Progress in the application of minimal residual disease detection in multiple myeloma

Xiaojia Zuo¹ · Dingsheng Liu²

Received: 18 November 2020 / Accepted: 17 December 2020 / Published online: 4 January 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature 2021

Abstract

Currently, multiple myeloma is an incurable malignant plasma cell neoplasm, and the incidence rate ranks second in terms of hematological malignancies. Most of the patients are diagnosed at an age between 65 and 74 years, with a median age of 69 years. After the introduction of novel drugs or therapeutic methods, the treatment efficacy became significantly improved in multiple myeloma patients. Minimal residual disease is considered to be the most important reason for relapse in multiple myeloma patients after complete remission, which is significantly related to progression-free survival and overall survival. Therefore, minimal residual disease testing is essential for multiple myeloma patients after treatment. Several high-sensitivity technologies are used to detect minimal residual disease both inside and outside of the bone marrow of multiple myeloma patients after treatment, including cytology technology, molecular biology, and imaging technologies. In recent years, most of these minimal residual disease monitoring assays have made great progress. In this review, we focus on the characteristics and progress of the technologies that have been applied in multiple myeloma patients.

Keywords Minimal residual disease · Flow cytometry · High-throughput sequencing · Mass spectrometry · Circulating tumor DNA · PET

Introduction

In the last two decades, an increasing number of therapeutic options have been applied in multiple myeloma (MM) patients, such as proteasome inhibitors, immunomodulators, monoclonal antibodies, hematopoietic stem cell transplantations, and chimeric antigen receptor T cell (CAR-T) immunotherapy, thus resulting in unprecedented complete remission (CR) rates as well as prolonged progression-free survival (PFS) and overall survival (OS). A growing number of reports [1–6] have confirmed that PFS and OS are closely related to the depth of remission; specifically, the greater the degree of remission is, the longer the OS is. Minimal residual disease (MRD) after treatment is considered to be the relevant

prognostic factor of PFS and OS in MM patients [1, 7]. When considering the crucial role of MRD testing, the International Myeloma Working Group (IMWG) [8] has recommended that MRD testing be included in the MM treatment efficacy evaluation system. The traditional efficacy criteria include stringent complete response (complete response as defined below plus normal free light chain ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio $\leq 4:1$ or $\geq 1:2$ for κ and λ patients, respectively, after counting ≥ 100 plasma cells)), complete response (negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and $< 5\%$ plasma cells in bone marrow aspirates), very good partial response, minimal response, stable disease, and progressive disease [8]. The detection requirement for MRD is to achieve complete remission in the assessment of efficacy response for MM patients after treatment [8]. Various traditional methods, including serum protein electrophoresis, immunofixation electrophoresis, serum free light chain detection, and bone marrow cell morphology, exhibit several disadvantages, such as low sensitivity and narrow applicability [9]. At present, several novel assays have been introduced in the MRD monitoring process of MM patients (both intramedullary and extramedullary processes),

✉ Dingsheng Liu
liudingsheng1973@163.com

¹ Clinical Medical School, Guizhou Medical University, Guiyang, China

² Department of Oncology and Hematology, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, Shanghai, China

such as cytology technology, molecular biology technology, and functional imaging technology [8, 10, 11]. This article summarizes the characteristics and the progress of these novel MRD monitoring approaches in MM patients.

Cytology technology

Flow cytometry

Flow cytometry is a highly sensitive and rapid quantitative detection method that is used to identify cell-specific immune markers. The principle of the MRD monitoring method is to recognize abnormal plasma cells from the background of normal plasma cells in MM bone marrow (Fig. 1). At present, more than 10 biomarkers can be applied to distinguish between the abnormal plasma cells and the normal plasma cells, including CD38, CD138, CD19, CD20, CD27, CD28, CD45, CD56, CD81, CD117, CD200, and cytoplasmic κ and λ immunoglobulin light chains [8, 11]. When compared with 4- and 6-color multiparameter flow cytometry (MFC) methods, the simultaneous assessment of ≥ 8 -color at a single tube can greatly improve the ability of identifying abnormal plasma cell markers, and the sensitivity can also be increased to one in 10^4 – 10^5 cells [1, 12]. Currently, MFC is becoming the most widely used method to monitor MRD in MM bone marrow, due to its high sensitivity, wide application, low cost, and short detection time [2].

However, the shortcomings of MFC should be noted. For example, there is an issue of a lack of standardization [8]. Barring this exception, expert consensus and guidelines have regulated and recommended the selection of biomarker combinations as well as the number of detectable cells and MRD positive standards. However, subjective factors still exist, such as expert-shared knowledge and experiences, which cannot completely solve the problems of both the specificities of the markers themselves and the lack of technical standardization [8, 13, 14].

Next-generation flow cytometry

Next-generation flow cytometry (NGF) has upgraded the use of MFC in terms of sensitivity and standardization. When compared with the previous generation of flow cytometry technologies, the advantages [2, 8, 12] of NGF include the following: (1) the number of obtained cells can be increased to millions for the initial improvement of the cell lysis technology, and a sensitivity of one in 10^5 – 10^6 cells can be obtained; (2) the data analysis software has been updated, which has strengthened the quality control of the method and has greatly improved the ability to automatically analyze cells; and (3) a reliance on more antibodies (more than 8 markers), the optimization of the combination (2-tube) of fluorescent

dyes and antibodies, the presence of cross-validated results, improved reproducibility and consistency, and the reliability of the test results. NGF has provided similar test results in different MM research centers, which confirmed the high standardization level of the method [2]. Furthermore, NGF is becoming a more attractive MRD detection assay due to its high sensitivity and the enhanced ability for automation in MM patients [8].

However, one of the potential flaws of NGF is the high cost that occurs with the detection of more reagents [8]. When considering the NGF method, some MM centers have suggested that single-tube 10-color MFC and 14-color MFC (cross-applied to MM patients with a sensitivity of one in 10^5 [or greater] cells) can reduce the cost, shorten the time of data collection, and achieve standardization [8].

The consensus guidelines [15] recommend that the best antigen combination for flow cytometry includes CD38, CD138, CD45, CD19, CD56, CD27, CD81, and CD117; routine testing of intracellular light chains (κ and λ) is not recommended, which cannot provide extra information for more than 97% of MM patients.

Notably, the cellular immune phenotype of MM patients will be changed after the treatment of MM patients with drugs including chemotherapy drugs and monoclonal antibodies, which will lead to both drug resistance and affect the selection and detection of immune markers in flow cytometry [16]. For instance, the level of CD38 expression was rapidly downregulated when daratumumab was introduced to treat MM patients, eventually leading to drug resistance [17]. Therefore, it is essential to explore novel and reliable immune markers for MRD monitor. Although CD229 and CD319 were expressed in different leukocyte subsets, the expression level was highest in plasma cells with good stability [18]. In addition, CD54 was highly expressed in malignant plasma cells, but not downregulated in MM patients after treatment [19]. Moreover, the level of CD54 expression was even significantly increased in malignant plasma cells of MM patients receiving chemotherapy, especially in tumors with a multidrug resistance phenotype [19]. Herein, CD54, CD229, and CD319 may become reliable alternative biomarkers for MRD monitor [18, 20].

Molecular biology technology

Allele-specific oligonucleotide real-time quantitative polymerase chain reaction

In recent years, polymerase chain reaction (PCR) technology, which amplifies specific DNA fragments in vitro, has been rapidly developed, and numerous types of derivative PCR technologies have emerged. One type of MRD detection PCR method that has been widely used in MM bone marrow

Fig. 1 The site or target of various detection methods applied in multiple myeloma patients. κ , cytoplasm kappa light chain; λ , cytoplasm lambda light chain; MFC, multiparameter flow cytometry; NGF, next-generation flow cytometry; BM, bone marrow; PB, peripheral blood; CDR, complementarity determining region; FR, framework; PCR, polymerase chain reaction; ASO-RQ-PCR, allele-specific oligonucleotide real-time

quantitative PCR; ddPCR, droplet digital PCR; HTS, high-throughput sequencing; C, constant region; V, variable region; L, light chain; H, heavy chain; Ig, immunoglobulin; MS, mass spectrometry; PET/CT, positron emission tomography/computed tomography; MRI, magnetic resonance imaging; PET/MRI, positron emission tomography/magnetic resonance imaging

detection is allele-specific oligonucleotide real-time quantitative PCR (ASO-RQ-PCR), which mainly uses ASO to identify plasma cell-specific monoclonal rearrangements of immunoglobulin heavy chain (IgH) genes in MM patients (Fig. 1). When compared with traditional quantitative and semiquantitative PCR methods, the advantages [1] of ASO-RQ-PCR technology include (1) the detection of DNA by fluorescence quantitative methods without post-PCR operations, as well as

improved accuracy; (2) the detection of extremely low numbers of plasma cells and an increased sensitivity of one in 10^5 – 10^6 cells; and (3) good repeatability.

However, the disadvantages [1, 4, 8, 21, 22] of ASO-RQ-PCR technology include (1) the requirement for the design of specific primers as well as a low and uncertain success rate of primer design (only 30–80%); (2) the requirement for calibration curves; (3) the cumbersome detection process; (4) the

significant extension of the detection time; (5) the high cost; and (6) the fact that the method is only suitable for a small number of MM patients.

Digital PCR

Digital PCR (dPCR) technology is an emerging DNA quantitative detection technology. The main principle of dPCR technology [21, 23] is to distribute single DNA molecules into isolated reactions, and the product (with a fluorescence signal) can be detected and analyzed after amplification, with positive reactions occurring with the target gene, and negative reactions occurring without the target gene. Thus, one can obtain the absolute quantification of a single-molecule (Fig. 1). With more detectable DNA obtained, the sensitivity can be increased to one in 10^6 DNA molecules. When compared with ASO-RQ-PCR, the advantages [7, 21, 24] of dPCR include (1) an independence on a calibration curve; (2) higher sensitivity; and (3) more direct and absolute quantification. With these advantages, dPCR can be widely applied to high-throughput sequencing and many other fields (i.e., gene mutation, among other fields). Additionally, the type of dPCR that can detect MRD is known as droplet dPCR (ddPCR) in MM patients. Li Q and colleagues [25] have reported that ddPCR applied for the detection of BRAF, KRAS, and NRAS gene mutations in MM bone marrow and circulating tumor cells [CTCs] and confirmed that MM patients with high variant allele frequencies are correlated with shortened OS.

However, the disadvantages [23] of ddPCR (similar to ASO-RQ-PCR) mainly include the following: (1) the required design of specific primers; (2) high cost; (3) low level of automation analysis; and (4) low experimental throughput. Additionally, neither ASO-RQ-PCR nor ddPCR technology evaluates MRD for IgH complementarity determining region III (CDR3 region) gene mutations [21].

High-throughput sequencing

High-throughput sequencing (HTS) technology is a high-throughput DNA or RNA quantitative deep sequencing technology that is derived from PCR technology. The core areas of MRD detection for HTS are the CDR3 region sequence (Fig. 1) at the intersection of the IgH variable region (V region), the diversity region (D region), and the adjoining region (J region). Additionally, the CDR3 region of the kappa and lambda light chains can be a secondary area in MM patients [26]. Extensive studies [27–30] have confirmed that the CDR3 region is shared among all tumor cells and reflects good stability and high tumor specificity. The operating procedures [26, 31] of HTS are to first obtain a sample of the baseline of the tumor cells, then to define the tumor-specific sequences for ultradeep sequencing, to amplify one or more immunoglobulin V regions by the use of multiple PCR and to finally perform the

PCR product ultradeep sequencing analysis processing. From these steps, the sensitivity can be as high as one in 10^6 cells or more. The advantages [8, 26, 30, 32] of HTS include the following: (1) an independence on the design of specific primers or standard curves; (2) a higher sensitivity and specificity; and (3) a wide application. With these advantages, HTS is currently evaluated as the most promising MRD test technology.

However, the shortcomings [26, 31] of HTS are also very prominent, including a dependence on diagnosis samples, a high test cost, a long test time, and the need for an identification of clone targets at baseline. It should be pointed out that somatic hypermutation mainly affects the CDR region but cannot be completely avoided, and this indirectly affects the framework region that is bound by PCR primers [26]. This situation may lead to low PCR efficiency and a failure to amplify tumor sequences, which cannot be distinguished from the normal polyclonal background. A possible effective way to improving PCR efficiency is to add additional primers during the HTS detection process to obtain more cloning targets [26].

Circulating tumor DNA

Circulating tumor DNA (ctDNA) is a part of the total cell-free DNA (cfDNA) segment in peripheral blood, and this DNA is mainly derived from CTCs that exist in the somatic changes of gene mutation, rearrangement, cloning, or epigenetic modification, which can be distinguished from nontumor cells [33, 34]. ctDNA can be used as a new MRD test target in MM patients, and the current technologies that use ctDNA as the detection target include ddPCR and HTS [25, 33]. The advantages [33–36] of using ctDNA as the MRD detection target include (1) this method being a relatively noninvasive method and possessing convenient continuous sampling; (2) gene point mutation; (3) the method being used as an indicator of the total tumor burden in MM patients; and (4) the method being used as an auxiliary test for extramedullary and oligosecretory MM patients. The potential value of ctDNA detection is high, but the sensitivity of the current test method is low; thus, further research is essential to improve the sensitivity [8, 33, 35, 37]. It can also allow us to seek novel test approaches, such as double-stranded sequencing assays, which can detect gene point mutations with a sensitivity of up to one in 10^7 and can have a high specificity, but there have been no reports about its application in MM patients [38, 39].

Mass spectrometry

MM tumor cells secrete an abnormal monoclonal immunoglobulin, which is known as the M-protein (Fig. 1). Conventional protein electrophoresis or immunofixation electrophoresis cannot be used for MRD detection in MM patients

mainly based on the following two points: (1) having a low sensitivity and (2) failing to distinguish between therapeutic antibodies and antibodies produced by the disease itself for MM patients after monoclonal antibody treatment, and false-positive results will occur, which will hinder the efficacy evaluation of patients and lead to excessive treatment [40]. Mass spectrometry (MS) is a quantitative detection method for determining the quality of unique proteins with specific amino acid sequences. Mass spectrometry includes two types: clonal peptide MS and intact protein MS [9].

The clonal peptide MS uses the CDR-specific peptides of each patient's M-protein as disease markers (a bottom-up proteomics method) and uses a liquid chromatography-tandem MS and a triple quadrupole mass spectrometer to analyze patient-specific peptide after purifying immunoglobulin decomposition [9]. The detection of MRD characteristics [9, 41] of clonal peptide MS includes (1) having a high sensitivity of up to at least 0.0001 g/dL, the most important point, which is about 2000 times higher than serum protein electrophoresis; (2) having a high specificity targeted selection of specific patient peptides and using of advanced bioinformatics and computational algorithms to identify unique peptides for each patient, and being less affected by the expression of polyclonal background proteins; (3) having a complicated workflow and being dependent on a high-quality diagnosis baseline (bone marrow fluid or a high concentration of serum M-protein, > 0.8 g/dL); and (4) having a long test time (up to several weeks) and a high cost.

The intact protein MS approach is the monoclonal immunoglobulin rapid accurate molecular mass that uses the accurate intact light chain mass of M-protein as a disease marker (a top-down method) and the heavy and light chains of a sample can be analyzed by liquid chromatography MS using a high-resolution mass spectrometer [9, 41, 42]. When compared with the clonal peptide MS, the MRD detection characteristics [9, 43] of intact protein MS include (1) being only dependent on a peripheral blood diagnosis baseline; (2) using a nontarget method to collect data, which can distinguish therapeutic antibodies from antibodies produced by the disease itself; (3) having an easy operation and no need patient-specific; (4) being easily affected by the expression of polyclonal background proteins, as well as having a lower sensitivity; and (5) having a certain guiding significance, even in MFC MRD-negative MM patient.

A more convenient method than the intact protein MS method is the high-throughput version that is MALDI-Tof MS, the characteristics [9, 44–46] of which include (1) having a shorter data collection time (< 1 min/sample); (2) having a lower cost; (3) both urine-based and blood-based samples are eligible, and having confirmed that blood-based samples may be most useful for peripheral blood MM recurrence; and (4) having low-quality resolution, lacks chromatographic separation before MS analysis, and having a low sensitivity.

In addition, false-positives will appear in the MS test results, which may be related to the time misalignment between the lysis of MM tumor cells and the elimination of M-proteins, due to the longer half-life of IgG or IgA antibodies that are secreted by MM tumor cells [9].

Imaging technology

Intramedullary MM tumor cells are distributed in patchy infiltrations due to their high degree of heterogeneity, which can be infiltrated either simultaneously or solely outside of the bone marrow. If only the bone marrow is detected, false-negative MRD results will occur. To more accurately evaluate the MRD of MM patients, the IMWG in 2019 recommended the uses of 18F-deoxyglucose positron emission tomography/computed tomography (FDG PET/CT) technology and magnetic resonance imaging (MRI) technology to evaluate MM both inside and outside of the bone marrow (Fig. 1) [10].

PET scanning

PET scanning is an imaging technology that can reflect the metabolic activity of different tissues, based on the high uptake of tracers by tumor cells and the accumulation of tracers in tumor tissues. PET scans can assess and grade the lesions depending on the maximum standardized uptake value (SUV_{max}) of different tissues, and it can also distinguish benign and malignant lesions based on metabolic activity [47]. The most commonly used PET tracer is 18F-FDG, and other tracers that have been applied in MM research and that have shown potential value [48] include C-11 choline [49, 50], 18F-fluorocholine [51], 18F-fluoro-alpha-methyltyrosine (FAMT) [52], C-11 methionine [50, 53], and 18F-NaF [54]. However, PET scans alone cannot provide morphological data. The hybrid imaging technology combining PET scans and CT or MRI scans can provide dual time point imaging, as well as tissue metabolic activity and morphological imaging [55]. The measurements of the SUV_{max} of multiple sequence intervals after radiotracer injections may help to distinguish between benign lesions and malignant lesions [55].

PET/CT

The advantages [56–60] of PET/CT in MM lesion evaluations include (1) the potential value of specificity and sensitivity is 80–100% when applied in the detection of bone lesions, and the minimum diameter can reach 4 mm; (2) the vital prognostic value can be provided by PET/CT, in terms of the evaluation of positive lesions in MM patients after treatment, and the correlation is strong between PET/CT-negative and high-quality treatment responses; and (3) a fairly early evaluation of the efficacy response for MM patients can be provided by

the use of PET/CT when referring to 18F-FDG uptake level and SUV_{max} . To date, PET/CT is considered to be the best imaging tool for evaluating MM tumor metabolic activity and for monitoring efficacy [47].

At the same time, the disadvantages of PET/CT cannot be ignored, such as the high costs, the fact that it is not yet standardized, the prevalence of false-positive results, and the prevalence of false-negative results [47]. The false-positive results may be related to factors such as inflammation, infection, mental implants, and tracer deposition in physiological areas, and the false-negative results may be related to high blood sugar or a recent use of high-dose steroid drugs [47].

MRI

MRI is a nonradiation imaging technology that selectively visualizes water, fat content, and tissue composition based on magnetic resonance signals. The MRI features of MM patient lesion areas show a high T_2 signal and a low T_1 signal, with different signal enhancements [55]. The advantages [60–64] of MRI detection in MM patients include having (1) a significant value for evaluating bone involvement in early or post-treatment MM patients; (2) a degree of assessment of myeloma cell infiltration before bone damage; (3) a high spatial resolution of nonenhanced MRI concerning the bone marrow distribution; (4) no ionizing radiation; (5) the ability of a functional MRI (e.g., diffusion-weighted imaging and apparent diffusion coefficient) to quantitatively detect bone damage involvement in MM patients; (6) the sensitivity of whole-body MRI in detecting intramedullary lesions being higher than that of PET/CT in all bodily areas except for the skull, especially in patients with a low proportion of plasma cells; and (7) the sensitivity of whole-body diffusion-weighted imaging in detecting extramedullary lesions being equivalent with PET/CT, with the techniques complementing each other to some extent.

However, the false-positive rate of MRI results is high, which may be mainly related to the necrosis of the lesion tissue in complete remission MM patients after treatment [60].

PET/MRI

The characteristics [65, 66] of PET/MRI evaluations in MM patients include (1) the diagnostic data being more accurate, depending on the data provided by both the PET scan and the MRI high-resolution morphology of the soft tissue; (2) additional information being obtained from MRI perfusion techniques and diffusion-weighted imaging; (3) the tracer SUV_{max} of PET/MRI being significantly lower than that of PET/CT, but a significant correlation existing between the two methods; and (4) the ability of PET/MRI to be comparable to PET/CT in detecting bone lesions in MM patients, as well as the ionizing radiation being less than the latter technique. At

present, there are few research reports on the use of PET/MRI in MM patients.

The comparison between each method monitoring MRD applied in MM patients after treatment shown in Table 1

Currently, the IMWG consensus guidelines recommend that the dichotomy standard for MRD detection and evaluation defined 10^{-5} as the threshold by NGF or HTS; that is, $< 10^{-5}$ is negative, $> 10^{-5}$ is positive, the specific assessment criteria include sustained MRD-negative, flow MRD-negative, sequencing MRD-negative, imaging-positive MRD-negative, and recurrence after MRD-negative [8]. Apart from this, two other views of MRD division are present. One is proposed the view of MRD triple-classification from Programa Para el Estudio de la Terapéutica en Hemopatías Malignas/Grupo Español de Mieloma (PETHEMA/GEM), and the specific classification criteria defined by NGF or HTS include $< 10^{-6}$, 10^{-6} – 10^{-5} , 10^{-5} – 10^{-4} , and confirmed that $< 10^{-6}$ could determine MM patients with very low recurrence rate [67]. And the other is the view of MRD four-classification proposed by American scholars, which defined by NGF or HTS include $< 10^{-6}$, 10^{-6} – 10^{-5} , 10^{-5} – 10^{-4} , and 10^{-4} – 10^{-2} , yet this view still needs further research and verification [67]. False-negative results more likely occur when only bone marrow MRD monitoring is performed, for the high heterogeneity of MM, and combined with imaging to detect extramedullary lesions is the optimal choice for the efficacy evaluation of MM patients [11].

Conclusion and future directions

In conclusion, the remission rate is gradually increasing in MM patients after treatment in the era of novel drug applications, and updates of the detection methods for remission after treatment highlight the important clinical significance. Negative MRD statuses are gradually becoming a new research and clinical efficacy evaluation endpoint. Increasing numbers of MRD detection methods are currently being used in MM patients after treatment, but these methods have their advantages and disadvantages.

Cytology detection (represented by flow cytometry) is currently the main assay of MRD detection in the bone marrow of MM patients after treatment. However, the expression of cell-specific immune markers is affected in MM patients after the application of chemotherapy, monoclonal antibodies, and CAR-T cell immunotherapy. Accordingly, it is necessary to identify new and more effective immune biomarkers for MFC. Molecular biology methods (represented by HTS) have been a hot research topic in recent years. Research reports are

Table 1 The comparison between each detection method applied in multiple myeloma patients after treatment. *MFC*, multiparameter flow cytometry; *NGF*, next-generation flow cytometry; *PCR*, polymerase chain reaction; *ASO-RQ-PCR*, allele-specific oligonucleotide real-time quantitative PCR; *ddPCR*, droplet digital PCR; *HTS*, high-throughput sequencing; *ctDNA*, circulating tumor DNA; *MS*, mass spectrometry; *BM*, bone marrow; *PB*, peripheral blood; -, no data or not applicable; * the cloning-type peptide MS needs high-quality diagnosis baseline

	Cytology technology (≥ 8 colors)				Molecular biology technology				Imaging technology		
	MFC	NGF	ASO-RQ-PCR	ddPCR	HTS	ctDNA	MS		PET/CT	MRI	
Appliance	$\leq 100\%$	$\leq 100\%$	60–70%	80%	$\geq 90\%$	-	-		100%	100%	
Sensitivity	10^{-4} – 10^{-5}	10^{-5} – 10^{-6}	10^{-5} – 10^{-6}	10^{-5} – 10^{-6}	10^{-6}	-	0.0001 g/dL		4 mm	4 mm or smaller	
Samples	BM/PB	BM/PB	BM/PB	BM/PB	BM/PB	PB	PB/Urine		-	-	
Level of detection	Cells	Cells	DNA	DNA	DNA	DNA	Protein		Local or systemic	Local or systemic	
Sample requirement	Fresh samples within 24–48 h	Fresh samples within 24–48 h	Both fresh and storage samples are available	Both fresh and storage samples are available	Both fresh and storage samples are available	Both fresh and storage samples are available	Both fresh and storage samples are available		-	-	
Diagnose sample	Important but not necessary	Important but not necessary	Mandatory	Mandatory	Mandatory	-	Mandatory*		Important but not necessary	Important but not necessary	
Detection time	3–5 h	3–5 h	3–4 weeks at diagnosis, followed-up ≥ 5 days	3–4 weeks at diagnosis, followed-up 2–3 h	≥ 7 days	-	Several weeks		2 h	Few hours	
Detection cost	Low	Midian	High	High	High	Low	Low		High	Midian	
Standardization	No	Yes	Yes	No	Yes	No	No		No	No	
Quantitation	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	

gradually increasing for the detection of the MRD bone marrow of MM patients after treatment, and MRD methods for detecting MM patients in the peripheral blood have attracted attention as well. MM patients often involve either simultaneous or solely extramedullary lesions, and the detection of extramedullary lesions is of great significance. The current extramedullary detection methods mainly involve imaging techniques (e.g., functional MRI, PET/CT, and PET/MRI), and they have a high potential application value in the future research of MM patients.

Authors' contributions Xiaojia Zuo is responsible for writing the whole article; Dingsheng Liu is responsible for proofreading the whole article and is the corresponding author.

Funding This work was supported by the Discipline Construction Program of Pudong New District Health Bureau of Shanghai (Grant No. PWZzk2017-31).

Data availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Code availability Not applicable.

References

- Paiva B, Van Dongen JJ, Orfao A (2015) New criteria for response assessment: Role of minimal residual disease in multiple myeloma. *Blood* 125(20):3059–3068. <https://doi.org/10.1182/blood-2014-11-568907>
- Flores-Montero J, Sanoja-Flores L, Paiva B, Puig N, Garcia-Sanchez O, Bottcher S, Van Der Velden VHJ, Perez-Moran JJ, Vidriales MB, Garcia-Sanz R, Jimenez C, Gonzalez M, Martinez-Lopez J, Corral-Mateos A, Grigore GE, Fluxa R, Pontes R, Caetano J, Sedek L, Del Canizo MC, Blade J, Lahuerta JJ, Aguilar C, Barez A, Garcia-Mateo A, Labrador J, Leoz P, Aguilera-Sanz C, San-Miguel J, Mateos MV, Durie B, Van Dongen JJM, Orfao A (2017) Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia* 31(10):2094–2103. <https://doi.org/10.1038/leu.2017.29>
- Lahuerta JJ, Paiva B, Vidriales MB, Cordon L, Cedena MT, Puig N, Martinez-Lopez J, Rosinol L, Gutierrez NC, Martin-Ramos ML, Oriol A, Teruel AI, Echeveste MA, De Paz R, De Arriba F, Hernandez MT, Palomera L, Martinez R, Martin A, Alegre A, De La Rubia J, Orfao A, Mateos MV, Blade J, San-Miguel JF, Group G P C S (2017) Depth of response in multiple myeloma: a pooled analysis of three pethema/gem clinical trials. *J Clin Oncol* 35(25):2900–2910. <https://doi.org/10.1200/JCO.2016.69.2517>
- Munshi NC, Avet-Loiseau H, Rawstron AC, Owen RG, Child JA, Thakurta A, Sherrington P, Samur MK, Georgieva A, Anderson KC, Gregory WM (2017) Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. *JAMA Oncol* 3(1):28–35. <https://doi.org/10.1001/jamaoncol.2016.3160>
- Rawstron AC, Gregory WM, De Tute RM, Davies FE, Bell SE, Drayson MT, Cook G, Jackson GH, Morgan GJ, Child JA, Owen RG (2015) Minimal residual disease in myeloma by flow cytometry: independent prediction of survival benefit per log reduction. *Blood* 125(12):1932–1935. <https://doi.org/10.1182/blood-2014-07-590166>
- Paiva B, Gutierrez NC, Rosinol L, Vidriales MB, Montalban MA, Martinez-Lopez J, Mateos MV, Cibeira MT, Cordon L, Oriol A, Terol MJ, Echeveste MA, De Paz R, De Arriba F, Palomera L, De La Rubia J, Diaz-Mediavilla J, Sureda A, Gorosquieta A, Alegre A, Martin A, Hernandez MT, Lahuerta JJ, Blade J, San Miguel JF, Groups P G C S (2012) High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. *Blood* 119(3):687–691. <https://doi.org/10.1182/blood-2011-07-370460>
- Takamatsu H, Wee RK, Zaimoku Y, Murata R, Zheng J, Moorhead M, Carlton VEH, Kong KA, Takezako N, Ito S, Miyamoto T, Yokoyama K, Matsue K, Sato T, Kurokawa T, Yagi H, Terasaki Y, Ohata K, Matsumoto M, Yoshida T, Faham M, Nakao S (2018) A comparison of minimal residual disease detection in autografts among ASO-qPCR, droplet digital PCR, and next-generation sequencing in patients with multiple myeloma who underwent autologous stem cell transplantation. *Br J Haematol* 183(4):664–668. <https://doi.org/10.1111/bjh.15002>
- Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, Munshi N, Lonial S, Blade J, Mateos MV, Dimopoulos M, Kastritis E, Boccadoro M, Orłowski R, Goldschmidt H, Spencer A, Hou J, Chng WJ, Usmani SZ, Zamagni E, Shimizu K, Jagannath S, Johnsen HE, Terpos E, Reiman A, Kyle RA, Sonneveld P, Richardson PG, Mccarthy P, Ludwig H, Chen W, Cavo M, Harousseau JL, Lentzsch S, Hillengass J, Palumbo A, Orfao A, Rajkumar SV, Miguel JS, Avet-Loiseau H (2016) International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol* 17(8):e328–e346. [https://doi.org/10.1016/S1470-2045\(16\)30206-6](https://doi.org/10.1016/S1470-2045(16)30206-6)
- Thoren KL (2018) Mass spectrometry methods for detecting monoclonal immunoglobulins in multiple myeloma minimal residual disease. *Semin Hematol* 55(1):41–43. <https://doi.org/10.1053/j.seminhematol.2018.02.008>
- Hillengass J, Usmani S, Rajkumar SV, Durie BGM, Mateos M-V, Lonial S, Joao C, Anderson KC, Garcia-Sanz R, Riva E, Du J, Van De Donk N, Berdeja JG, Terpos E, Zamagni E, Kyle RA, San Miguel J, Goldschmidt H, Giral S, Kumar S, Raju N, Ludwig H, Ocio E, Schots R, Einsele H, Schjesvold F, Chen W-M, Abildgaard N, Lipe BC, Dytfield D, Wirk BM, Drake M, Cavo M, Lahuerta JJ, Lentzsch S (2019) International Myeloma Working Group consensus recommendations on imaging in monoclonal plasma cell disorders. *Lancet Oncol* 20(6):e302–e312. [https://doi.org/10.1016/S1470-2045\(19\)30309-2](https://doi.org/10.1016/S1470-2045(19)30309-2)
- Caers J, Garderet L, Kortum KM, O'dwyer ME, Van De Donk N, Binder M, Dold SM, Gay F, Corre J, Beguin Y, Ludwig H, Larocca A, Driessen C, Dimopoulos MA, Boccadoro M, Gramatzki M, Zweegman S, Einsele H, Cavo M, Goldschmidt H, Sonneveld P, Delforge M, Auner HW, Terpos E, Engelhardt M (2018) European Myeloma Network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when. *Haematologica* 103(11):1772–1784. <https://doi.org/10.3324/haematol.2018.189159>
- Soh KT, Wallace PK (2019) Monitoring of measurable residual disease in multiple myeloma by multiparametric flow cytometry. *Curr Protoc Cytom* 90(1). <https://doi.org/10.1002/cpcy.63>

13. Flanders A, Stetler-Stevenson M, Landgren O (2013) Minimal residual disease testing in multiple myeloma by flow cytometry: major heterogeneity. *Blood* 122(6):1088–1089. <https://doi.org/10.1182/blood-2013-05-506170>
14. Salem D, Stetler-Stevenson M, Yuan C, Landgren O (2016) Myeloma minimal residual disease testing in the united states: evidence of improved standardization. *Am J Hematol* 91(12):E502–E503. <https://doi.org/10.1002/ajh.24540>
15. Stetler-Stevenson M, Paiva B, Stoolman L, Lin P, Jorgensen JL, Orfao A, Van Dongen J, Rawstron AC (2016) Consensus guidelines for myeloma minimal residual disease sample staining and data acquisition. *Cytometry B Clin Cytom* 90(1):26–30. <https://doi.org/10.1002/cyto.b.21249>
16. Paiva B, Corchete LA, Vidriales MB, Puig N, Maiso P, Rodriguez I, Alignani D, Burgos L, Sanchez ML, Barcena P, Echeveste MA, Hernandez MT, Garcia-Sanz R, Ocio EM, Oriol A, Gironella M, Palomera L, De Arriba F, Gonzalez Y, Johnson SK, Epstein J, Barlogie B, Lahuerta JJ, Blade J, Orfao A, Mateos MV, San Miguel JF (2016) Spanish Myeloma Group / Program for the Study of Malignant Blood Diseases Therapeutics Cooperative Study G. Phenotypic and genomic analysis of multiple myeloma minimal residual disease tumor cells: a new model to understand chemoresistance. *Blood* 127(15):1896–1906. <https://doi.org/10.1182/blood-2015-08-665679>
17. Krejci J, Frerichs KA, Nijhof IS, Van Kessel B, Van Velzen JF, Bloem AC, Broekmans MEC, Zweegman S, Van Meerloo J, Musters RJP, Poddighe PJ, Groen RWJ, Chiu C, Plesner T, Lokhorst HM, Sasser AK, Mutis T, Van De Donk N (2017) Monocytes and granulocytes reduce CD38 expression levels on myeloma cells in patients treated with daratumumab. *Clin Cancer Res* 23(24):7498–7511. <https://doi.org/10.1158/1078-0432.CCR-17-2027>
18. Soh KT, Tario JD Jr, Hahn T, Hillengass J, McCarthy PL, Wallace PK (2020) CD319 (SLAMF7) an alternative marker for detecting plasma cells in the presence of daratumumab or elotuzumab. *Cytometry B Clin Cytom*. <https://doi.org/10.1002/cyto.b.21961>
19. Klausz K, Cieker M, Kellner C, Rosner T, Otte A, Krohn S, Lux A, Nimmerjahn F, Valerius T, Gramatzki M, Peipp M (2020) Fc-engineering significantly improves the recruitment of immune effector cells by anti-ICAM-1 antibody MSH-TP15 for myeloma therapy. *Haematologica*. <https://doi.org/10.3324/haematol.2020.251371>
20. Pojero F, Flores-Montero J, Sanoja L, Perez JJ, Puig N, Paiva B, Bottcher S, Van Dongen JJ, Orfao A, Euroflow G (2016) Utility of CD54, CD229, and CD319 for the identification of plasma cells in patients with clonal plasma cell diseases. *Cytometry B Clin Cytom* 90(1):91–100. <https://doi.org/10.1002/cyto.b.21269>
21. Takamatsu H (2017) Comparison of minimal residual disease detection by multiparameter flow cytometry, ASO-qPCR, droplet digital PCR, and deep sequencing in patients with multiple myeloma who underwent autologous stem cell transplantation. *J Clin Med* 6(10). <https://doi.org/10.3390/jcm6100091>
22. Ladetto M, Pagliano G, Ferrero S, Cavallo F, Drandi D, Santo L, Crippa C, De Rosa L, Pregno P, Grasso M, Liberati AM, Caravita T, Pisani F, Guglielmelli T, Callea V, Musto P, Cangialosi C, Passera R, Boccadoro M, Palumbo A (2010) Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. *J Clin Oncol* 28(12):2077–2084. <https://doi.org/10.1200/JCO.2009.23.7172>
23. Lin J, Su G, Su W, Zhou C (2017) Progress in digital PCR technology and application. *Sheng Wu Gong Cheng Xue Bao* 33(2):170–177. <https://doi.org/10.13345/j.cjb.160269>
24. Pott C, Bruggemann M, Ritgen M, Van Der Velden VHJ, Van Dongen JJM, Kneba M (1956) Mrd detection in B-cell non-Hodgkin lymphomas using ig gene rearrangements and chromosomal translocations as targets for real-time quantitative PCR. *Methods Mol Biol* 2019:199–228. https://doi.org/10.1007/978-1-4939-9151-8_9
25. Li Q, Huang HJ, Ma J, Wang Y, Cao Z, Karlin-Neumann G, Janku F, Liu Z (2020) Ras/raf mutations in tumor samples and cell-free DNA from plasma and bone marrow aspirates in multiple myeloma patients. *J Cancer* 11(12):3543–3550. <https://doi.org/10.7150/jca.43729>
26. Rustad EH, Boyle EM (2020) Monitoring minimal residual disease in the bone marrow using next generation sequencing. *Best Pract Res Clin Haematol* 33(1):101149. <https://doi.org/10.1016/j.beha.2020.101149>
27. Briney B, Inderbitzin A, Joyce C, Burton DR (2019) Commonality despite exceptional diversity in the baseline human antibody repertoire. *Nature* 566(7744):393–397. <https://doi.org/10.1038/s41586-019-0879-y>
28. Ho C, Arcila ME (2018) Minimal residual disease detection of myeloma using sequencing of immunoglobulin heavy chain gene VDJ regions. *Semin Hematol* 55(1):13–18. <https://doi.org/10.1053/j.seminhematol.2018.02.007>
29. Puig N, Conde I, Jimenez C, Sarasquete ME, Balanzategui A, Alcoceba M, Quintero J, Chillon MC, Sebastian E, Corral R, Marin L, Gutierrez NC, Mateos MV, Gonzalez-Diaz M, San-Miguel JF, Garcia-Sanz R (2015) The predominant myeloma clone at diagnosis, CDR3 defined, is constantly detectable across all stages of disease evolution. *Leukemia* 29(6):1435–1437. <https://doi.org/10.1038/leu.2015.7>
30. Rustad EH, Misund K, Bernard E, Coward E, Yellapantula VD, Hultcrantz M, Ho C, Kazandjian D, Korde N, Mailankody S, Keats JJ, Akhlaghi T, Viny AD, Mayman DJ, Carroll K, Patel M, Famulare CA, Op Bruinink DH, Hutt K, Jacobsen A, Huang Y, Miller JE, Maura F, Papaemmanuil E, Waage A, Arcila ME, Landgren O (2019) Stability and uniqueness of clonal immunoglobulin CDR3 sequences for mrd tracking in multiple myeloma. *Am J Hematol* 94(12):1364–1373. <https://doi.org/10.1002/ajh.25641>
31. Perrot A, Lauwers-Cances V, Corre J, Robillard N, Hulin C, Chretien ML, Dejoie T, Maheo S, Stoppa AM, Pegourie B, Karlin L, Garderet L, Arnulf B, Doyen C, Meuleman N, Royer B, Eveillard JR, Benboubker L, Dib M, Decaux O, Jaccard A, Belhadj K, Brechignac S, Kolb B, Fohrer C, Mohty M, Macro M, Richardson PG, Carlton V, Moorhead M, Willis T, Faham M, Anderson KC, Harousseau JL, Leleu X, Facon T, Moreau P, Attal M, Avet-Loiseau H, Munshi N (2018) Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. *Blood* 132(23):2456–2464. <https://doi.org/10.1182/blood-2018-06-858613>
32. Takamatsu H (2020) Clinical value of measurable residual disease testing for multiple myeloma and implementation in Japan. *Int J Hematol* 111(4):519–529. <https://doi.org/10.1007/s12185-020-02828-7>
33. Pugh TJ (2018) Circulating tumour DNA for detecting minimal residual disease in multiple myeloma. *Semin Hematol* 55(1):38–40. <https://doi.org/10.1053/j.seminhematol.2018.03.002>
34. Oberle A, Brandt A, Voigtlaender M, Thiele B, Radloff J, Schulenkorf A, Alawi M, Akyuz N, Marz M, Ford CT, Krohn-Grimberghe A, Binder M (2017) Monitoring multiple myeloma by next-generation sequencing of V(D)J rearrangements from circulating myeloma cells and cell-free myeloma DNA. *Haematologica* 102(6):1105–1111. <https://doi.org/10.3324/haematol.2016.161414>
35. Long X, Xu Q, Lou Y, Li C, Gu J, Cai H, Wang D, Xu J, Li T, Zhou X, Xiao M, Wang Y, Mao X, Zhou J, Chen L (2020) The utility of non-invasive liquid biopsy for mutational analysis and minimal residual disease assessment in extramedullary multiple myeloma. *Br J Haematol* 189(2):e45–e48. <https://doi.org/10.1111/bjh.16440>

36. Mithraprabhu S, Sirdesai S, Chen M, Khong T, Spencer A (2018) Circulating tumour DNA analysis for tumour genome characterisation and monitoring disease burden in extramedullary multiple myeloma. *Int J Mol Sci* 19(7). <https://doi.org/10.3390/ijms19071858>
37. Biancon G, Gimondi S, Vendramin A, Carniti C, Corradini P (2018) Noninvasive molecular monitoring in multiple myeloma patients using cell-free tumor DNA: a pilot study. *J Mol Diagn* 20(6):859–870. <https://doi.org/10.1016/j.jmoldx.2018.07.006>
38. Pel J, Choi WWY, Leung A, Shibahara G, Gelinas L, Despotovic M, Ung WL, Marziali A (2018) Duplex proximity sequencing (Pro-Seq): a method to improve DNA sequencing accuracy without the cost of molecular barcoding redundancy. *PLoS One* 13(10):e0204265. <https://doi.org/10.1371/journal.pone.0204265>
39. Kennedy SR, Schmitt MW, Fox EJ, Kohm BF, Salk JJ, Ahn EH, Prindle MJ, Kuong KJ, Shen JC, Risques RA, Loeb LA (2014) Detecting ultralow-frequency mutations by duplex sequencing. *Nat Protoc* 9(11):2586–2606. <https://doi.org/10.1038/nprot.2014.170>
40. Zajec M, Jacobs JFM, Groenen P, De Kat Angelino CM, Stingl C, Luidert TM, De Rijke YB, Vanduijn MM (2018) Development of a targeted mass-spectrometry serum assay to quantify M-protein in the presence of therapeutic monoclonal antibodies. *J Proteome Res* 17(3):1326–1333. <https://doi.org/10.1021/acs.jproteome.7b00890>
41. Bergen HR 3rd, Dasari S, Dispenzieri A, Mills JR, Ramirez-Alvarado M, Tschumper RC, Jelinek DF, Barnidge DR, Murray DL (2016) Clonotypic light chain peptides identified for monitoring minimal residual disease in multiple myeloma without bone marrow aspiration. *Clin Chem* 62(1):243–251. <https://doi.org/10.1373/clinchem.2015.242651>
42. Barnidge DR, Dasari S, Botz CM, Murray DH, Snyder MR, Katzmann JA, Dispenzieri A, Murray DL (2014) Using mass spectrometry to monitor monoclonal immunoglobulins in patients with a monoclonal gammopathy. *J Proteome Res* 13(3):1419–1427. <https://doi.org/10.1021/pr400985k>
43. Martins CO, Huet S, Yi SS, Ritorto MS, Landgren O, Dogan A, Chapman JR (2020) Mass spectrometry-based method targeting Ig variable regions for assessment of minimal residual disease in multiple myeloma. *J Mol Diagn* 22:901–911. <https://doi.org/10.1016/j.jmoldx.2020.04.002>
44. Eveillard M, Rustad E, Roshal M, Zhang Y, Ciardiello A, Korde N, Hultcrantz M, Lu S, Shah U, Hassoun H, Smith E, Lesokhin A, Mailankody S, Landgren O, Thoren K (2020) Comparison of MALDI-Tof mass spectrometry analysis of peripheral blood and bone marrow-based flow cytometry for tracking measurable residual disease in patients with multiple myeloma. *Br J Haematol* 189(5):904–907. <https://doi.org/10.1111/bjh.16443>
45. Long S, Qin Q, Wang Y, Yang Y, Wang Y, Deng A, Qiao L, Liu B (2019) Nanoporous silica coupled MALDI-Tof ms detection of bence-jones proteins in human urine for diagnosis of multiple myeloma. *Talanta* 200:288–292. <https://doi.org/10.1016/j.talanta.2019.03.067>
46. Mills JR, Barnidge DR, Dispenzieri A, Murray DL (2017) High sensitivity blood-based m-protein detection in sCR patients with multiple myeloma. *Blood Cancer J* 7(8):e590. <https://doi.org/10.1038/bcj.2017.75>
47. Cavo M, Terpos E, Nanni C, Moreau P, Lentzsch S, Zweegman S, Hillengass J, Engelhardt M, Usmani SZ, Vesole DH, San-Miguel J, Kumar SK, Richardson PG, Mikhael JR, Da Costa FL, Dimopoulos M-A, Zingaretti C, Abildgaard N, Goldschmidt H, Orlowski RZ, Chng WJ, Einsele H, Lonial S, Barlogie B, Anderson KC, Rajkumar SV, Durie BGM, Zamagni E (2017) Role of 18F-FDG PET/CT in the diagnosis and management of multiple myeloma and other plasma cell disorders: a consensus statement by the international myeloma working group. *Lancet Oncol* 18(4):e206–e217. [https://doi.org/10.1016/s1470-2045\(17\)30189-4](https://doi.org/10.1016/s1470-2045(17)30189-4)
48. Shah SN, Oldan JD (2017) PET/MR imaging of multiple myeloma. *Magn Reson Imaging Clin N Am* 25(2):351–365. <https://doi.org/10.1016/j.mric.2017.01.003>
49. Jamet B, Bailly C, Carlier T, Touzeau C, Nanni C, Zamagni E, Barre L, Michaud AV, Chereil M, Moreau P, Bodet-Milin C, Kraeber-Bodere F (2019) Interest of PET imaging in multiple myeloma. *Front Med (Lausanne)* 6:69. <https://doi.org/10.3389/fmed.2019.00069>
50. Lapa C, Kircher M, Da Via M, Schreder M, Rasche L, Kortum KM, Einsele H, Buck AK, Hanscheid H, Samnick S (2019) Comparison of 11C-choline and 11C-methionine PET/CT in multiple myeloma. *Clin Nucl Med* 44(8):620–624. <https://doi.org/10.1097/RLU.0000000000002638>
51. Cassou-Mounat T, Balogova S, Nataf V, Calzada M, Huchet V, Kerrou K, Devaux JY, Mohty M, Talbot JN, Garderet L (2016) 18F-fluorocholine versus 18F-fluorodeoxyglucose for PET/CT imaging in patients with suspected relapsing or progressive multiple myeloma: a pilot study. *Eur J Nucl Med Mol Imaging* 43(11):1995–2004. <https://doi.org/10.1007/s00259-016-3392-7>
52. Isoda A, Higuchi T, Nakano S, Arisaka Y, Kaira K, Kamio T, Mawatari M, Matsumoto M, Sawamura M, Tsushima Y (2012) 18F-FAMT in patients with multiple myeloma: clinical utility compared to 18F-FDG. *Ann Nucl Med* 26(10):811–816. <https://doi.org/10.1007/s12149-012-0645-9>
53. Caldarella C, Annunziata S, Pagano L, De Stefano V, Rufini V (2017) Improved detection of minimal residual disease by 11C-methionine PET/CT in a young patient with unusual extramedullary presentation of recurrent multiple myeloma. *Clin Nucl Med* 42(2):e130–e134. <https://doi.org/10.1097/RLU.0000000000001462>
54. Sachpekidis C, Hillengass J, Goldschmidt H, Wagner B, Haberkorn U, Kopka K, Dimitrakopoulou-Strauss A (2017) Treatment response evaluation with (18)F-FDG PET/CT and (18)F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation. *Eur J Nucl Med Mol Imaging* 44(1):50–62. <https://doi.org/10.1007/s00259-016-3502-6>
55. Behzadi AH, Raza SI, Carrino JA, Kosmas C, Gholamrezaezhad A, Basques K, Matcuk GR Jr, Patel J, Jadvar H (2018) Applications of PET/CT and PET/MR imaging in primary bone malignancies. *PET Clin* 13(4):623–634. <https://doi.org/10.1016/j.cpet.2018.05.012>
56. Sachpekidis C, Mai EK, Goldschmidt H, Hillengass J, Hose D, Pan L, Haberkorn U, Dimitrakopoulou-Strauss A (2015) (18)F-FDG dynamic PET/CT in patients with multiple myeloma: patterns of tracer uptake and correlation with bone marrow plasma cell infiltration rate. *Clin Nucl Med* 40(6):e300–e307. <https://doi.org/10.1097/RLU.0000000000000773>
57. Sager S, Ergul N, Ciftci H, Cetin G, Guner SI, Cernik TF (2011) The value of FDG PET/CT in the initial staging and bone marrow involvement of patients with multiple myeloma. *Skelet Radiol* 40(7):843–847. <https://doi.org/10.1007/s00256-010-1088-9>
58. Spinnato P, Bazzocchi A, Brioli A, Nanni C, Zamagni E, Albinis U, Cavo M, Fanti S, Battista G, Salizzoni E (2012) Contrast enhanced MRI and (18)F-FDG PET/CT in the assessment of multiple myeloma: a comparison of results in different phases of the disease. *Eur J Radiol* 81(12):4013–4018. <https://doi.org/10.1016/j.ejrad.2012.06.028>
59. Waheed S, Mitchell A, Usmani S, Epstein J, Yaccoby S, Nair B, Van Hemert R, Angtuaco E, Brown T, Bartel T, McDonald J, Anaissie E, Van Rhee F, Crowley J, Barlogie B (2013) Standard and novel imaging methods for multiple myeloma: correlates with prognostic laboratory variables including gene expression profiling data. *Haematologica* 98(1):71–78. <https://doi.org/10.3324/haematol.2012.066555>

60. Zamagni E, Tacchetti P, Cavo M (2019) Imaging in multiple myeloma: how? When? *Blood* 133(7):644–651. <https://doi.org/10.1182/blood-2018-08-825356>
61. Dimopoulos MA, Hillengass J, Usmani S, Zamagni E, Lentzsch S, Davies FE, Raje N, Sezer O, Zweegman S, Shah J, Badros A, Shimizu K, Moreau P, Chim CS, Lahuerta JJ, Hou J, Jurczyszyn A, Goldschmidt H, Sonneveld P, Palumbo A, Ludwig H, Cavo M, Barlogie B, Anderson K, Roodman GD, Rajkumar SV, Durie BG, Terpos E (2015) Role of magnetic resonance imaging in the management of patients with multiple myeloma: a consensus statement. *J Clin Oncol* 33(6):657–664. <https://doi.org/10.1200/JCO.2014.57.9961>
62. Chen J, Li C, Tian Y, Xiao Q, Deng M, Hu H, Wen B, He Y (2019) Comparison of whole-body DWI and (18)F-FDG PET/CT for detecting intramedullary and extramedullary lesions in multiple myeloma. *AJR Am J Roentgenol* 213(3):514–523. <https://doi.org/10.2214/AJR.18.20989>
63. Rasche L, Alapat D, Kumar M, Gershner G, McDonald J, Wardell CP, Samant R, Van Hemert R, Epstein J, Williams AF, Thanendrarajan S, Schinke C, Bauer M, Ashby C, Tytarenko RG, Van Rhee F, Walker BA, Zangari M, Barlogie B, Davies FE, Morgan GJ, Weinhold N (2019) Combination of flow cytometry and functional imaging for monitoring of residual disease in myeloma. *Leukemia* 33(7):1713–1722. <https://doi.org/10.1038/s41375-018-0329-0>
64. Stecco A, Buemi F, Iannesi A, Carriero A, Gallamini A (2018) Current concepts in tumor imaging with whole-body MRI with diffusion imaging (Wb-MRI-DWI) in multiple myeloma and lymphoma. *Leuk Lymphoma* 59(11):2546–2556. <https://doi.org/10.1080/10428194.2018.1434881>
65. Riola-Parada C, Garcia-Canamaque L, Perez-Duenas V, Garcerant-Tafur M, Carreras-Delgado JL (2016) Simultaneous pet/mri vs pet/ct in oncology. A systematic review. *Rev Esp Med Nucl Imagen Mol* 35(5):306–312. <https://doi.org/10.1016/j.remnm.2016.06.001>
66. Sachpekidis C, Hillengass J, Goldschmidt H, Mosebach J, Pan L, Schlemmer HP, Haberkorn U, Dimitrakopoulou-Strauss A (2015) Comparison of (18)F-FDG PET/CT and PET/MRI in patients with multiple myeloma. *Am J Nucl Med Mol Imaging* 5(5):469–478
67. Bal S, Weaver A, Cornell RF, Costa LJ (2019) Challenges and opportunities in the assessment of measurable residual disease in multiple myeloma. *Br J Haematol* 186(6):807–819. <https://doi.org/10.1111/bjh.16130>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.