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Diagnosis and Laboratory Follow-Up of Patients with Multiple Myeloma: Guidelines from the Portuguese Multiple Myeloma Group

Diagnóstico e Seguimento Laboratorial de Doentes com Mieloma Múltiplo: Recomendações do Grupo Português do Mieloma Múltiplo

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ABSTRACT

Multiple myeloma is a neoplasm of plasma cells that in most cases is associated with the secretion of monoclonal immunoglobulins and can involve multiple organs. Its timely diagnosis is essential to limit or avoid irreversible damage and dysfunction of target organs. Appropriate initial stratification of patients allows for optimization in the selection and sequence of therapy, as well as proper follow-up during treatment and monitoring, impacting survival. These laboratory guidelines from the Portuguese Multiple Myeloma Group provide recommendations for the diagnosis and laboratory follow-up of patients with multiple myeloma. The follow-up and diagnosis of patients with other clinically significant monoclonal gammopathies were not included in this text. This article was based on international guidelines, scientific publications, and the experience of a panel of specialists in clinical and laboratory fields dedicated to the study and treatment of multiple myeloma.

Keywords: Clinical Laboratory Techniques; Multiple Myeloma/blood; Multiple Myeloma/diagnosis; Portugal; Practice Guidelines as Topic

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RESUMO

O mieloma múltiplo é uma neoplasia de plasmócitos que, na maioria dos casos, se associa à secreção de imunoglobulinas monoclonais e que pode cursar com um atingimento multiorgânico. O diagnóstico atempado do mieloma múltiplo é essencial para evitar ou limitar danos irreversíveis e disfunção dos órgãos-alvo. A apropriada estratificação inicial dos doentes permite otimizar a seleção e a sequência da terapêutica, assim como o correto seguimento durante o tratamento, com impacto na sobrevivência. As presentes recomendações laboratoriais do Grupo Português do Mieloma Múltiplo oferecem orientações para o diagnóstico e seguimento laboratorial dos doentes com mieloma múltiplo. O seguimento e diagnóstico de doentes com outras gamapatias monoclonais de significado clínico não foram incluídos neste texto. A sua elaboração tem por base orientações internacionais, publicações científicas e experiência de um painel de especialistas nacionais das áreas clínicas e laboratoriais dedicados ao estudo e tratamento do mieloma múltiplo.

Palavras-chave: Guias de Prática Clínica; Mieloma Múltiplo/diagnóstico; Mieloma Múltiplo/sangue; Portugal; Técnicas de Laboratório Clínico

INTRODUCTION

Multiple myeloma (MM) is a hematologic neoplasm characterized by the proliferation of clonal plasma cells in the bone marrow (BM). It is often associated with the production of large quantities of monoclonal immunoglobulin (Ig), commonly referred to as monoclonal protein (MP), M-protein, or paraprotein. The MP can consist of intact Ig (the pair composed of both light and heavy chains) or, in some cases, only the Ig light chain. In rare cases (3% - 5%), MM may be non-secretory or oligo-secretory (it either does not produce, does not secrete, or secretes minimal amounts of MP). The diagnostic criteria for plasma cell dyscrasias are based on the guidelines from the International Myeloma Working Group (IMWG) (Table 1).[1]

Multiple myeloma is typically preceded by a pre-malignant stage called monoclonal gammopathy of undetermined significance (MGUS), followed by an intermediate, still asymptomatic stage known as smoldering MM (SMM), which is associated with a higher risk of progressing to symptomatic MM.[2-4] The term “monoclonal gammopathy of clinical significance” was introduced to describe monoclonal gammopathies with target organ involvement that do not meet MM or AL amyloidosis criteria. Its designation specifies the target organ affected, such as renal, cutaneous, or neurological monoclonal gammopathy.[5] Other plasma cell dyscrasias include solitary bone or extramedullary plasmacytoma, heavy or light chain deposition diseases, and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes), which are not covered in these recommendations. These guidelines specifically focus on the diagnosis and laboratory follow-up of MM.

The diagnosis of MM can be a lengthy and complex process, requiring the presence of clonal plasma cells in the bone marrow (≥ 10%) and the identification of disease markers that define myeloma-defining events (Table 1).[1] However, clinical symptoms often appear late and overlap with those of more common conditions, particularly in the elderly, such as bone pain, which can hinder early diagnosis and delay the start of MM treatment, negatively impacting prognosis and patient quality of life. Therefore, a well-targeted laboratory investigation is essential for an early and differential diagnosis of MM.

Due to the heterogeneous clinical course of MM, it is crucial to establish an algorithm for its diagnosis and staging, identify biomarkers for proper risk stratification, evaluate treatment response during different phases of the disease, and manage potential toxicities during or after treatment. While the laboratory plays a significant role in this process, the clinical information provided is equally important for selecting and optimizing the tests performed at each stage of disease evaluation.

Higher sensitivity and specificity may lead to quicker and more precise recognition of the disease, as well as to more individualized treatments regarding the patient and the MM subtype, while maximizing efficacy and minimizing adverse effects.

Despite the extensive information available on laboratory methods for the diagnosis and follow-up of MM, there is a lack of harmonization in the methodologies used, their interpretation, and the presentation of results.

In this article, the Portuguese Multiple Myeloma Group (PMMG) offers its recommendations for the laboratory diagnosis and follow-up of MM patients, providing a valuable tool to support timely and informed decision-making. These recommendations are based on international guidelines, scientific publications, and the expertise of clinical and laboratory specialists dedicated to the study and treatment of MM.

Most of the recommendations presented are grade C, based on level 4 evidence (evidence from expert committee reports and/or clinical experiences of respected authorities). When possible, level 1 - 3 evidence (based on randomized controlled trials, or well conducted non-randomized studies) was sought, with supporting references provided.

LABORATORY DIAGNOSIS

Significant advances in the understanding of MM biology have led to the development of various innovative therapies. Additionally, recently introduced methodologies enable more accurate evaluations, including the identification of prognostic factors that help recognize high-risk patients.

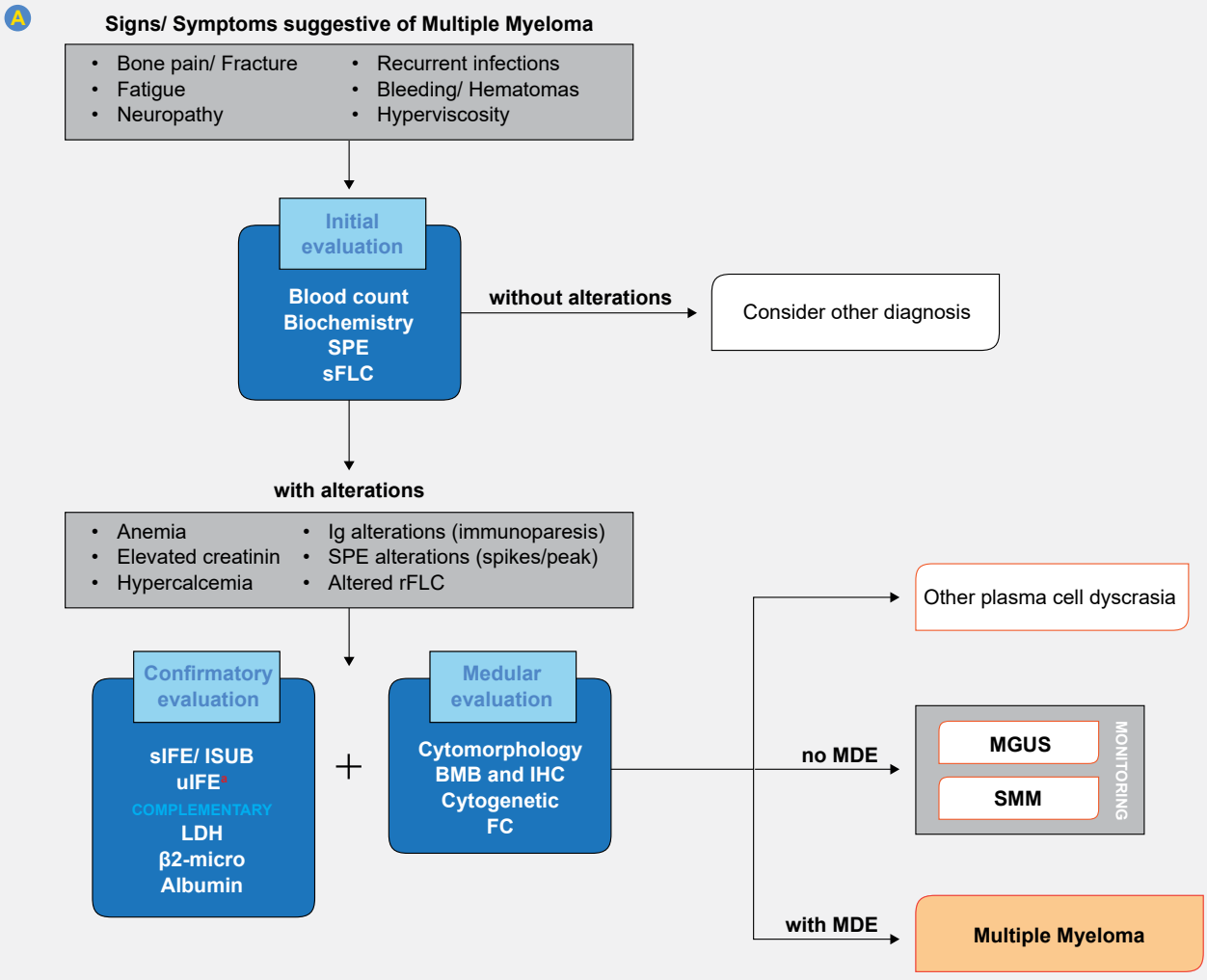
Clinical suspicion of MM serves as the starting point for its diagnostic algorithm (Fig. 1). This suspicion may be

Table 1 – Diagnostic criteria according to the International Myeloma Working Group (IMWG), adapted from Rajkumar *et al*, 2014.[1]

A	
Disorder	**Diagnostic criteria**
Monoclonal gammopathy of undetermined significance (MGUS)[a]	All criteria must be met: Non-IgM MGUS - Serum MP (non-IgM) < 30 g/L - Clonal BM plasma cells < 10% - Absence of myeloma defining events (MDE) Light chain MGUS - No Ig heavy chain expression on SIF - Abnormal FLC ratio (< 0.26 or > 1.65) - Urinary MP < 500 mg/24 hours - Clonal BM plasma cells < 10% - Absence of MDE IgM MGUS - Serum IgM MP < 30 g/L - BM lymphoplasmacytic infiltration < 10% - No evidence of LPD, AL amyloidosis or other lesions associated with light chains, heavy chains, or Ig
Smoldering multiple myeloma (SMM)	Both criteria: - Serum MP ≥ 30 g/L or urinary MP ≥ 500 mg/24 hours and/or clonal BM plasma cells 10% - 60% - Absence of MDE or amyloidosis
Multiple myeloma (MM)	Both criteria: - Clonal BM plasma cells ≥ 10% or BMB-proven bony or extramedullary plasmacytoma - Any of the MDE
Solitary plasmacytoma (SP)	All criteria must be met: - Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells - Absence of clonal BM plasma cells - No osteolytic lesions on skeletal radiography, CT or PET-CT - Absence of CRAB or amyloidosis
SP with minimal marrow involvement	Same as SP except: - BM clonal plasma cells < 10%
Non-secretory MM	- BM plasma cells ≥ 10% or biopsy-proven bone lesion - CRAB - Absence of serum or urinary MP
Plasma cell leukemia (PCL)	Diagnosis of MM and PB plasma cells ≥ 5%[b]

B		
Myeloma defining events (MDE)		
SLiM	(S) Clonal BM plasma cells ≥ 60%[c]	
	(Li) Involved/ uninvolved serum FLC ratio ≥ 100[d]	
	(M) > 1 focal lesion in MRI[e]	
CRAB	(C) Hypercalcemia	Serum calcium > 0.25 mmol/L (> 1 mg/dL) higher than the upper limit of normal or > 2.75 mmol/L (> 11 mg/dL)
	(R) Renal failure	eGFR < 40 ml/min/1.73 m^2 or serum creatinin > 177 µmol/L (> 2 mg/dL)
	(A) Anemia	Hb < 10 g/dL or > 2 g/dL below the lower limit of normal
	(B) Bone lesions	One or more osteolytic lesions on skeletal radiography, CT or PET-CT

a: MGUS comprises three variants: non-IgM, Light chain, IgM.
b: Fernández de Larrea *et al*, 2021.[30]
c: Clonality established by light chain κ/λ restriction using flow cytometry, immunohistochemistry or immunofluorescence.
d: Involved FLC must be ≥ 100 mg/L.
e: Each focal lesion must be ≥ 5 mm. If BM clonal plasma cell < 10%, more than one lesion is necessary to distinguish solitary plasmacytoma from minimal marrow involvement.
BM: bone marrow; BMB: bone marrow biopsy; CT: computed tomography; eGFR: estimated glomerular filtration rate; FLC: free light chain; Hg: hemoglobin; Ig: immunoglobulin; LPD: lymphoproliferative disorders; MM: multiple myeloma; MP: M-protein; MRI: magnetic resonance imaging; PB: peripheral blood; PET-CT: positron emission tomography-computed tomography; SIF: serum immunofixation.



B

Tests \ Stage	Diagnosis	Follow-up[b]	Response evaluation	Relapse
SPE	x	x	x	x
sFLC	x	x	x	x
sIFE	x		x	
uIFE	x[c]		x[c]	x[c]
Morphology study (BM)	x	x[d]	x	x[d]
BMB	x			
Cytogenetic	x			x
FC	x	x[d]	x	x[d]
LDH	x			x
β2-micro	x			x

Figure 1 – Diagnostic algorithm for patients with suspected multiple myeloma (A) and summary table of tests/parameters to be conducted at different stages of patient evaluation (B)

a: Light chain MM and AL amyloidosis.
b: Any analysis that is not informative in the evaluation of MP can be excluded from the follow-up panel for that patient (example: SPE in the follow-up of a patient with light chain MM).
c: In the presence of proteinuria, confirmation of clonality may be necessary in light chain MM.
d: In patients with non-quantifiable MP, non-secretory/oligo-secretory MM.
BM: bone marrow; BMB: bone marrow biopsy; FC: flow cytometry; FISH: fluorescence in situ hybridization; Ig: immunoglobulins; IHC: immunohistochemistry; LDH: lactate dehydrogenase; MDE: myeloma-defining events; MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; sFLC: serum free light chain; sIFE: serum immunofixation; SMM: smoldering MM; SPE: serum protein electrophoresis; rFLC: free light chain ratio; ISUB: immunosubtraction; uIFE: urine immunofixation

triggered by findings from 'routine' analytical studies (e.g., presence of MP, anemia, hypercalcemia, or renal dysfunction) and/or nonspecific but compatible clinical signs and symptoms (e.g., asthenia, recurrent infections, or back pain). The initial assessment includes identifying parameters to determine which patients need to begin therapy. This decision is guided by the presence of myeloma-defining events, known as the SLiM-CRAB criteria, as outlined in section B of Table 1.

Monoclonal protein is a highly specific and sensitive serum tumor marker for MM, offering a key advantage in differential diagnosis and patient follow-up. However, there are several forms of disease presentation, as well as different types and concentrations of MP, making its accurate identification challenging. Proper identification of MP depends on the sample quality, the clinical information provided, and the chosen analytical methods.

When MP is detected, either through changes in the serum protein electrophoresis (SPE) spectrum or an abnormal free light chain (FLC) ratio (which indicates an excess of one light chain type (κ or λ) *versus* the other), it is mandatory to characterize the Ig to confirm monoclonality by serum immunofixation (sIFE) or immunosubtraction. Immunosubtraction, a laboratory technique performed by capillary zone electrophoresis, may be used whenever sensitive. However, when in doubt, the reference method (sIFE) should be used.[6-8] Serum immunofixation screening is typically performed using antisera for IgG, IgA, IgM, κ, and λ. In cases where only a light chain is detected, testing with IgD and IgE antisera is recommended to exclude the rarer forms of IgD and IgE MM. If no changes are observed in the SPE spectrum but clinical suspicion of MM remains, sIFE should still be performed. This technique is more sensitive than SPE and can detect smaller amounts of MP.[9,10] Simultaneous assessment of SPE and FLC quantification (amount of free light chains produced by plasma cells) ensures effective screening for serum MP.[11] Moreover, FLC quantification is valuable in cases of suspected non-secretory/oligo-secretory MM and AL amyloidosis.

Testing for MP in a 24-hour urine sample (U24h) has traditionally been used for the characterization and follow-up of light chain MM and AL amyloidosis. However, recent studies suggest that serum FLC quantification can effectively replace U24h evaluation in most patients.[12-14] While renal function affects serum FLC concentration, there are adjusted reference intervals for the FLC ratio based on estimated glomerular filtration rate, which can be useful when applying this test to chronic kidney disease cases.[15,16] It is important to note that these intervals are assay-specific, and the patient's clinical status should be considered in each evaluation. As a result, U24h collection may be used more selectively. Although still referenced in IMWG recommendations, urine protein electrophoresis is a less sensitive method for detecting low MP concentrations, making it redundant in follow-up. In cases of discrepancies or diagnostic uncertainty, urine immunofixation (uIFE) is recommended.

Total Ig quantification includes both monoclonal and polyclonal Ig and is therefore not recommended for identifying and quantifying MP.[9] Nevertheless, total Ig quantification can indicate which Ig is increased and provide insights into the patient's immunoparesis status (suppression of normal Ig production), which is important in MM patient evaluation. Quantifying Ig based on the heavy/light chain pair of Ig has become possible with the recent development of a specific immunoassay (Hevylite®) for the IgGκ/IgGλ, IgAκ/IgAλ, or IgMκ/IgMλ chain pairs. This tool offers advantages over traditional techniques by detecting MP that migrates in the beta fractions or several peaks of the same MP resulting from polymerization.[17] This tool also helps in assessing specific immunoparesis for specific Ig heavy/light chain pairs.[18-21]

Bone marrow morphological assessment, performed via bone marrow aspirate (BMA) and/or core needle bone marrow biopsy (BMB), allows for the identification and quantification of plasma cell infiltration. Simultaneous BMA and BMB are recommended at diagnosis,[22,23] and when there is a discrepancy in plasma cell counts between the two, the higher value should be considered.[1] Plasma cell clonality can be determined through immunophenotyping by flow cytometry (FC) on the BMA sample or by immunohistochemistry in the case of BMB.[24] In the minority of patients with non-secretory MM (with negative MP by SPE/sIFE, UPE/uIFE and sFLC), medullary plasmacytosis is the only measurable marker both at diagnosis and follow-up.

If the BMA is hemodiluted, unrepresentative, or shows plasma cell counts below 10%, but there is still a strong suspicion of MM, additional BM samples should be obtained from a different anatomical site, or a biopsy guided by imaging studies should be performed.[22,24,25] For a more comprehensive assessment and to resolve potential discrepancies, it is beneficial to integrate BMA and BMB results into a single report.[26] However, this is challenging due to differences in turnaround times, their execution in separate laboratories, and the involvement of different specialties.

The cell morphology findings in BMA, observed in at least 200 cells, range from morphologically normal plasma cells to those with varying degrees of cellular maturation or nuclear (sometimes multinucleated) and cytoplasmic alterations. The most common morphology findings in abnormal plasma cells include hyperbasophilic cytoplasm and a rounded nucleus with 'clock-face' or 'spoked-wheel' chromatin, eccentrically located, accompanied by marked perinuclear clearing.[22,24-27]

In peripheral blood smears, characteristic features of

MM can also be observed, such as erythrocyte stacking due to paraproteinemia (unbalanced production of a single type of MP), and the presence of abnormal plasma cells in circulation. If these plasma cells exceed 5% of the total leukocytes, it may indicate plasma cell leukemia, a rare and very aggressive entity.[28-30]

Flow cytometry plays a role in diagnosing MM by identifying and quantifying neoplastic plasma cells using specific antibodies (CD138, CD38), assessing phenotypic abnormalities (CD19, CD56, CD27, CD28, CD45, CD117, CD81, Beta2-microglobulin), and confirming clonality through intracellular κ and λ light chain restriction. Widely validated FC procedures and antibody panels, such as those developed by the Euroflow cooperative group, are widely used in clinical practice.[31]

It is worth noting that the quality of the BM sample can affect FC results, often leading to discrepancies with BMA findings due to sample hemodilution. Despite this limitation, FC remains an effective method for assessing plasma cell clonality and identifying phenotypic alterations associated with MM.[32]

Although cytogenetic abnormalities (CA) are not part of MM diagnostic criteria, their identification by fluorescence *in situ* hybridization (FISH) should be performed at diagnosis, given its prognostic impact, discussed later in these guidelines. This assessment should be conducted on BM samples with ≥ 10% plasma cells and/or whenever MM diagnosis is confirmed. To accurately detect CA, an analysis of at least 100 plasma cell nuclei is recommended, and for this, it is crucial first to isolate plasma cells using magnetic bead separation (antibody-coated magnetic beads specifically bind to plasma cells; by applying a magnetic field to the sample the magnetically labeled cells are retained while unlabeled cells are removed) or fluorescence-activated cell sorting (FACS) (a subtype of flow cytometry that allows plasma cells present in the sample to be separated by labeling them with specific fluorescence-conjugated antibodies; when passing through a laser beam the fluorochromes emit light captured by detectors; post-detection, the identified plasma cells are electronically charged and pass through an electromagnetic field that diverts them). This approach significantly increases sensitivity and ensures the identification of only MM-specific CA.[33]

Table 2 outlines the recommended tests and laboratory parameters to be evaluated at different stages for patients with suspected or confirmed MM.

STAGING AND RISK STRATIFICATION

The course of MM is highly heterogeneous among patients. Thus, it is essential to identify prognostic factors that can stratify patients into different risk groups, guiding therapeutic decisions. These factors should be evaluated during the initial process. The most used models in clinical practice are the International Staging System for Multiple Myeloma (ISS) and the Revised ISS (R-ISS) (Table 3). The R-ISS incorporates the traditional ISS markers (serum beta2-microglobulin and albumin), along with serum lactate dehydrogenase levels and CA with adverse prognostic value. The presence of these CA is associated with reduced overall survival (OS), with significantly lower survival rates in patients harboring two or more high-risk CA. These patients are classified as ultra-high-risk (≥ 2 high-risk CA), with a median OS of only nine months.[34]

While certain genetic mutations have been associated with poorer outcomes, routine screening for these mutations is not currently recommended in clinical practice.[34,35] Similarly, recent studies suggest that quantifying circulating tumor plasma cells in peripheral blood using Next Generation Flow (NGF) may also indicate a worse prognosis, but this method is not yet recommended for routine clinical assessment.[36]

FOLLOW-UP AND RESPONSE EVALUATION

The follow-up and evaluation of treatment response in patients with MM have evolved alongside significant advances in available therapeutic options.[37,38] After each treatment cycle, the patient's response should be assessed, considering both the diagnostic test results and current disease status, to ensure an accurate evaluation of the response (Table 2).[23]

The definition of treatment response includes the assessment of MP, BMA/BMB, and imaging studies (outside the scope of these recommendations), as well as parameters related to myeloma-defining events and potential treatment-related complications (Table 4).

Serum MP quantification remains a critical tool for monitoring changes in tumor burden during treatment. It is important to choose the biomarkers that best detect MP to ensure an accurate interpretation of the patient's condition. Serum immunofixation should only be performed when MP is not measurable and/or when new suspicious changes in the electrophoretic pattern emerge. Similarly, uIFE should only be performed if MP becomes undetectable or if there are doubts regarding clonality. Tests that do not add value to the known information, such as U24h, should be avoided to prevent unnecessary patient discomfort.

Bone marrow studies are valuable for confirming complete remission (following negative sIFE results), assessing measurable residual disease (MRD) in these patients, evaluating response in non-secretory MM, and investigating unexplained cytopenias.

In relapse/progression, a new BM evaluation may be beneficial for assessing CA via FISH, particularly if a complete cytogenetic study was not performed at diagnosis, or

Table 2 – Tests and laboratory parameters to investigate at different stages for patients with suspected or diagnosed multiple myeloma

	Sample	Collection tube/ container[a]	Tests	Observations
Diagnosis	PB	EDTA tube	Blood count	
		Dry tube with separation gel	Uremia, Creatinine, Calcium, Total Proteins, Albumin, LDH[b], β2m[b] and Ig	
			SPE	MP quantification
			sIFE/ISUB	Characterization of Ig isotype
			sFLC	
	BM aspirate	Slide	Cytomorphologic study	
		EDTA tube	Immunophenotype by FC	
		Lithium/sodium heparin tube	Cytogenetic study[b]	Analysis of AC by FISH
	BM biopsy	Flask with formaldehyde	Histological and immunohistochemical study	
	Urine (24h)	Dry flask	UTP	Assess the presence of glomerular proteinuria
			UPE	MP quantification
			uIFE	Characterization of Ig isotype
Monitoring (MGUS/SMM)	Repeat the tests performed at diagnosis in PB, without BM assessment or urine (24h)			
Follow-up/response evaluation[c]	PB	See diagnosis	Blood count	
			Creatinine Calcium and Ig	
			SPE	
			sIFE	If negative SPE
			sFLC	
	Urine		UPE	If positive MP (≥ 200 mg/24h) at diagnosis
			uIFE	If negative UPE
	BM aspirate[d]		Morphology and immunophenotypic study by FC	Confirmation of CR, study of cytopenias and MRD assessment[e]
Suspicion of relapse/ progresion	Repeat the tests performed at diagnosis. Consider performing a BM study (an increase in clonal plasma cells may help in deciding to start new treatment in patients where progression is uncertain, allows the identification of any new CA with an impact on therapeutic decisions, or establishes eligibility for clinical trials)			

a: The collection and container used are essential to ensure the quality of the sample and avoid interferences in the methodologies performed. Although there are alternatives to some of the collection tubes, the same characteristics should be maintained for the diagnosis and monitoring of the patient.
b: Prognostic value, see Table 3.
c: Response evaluation during treatment: before each cycle, post-induction/pre-transplant, and post-transplant (adapted to patient's characteristics, risk stratification and treatment – see observations).
d: In response evaluation post-induction/pre-transplant, and post-transplant/maintenance in patients in complete remission, to establish sustained MRD, see Table 4.
e: For MRD evaluation, see Table 4.
β2m: beta2 microglobulin; BM: bone marrow; CA: cytogenetic abnormalities; CR: complete response; EDTA: ethylenediaminetetraacetic acid; FC: flow cytometry; FISH: fluorescence in situ hybridization; FLC: free light chain; Ig: immunoglobulin(s); ISUB: immunosubtraction; LDH: lactate dehydrogenase; MP: M-Protein; MRD: measurable residual disease; PB: peripheral blood; sFLC: serum FLC; sIFE: serum Immunofixation; SPE: serum protein electrophoresis; uIFE: urine immunofixation; UPE: urine protein electrophoresis; UTP: urinary total protein.

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to detect newly emerging CA with therapeutic relevance.[39]

With the advent of more effective therapies that achieve deeper responses, the response criteria have been updated to include assessment of MRD (number of myeloma cells remaining after treatment) in the BM of patients in complete remission.[10] New-generation methodologies, such as NGF and next-generation sequencing (NGS), are now the reference methods due to their higher sensitivity (≤ 10^{-5}).[40] The NGF approach is validated and accessible in most clinical FC laboratories. The first BM aspirate sample (first pull) should be used [low sample quality and quantity (examples: coagulated, collected more than 48h, or hemodiluted) can lead to false negative results], specific populations whose absence/decreased values indicate hemodilution (mast cells, erythroblasts, myeloid and B lymphoid precursors) should be identified and comparison with reference values should be done (Table 4). Automated analysis with reference databases accelerates the process and avoids operator bias. Next-generation sequencing is more time-consuming, has a lower applicability rate than NGF, and is not widely available in most clinical laboratories. This methodology involves studying the specific rearrangement pattern of the *IgH* gene (*VDJ, DJ, Igκ*, and *Igλ*) present in each patient at diagnosis, although it can be performed on cryopreserved samples (Table 4).

Achieving MRD negativity is associated with improved overall survival (OS),[41] making this level of depth of response an important treatment goal. Correct identification of patients achieving MRD negativity is essential, as their responses are both deeper and more durable.[42] In addition to being a surrogate marker for OS, MRD negativity has also been approved by the Food and Drug Administration as a valid endpoint in MM clinical trials, further emphasizing its significance in patient management.[43]

PRESENTATION OF RESULTS/REPORT

Harmonizing laboratory activities is essential to ensure that each patient receives the maximum benefit from the information provided. Results generated by different laboratories must be comparable in terms of terminology, units of measurement, reference ranges, decision limits, and report formats, in addition to the accuracy of analytical results.[44]

Table 3 – Recommended laboratory evaluation for risk stratification in multiple myeloma

Variables	Laboratory parameters	Stages	References
ISS	Serum β2m Serum albumin	I: serum β2m < 3.5 mg/L, serum albumin ≥ 3.5 g/dL II: neither I nor III III: serum β2m > 5.5 mg/L	Greipp *et al*, 2005
CA[a]	del 17p13 (TP53) t(4;14)(p16;q32) (IGH::FGFR3/NSD2) t(14;16)(q32;q23) (IGH::MAF) t(11;14)(q13;q32) (IGH::CCND1) 1p32/1q21 alterations (1q21 amp/gain: *MCL1, CKS1B, ANP32E, BCL9*[b]; del(1p): *CDKN2C, MTF2, FAM46C*)[c]	Adverse risk: del 17p13 (TP53), t(4;14)(p16;q32) (IGH::FGFR3/NSD2), t(14;16)(q32;q23) (IGH::MAF), 1p32/1q21 alterations (1q21 amp/gain: *MCL1, CKS1B, ANP32E, BCL9*[b]; del(1p): *CDKN2C, MTF2, FAM46C*)	Fonseca *et al*, 2009
LDH	LDH levels	High: Serum LDH > upper normal limit	Terpos *et al*, 2010
R-ISS	In risk stratification, the three previous prognostic factors are included (ISS stage, CA, and LDH)	I: ISS I and no high-risk CA and normal LDH II: neither I nor III III: ISS III and high-risk CA or abnormal LDH	Palumbo *et al*, 2015
R2-ISS	1q+ (0.5 points) ISS II (1 points) ISS III (1.5 points) del(17p) (1 points) Abnormal LDH (1 point) t(4;14) (1 point)	Low: R2-ISS I (0 points) Low-intermediate: R2-ISS II (0.5 - 1 points) High-intermediate: R2-ISS III (1.5 - 2.5 points) High: R2-ISS IV (3 - 5 points)	D'Agostino *et al*, 2022

a: Cytogenetic study performed in purified plasma cells from bone marrow aspirate [CD38+ cells magnetically separated (*beads*) or by FACS].
b: Gain of 1q represents an extra copy of the long arm of chromosome 1 (three copies of 1q), while amplification of 1q is defined as the presence of two or more additional copies (≥ 4 copies of 1q).
c: Additional CA that can be identified are t(14;20)(q32;q12) (IGH::MAFB) (present in 2% - 3%; adverse risk) and t(6;14)(p21;132) (IGH::CCND3) (present in 5%; adverse risk) (Sonneveld *et al*, 2016; Walker *et al*, 2019).[16,50]
1q+: 1q gain/amplification; β2m: beta2 microglobulin; CA: cytogenetic abnormalities; del: deletion; FACS: fluorescence-activated cell sorting; FISH: fluorescence in situ hybridization; ISS: international staging system; LDH: lactate dehydrogenase; R-ISS: 1st revision of ISS; R2-ISS: 2nd revision of ISS; t: translocation.

Table 4 – Response criteria in multiple myeloma, adapted from Kumar *et al*, 2016[10] (section 1 of 2)

Response	Criteria	Additional recommendations
Sustained MRD negative	MRD negativity in bone marrow (by NGF, NGS, or both) and by imaging confirmed with a minimum of one year between assessments (subsequent evaluations can be used to define the duration of response negativity)	The method used to define the response (sustained MRD negativity by NGF; sustained MRD negativity by NGS) must be specified
Imaging MRD negative[a]	Absence of bone lesions by PET-CT	
Sequencing MRD negative	Absence of clonal plasma cells by NGS in BM aspirates using a validated methodology with a sensitivity of ≥ 10^{-5}	The presence of a clone is defined as < 2 identical DNA sequences
Flow MRD negative	Absence of phenotypically aberrant clonal plasma cells by NGF in BM aspirates following the EuroFlow methodology (or equivalent validated methodology) with a sensitivity of ≥ 10^{-5}	The reference NGF method consists of 2 tubes of 8 colors with the evaluation of at least 5 million cells[51]
S*tringent* complete Response (sCR)	CR, normal FLC ratio, absence of clonal plasma cells in BM	Presence/absence of clonal plasma cells on BM based on a count of ≥ 100 plasma cells (κ/λ ratio ≤ 4:1 or ≥ 1:2 for κ and λ patients respectively)
Complete response (CR)	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and < 5% plasma cells in bone marrow (non-secretary MM)	In patients without detectable MP in serum or urine, a normal FLC ratio is required
Very good partial response (VGPR)	Serum and urine MP detectable by immunofixation but not on electrophoresis or > 90% reduction in serum MP plus urine MP level < 100 mg/24h	
Partial response (PR)	≥ 50% reduction in serum MP, > 90% reduction in urine < 200 mg/24h	In patients without quantifiable MP in serum or urine, ≥ 50% reduction in the difference between involved FLC and uninvolved FLC is required. In patients without quantifiable MP in serum or urine and not evaluable by sFLC, but with > 30% clonal plasma cells in BM at diagnosis, a ≥ 50% reduction in plasma cells in BM is required. In patients with plasmacytomas at diagnosis, a reduction of ≥ 50% in size is also necessary.
Minimal response	≥ 25% but ≤ 49% reduction in serum MP, 50% - 89% reduction in urinary MP (24h)	In patients with plasmacytomas at diagnosis, a reduction of ≥ 50% in size is also necessary
Stable disease	Not meeting criteria for CR, VGPR, PR, or progressive disease	

a: Outside the scope of these recommendations.
BM: bone marrow; CRAB: hypercalcemia, renal insufficiency, anemia, bone lesions; CR: complete response; DNA: deoxyribonucleic acid; FLC: free light chain(s); IHC: immunohistochemistry; MP: M-protein; MRD: measurable residual disease; NGF: next generation flow; NGS: next generation sequencing; PET-TC: positron emission tomography-computerized tomography.

While the IMWG has developed recommendations for classification, diagnosis, and response evaluation, it does not provide guidance on standardizing reporting in this area. However, some groups have been working towards this goal.[45-48]

Diagnosis and response evaluations for patients in follow-up should be conducted in the same laboratory using the same methodology. Additionally, reports should contain the same amount of information and be presented in a uniform format, regardless of who produces them. Specifying the methodologies used is crucial, given the variety of available options and the inherent sensitivity differences between them.

Given the potential severity of MM, especially in the diagnostic context, it is vital to ensure the timely delivery of samples to the laboratory, their rapid processing, and acceptable turnaround times tailored to each laboratory methodologies used.

Table 5 compiles suggestions for the presentation of results/report.

Table 4 – Response criteria in multiple myeloma, adapted from Kumar *et al*, 2016[10] (section 2 of 2)

Response	Criteria	Additional recommendations
Progressive disease	Increase of > 25% from the lowest response value in any one or more of the following:	
	- Serum MP (absolute increase ≥ 0.5 g/dL)	Serum MP increases of > 1 gm/dL are sufficient to define relapse if starting MP is > 5 g/dL.
	- Urinary MP (absolute increase ≥ 200 mg/24h)	
	- Difference between involved and uninvolved FLC levels (absolute increase > 10 mg/dL)	Only in patients without measurable serum and urine MP levels
	- BM plasma cell percentage (absolute percentage > 10%)	In patients without quantifiable serum or urinary MP and not evaluable by FLC
	Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas (≥ 50% in size of > 1 lesion or in the diameter of a pre-existing lesion with > 1cm of smaller axis	
	Increase of ≥ 50% of circulating plasma cells (≥ 200/uL)	When this is the only way to assess tumor burden
	Development of hypercalcemia (corrected calcium > 11.5 mg/dL or 2.65 mmol/L)	Attributed solely to the plasma cell proliferative disorder
Relapse	Requires ≥ 1:	
	- Direct indicators of increasing disease and/or end organ dysfunction (CRAB features)	
	- New soft tissue plasmacytomas or bone lesions	
	- Definite increase in the size of existing plasmacytomas or bone lesions (≥ 50% and ≥ 1 cm, respectively)	
	- Hypercalcemia (corrected calcium > 11.5 mg/dL or 2.65 mmol/L)	
	- Decrease in haemoglobin of > 2 g/dL or 1.25 mmol/L) attributed to the disease	
	- Rise in serum creatinine by ≥ 2 mg/dL or ≥ 177 mmol/L	
	- Hyperviscosity symptoms secundary to MP	

a: Outside the scope of these recommendations.
BM: bone marrow; CRAB: hypercalcemia, renal insufficiency, anemia, bone lesions; CR: complete response; DNA: deoxyribonucleic acid; FLC: free light chain(s); IHC: immunohistochemistry; MP: M-protein; MRD: measurable residual disease; NGF: next generation flow; NGS: next generation sequencing; PET-TC: positron emission tomography-computerized tomography.

FINAL REMARKS

Recent advancements in the treatment and management of MM have led to the development of more precise and specific laboratory tests, requiring careful consideration regarding their integration into routine diagnostic processes. In addition to the methodologies currently available, others such as the detection and characterization of monoclonal proteins using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and liquid chromatography mass spectrometry (LC-MS), as well as the identification of circulating tumor components through liquid biopsy techniques, such as the detection of circulating pathological plasma cells, nucleic acids (circulating tumor DNA or microRNA), and extracellular vesicles may have the potential to become part of standard laboratory testing in the future.

It is important to note that diagnosing MM requires additional diagnostic tests, including imaging studies, which fall outside the scope of these laboratory recommendations. Given that this diagnosis involves various clinical and laboratory areas, it should be reviewed and discussed in a multidisciplinary meeting.

These recommendations from the PMMG provide key guidance for the diagnosis and follow-up of patients with MM, emphasizing the importance of using the most appropriate laboratory tests at each stage of evaluation.

Table 5 – Recommendation on information to include in a laboratory report in the context of multiple myeloma (section 1 of 2)

Test	Important information to include in the report	Comments
SPE	Methodology used	
	Electrophoresis profile	• Include all determined fractions, their concentration, and respective reference values. Presenting the percentage values of the fractions is optional.
	MP when present, quantified in g/L or g/dL (with one decimal place) and reported separately from other fractions	• Characterize the isotype of the MP in the first sample where it is identified. • If it is not possible to identify the isotype of the MP, mention the need for a confirmatory test and identification of monoclonal Ig isotype. • In the electrophoresis profile, shade the area corresponding to the MP migration, obtained by the perpendicular drop method. • If more than one monoclonal isotype is present, identify and quantify each one independently, indicating their respective mobilities in the profile. • If the quantification is below the limit of detection (LoD) of the technique, report the value as "< LoD g/L." • In cases of Ig polymerization, characteristic of IgA, the MP quantification corresponds to the sum of the peaks present in the profile. • The presence of small peaks may be transient and should therefore be evaluated in the clinical and therapeutic context, especially after bone marrow transplant or the start of monoclonal antibody treatment. • Clarify any type of interference that could affect the interpretation of the results in the comments section.
	Total serum protein value	• Present this value, which is necessary for calculating the concentration of the fractions, and specify the determination method.
UPE	Methodology used	
	Total protein value in 24-hour urine	• Present values in g/24h or mg/24h, along with the respective determination method; note that the test may not be informative in the absence of proteinuria.
	Electrophoresis profile, if relevant	• If the profile is included, quantify the fractions found and the M-protein, if present, in g/L; the M-protein should be identified.
sIFE/ ISUB/ uIFE	Methodology used	
	Presence or absence of MP	• When MP is present, identify the isotype(s) and their respective mobility zones.
sFLC	Methodology used	
	Results for both light chains and their ratio	• If the Freelite® assay is used, it is possible to indicate the existence of a reference range for patients with impaired renal function (reduced eGFR).[15,16]
Bone marrow aspirate	Anatomical site and difficulty of the aspiration	
	Adequacy and cellularity of the aspirate	
	Differential count of nucleated cells, total number of cells counted, and erythroid:myeloid ratio	• Include quantitative and qualitative comments regarding all cell lineages and any abnormal cells. For example, quantification and description of the plasma cells found.
	Iron deposition staining and cytochemical studies, if performed	
	A conclusion	

CG: cytogenetics; FC: flow cytometry; FISH: fluorescence in situ hybridization; FLC: free light chains; ICD: international classification of diseases; ISCN: The International System for Human Cytogenomic Nomenclature; ISUB: immunosubtraction; LoD: limit of detection; LQ: limit of quantification; eGFR: estimated glomerular filtration rate; MG: molecular genetics; MoAb: monoclonal antibodies; MP: M-protein; MRD: measurable residual disease; Ig: immunoglobulin; SNOMED CT: systematized nomenclature of medicine; SPE: serum protein electrophoresis; sIFE: serum immunofixation; uIFE: urinary immunofixation; UPE: urinary protein electrophoresis

Table 5 – Recommendation on information to include in a laboratory report in the context of multiple myeloma (section 2 of 2)

Test	Important information to include in the report	Comments
Bone marrow biopsy	Adequacy, macroscopic appearance, and length of the core biopsy	
	Percentages and pattern of cellularity	
	Bone architecture and reticulin staining	
	Characterization of different cell lineages	• Include location, number, morphology, and differentiation pattern.
	Characterization of abnormal cells and/or infiltrates	
	Immunohistochemistry results	
	A conclusion	• Include disease classification/differential diagnosis and its SNOMED CT or ICD coding.
FC	The identified populations and their respective percentages in the total cellularity	• In response evaluation (MRD), include erythroblasts, B lymphoid precursors, and mast cells (indicators of sample hemodilution in bone marrow).
	Percentage of plasma cells	• Indicate the total value of analyzed plasma cells and neoplastic plasma cells (in the total cellularity and total plasma cell population).
	Description of the neoplastic plasma cell phenotype	• Include clonality.
	LoD and LQ (sensitivity)	• Include in response evaluation (MRD), calculated based on the total number of acquired events and the number of neoplastic plasma cells, with a minimum of 30 cells for LoD and 50 for LQ, respectively[52]
	A conclusion	• Indicate the cellularity pattern and sample adequacy (e.g., hypocellular, hemodiluted sample).
CG	Methodology and probes used	
	Clear description of the results obtained	• Following the latest version of the International System for Human Cytogenomic Nomenclature (ISCN) – http//iscn.karger.com/
	Interpretation of the results	• Indicate the clinical significance of the results, including prognosis.
MG	Methodology used	
	Interpretation of the results	• Indicate the clinical significance of the results

CG: cytogenetics; FC: flow cytometry; FISH: fluorescence in situ hybridization; FLC: free light chains; ICD: international classification of diseases; ISCN: The International System for Human Cytogenomic Nomenclature; ISUB: immunosubtraction; LoD: limit of detection; LQ: limit of quantification; eGFR: estimated glomerular filtration rate; MG: molecular genetics; MoAb: monoclonal antibodies; MP: M-protein; MRD: measurable residual disease; Ig: immunoglobulin; SNOMED CT: systematized nomenclature of medicine; SPE: serum protein electrophoresis; sIFE: serum immunofixation; uIFE: urinary immunofixation; UPE: urinary protein electrophoresis

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AUTHOR CONTRIBUTIONS

AMP, JPB, JC: Study design, writing and critical review of the manuscript.

MJS, CG, BF, MC, SC, HC, CC, APC, MC, MRC, NC, PF, JGF, RH, SL, PL, AP, CP, IR, ABS, PS, JS, MJRS, SS, TS, MT, FT, RB, AR, CJ: Writing and critical review of the manuscript.

All authors approved the final version to be published.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in October 2024.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

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REFERENCES

1. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-48.
2. Rajkumar SV, Landgren O, Mateos MV. Smoldering multiple myeloma. Blood. 2015;125:3069-75.
3. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak

MF, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. N Engl J Med. 2002;346:564-9.
4. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med. 2007;356:2582-90.
5. Fermand JP, Bridoux F, Dispenzieri A, Jaccard A, Kyle RA, Leung N, et al. Monoclonal gammopathy of clinical significance: a novel concept with therapeutic implications. Blood. 2018;132:1478-85.
6. Thoren KL, McCash SI, Murata K. Immunotyping provides equivalent results to immunofixation in a population with a high prevalence of monoclonal gammopathies. J Appl Lab Med. 2021;6:1551-60.
7. Yang Z, Harrison K, Park YA, Chaffin CH, Thigpen B, Easley PL, et al. Performance of the sebia CAPILLARYS 2 for detection and immunotyping of serum monoclonal paraproteins. Am J Clin Pathol. 2007;128:293-9.
8. McCudden CR, Mathews SP, Hainsworth SA, Chapman JF, Hammett-Stabler CA, Willis MS, et al. Performance comparison of capillary and agarose gel electrophoresis for the identification and characterization of monoclonal immunoglobulins. Am J Clin Pathol. 2008;129:451-8.
9. Willrich MA, Katzmann JA. Laboratory testing requirements for diagnosis and follow-up of multiple myeloma and related plasma cell dyscrasias. Clin Chem Lab Med. 2016;54:907-19.
10. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International myeloma working group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17:e328-46.
11. Katzmann JA, Kyle RA, Benson J, Larson DR, Snyder MR, Lust JA, et al. Screening panels for detection of monoclonal gammopathies. Clin Chem. 2009;55:1517-22.
12. Dejoie T, Corre J, Caillon H, Moreau P, Attal M, Loiseau HA. Responses in multiple myeloma should be assigned according to serum, not urine, free light chain measurements. Leukemia. 2019;33:313-8.
13. Nowrousian MR, Brandhorst D, Sammet C, Kellert M, Daniels R, Schuett P, et al. Serum free light chain analysis and urine immunofixation electrophoresis in patients with multiple myeloma. Clin Cancer Res. 2005;11:8706-14.
14. Tschautscher M, Rajkumar V, Dispenzieri A, Lacy M, Gertz M, Buadi F, et al. Serum free light chain measurements to reduce 24-h urine monitoring in patients with multiple myeloma with measurable urine monoclonal protein. Am J Hematol. 2018;93:1207-10.
15. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol. 2008;9:11.
16. Long TE, Indridason OS, Palsson R, Rognvaldsson S, Love TJ, Thorsteinsdottir S, et al. Defining new reference intervals for serum free light chains in individuals with chronic kidney disease: results of the iStopMM study. Blood Cancer J. 2022;12:133.
17. Ríos-Tamayo R, Puig N, Algarín M, García de Veas Silva JL, Barbosa N, Encinas C, et al. The current role of the heavy/light chain assay in the diagnosis, prognosis and monitoring of multiple myeloma: an evidence-based approach. Diagnostics. 2021;11:2020.
18. Harutyunyan NM, Vardanyan S, Ghermezi M, Gottlieb J, Berenson A, Andreu-Vieyra C, et al. Levels of uninvolved immunoglobulins predict clinical status and progression-free survival for multiple myeloma patients. Br J Haematol. 2016;174:81-7.
19. Ludwig H, Milosavljevic D, Berlanga O, Zojer N, Hübl W, Fritz V, et al. Suppression of the noninvolved pair of the myeloma isotype correlates with poor survival in newly diagnosed and relapsed/refractory patients with myeloma. Am J Hematol. 2016;91:295-301.
20. Lakhwani S, Rosiñol L, Puig N, Pico-Picos MA, Medina-González L, Martínez-López J, et al. Recovery of uninvolved heavy/light chain pair immunoparesis in newly diagnosed transplant-eligible myeloma patients complements the prognostic value of minimal residual disease detection. Haematologica. 2024;109:1909-17.
21. Lin Y, Qiu L, Usmani S, Joo CW, Costa L, Derman B, et al. Consensus guidelines and recommendations for the management and response assessment of chimeric antigen receptor T-cell therapy in clinical practice for relapsed and refractory multiple myeloma: a report from the International Myeloma Working Group Immunotherapy Committee. Lancet Oncol. 2024;25:e374-87.
22. Bain BJ, Clark DM, Wilkins BS. Bone marrow pathology. New Jersey: Wiley-Blackwell; 2019.
23. Caers J, Garderet L, Kortüm KM, O'dwyer ME, van de Donk NW, Binder M, et al. European myeloma network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when. Haematologica. 2018;103:1772-84.
24. Ribourtout B, Zandecki M. Plasma cell morphology in multiple myeloma and related disorders. Morphologie. 2015;99:38-62.
25. Landgren O, Rajkumar SV. New developments in diagnosis, prognosis, and assessment of response in multiple myeloma. Clin Cancer Res. 2016;22:5428-33.
26. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC. ICSH guidelines for the standardization of bone marrow specimens and reports. Int J Lab Hematol. 2008;30:349-64.
27. Takakuwa T, Araki T, Nakamura K, Fukuyama T, Miura A, Fujitani Y, et al. Morphologic classification is an important prognostic factor in patients with newly diagnosed multiple myeloma treated with bortezomib or lenalidomide. Ann Clin Lab Sci. 2020;50:333-41.
28. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364:1046-60.
29. Fernández de Larrea C, Kyle RA, Durie BG, Ludwig H, Usmani S, Vesole DH, et al. Plasma cell leukemia: consensus statement on diagnostic requirements, response criteria and treatment recommendations by the International Myeloma Working Group. Leukemia. 2013;27:780-91.
30. Fernández de Larrea C, Kyle R, Rosiñol L, Paiva B, Engelhardt M, Usmani S, et al. Primary plasma cell leukemia: consensus definition by the International Myeloma Working Group according to peripheral blood plasma cell percentage. Blood Cancer J. 2021;11:192.
31. van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26:1908-75.
32. Rawstron AC, Orfao A, Beksac M, Bezdickova L, Brooimans RA, Bumbea H, et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica. 2008;93:431-8.
33. Ross FM, Avet-Loiseau H, Ameye G, Gutierrez NC, Liebisch P, O'Connor S, et al. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. Haematologica. 2012;97:1272-7.
34. Shah V, Sherborne AL, Walker BA, Johnson DC, Boyle EM, Ellis S, et al. Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. Leukemia. 2018;32:102-10.
35. Walker BA, Boyle EM, Wardell CP, Murison A, Begum DB, Dahir NM, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. J Clin Oncol. 2015;33:3911-20.
36. Gonsalves WI, Jevremovic D, Nandakumar B, Dispenzieri A, Buadi FK, Dingli D, et al. Enhancing the R-ISS classification of newly diagnosed multiple myeloma by quantifying circulating clonal plasma cells. Am J Hematol. 2020;95:310-5.
37. Kumar SK, Dispenzieri A, Lacy MQ, Gertz MA, Buadi FK, Pandey S, et al. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia. 2014;28:1122-8.
38. Binder M, Nandakumar B, Rajkumar SV, Kapoor P, Buadi FK, Dingli D, et al. Mortality trends in multiple myeloma after the introduction of novel therapies in the United States. Leukemia. 2022;36:801-8.
39. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, et al. International myeloma working group molecular classification of multiple myeloma: spotlight review. Leukemia. 2009;23:2210-21.
40. Caetano J, Barahona F, Lúcio P, João C. Measurable residual disease assessment in multiple myeloma: how deep is enough? Hemato. 2022;3:385-413.
41. Munshi NC, Avet-Loiseau H, Anderson KC, Neri P, Paiva B, Samur M, et al. A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma. Blood Adv. 2020;4:5988-99.
42. Lahuerta JJ, Paiva B, Vidriales MB, Cordón L, Cedena MT, Puig N, et al. Depth of response in multiple myeloma: a pooled analysis of three

PETHEMA/GEM clinical trials. J Clin Oncol. 2017;35:2900-10.
43. Food and Drug Administration. April 12, 2024 meeting of the oncologic drugs advisory committee meeting announcement. [cited 2024 Jun 23]. Available from: https://www.fda.gov/advisory-committees/advisory-committee-calendar/april-12-2024-meeting-oncologic-drugs-advisory-committee-meeting-announcement-04122024.
44. Zaninotto M, Graziani MS, Plebani M. The harmonization issue in laboratory medicine: the commitment of *CCLM*. Clin Chem Lab Med. 2023;61:721-31.
45. Singh G. Serum and urine protein electrophoresis and serum-free light chain assays in the diagnosis and monitoring of monoclonal gammopathies. J Appl Lab Med. 2020;5:1358-71.
46. Booth RA, McCudden CR, Balion CM, Blasutig IM, Bouhtiauy I, Rodriguez-Capote K, et al. Candidate recommendations for protein electrophoresis reporting from the Canadian Society of Clinical Chemists Monoclonal Gammopathy Working Group. Clin Biochem. 2018;51:10-20.
47. Tate JR, Keren DF, Mollee P. A global call to arms for clinical laboratories – harmonised quantification and reporting of monoclonal proteins. Clin Biochem. 2018;51:4-9.
48. Moss MA. Moving towards harmonized reporting of serum and urine protein electrophoresis. Clin Chem Lab Med. 2016;54:973-9.
49. Sonneveld P, Avet-Loiseau H, Lonial S, Usmani S, Siegel D, Anderson KC, et al. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. Blood. 2016;127:2955-62.
50. Walker BA, Mavrommatis K, Wardell CP, Ashby TC, Bauer M, Davies F, et al. A high-risk, double-hit, group of newly diagnosed myeloma identified by genomic analysis. Leukemia. 2019;33:159-70.
51. Flores-Montero J, Sanoja-Flores L, Paiva B, Puig N, García-Sánchez O, Böttcher S, et al. Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia. 2017;31:2094-103.
52. Arroz M, Came N, Lin P, Chen W, Yuan C, Lagoo A, et al. Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting. Cytometry B Clin Cytom. 2016;90:31-9.