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European Myeloma Network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when

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ABSTRACT

The diagnosis of multiple myeloma can be challenging, even for experienced physicians, and requires close collaboration between numerous disciplines (orthopedics, radiology, nuclear medicine, radiation therapy, hematology and oncology) before the final diagnosis of myeloma is made. The definition of multiple myeloma is based on the presence of clinical, biochemical, histopathological, and radiological markers of disease. Specific tests are needed both at presentation and during follow-up in order to reach the correct diagnosis and characterize the disease precisely. These tests can also serve prognostic purposes and are useful for follow-up of myeloma patients. Molecular analyses remain pivotal for defining high-risk myeloma and are used in updated patient stratifications, while minimal residual disease assessment via flow cytometry, molecular techniques and radiological approaches provides additional prognostic information on patients' long-term outcome. This pivotal information will guide our future treatment decisions in forthcoming clinical trials. The European Myeloma Network group updated their guidelines on different diagnostic recommendations, which should be of value to enable appropriate use of the recommendations both at diagnosis and during follow-up.

Introduction

The classification and differential diagnosis of monoclonal gammopathies is based on clinical, biological and radiological criteria but remains challenging in certain cases. Multiple myeloma (MM) is the most common malignant gammopathy and is associated with a wide spectrum of signs and symptoms.¹ In the past decade, the treatment options for patients with MM have increased considerably. Together with improved supportive care, these new regimens significantly prolong the survival of both younger and older patients.² The 2014 revision of the diagnostic criteria for MM allows the initiation of treatment in patients defined only by biomarkers, annotated as SLIM criteria [bone marrow (BM) infiltration >60%, involved/uninvolved serum free light-chain (SFLC) ratio >100 or >1 focal lesion >5 mm as determined by magnetic resonance imaging (MRI)], without waiting for conventional CRAB criteria (hypercalcemia, renal impairment, anemia, bone disease) to occur.^{3,4} Both the SLIM biomarker and CRAB criteria are listed in Figure 1. Given the recent evolution in diagnosis and response assessment, members of the European Myeloma Network (EMN) agreed to review and recommend diagnostic and response criteria to allow their discriminating use in daily practice and current care of patients⁷.

Methodology

These recommendations were developed by a panel of clinical experts on MM based on evidence of published data through August 2017. Expert consensus was used to suggest recommendations, where sufficient data were lacking. The final recommendations were classified based

on the GRADE criteria,⁵ which incorporates the strength and quality of evidence (*Online Supplementary Table S1*). Based on discussions at the 2017 EMN Trialist meeting (Baveno, Italy) guidelines were prepared and circulated among all panel members. The manuscript subsequently underwent revision in three rounds until the EMN experts reached consensus. In line with the guidelines of the International Committee of Medical Journal Editors, authorship was based on active contribution during discussion, writing and revision of the guidelines.

European Myeloma Network recommendations

Diagnostic tools

Blood tests

A defining feature of plasma cell (PC) disorders is the secretion of monoclonal immunoglobulins, often referred to as a monoclonal M-protein, which can be used as a diagnostic marker, but also for the follow-up of the disease. Its heavy- and light-chain components can be identified by immunofixation and further quantified by serum protein electrophoresis and/or a serum free light-chain (SFLC) assay. It should be kept in mind that with persisting disease and possible de-differentiation of myeloma cells, the capacity to produce M-proteins may decrease or be completely lost (light-chain escape). In those patients, low M-protein levels, the presence of light chains only, or even complete absence of M-proteins and light chains may be mistaken as an ongoing or evolving response.⁶

Of note, immunofixation is approximately 10-fold more sensitive than serum protein electrophoresis and is required at diagnosis to characterize the phenotype of the M-protein, and for confirmation of a complete response, which is defined as being immunofixation-negative.⁷

	MGUS	SMM	MM	
			Biomarker	CRAB
M-Protein < 30 g/l	} →			
BM PC < 10%				
M-Protein > 30 g/l		→		
BM PC > 10%		→		
BM PC > 60%			→	
FLC ratio > 100			→	
MRI ≥ 2 focal lesions			→	
Hypercalcemia				→
Renal failure				→
Anemia				→
Bone disease				→

Figure 1. The differential diagnosis between monoclonal gammopathy of undetermined significance, smoldering myeloma and multiple myeloma. The discrimination between these monoclonal gammopathies is based on: (i) the plasma cell infiltration in the bone marrow, (ii) the presence of clinical symptoms related to myeloma disease and (iii) the existence of biomarkers of disease that allow initiation of treatment. MGUS: monoclonal gammopathy of undetermined significance; SMM: smoldering multiple myeloma; MM: multiple myeloma; BM: bone marrow; PC: plasma cells; FLC: free light chain; MRI: magnetic resonance imaging.

Serum electrophoresis and immunofixation may not be able to detect light-chain aberrations in patients with oligo-secretory disease, such as light-chain MM. Due to their low molecular weight, these SFLC are rapidly cleared by the kidneys. In such cases, the monoclonal burden should be measured in a 24 h urine collection or in the serum by an automated SFLC immunoassay (Grade 1A), the latter having a higher sensitivity to detect and quantify the involved free light chains.⁷ In concordance with the International Myeloma Working Group (IMWG), we recommend the performance of serum immunofixation and electrophoresis on serum and urine samples and a SFLC assay for the diagnosis of a monoclonal PC disorder (Grade 1A).

Additional laboratory tests should be performed for the diagnosis and follow-up of MM patients, such as a complete blood count to evaluate possible cytopenias, blood smears to look for circulating PC and general biochemistry tests (renal and liver function tests, calcium, phosphate, uric acid, albumin, creatinine, lactate dehydrogenase, C-reactive protein, β 2-microglobulin). Quantification of serum immunoglobulins by nephelometry enables an indirect measurement of the M-protein or the recognition of a secondary hypogammaglobinemia and is recommended for any patient presenting with a gammopathy (oligo-, poly- or monoclonal) (Grade 1A).

The HevyLite[®] immunoassay quantifies both the involved and uninvolved intact immunoglobulin chains and quantifies them separately (IgG κ / λ , IgA κ / λ , IgM κ / λ).⁸ This assay has prognostic value for progression-free and overall survival⁹ and seems particularly useful when the M-protein is difficult to measure via serum protein electrophoresis. This assay is not yet part of the routine workup of MM patients, but may be of value in the follow-up of patients and has been included in clinical trials.¹⁰

Urine analysis

Proteinuria should be assessed on urine samples from all patients at diagnosis and during the follow-up. If proteinuria is present, it should be quantified in a 24 h urine collection. Total 24 h protein and Bence-Jones proteinuria should be evaluated by densitometry, electrophoresis and immunofixation. To detect low amounts of monoclonal proteins, it is recommended that the urine is concentrated 200-fold.¹¹ These 24 h urine collections are often inconsistently performed, resulting in incomplete urine collection. In addition, renal function can influence the accuracy of the results, a fact which should be taken into consideration when interpreting laboratory values. The SFLC assay can be used for the follow-up of patients with light-chain MM. A recent French study, focusing on patients with light-chain MM, demonstrated that the SFLC assay is superior to 24 h urine collection for: (i) identifying patients with measurable disease, (ii) following their response to initial therapy, and (iii) giving a prognostic indication of the patients' response and overall survival.¹² This study included 113 patients with light-chain MM, all of whom had an abnormal SFLC ratio and measurable disease parameters in serum, while only 64% patients had measurable M-proteins in the urine, as determined by urine protein electrophoresis. Similar results were found in 576 patients with light-chain MM from the UK Myeloma IX and XI trials. The disease burden of the patients with light-chain MM could be measured and monitored by urine protein electrophoresis in 80% of

cases. Of the remaining patients 113 (97%) had involved free light chains >100 mg/L, which was sufficient to measure response to therapy.¹³ These two studies confirmed the importance of SFLC measurements to diagnose and monitor patients with light-chain or oligosecretory MM. The replacement of urine studies by the SFLC assay for all myeloma patients remains controversial, since an Eastern Cooperative Oncology Group study on 399 MM patients (of whom only a minority had light-chain MM disease) found only a weak correlation between results of the SFLC assay and 24 h protein analysis.¹⁴

In line with the IMWG guidelines,¹⁵ we recommend the SFLC assay for the diagnosis and monitoring of patients with oligosecretory disease (Grade 2B). However, for patients with measurable urinary M-proteins, MM should be monitored by 24 h urine collections. When albumin is the dominant protein found in the urine, a glomerulopathy (such as AL-amyloidosis or light-chain deposition disease) should be excluded. The 24 h urine collection remains important when results are discordant.

Bone marrow studies

A BM aspirate enables quantification of infiltrating PC and cytogenetic studies on purified PC. Unfortunately, dilution by peripheral blood during aspiration or the presence of patchy disease (uneven distribution of MM cells throughout the BM) may result in an underestimation of tumor infiltration.¹⁶ We therefore recommend an additional BM trephine biopsy, which may generate complementary information (Grade 1B). A BM biopsy correctly identified MM disease in 95% of symptomatic patients with a low PC count on the initial BM smears.¹⁶ The correct quantification of BM PC is also important because of the 60% cut-off as a biomarker of malignancy. The IMWG earlier recommended retaining the highest PC infiltration in case of discrepancy. Finally, the monoclonality of PC in the diagnostic sample should be confirmed by multiparameter flow cytometry or by immunohistochemistry confirming light-chain restriction.

Flow cytometry of bone marrow cells

In cases of monoclonal gammopathies, the most relevant information provided by multiparameter flow cytometry is the identification and enumeration of neoplastic *versus* polyclonal BM PC. Regardless of the disease category, these neoplastic PC share similar immunophenotypic features, which are distinct from those of normal PC. Typically, CD38, CD138 and CD45 (together with light scatter characteristics) are the best backbone markers for the discrimination of PC. In addition, expression of CD19, CD56, CD117, CD20, CD28, CD27 and CD81, together with cytoplasmic immunoglobulin light-chain restriction, allows a clear discrimination between normal *versus* monoclonal PC¹⁷ and was used by the EuroFlow consortium to create a standardized panel allowing the quantification and immunophenotypic characterization of neoplastic PC.¹⁸

Due to dilution and the sometimes patchy disease distribution, multiparameter flow cytometry often underestimates the infiltration but remains important for detection of monoclonal PC in the peripheral blood and for the detection of minimal residual disease (MRD) in the BM. The Mayo Clinic group reported on the prognostic importance of circulating neoplastic cells in patients with

newly diagnosed or relapsing MM.^{19,20} They recently monitored circulating MM cells at diagnosis and after induction therapy by multiparameter flow cytometry and confirmed inferior progression-free and overall survival for patients with persistent circulating MM cells before transplantation.²¹

Molecular studies

Cytogenetics

MM remains a heterogeneous disease with some patients progressing rapidly, while others survive more than 10 years. This clinical diversity is mainly driven by genetic abnormalities affecting the biological characteristics of MM cells.²² These alterations, summarized in Table 1, are important prognostic factors and can be divided into primary, disease-initiating abnormalities (hyperdiploidy and translocations involving the *IGH* locus) and secondary events, related to further progression of the disease.²³ Fluorescence *in situ* hybridization on interphase cells, performed after purification of CD138⁺ cells or after counterstaining for the monoclonal light chains, is the technique required to detect these abnormalities.²⁴ Alternative techniques that can be used are single-nucleotide polymorphism arrays, which are able to detect loss of heterozygosity and numerical chromosome abnormalities, and comparative genomic hybridization arrays, which mainly reveal numerical abnormalities.

Up to 65% of patients with MM have translocations that involve the immunoglobulin heavy chain gene (*IGH*) on chromosome 14q32. The prevalence and prognostic impact of these *IGH* translocations vary according to the partner chromosome (Table 1). Hyperdiploidy generally consists of numerical gains (of the odd chromosomes)

with a few structural changes, and is usually associated with longer overall survival. Not all trisomies have the same prognostic impact: trisomy 21 impairs, while trisomies 3 and 5 improve overall survival and may partially abrogate the negative impact of del17p and t(4;14).²⁵

The most recurrent secondary alterations are deletion/monosomy of chromosome 13, deletion of chromosome 17p13, chromosome 1 abnormalities (1p deletions and 1q gains/amplifications), and *C-MYC* translocations. Deletion 17p13 is considered the most detrimental prognostic factor (due to short remission after high-dose therapy and an increased incidence of extramedullary disease) and is present in 8-10% of untreated patients.²⁶ This deletion becomes clinically relevant when identified in the majority of PC. Different percentages (varying from 10%-60%) have been proposed to define a threshold that is associated with an impaired prognosis.²⁷⁻²⁹ The presence of a biallelic inactivation (i.e. by an additional mutation) of *TP53* may particularly shorten overall survival.³⁰ Aberrations of chromosome 1 (either 1q21 gains/amplifications or deletions of 1p32) are common and associated with shorter progression-free and overall survival, particularly the less frequent del(1p32).³¹ Patients with adverse cytogenetics may have additional aberrations: in the British MRC IX trial patients with two adverse cytogenetic lesions had a median overall survival of 2 years, while the survival of patients with three aberrations (an adverse *IGH* translocation, +1q21 and del17p13) was only 9 months.³² This inferior survival of patients with additional genetic abnormalities was also found in an *Intergroupe Francophone du Myelome* study that focused on patients with either del17p13 or t(4;14): for patients harboring t(4;14), multivariate analyses showed

Table 1. Recommended cytogenetic studies with implicated gene alterations and related prognosis.

Cytogenetics	Genetic event	Frequency	Prognosis	Response to PI	Response to IMiD	Remarks	Ref
Del17p13	<i>P53</i>	5-15%	Independent marker, with negative impact on PFS and OS	Negative prognostic factor	Negative prognostic factor Pomalidomide seems beneficial	Most important prognostic factor	31, 60, 97
t(4;14)(p16.3;q32)	<i>FGR3</i> <i>MMSET</i>	15%	Independent marker, with negative impact on PFS and OS	Improves survival compared to classic agents	Unfavorable for any IMiD		27, 98-100
Gain 1q21	<i>CKS1B</i>	34-40%	Independent marker, with negative impact on PFS and OS	Negative prognostic factor	Negative prognostic factor	Might be directly implicated in bortezomib resistance	101
Del 1p32, Del 1p22	<i>CDKN2C</i>	7-17%	Independent marker, with negative impact on PFS and OS	Negative prognostic factor			102
t(11;14)(q13;q32)	<i>CCND1</i>	20%	Good prognosis	Good prognosis	Good prognosis	Sensitive to venetoclax	103
t(14;16)(q32;q23)	<i>CMAF</i>	2-3%	Controversial			Considered as a negative prognostic factor, but not confirmed in IFM study	104
Hyperdiploidy of odd chromosomes		60%	Standard prognosis, unless associated with other negative prognostic markers	Standard prognostic factor		May neutralize the negative prognostic impact of del17p or t(4;14)	25

PFS: progression free survival, OS overall survival, *FGFR3*: fibroblast growth factor receptor 3, *MMSET*: multiple myeloma SET domain, PI proteasome inhibitor, IMiD: immunomodulatory drug, *CKS1B*: CDC28 protein kinase regulatory subunit 1B, *CDKN2C*: cyclin-dependent kinase 4 inhibitor C, *CCND1*: cyclin-D1, *MAF*: musculoaponeurotic fibrosarcoma, IFM: *Intergroupe Francophone du Myelome*

a shorter overall survival for patients with a combined del(13q14) or del(1p32). Among patients with del17p13, overall survival was shorter in those with del(1p32).³³

Next-generation genome sequencing

Next-generation sequencing allows the detection of baseline clonal heterogeneity,³⁴ clonal tiding³⁵ and linear and branching evolution and contributes to a better understanding of MM disease biology.³⁶ The availability of more than 2000 sequenced MM genomes has essentially defined the genomic landscape. These data revealed a high incidence of clinically relevant genomic aberrations, including oncogenic RAS mutations, but also a number of rarer and potentially actionable lesions, such as BRAF mutations.^{37,38} Of note, the vast majority of available genomic data in MM is still derived from samples obtained at diagnosis and does not, therefore, necessarily reflect the situation during disease progression. In addition, the clinical relevance of most mutations has not yet been determined and is undergoing investigation in large sequencing programs (CoMMpass, The Myeloma Genome Project and others).^{39,40} No mutation screening has yet been implemented in standard clinical workflows, but mutational analyses may help to identify potential therapeutic targets (such as BRAF mutations) and to stratify of patients in clinical trials.

Gene expression profiling

Based on microarrays to study mRNA expression, gene expression profiling gives a global snapshot of disease biology and may help clinicians to classify patients into separate groups with distinct outcomes. The University of Arkansas pioneered this technique to stratify MM patients

and to characterize individuals' disease at the molecular level.⁴¹ They identified gene expression profiling patterns that allowed MM patients to be grouped in seven disease classes. Further correlation of their microarray results with survival data of individual patients identified a list of 70 genes (GEP70) that had strong prognostic information.⁴¹ Similarly, the HOVON group identified a 92-gene signature (termed SKY92), based on the gene expression profiling results of the Hovon-65 trial.^{42,43} Other gene expression profiling-based risk models have been developed, such as the IFM-15 and MRC-IX-6 gene signatures^{44,45} Although not routinely determined in the majority of laboratories within or outside Europe, both the GEP70 and the SKY92 profiles are commercially available.

Imaging

Traditionally, osteolytic bone disease was investigated by conventional skeletal radiography. The 2014 IMWG disease criteria also considered small osteolytic lesions (≥5 mm), detected by computed tomography (CT) or combined ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET/CT) as symptoms of myeloma-induced bone disease.³ Taking into account these definitions, in 2015 the EMN proposed a relevant algorithm for guiding the choice of imaging technique.^{46,47} Different European centers have integrated CT into their diagnostic work-up based on its superior sensitivity and its ease of operation. This choice was supported by the recent implementation as a national standard of care in the diagnostic workup of patients with suspected MM in the UK and elsewhere.⁴⁸ Whole-body CT has also been integrated into the diagnostic work-up of the European Society of Medical Oncology⁴⁹ and the upcoming IMWG guidelines.

Table 2. Recommendations on further examinations at diagnosis, for response assessment, during follow-up and at relapse.

Diagnostic site	Tool	Diagnosis	At response	At follow-up	At relapse
Bone marrow	BM cytology and biopsy to confirm plasmacytosis and monoclonality	Obligatory	Obligatory*	Not required	Obligatory**
	Flow cytometry	Recommended	Optional	Not required	Optional
	Cytogenetics	Obligatory	Not required	Not required	Optional
Blood	Advanced techniques: GEP, NGS	Optional	Not required	Not required	Not required
	Blood count and blood smear	Obligatory	Obligatory	Obligatory	Obligatory
	Serum electrophoresis and IF	Obligatory	Obligatory	Obligatory	Obligatory
	Serum free light chain	Recommended***	Recommended***	Recommended***	Recommended***
	Serum immunoglobulin levels	Obligatory	Obligatory	Obligatory	Obligatory
	Renal and liver function tests	Obligatory	Obligatory	Obligatory	Obligatory
	Calcium	Obligatory	Obligatory	Obligatory	Obligatory
	Lactate dehydrogenase	Obligatory	Obligatory	Obligatory	Obligatory
Urine	Albumin, β2-microglobulin	Obligatory	Recommended	Recommended	Obligatory
	Urine sample to check for proteinuria and Bence-Jones proteins	Obligatory	Obligatory	Obligatory	Obligatory
Imaging	24 h urine collection	Recommended†	Recommended†	Recommended†	Recommended†
	Low dose whole-body CT	Recommended††	Not required	When symptomatic	Recommended
	PET/CT	Optional	Optional†††	When symptomatic	Optional
	Whole-body MRI	Optional	Not required	When symptomatic	Optional

BM: bone marrow; GEP: gene expression profiling; IF: immunofixation; NGS: next generation sequencing; CT: computed tomography; PET: positron emission tomography; MRI: magnetic resonance imaging; *Obligatory for patients in complete response. **Obligatory for patients with light chain escape, oligosecretory disease, *** SFLC monitoring is obligatory for patients with light-chain disease. †Obligatory in the case of proteinuria. ††Obligatory when radiographs do not show osteolytic lesions †††PET/CT is required for confirmation of minimal residual disease negativity.

The risk of pathological fractures or neurological complications should be assessed in patients with lytic lesions. In this regard, MRI is the preferred examination to detect spinal cord compression. If whole-body CT is not available, conventional radiographs can still be used but must be interpreted with their limited sensitivity in mind. In asymptomatic patients without lytic lesions, axial MRI or whole-body MRI should be considered to assess the presence of focal lesions (Grade 1B). Addition of dynamic contrast enhancement or diffusion weighted imaging to a whole-body MRI protocol provides additional information on BM vascularization, cellularity, and composition and improves the sensitivity of MRI.^{50,51} Two or more focal lesions on MRI are considered as a MM-defining biomarker.⁵² ¹⁸F-FDG PET/CT can replace whole-body CT, if image acquisition of CT allows a detailed evaluation of the bone structures from vertex to knees, including both arms (Grade 1B).⁵³ ¹⁸F-FDG PET/CT is important to assess the presence of extramedullary disease, known to be an independent prognostic factor.⁵⁴ The integration of MRI, PET-CT and whole-body CT always requires experience, interdisciplinary consensus and reflection and needs to be correlated with blood, urine and BM results. Finally, baseline ¹⁸F-FDG PET/CT scans enable post-treatment follow-up of hypermetabolic regions with a greater sensitivity than MRI.^{54,55} PET/CT is also useful in confirming MRD.⁵⁶

European Myeloma Network recommendations for the diagnosis of multiple myeloma:

The initial work-up should include: complete blood count, kidney function tests, serum protein electrophoresis with immunofixation, serum albumin, β 2-microglobulin, lactate dehydrogenase, C-reactive protein, calcium, serum free light chains (especially useful in the case

of light-chain multiple myeloma), 24 h protein collection with protein quantification, electrophoresis and urine immunofixation, and bone marrow (aspiration only is acceptable) studies to quantify and characterize abnormal plasmacytosis (Table 2). The intervals between follow-up studies depend on the response obtained and the patients' characteristics, as proposed in Table 3 (Grade 2C). After CD138+ plasma cell sorting, fluorescence in situ hybridization analysis should include at least t(4;14) and del17p; analysis of t(14;16), 1q21 gain and del(1p32) are also recommended. In addition, bone integrity needs to be evaluated with whole-body computed tomography and/or whole-body magnetic resonance imaging (at least axial). Quantification of the level of plasma cell infiltration, serum free light chains and magnetic resonance imaging assessment are required to assess the SLiM-CRAB biomarkers that define early active multiple myeloma. At relapse, the extent of myeloma-induced bone disease should be re-evaluated, especially if the relapse occurs late after the initial diagnosis.

Staging and prognosis

Disease-specific prognostic scores

The variable outcome of MM patients depends on differences in disease biology, global disease burden and the health status of the patient. Researchers have developed clinical scoring systems in order to estimate individual prognosis. The degree of anemia, renal failure and osteolysis were the first disease-related prognostic biomarkers described in MM and were all included in the Salmon & Durie staging system. Subsequently, β 2-microglobulin, albumin and C-reactive protein levels, and proliferative activity of MM cells were found to be additional prognostic factors, and albumin and β 2-microglobulin levels were incorporated in the International Staging System (ISS) in

Table 3. Follow-up of multiple myeloma patients according to response and patients' characteristics (general strength of these recommendation GRADE 2C).

Patient risk group	Response to prior treatment	Blood and urine tests	Imaging
General myeloma population	In CR or VGPR	Follow-up with blood and urine samples, initially every 1-3 months* with gradually increasing intervals (max 6 months)	Imaging studies should be performed when there are signs of bone disease. Consider PET/CT for high-risk patients
	In PR	Follow-up with blood and urine samples, initially every 1-2 months* with gradually increasing intervals (max 6 months)	Imaging studies should be performed when there are signs of bone disease. Consider PET/CT for high-risk patients.
	With biological progression	Regular follow-up with blood and urine samples, initially every month. Consider treatment initiation for patients with high-risk disease	Imaging using the whole body approach is recommended.
Special patient groups	Frail patients	Follow-up with blood and urine samples, every 2 months; if stable increase intervals to 3 months. Involve family doctor for follow up	Imaging directed to the affected region only when signs of progressive bone disease.
	LC-MM and patients with renal failure	Blood, SFLC and 24 h urine collection.	Imaging studies should be performed when there are signs of bone disease
	Patients with extramedullary disease	Follow-up with SPEP, UPEP and SFLC every month; if stable, increase intervals to 3 months	PET/CT is the preferred technique for follow-up. Recommended every 6 months and obligatory in the case of progression

CR: complete response; VGPR: very good partial response; PR: partial response, PET/CT: positron emission tomography-computed tomograph; max: maximum, SPEP: serum protein electrophoresis; UPEP: urine protein electrophoresis; SFLC: serum free light chain, LC-MM: light-chain multiple myeloma, 24 h: 24 hours * The monitoring intervals are generally shorter for patients with high-risk disease (monthly follow-up) than for patients with standard-risk disease (every 2 to 3 months).

Table 4. The Revised-International Staging System is one of the best stratification methods; it is based on routinely available cytogenetic and biochemistry tests (Palumbo *et al.*).⁵⁶

R-ISS definitions	Determinants	Number	OS (5 years)	Median OS	PFS (5 years)	Median PFS
R-ISS stage I	ISS stage I, no high-risk CA, and normal LDH	871 (28%)	82%	NR	55%	66 months
R-ISS stage II	Other combinations	1894 (62%)	62%	83 months	36%	42 months
R-ISS stage III	ISS stage III plus high-risk CA or high LDH	295 (10%)	40%	43 months	24%	29 months

R-ISS Revised-International Staging System; ISS: International Staging System; OS: overall survival; PFS: progression free survival; CA: cytogenetic abnormalities; LDH: lactate dehydrogenase; NR: not reported.

2005. Other biomarkers have been identified and include markers of tumor cell proliferation, cytokines, pro-angiogenic factors, indicators of bone remodeling, circulating (exosomal) miRNA and imaging abnormalities. Another promising biomarker is the serum level of shed B-cell maturation antigen, which correlates well with BM PC infiltration and declines according to tumor response.⁵⁷ Follow-up of serum B-cell maturation antigen levels seems of interest in patients with non-secretory disease, for whom we lack reliable parameters in the blood; future studies are therefore warranted.

Apart from the often still reported Salmon & Durie staging system, the ISS and revised (R)-ISS are frequently used as staging systems; in the latter systems, the β_2 -microglobulin and albumin levels reflect patients' tumor burden, turnover rate, presence of renal impairment, and nutritional and performance status.⁵⁸ In order to improve the prognostic performance of the ISS score, the IMWG updated it, adding high-risk cytogenetics [t(4;14), t(14;16), and del17p determined by interphase fluorescence *in situ* hybridization] and elevated serum lactate dehydrogenase (Table 4).⁵⁹ These factors had been previously identified as relevant risk factors for early progression after autologous transplantation.⁶⁰ Of note, the ISS and R-ISS give prognostic information at diagnosis, but have not been validated in relapsed/refractory MM.

Frailty and co-morbidities

Once a treatment has started, adherence to the established protocol remains a major clinical concern in elderly and frail patients. This requires an individualized approach in which therapeutic decisions should be driven by both disease features and the patient's characteristics. As in other malignancies, comprehensive geriatric assessments have been evaluated to assess patients' functional, cognitive and mental status, comorbidities, nutrition and presence of geriatric symptoms. Palumbo and co-workers developed a retrospective simplified geriatric assessment, named the IMWG-Frailty Index, in which age, the Charlson Comorbidity Index, activities of daily living and instrumental activities of daily living were used to discriminate between fit, intermediate-fit and frail patients, showing different incidences of severe adverse events, progression-free survival and overall survival.⁶¹ Expectedly, more severe adverse events and treatment discontinuations were reported in frail patients. The most extensive retrospective and prospective tests and validation analyses were performed within the German study group, who prospectively assessed the IMWG-Frailty Index with the revised Myeloma Comorbidity Index (R-MCI) and other comorbidity indices.⁶² A second prospective German

study including 801 MM patients determined that impaired renal and pulmonary function, poorer Karnofsky performance status, frailty and age were independent, multivariate risk factors for overall survival. Addition of cytogenetic abnormalities resulted in the weighted revised Myeloma Comorbidity Index, which is able to assess patients' physical condition accurately and is simple to apply in the clinic.⁶³ Although not yet proven via randomized treatment algorithms, there is circumstantial evidence that limited induction therapy, careful dose modifications and reductions, sensible use of supportive care and watchful surveillance of unfit and frail patients may improve patients' outcome further.⁶⁴ The EMN insists on developing trials, specifically designed for frail patients, for further refinement of frailty-related diagnostics and best treatment selection.⁶⁵

Drug-related biomarkers

While prognostic factors regarding disease evaluation are listed above, drug-related biomarkers are being assessed to predict response to treatment and, possibly, to facilitate optimal treatment while avoiding ineffective therapies and unnecessary toxicity. Recent pharmacogenomic studies revealed gene signatures that could predict the clinical outcome after treatments based on immunomodulatory drugs⁶⁶ or bortezomib.⁶⁷ The expression of cereblon, an intracellular binding partner of immunomodulatory drugs has been intensively studied as a biomarker and initial studies correlated cereblon levels with the outcome of MM patients receiving treatment with such drugs.⁶⁸⁻⁷¹ These studies used quantitative real time PCR analysis, gene expression profiling or immunohistochemistry to quantify cereblon expression and showed that loss of cereblon expression was associated with resistance to immunomodulatory drugs. Further investigations revealed limitations of these assays, because both splice variants of cereblon and point mutations were described.^{72,73} When exploring predictors for tumor responses to daratumumab, higher CD38 expression was found on MM cells of responsive patients. However, good responses were also seen in patients with lower CD38 expression, an observation confirmed in a second study that showed that CD38 expression level was not necessarily predictive of response in advanced MM; nevertheless attempts to assess agents that keep CD38 upregulation increased, e.g., all-*trans* retinoic acid and histone deacetylase inhibitors, are being pursued pre-clinically and clinically.^{74,75}

Finally, expression of anti-apoptotic proteins, BCL-2, BCL-XL or MCL-1, measured by quantitative real-time PCR, predict pharmacological responses to the bcl-2 inhibitor venetoclax, which is mostly active in patients harboring t(11,14) translocations.⁷⁶

European Myeloma Network recommendations:

The International Staging System score and, whenever possible, the Revised International Staging System score, should be determined at diagnosis to assess prognosis. At least a minimal frailty assessment should be performed to aid the choice of induction therapy, dose amendments and supportive care. Although of interest due to their prognostic and predictive value, biomarkers, such as cereblon and CD38 protein expression, are not routinely assessed in daily multiple myeloma care, while fluorescence in situ hybridization for *t(11;14)* should be performed if treatment with venetoclax is a clinical option.

Response assessment

The implication of the results of an SFLC assay and MRD assessment prompted the IMWG to update MM response criteria.⁷⁷ In 2011, two new categories, stringent complete response and very good partial response, were added. Correct disease assessment is not only crucial for reporting in clinical trials, it also indicates prognosis in individual cases.^{77,78} It is well known that patients who obtain a complete response following induction have improved progression-free and overall survival after intensive treatment.⁷⁹

Patients should, therefore, be evaluated before initiation of each treatment cycle to determine their response to therapy. For MM patients with intact immunoglobulins, the recommended method for monitoring is quantification of serum and urinary M-protein. Whether all serum (and urine) parameters have to be checked after each cycle, rather than after every two or three cycles is left to the discretion of each physician, taking into account disease aggressiveness, organ (i.e. renal) impairment and various other factors. To confirm a stringent complete response, normalization of the SFLC values and disappearance of monoclonal PC infiltration in the BM should be added to negative immunofixation on serum and urine samples. The BM must be evaluated in order to confirm a complete response, but this can be done at some time after the end of treatment, allowing full recovery of the BM. Of note, BM infiltration can be heterogeneous with persisting focal lesions in an otherwise recovered BM (earlier referred to as patchy disease). The follow-up of patients with light-chain MM and measurable M-protein levels in urine should include 24 h urine collections. The SFLC assay generally allows response assessment in patients with oligosecretory disease with unmeasurable serum and urine M-protein levels [serum M-protein <1 g/dL (10 g/L) or urine M-protein <200 mg/24 h]. If the SFLC assay is not informative, BM plasmacytosis should be assessed.⁷⁷

The definition of relapse applies to a patient in complete response who experiences reappearance of MM, while progression refers to patients with an increasing disease burden from a baseline or persistent residual disease. An additional assessment for confirmation is mandatory before initiating a new line of therapy. MM progression can be determined biochemically (increase in an existing monoclonal peak), or by radiological and clinical criteria. The interested reader can find the criteria for relapse and disease progression recently described by the IMWG.⁷⁷ Response assessment can be challenging, especially in cases of deep response after the use of monoclonal antibodies, which may interfere with quantification of the M-protein and may require specific assays.⁸⁰

Minimal residual disease

Current induction regimens, in association with autologous stem cell transplantation, achieve very high

response rates and the responses are often deep. Unfortunately, however, MM often recurs due to residual MM cells, drug resistance and/or persistence of resistant dormant subclones.⁸¹ MRD can be assessed by multiparameter flow cytometry, polymerase chain reaction (PCR)-based methods or next-generation sequencing to identify persistent clonal cells. Recent studies, listed in Table 5, confirmed the prognostic impact of MRD status as an independent variable for outcome.⁸² In the future, MRD will be more widely used in clinical trials to guide treatment choices and probably as a surrogate marker for progression-free and overall survival.⁵⁶

Conventional flow-MRD approaches, based on multiple institutional non-standardized protocols, can reliably identify malignant PC and discriminate aberrantly expressed cell surface markers in approximately 90% of patients (with a sensitivity of detecting 10^{-4} atypical PC in normal BM). Recent studies conducted by Spanish and UK groups have shown that negative MRD by multiparameter flow cytometry is predictive for both progression-free survival and overall survival, even in patients who achieved a complete response.^{83,84} Recent technical advances have increased the sensitivity of next-generation flow cytometry protocols down to the 10^{-6} range.⁸⁵

MRD analysis by PCR detects persistent residual tumor cells through the amplification of a tumor-specific molecular marker. The *IGH* rearrangement is used as a marker of clonality in various B-cell malignancies.⁸⁶ Allele-specific oligonucleotide PCR with primers complementary to the heavy chain variable sequence remains one of the most sensitive approaches to detect residual malignant PC, reaching a sensitivity of 10^{-5} .⁸⁷ Unfortunately, it is a laborious, time-consuming approach that is not widely available because of its dependence on patient-specific primers and probes for quantitative PCR.

Next-generation sequencing of the *IGH* rearrangement segments provides insights into the architecture of the B-lineage repertoire with consensus primers. Since the B-lineage repertoire includes the malignant PC clone in BM, next-generation sequencing of *IGH* enables a quantitative determination of MRD, without per-patient customization, provided that the malignant clone was identified in a diagnostic sample or a sample taken during active disease.⁸⁸ Results from next-generation sequencing are highly concordant with flow-based MRD detection, highly reproducible and reach a sensitivity of 10^{-6} .^{89,91} A lack of standardization and limited commercial availability are the main restraints for next-generation sequencing. Flow cytometry and molecular techniques both require an appropriate BM sample. Heterogeneous BM infiltration and peripheral blood dilution can be major hurdles to the evaluation of MRD. Since neither of these techniques is able to detect extramedullary disease, they should be combined with imaging studies.

Imaging is a third approach to evaluate MRD in MM. Both PET/CT and MRI have been evaluated in this setting.^{53,55} Regarding PET/CT, two large studies assessed the prognostic value of negative PET/CT after induction and autologous stem cell transplantation.^{55,92} Both studies found that PET/CT-negative patients had a better progression-free and overall survival compared to PET/CT-positive patients (52 versus 38 months and 5-year estimates of 90% versus 71%, respectively).⁹² In the French IMAJEM study, MRD was evaluated in 86 patients via PET-CT and flow cytometry. Although the concordance

Table 5. Recent studies on minimal residual disease and the implications for progression-free and overall survival of patients with multiple myeloma.

N.	Reference	Number of patients	Method used for MRD assessment	Study question	Patient cohorts	Time point of MRD assessment	Results	Lesson learnt
#1	Lahuerta <i>et al.</i> , JCO 2017. ¹⁰⁵	609	MFC	PFS and OS in MRD ⁻ pts	NDMM	9 months after treatment	MRD ⁻ prolonged PFS and OS; MRD ⁺ in CR similar PFS and OS to MRD ⁻ in nCR and PR.	MRD ⁻ status surpasses CR in all risk groups. MRD negativity most relevant endpoint for elderly fit pts with ASCT.
#2	Chakraborty <i>et al.</i> , Biol Blood Marrow Transplant 2017. ¹⁰⁶	185	MFC	PFS and OS in post-transplant sCR patients with HR cytogenetic MM	NDMM	3 months post-ASCT	56% MRD ⁻ with superior OS and PFS; del17 pts no difference in PFS and OS between sCR and MRD ⁻ ; t(4;14) pts superior PFS and OS in MRD ⁻ pts than sCR	MRD status important markers for survival but differs according to cytogenetics
#3	Nadiminti <i>et al.</i> , OncoTargets and Therapy 2017. ¹⁰⁷	100	MFC	Toxicity, safety, PFS and OS of VTD + HD melphalan	NDMM and pre-treated	6 months post-ASCT	MRD ⁻ in 85% 6 months after transplantation, sCR in 56% and CR in 20%	MRD and sCR excellent markers for PFS and OS
#4	Paiva <i>et al.</i> , Blood 2016. ¹⁰⁸	162	MFC	Monitor MRD in transplant-ineligible pts	NDMM elderly	At ID after 9,18 cycles	Determines 3 MRD groups, with significant longer PFS and OS for MRD ⁻ group	MRD is a relevant prognostic factor in elderly and MRD status correlates with OS and PFS
#5	Ludwig <i>et al.</i> , BJH 2015. ¹⁰⁹	93	MFC	VTD and VTCD induction in NDMM	Untreated MM, elderly	At baseline and at suspected CR	OS and PFS longer in MRD ⁻ vs. MRD ⁺ pts with BM CR. Same results for MRD ⁻ in VGPR or PR group	MRD surpasses conventional SFLC and BM CR
#6	Roussel <i>et al.</i> , JCO 2014. ¹¹⁰	31	MFC	VRD induction and consolidation for ASCT pts	Untreated MM, <65 years	ID + pre-ASCT, post-ASCT, post-consolidation, end of treatment	58% in CR, 68% MRD ⁻ .	None of the MRD ⁻ group relapsed during a FU of 39 months
#7	Mateos <i>et al.</i> , Blood 2014. ¹¹¹	260	MFC	VMP vs. VTP as induction	NMDD, >65 years	6 cycles of induction	22% MRD ⁻ with longer PFS and OS. VMP better than VTP. 70% in CR after VMP also MRD ⁻ only 45% in VTP group.	MRD surpasses CR and is a prognostic factor for OS and PFS
#8	Oliva <i>et al.</i> , Oncotarget 2017. ¹¹²	50	MFC and ASO-RQ-PCR	Consolidation with ASCT or CRD plus R maintenance → MRD	NDMM	After 3 and 6 courses of maintenance and then every 6 months till PD	Lower MRD in ASCT vs. CRD pts. Differences between HR vs. SR and relapse vs. non-relapse pts	MRD identifies a low-risk group, response independently, better characterizes activity of treatment.
#9	Puig <i>et al.</i> , Leukemia 2014. ¹¹³	170	ASO RQ-PCR; MFC	Applicability, sensitivity and prognostic value of ASO RQ-PCR	NDMM with and without ASCT	ID and after treatment	MRD assessed in 103 pts.; 54% MRD ⁻ by PCR, 46% by MFC. MRD ⁻ pts had prolonged PFS and OS	ASO RQ-PCR less applicable than MFC but powerful to assess treatment efficacy and risk stratification
#10	Ferrero <i>et al.</i> , Leukemia 2015. ¹¹⁴	39	PCR	MRD kinetics' impact on survival	NDMM with ASCT	ID, study entry, after 2 cycles CTD, end of treatment, every 6 months until PD	OS 72% at 8 years median FU in MRD ⁻ and 48% in MRD ⁺ pts. PFS for MRD ⁻ 38 months and MRD ⁺ 9 months	Long-term MRD monitoring is useful and maintenance therapy ensures responses
#11	Martinez-Lopez <i>et al.</i> , Blood 2014. ⁹⁰	133	MFC and NGS	Prognostic value of MRD in pts with VGPR after front-line therapy	NDMM	After induction for elderly, after ASCT for <65 years	MRD ⁻ pts had significantly longer PFS and OS; median PFS: MRD ^{≥10⁻³} 27 months, 10 ⁻³ -10 ⁻⁵ 48 months; <10 ⁻⁵ 80 months.	MRD surpasses CR and depth of MRD level showed significant differences in pts with CR
#12	Chari <i>et al.</i> , Blood 2017. ¹¹⁵	103	NGS	Safety, OS/PFS and MRD	≥2 treatment lines	Pts in CR or better after treatment	29% of pts in CR are MRD ⁻ ;	MRD surpasses CR
#13	Korde <i>et al.</i> , JAMA Oncol. 2015. ¹¹⁶	45	MFC, NGS, FGD-PET/CT	Tolerability and impact on MRD negativity	NDMM and HR SMM	ID, achievement of CR and/or completion of cycles 8, 20 and 32, end of treatment	nCR or better pts were MRD ⁻ in 100% NDMM /92% SMM by MFC, 67%/75% by NGS and 41%/26% by FGD-PET/CT	High rates of MRD negativity with longer PFS in NDMM/HR SMM

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N.	Reference	Number of patients	Method used for MRD assessment	Study question	Patients cohort	Time points of MRD assessment	Results	Lesson learnt
#14	Zagmani <i>et al.</i> ; Clin Cancer Res 2015. ⁹²	76	FGD-PET/CT	Role of FGD-PET/CT on PFS and OS	NDMM	Baseline, during post-treatment FU, PD	PET-negativity post-treatment in 70%, CR in only 53%. PET-negativity influenced PFS and OS favorably.	PET-CT more careful evaluation of CR. PET-negativity independent predictor of prolonged PFS/OS
#15	Patriarca <i>et al.</i> , Biol Blood Marrow Transplant 2015. ¹¹⁷	54	FGD-PET/CT	Prognostic significance of PET/CT	AlloSCT pts	Before and/or within 6 months after alloSCT.	Persistence of EMD at transplantation → poor PFS. EMD and <CR or VGPR after alloSCT → shorter PFS/OS. PET CR pts prolonged PFS/OS.	PET/CT imaging significantly associated with outcome.
#16	Lapa <i>et al.</i> ; Oncotarget 2014. ¹¹⁸	37	FGD-PET/CT	Prognostic value of FGD-PET/CT	Pretreated, and progression after SCT	At relapse, after treatment	Absence of foci positive factor for PFS/OS. EMD and intense uptake associated with shorter PFS/OS. 30% of pts' management changed after PET/CT	Prognostic value of PET/CT in post-SCT relapse patients important and significant impact on pt management

AlloSCT: allogenic stem cell transplantation; ASCT: autologous stem cell transplantation; ASO RQ-PCR: allele-specific oligonucleotide real-time quantitative polymerase chain reaction; BM: bone marrow; CR: complete remission; CRD: cyclophosphamide, lenalidomide, dexamethasone; d: day; EMD: extramedullary disease; FGD-PET/CT: fluorodeoxyglucose positron emission tomography-computed tomography; FU: follow-up; HD: high dose; HR: high risk; ID: initial diagnosis; MFC: multiparameter flow cytometry; MM: multiple myeloma; MRD: minimal residual disease; nCR: near-complete remission; NGS: next generation sequencing; NDMM: newly diagnosed multiple myeloma; OS: overall survival; PCR: polymerase chain reaction; PD: progressive disease; PFS: progression free survival; PR: partial remission; pts: patients; R: lenalidomide; sCR: stringent complete remission; SCT: stem cell transplantation; SFCL: serum free light chain; SMM: smouldering multiple myeloma; SR: standard risk; TTP: time to tumor progression; VGPR: very good partial remission; VMP: bortezomib, melphalan, prednisone; VRD: bortezomib, lenalidomide, dexamethasone; *vs.*: versus; VTCD: bortezomib, thalidomide, cyclophosphamide, dexamethasone; VTD: bortezomib, thalidomide, dexamethasone; VTP: bortezomib, thalidomide, prednisone.

between the two tests was low, progression-free survival was better in patients who were negative according to both techniques compared to those who were positive by PET and/or flow cytometry (3-year progression-free survival, 86.8% versus 52.9%), indicating that both techniques are complementary. A major advantage of PET/CT is its capacity to assess MRD outside the BM; its disadvantages are high cost and the lack of reimbursement in certain countries, insufficient standardization and reduced tracer uptake in some MM patients. Evaluation via PET/CT has been incorporated into the new IMWG MRD criteria.⁷⁷ In the future, the increased capabilities of diffusion weighted MRI to detect small lesions and diffuse infiltration may offer advantages that merit prospective evaluation in MRD assessment studies.⁵⁰

In addition, recent studies have demonstrated that circulating DNA fragments carrying tumor-specific sequence alterations can be detected and quantified in the blood of patients with solid tumors.^{93,94} In MM, various studies have provided evidence that - much like in solid tumors - MM-specific alterations (VDJ rearrangements or somatic genomic alterations) can also be identified and tracked in cell-free DNA circulating in blood.^{95,96}

European Myeloma Network recommendations:

Response assessment is an essential part of myeloma management. Patients under treatment should be evaluated before the initiation of each cycle and according to international guidelines. Minimal residual disease testing is not currently recommended in routine follow-up of patients but is likely to be incorporated in standard response/progression evaluation soon. Valid options for the assessment of minimal residual disease are based on bone marrow cells (next-generation flow cytometry) or molecular analysis (next-

generation sequencing), often also combined with an imaging-based evaluation. These methods require appropriate expertise.

Conclusion

While novel agents have certainly improved the outcomes of patients with myeloma, prompt diagnosis and close follow-up of MM patients remain highly relevant and contribute to better survival. In most cases, the diagnosis of MM is straightforward, being based on biological and radiological evidence when evocative clinical signs are present. During response assessment, the evaluation of MRD will become increasingly important and, within the next few years, will guide treatment choices in clinical trials and possibly also outside trial scenarios. International efforts are needed to standardize the different techniques that can be used to evaluate MRD. Guidelines on appropriate follow-up and patient-tailored monitoring have been updated in this EMN consensus paper and should help to improve the outcome and prognosis of our patients.

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