in myeloma using next-generation sequencing clonality testing: Feasibility, challenges, and direct comparison with high-sensitivity flow cytometry. J Mol Diagn 2021; 23:181–99.
27. 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.
28. De Tute RC, Rawstron A, Pawlyn C, Davies F, Jones J, Kaiser M, et al. Sequential minimal residual disease (MRD) monitoring: results from the UK Myeloma XI trial. Clin Lymphoma Myeloma Leuk 2019;19:e45–6.

Downloaded from https://academic.oup.com/clinchem/article/67/12/1689/6395265 by guest on 25 November 2022