Early identification of functional high-risk multiple myeloma patients after transplant: the predictive power of fat fraction and Response Assessment Category score in diffusion-weighted whole-body magnetic resonance imaging

Angelo Belotti,[1] Barbara Frittoli,[2] Sofia Terlizzi,[3] Rossella Ribolla,[1] Claudia Crippa,[1] Chiara Saeli,[2] Samantha Ferrari,[1] Nicola Bianchetti,[1] Chiara Bottelli,[1] Chiara Cattaneo,[1] Carmela Carbone,[3] Alessandro Gullino,[3] Marco Chiarini,[4] Viviana Giustini,[4] Aldo Roccaro,[5] Luigi Grazioli[2] and Alessandra Tucci[1]

[1]Department of Hematology, ASST Spedali Civili di Brescia; [2]Department of Radiology, ASST Spedali Civili di Brescia; [3]University of Brescia; [4]Clinical Chemistry Laboratory/Diagnostic Department and [5]Clinical Research Development and Phase I Unit – ASST Spedali Civili di Brescia, Brescia, Italy

Correspondence: A. Belotti
ange.belotti@gmail.com

Received: January 19, 2025.
Accepted: March 27, 2025.
Early view: April 3, 2025.

https://doi.org/10.3324/haematol.2025.287409

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Abstract

Functional high-risk (FHR) multiple myeloma (MM) patients, defined as those with early relapse despite optimal initial therapy, represent an unmet clinical need. Diffusion-weighted whole-body MRI (DW-MRI) is increasingly used in MM management due to its high sensitivity in assessing treatment response. The Myeloma Response Assessment and Diagnosis System (MY-RADS) established the Response Assessment Category (RAC), a 5-point scale ranging from complete response (RAC 1) to progressive disease (RAC 5), which independently stratifies patients with different outcomes after autologous stem cell transplantation (ASCT). The relative fat fraction (rFF), derived from DW-MRI, provides additional prognostic insights into bone marrow composition. This study aimed to evaluate whether the combined assessment of RAC and rFF could identify FHR MM patients at risk of early relapse, defined as progression within 18 months post-autologous stem cell transplantation (ASCT). Ninety-seven MM patients were retrospectively analyzed after ASCT, before maintenance, with a median follow-up of 47 months. An rFF threshold of 17.2% predicted early relapse with 83% sensitivity and 85% specificity. Patients with rFF >17.2% had significantly improved post-ASCT progression-free survival (PFS, median not reached [NR] *vs*. 13.7 months, HR 0.18; 95% CI: 0.08-0.43) and overall survival (OS, 3-year rate: 96% *vs*. 62%, HR 0.12; 95% CI: 0.03-0.45) compared to rFF ≤17.2%. Patients with RAC 1/rFF High had the best outcomes, while RAC ≥2/rFF Low had the worst prognosis (median PFS: NR *vs*. 12.3 months, HR 0.21; 95% CI: 0.07-0.62). rFF complements RAC for response assessment after ASCT, enabling early identification of FHR patients with poor prognosis.

Introduction

Patients with functional high-risk (FHR) multiple myeloma (MM) represent a difficult to treat population of patients characterized by a high risk of early relapse despite an optimal induction treatment with currently available therapies, irrespective of their baseline cytogenetic or molecular risk profile. Unlike traditional high-risk classifications based on cytogenetic abnormalities, FHR is defined by dynamic clinical and biological features, including early relapse, often within 12-18 months of initiating therapy, indicating aggressive disease biology.[1] This feature highlights the functional behavior of the disease, which may not be fully captured by standard staging systems or genetic profiling. FHR patients exhibit significantly worse survival outcomes, making early identification critical for optimizing therapeutic strategies. Hence, beyond the evaluation of established high-risk markers at diagnosis, it is particularly important to assess residual disease after treatment, both within and outside the bone marrow (BM). In this context, diffusion-weighted whole-body magnetic resonance imaging (DW-MRI) is increasingly used in the management of MM due to its high sensitivity in the detection of BM infiltration and in the evaluation of extramedullary disease.[2] The Myeloma Response Assessment

and Diagnosis System (MY-RADS) imaging guidelines have established criteria for a response assessment category (RAC) score with a 5-point scale defining complete imaging response (i.e., RAC 1) or residual or progressive disease after therapy (i.e., RAC 2-5). The assignment of RAC is based on specific morphological findings and quantitative calculation of the apparent diffusion coefficient (ADC) after treatment.[3] Briefly, the ADC reflects the motion of water molecules at the cellular level within tissues, directly correlating with cell density. We have previously shown that RAC criteria were able to independently stratify patients with different outcomes after autologous stem cell transplantation (ASCT).[4] Another crucial parameter for radiologists in the interpretation of magnetic resonance imaging (MRI) images, both at diagnosis and after treatment, is the relative fat fraction (rFF), which is a quantitative biomarker that measures the percentage of fat cells within tissues, particularly in the BM. It offers important information about the composition of bone lesions, effectively distinguishing normal BM, characterized by high fat content, from cancerous or infiltrated bone, where fat content is significantly reduced.[5] Current literature provides valuable insights, albeit in heterogeneous patient populations primarily affected by solid tumors. Several works have suggested that a rFF lower than 20% is likely indicative of neoplastic marrow replacement rather than a benign process.[5-7] This cut-off provides a practical method for clinicians to differentiate between benign marrow and malignant lesions, making it an important tool in the diagnostic pathway for cancers that involve the bone, such as MM and metastatic cancers from breast and prostate origins. Higher rFF percentages can indicate treatment response through the restoration of marrow fat in cancer patients; hence, the combination of DW-MRI and rFF allows for the identification of viable bone metastases.[8] Similarly, in MM, the response of bone disease to treatment has been shown to correlate with an increase in the rFF of involved BM.[9] The available data primarily focus on solid tumor cases and newly diagnosed patients, underscoring the need for dedicated studies on MM. Notably, no specific rFF cut-off has been identified or validated in MM for assessing focal lesions or BM infiltration, particularly regarding the optimal rFF threshold predictive of outcomes after ASCT. The present study aimed to evaluate the predictive role of rFF, determine an optimal threshold for identifying FHR patients post ASCT, and assess whether combining rFF with RAC score improves risk stratification. With this aim, we sought to evaluate the optimal rFF cut-off threshold in residual bone lesions after ASCT, prior to maintenance therapy, in order to identify FHR MM patients at risk of early relapse, defined in our study as relapse occurring within 18 months after transplant.

Methods

This retrospective study analyzed 97 consecutive MM patients diagnosed between January 2018 and December 2022 who underwent DW-MRI evaluation after ASCT but prior to maintenance therapy. Details of the MRI protocol and the application of RAC criteria in our external validation of the MY-RADS guidelines in clinical practice have been published previously.[4] The protocol includes scanning from vertex to toes in 4-5 slabs depending on patient height, with the axial acquisition of morphological sequences as T1 DIXON and T2 HASTE images, T1 TSE and STIR sagittal images on the whole spine and functional axial DWI/ADC images. Total imaging time for the study is about 40 minutes (min) and no contrast media is administered. Lesions are classified as restricting (low ADC values and signal increase in DW-MRI) and non-restricting (high ADC values and signal decrease in DW-MRI): a target lesion is defined as a lesion >5 mm identified on T1- and T2-weighted morphological sequences, with high signal intensity on high b-value images (900 second [s]/mm^2) and ADC values <1,400 μm^2/s. When multiple lesions were identified, the final attribution of RAC was referred to the main 5 target lesions. The rFF was specifically assessed using the Dixon sequence, as outlined in the MY-RADS protocol.[3] Post-ASCT progression-free survival (PFS) was calculated from the date of the last ASCT (the second in case of double ASCT) until progression or death from any cause. Post-ASCT overall survival (OS) was calculated until death from any cause or last follow up. RAC score was assigned based on MY-RADS criteria and outcome was analyzed according to imaging response. In addition to the RAC score, the rFF of up to 5 target lesions was evaluated for each patient, along with 2 measurements on the bilateral iliac crest, to account for patients with a diffuse infiltration pattern in the absence of focal lesions at diagnosis. Receiver Operating Characteristic (ROC) curve analysis was performed in order to select the optimal threshold value for rFF predictive for early relapse (within 18 months). The Youden Index was utilized to determine the threshold that maximized the combined sensitivity and specificity. Post-ASCT PFS and post ASCT OS were, therefore, analyzed according to the identified threshold and after combining RAC score. In patients with available minimal residual disease (MRD) evaluation from BM aspirates prior to maintenance, assessed using multiparametric flow cytometry (MFC, with a sensitivity of 10^{-5}), a combined analysis with imaging response was also performed. This study was carried out in accordance with the principles of the Declaration of Helsinki and has been approved by our institutional ethics committee.

Statistical analysis

The log-rank (Mantel-Cox) test, applied to the Kaplan-Meier method, was used to estimate survival curves according to the RAC score and the cut-off identified by the ROC analysis. Fisher's exact test was performed to compare the frequency distributions of patient subgroups based on the RAC score, rFF, and MRD results. Additionally, Fisher's

exact test was used to assess the distribution of RAC and rFF values in relation to prognostic factors at diagnosis, including high-risk fluorescence *in situ* hybridization (FISH) abnormalities, revised International Staging System (R-ISS) stage, and the presence of extramedullary disease (EMD). Cohen's kappa statistics were calculated to assess the level of agreement between marrow MRD results and the RAC score, as well as between MRD results and rFF in residual bone lesions after ASCT. Multivariate analysis using Cox proportional hazard models was performed to identify independent predictors of post-ASCT PFS and OS. Statistical analyses were conducted using GraphPad Prism software.

Results

Baseline characteristics

The following induction regimens were used in the 97 patients studied: bortezomib, thalidomide, dexamethasone (VTD) in 58 (60%), bortezomib, lenalidomide, dexamethasone (VRD) in 6 (6%), daratumumab, bortezomib, lenalidomide, dexamethasone (Dara-VRD) in 5 (5%), daratumumab, cyclophosphamide, dexamethasone (Dara-VCD) in 6 (6%), daratumumab, bortezomib, thalidomide, dexamethasone (Dara-VTD) in 21 (22%), isatuximab, carfilzomib, lenalidomide, dexamethasone (Isa-KRD) in one (1%). Fifty-six patients (58%) received single ASCT (MEL200 mg/m^2 conditioning), whereas double ASCT was performed in 41 (42%) cases. Regarding maintenance, 89 (92%) patients received lenalidomide until progression, one (1%) patient received daratumumab and lenalidomide, 4 (4%) patients received daratumumab and ixazomib, 3 (3%) patients received ixazomib. Median follow up was 47 months. Patients' characteristics are shown in Table 1. International Myeloma Working Group (IMWG) responses before maintenance were: 5 partial response (PR) (5%), 32 very good partial response (VGPR) (33%), 42 complete response (CR) (43%), and 18 stringenet CR (sCR) (19%). MRD assessment before maintenance with MFC was available in 79 patients and was negative in 52 (66%) cases.

Response assessment category score and relative fat fraction

Complete imaging response after ASCT according to MY-RADS was observed in 66 patients (RAC1: 68%), whereas residual disease was observed in 31 patients (RAC ≥2: 32%; RAC2: 29%; RAC3: 3%; respectively). No correspondence was observed between the depth of response according to the IMWG criteria and the distribution of patients with RAC1 and RAC ≥2 across the different response levels. A total of 416 areas on DW-MRI before maintenance were drawn and reviewed to calculate rFF values. Mean rFF ROC

Table 1. Patients' characteristics.

Characteristics	Values
Patients, N	97 (41 female, 56 male)
Median age in years (range)	61 (34-73)
ISS Stage III, N (%)	33 (34)
R-ISS Stage III, N (%)	11 (11)
R2-ISS High, N (%)	5 (5)
High-risk cytogenetic profile, N (%)	34 (35)
Extramedullary disease, N (%)	5 (5)
Induction treatment, N (%) VTD VRD Daratumumab-VRD Daratumumab-VCD Daratumumab-VTD Isatuximab-KRD Single ASCT Double ASCT	 58 (60) 6 (6) 5 (5) 6 (6) 21 (22) 1 (1) 56 (58) 41 (42)
Maintenance, N (%) Lenalidomide Daratumumab-lenalidomide Daratumumab-ixazomib Ixazomib	 89 (92) 1 (1) 4 (4) 3 (3)

Patients with high-risk cytogenetics had t(4;14), t(14;16) or del17p and/or 1q gain/amp1q. N: number; ASCT: autologous stem cell transplantation; ISS: International Staging System; R-ISS: Revised International Staging System; R2-ISS: Revised 2 International Staging System; VTD: bortezomib, thalidomide, dexamethasone; VCD: bortezomib, cyclophosphamide, dexamethasone; VRD: bortezomib, lenalidomide, dexamethasone; KRD: carfilzomib, lenalidomide, dexamethasone.

curve analysis revealed an AUC of 0.84 (95% CI: 0.72-0.95, *P*<0.0001); a cut-off threshold of 17.2% for rFF showed the highest Youden Index and identified early relapse with 83% (95% CI: 0.61-0.94) sensitivity, 85% (95% CI: 0.75-0.90%) specificity, a positive predictive value (PPV) of 55% (95% CI: 36-74%) and a negative predictive value (NPV) of 96% (95% CI: 91-100%). Considering the identified threshold of 17.2% for rFF, 27 patients (28%) presented with rFF values below the threshold on residual bone lesions after ASCT, while 70 patients (72%) had rFF values >17.2%. Our analysis revealed a significant association between rFF <17.2% and RAC ≥2 (*P*<0.0001). Among patients with rFF <17.2%, 19 (70.4%) had RAC ≥2, while among those with rFF >17.2%, only 12 (17.1%) had RAC ≥2. The overall concordance between these two imaging parameters was 79.4%, with a moderate agreement (Cohen's kappa = 0.51).

Correlation between response assessment category score, relative fat fraction, and prognostic factors

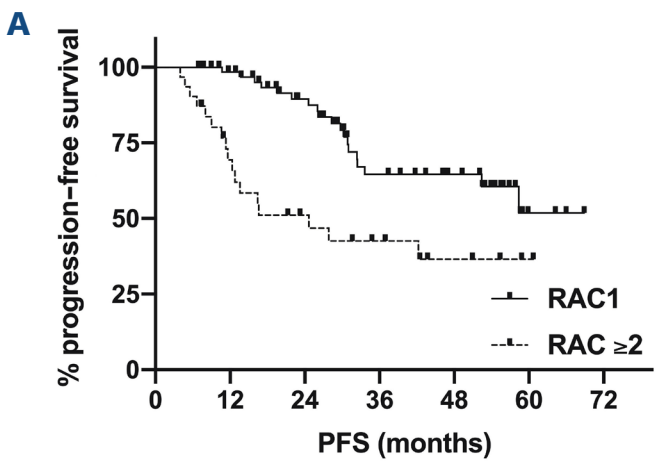
Our analysis revealed that neither high-risk FISH abnormalities (t(4;14), t(14;16), del17p, amp1q) nor R-ISS stage (1-2 *vs*. 3) nor R2-ISS (non-high *vs*. high) were significantly associated with post-treatment RAC score (RAC1 *vs*. RAC ≥2) or rFF in residual bone lesions after ASCT (≥17.2% *vs*. <17.2%). EMD was present in 5% of patients at diagnosis, with no statistically significant differences in the distribution of patients based on post-treatment imaging parameters.

Survival outcomes

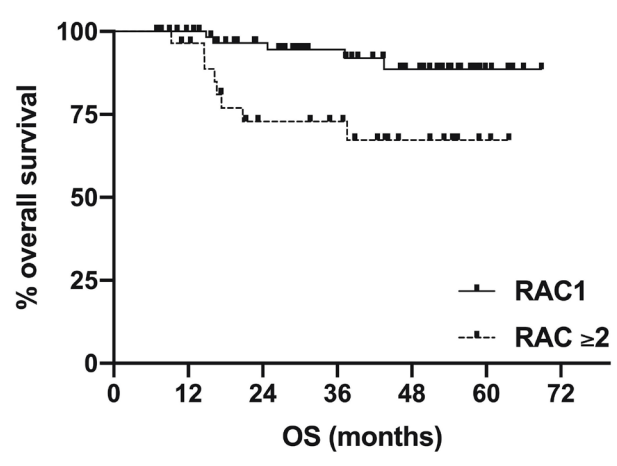
Patients with RAC1 achieved significantly longer post-ASCT PFS, with respect to patients with RAC≥2: median not reached (NR) *vs*. 24.6 months, *P*=0.0009, HR 0.27 (HR 0.27; 95% CI: 0.13-0.58), as well as post-ASCT OS: the 3-year post-ASCT survival rate was 95% *versus* 73%, *P*=0.0072, HR 0.24 (95% CI: 0.07-0.81) (Figure 1). Considering the identified threshold of 17.2% for rFF, 27 patients (28%) presented with rFF values below the threshold on residual bone lesions after ASCT, while 70 patients (72%) had rFF values >17.2%. Post-ASCT PFS was significantly superior in patients with rFF >17.2% *versus* rFF <17.2% in residual bone lesions after transplant, defined below as rFF High *versus* rFF Low: median NR 13.7 months, *P*<0.0001, HR 0.18 (95% CI: 0.08-0.43), as well as post-ASCT OS: the 3-year post ASCT survival rate was 96% *versus* 62%, *P*<0.0001, HR 0.12 (95% CI: 0.03-0.45) (Figure 2). Out of 97 patients, 18 (18.5%) experienced relapse within 18 months post-ASCT and were classified FHR: 15 out of these 18 patients (83%) had an rFF below the identified cut-off of 17.2%. Combining RAC score and rFF, three different risk groups were identified: RAC1/rFF High *vs*. RAC ≥2/rFF Low *vs*. RAC ≥2/rFF High or RAC1/rFF Low; these had significantly different post-ASCT PFS (*P*<0.0001) and post-ASCT OS (*P*=0.0002) (Figure 3). In particular, significantly superior post-ASCT PFS was observed in patients with RAC1/rFF High before maintenance, with respect to patients with RAC ≥2/rFF Low (PFS NR *vs*. 12.3 months, *P*<0.0001, HR 0.21, 95% CI: 0.07-0.62). An intermediate post-ASCT PFS (median 42 months) was observed in patients with either RAC ≥2/rFF High or RAC1/rFF Low, which was significantly lower compared to the RAC1/rFF High group (*P*=0.021, HR 0.52, 95% CI: 0.20-1.40). Similarly, superior post-ASCT OS was observed in RAC1/rFF High patients compared to those with RAC ≥2/rFF Low before maintenance (3-year survival rate: 98% *vs*. 59%, *P*<0.0001, HR 0.1, 95% CI: 0.02-0.47). An intermediate outcome was observed in patients with either RAC ≥2/rFF High or RAC1/rFF Low, which was inferior compared to the RAC1/rFF High group (3-year post-ASCT OS rate: 83%, *P*=0.031, HR 0.28, 95% CI: 0.04-1.94).

Imaging and marrow minimal residual disease: combined evaluation

Comparing DW-MRI assessment before maintenance with



	RAC 1	RAC ≥ 2	HR (95% CI)	*P*
Median PFS (months)	NR	24.6	0.27 (0.13-0.58)	0.0009



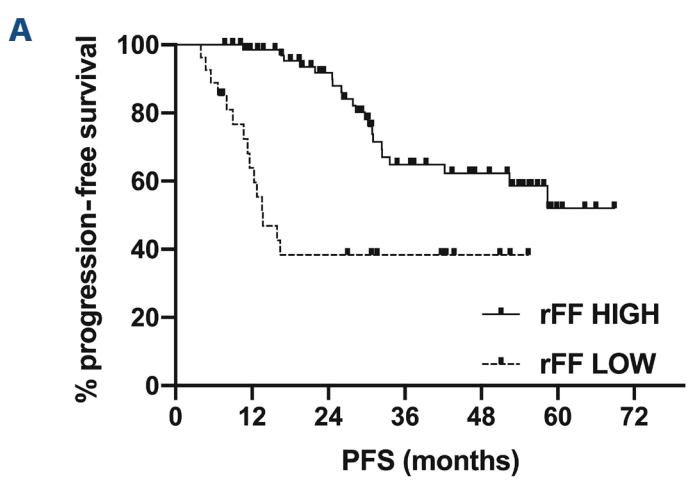
	RAC 1	RAC ≥ 2	HR (95% CI)	*P*
3y OS	95%	73%	0.24 (0.07-0.81)	0.0072

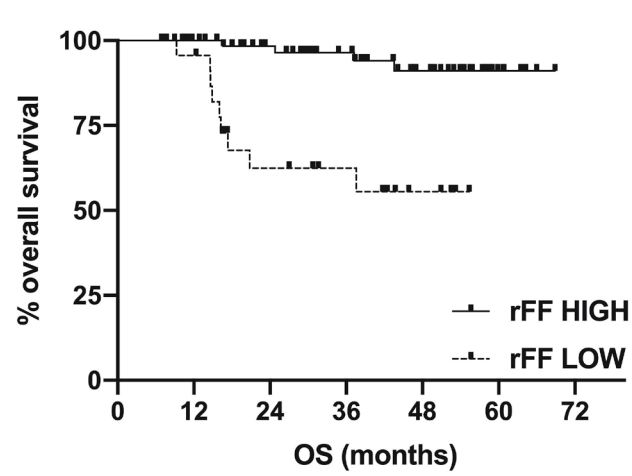
Figure 1. Kaplan-Meier curves according to response assessment category score: 1 *versus* ≥2. (A) Post-autologous stem cell transplantation (ASCT) progression-free survival (PFS). (B) Post-ASCT overall survival (OS). RAC: response assessment category. CI: Confidence Interval; HR: Hazard Ratio; y: years.

marrow MFC results in the 79 patients with available MRD data, a low concordance between the two techniques was observed. This was evident both for the RAC score (concordance: 58%, kappa: 0.046) and for rFF in residual bone lesions after transplant, using the identified threshold of 17.2% (concordance: 61%, kappa: 0.087). Notably, patients with marrow MRD positivity and low rFF before maintenance experienced extremely poor outcomes, with a median post-ASCT PFS of 11.3 months, compared to MRD-negative / rFF High patients (median post-ASCT PFS: not reached, *P*<0.0001) (Figure 4). Table 3 summarizes the distribution of RAC score and rFF according to IMWG response categories and MRD status.

Multivariate analysis

Among prognostic parameters (ISS stage 3, cytogenetic risk profile, MRD status before maintenance, RAC score, and rFF in residual bone lesions after ASCT), multivariate analysis revealed that post-ASCT PFS was independently influenced by high-risk cytogenetic profile, MRD positivity, and rFF <17.2%. Similarly, post-ASCT OS was independently associated with high-risk cytogenetic profile and rFF <17.2% (Table 2).

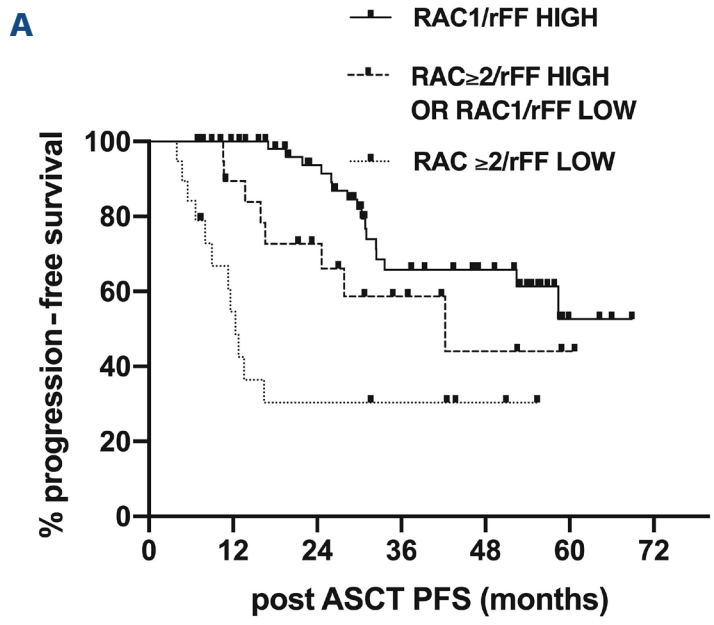


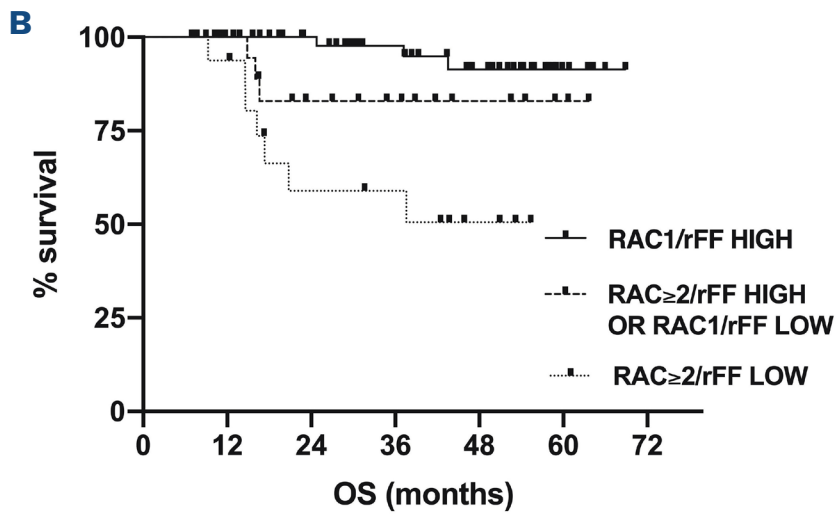


	rFF High	rFF Low	HR (95% CI)	*P*
Median PFS (months)	NR	13.7	0.18 (0.08-0.43)	<0.0001

	rFF High	rFF Low	HR (95% CI)	*P*
3y OS	96%	62%	0.12 (0.03-0.45)	<0.0001

Figure 2. Kaplan-Meier curves according to relative fat fraction in residual bone lesions after transplant: High *versus* Low. (A) Post-autologous stem cell transplantation (ASCT) progression-free survival (PFS). (B) Post-ASCT overall survival (OS). CI: Confidence Interval; HR: Hazard Ratio; rFF: relative fat fraction.





	RAC1/ rFF High	RAC ≥ 2/rFF High OR RAC1/rFF Low	RAC ≥ 2/ rFF Low	*P*
Median PFS (months)	NR	42	12.3	<0.0001

	RAC1/ rFF High	RAC ≥ 2/rFF High OR RAC1/rFF Low	RAC ≥ 2/ rFF Low	*P*
3y OS	98%	83%	59%	0.0002

Figure 3. Kaplan-Meier curves according to response assessment category score and relative fat fraction in residual bone lesions after transplant. (A) Post-autologous stem cell transplantation (ASCT) progression-free survival (PFS). (B) Post-ASCT overall survival (OS). RAC: response assessment category; rFF: relative fat fraction.

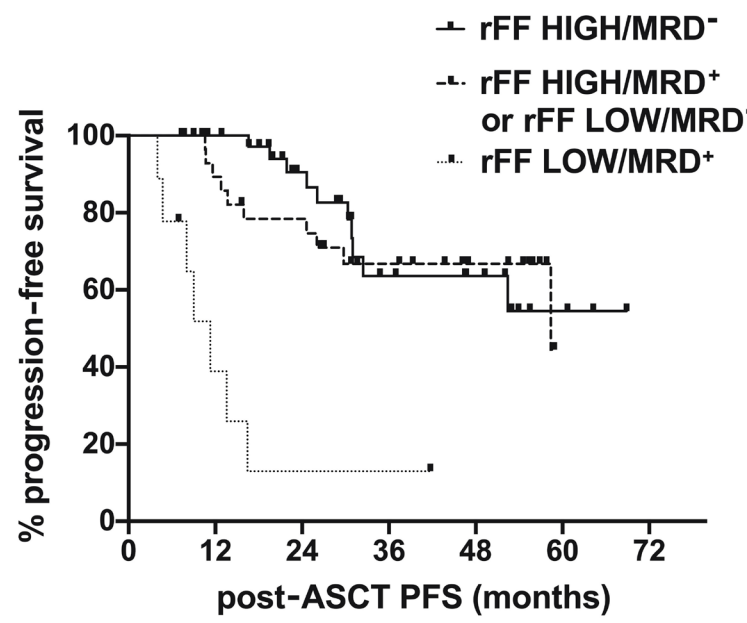
Discussion

Functional high-risk MM represents a challenging subgroup of patients with aggressive disease, characterized by early relapse despite optimal induction therapies. Notably, these patients may not exhibit conventional high-risk cytogenetic or molecular abnormalities at diagnosis, meaning that traditional risk stratification methods are not sufficient to identify them. In fact, early identification of these patients is critical to guide therapeutic strategies and improve outcomes. In this study, we demonstrate that the combined use of the RAC score and rFF, derived from DW-MRI, provides a robust tool for identifying FHR MM patients after ASCT. Specifically, our results show that patients with RAC ≥2 and rFF <17.2% are at a markedly higher risk of early relapse, confirming the ability of functional imaging techniques to capture residual disease burden and differentiate high-risk patients from those with excellent outcome. The rFF is a quantitative biomarker recommended by the MY-RADS guidelines for the acquisition and interpretation of DW-MRI images. It plays a crucial role in distinguishing normal BM from malignant infiltration and in assessing changes in marrow composition during treatment. However, the thresholds for pathological rFF values currently in use are primarily derived from studies conducted on heterogeneous populations of patients with solid tumors.[5-7] In these studies, a cut-off of 20% at diagnosis has emerged as a pragmatic threshold for differentiating malignant from benign BM patterns, making it a useful parameter in clinical practice. In the context of MM, existing studies have mainly focused on rFF measurements at diagnosis to evaluate the degree of BM infiltration.[9,10]

Table 2. Multivariate analysis.

	HR (95% CI)	*P*
PFS		
rFF Low	0.29 (0.10-0.84)	0.022
RAC ≥2	0.60 (0.21-1.69)	0.336
High-risk cytogenetic	0.15 (0.05-0.42)	0.0001
ISS-3	0.90 (0.58-1.41)	0.66
MRD positivity	0.31 (0.12-0.78)	0.013
OS		
rFF Low	0.08 (0.01-0.53)	0.008
RAC ≥2	1.47 (0.23-9.44)	0.684
High-risk cytogenetic	0.09 (0.01-0.55)	0.01
ISS-3	1.95 (0.31-12.28)	0.475
MRD positivity	0.77 (0.12-4.89)	0.784

Data are presented as Hazard Ratio (HR). CI: Confidence Interval; ISS: International Staging System; MRD: minimal residual disease; PFS: progression-free survival; OS: overall survival; RAC: Response Assessment Category score; rFF: relative fat fraction.



	rFF High/MRD-	rFF High/MRD+ OR rFF Low/MRD-	rFF Low/MRD+	*P*
Median PFS (months)	NR	58.4	11.3	<0.0001

Figure 4. Post-autologous stem cell transplantation progression-free survival according to minimal residual disease and relative fat fraction in residual bone lesions after transplant. ASCT: autologous stem cell transplantation; MRD: minimal residual disease; NR: not reached; PFS: progression-free survival; rFF: relative fat fraction.

Table 3. Distribution of Response Assessment Category and relative fat fraction according to International Myeloma Working Group response and minimal residual disease status.

IMWG response (N)	RAC1 N=66	RAC ≥2 N=31	*P*	rFF Low N=27	rFF High N=70	*P*
sCR (18)	12	6	0.99	5	13	0.99
CR (42)	30	12	0.66	11	31	0.82
VGPR (32)	22	10	0.99	7	25	0.47
PR (5)	2	3	0.32	4	1	0.02
MRD negative (52/79)	37	15	0.79	13	39	0.44

CR: complete response; IMWG: International Myeloma Working Group; MRD: minimal residual disease; N: number; PR: partial response; RAC: Response Assessment Category score; rFF: relative fat fraction; sCR: stringent complete response; VGPR: very good partial response.

Moreover, preliminary data suggest that rFF undergoes significant and early changes during treatment, potentially serving as a sensitive indicator of therapeutic response.[11-14] To the best of our knowledge, no rFF cutoff has been established for MM in the post-ASCT setting, particularly for identifying FHR patients at risk of early relapse. The identification of a rFF threshold of 17.2% in residual focal lesions after transplant represents a novel contribution to the field, as this biomarker reflects the composition of BM lesions and their response to treatment. The identified cut-off value of 17.2% demonstrated great predictive accuracy. Notably, the NPV of 96% highlights the robustness of rFF in reliably ruling out low-risk patients. This finding underscores the potential of rFF, not only as a diagnostic tool, but also as a prognostic marker in post-ASCT risk stratification. However, further validation in larger prospective studies is required to determine whether it could ultimately influence clinical decision-making. Furthermore, our findings demonstrate a significant association between rFF <17.2% and RAC ≥2, with an overall concordance of 79.4% and a moderate agreement. This suggests that rFF and RAC are complementary but not fully overlapping, reinforcing the concept that rFF provides additional biological insights beyond RAC-based response assessment. Our findings confirm that 83% of patients classified as FHR exhibited an rFF below the identified 17.2% cut-off. This high sensitivity underscores the ability of this threshold to effectively identify patients at increased risk of early relapse. By integrating rFF with RAC score, we further demonstrate the ability to stratify patients into distinct risk categories, offering a more comprehensive assessment of residual disease burden and FHR status. The stronger prognostic impact of rFF <17.2% compared to RAC ≥2 on PFS can be explained by the methodology used to define the rFF threshold. Specifically, the 17.2% cut-off was selected to identify FHR patients who experienced relapse within 18 months post ASCT. Since this threshold was optimized for early relapse prediction, it is expected that patients classified as rFF <17.2% have a significantly shorter PFS compared to those with RAC ≥2. Conversely, the RAC score captures a broader imaging response assessment, reflecting both focal lesion evolution and diffuse marrow involvement. While RAC ≥2 also identifies patients with residual disease, its categorical nature may not capture the same level of granularity as rFF, which provides a quantitative measure of marrow composition and treatment response. Importantly, unlike our previously published experience,[4] where RAC score emerged as an independent prognostic factor, in the present multivariate analysis, RAC ≥2 did not retain statistical significance for PFS and OS. This difference may be explained by the larger number of co-variates included in the current model (6 *vs.* 3 in the previous study), which inherently affects the statistical power of individual predictors. Moreover, the design of this study specifically aimed to identify FHR patients with early relapse, and the rFF <17.2% cut-off was optimized for this purpose. This differs from our previously published experience, where RAC score was assessed in a broader prognostic context, evaluating overall imaging response rather than focusing on early relapse prediction. It is known that MM is characterized by the co-existence of multiple clones with variable distribution.[15] This clonal and spatial heterogeneity underlies the variability in treatment response and is a key driver of the disease's natural history, marked by continuous relapses. For this reason, in addition to BM MRD assessment, it is particularly important to evaluate residual disease using functional imaging techniques. Studies conducted in recent years on positron emission tomography for the evaluation of skeletal and extramedullary MRD have consistently reported low concordance with BM-based MRD methods.[16-18] Similarly, as already observed in our previously published experience with post-ASCT DW-MRI,[4] the present study also confirms low concordance between MRD assessed by MFC and imaging response. This finding highlights the importance of a combined evaluation of both techniques to comprehensively assess treatment response and residual disease burden. Notably, our results demonstrate that the integration of marrow MRD with rFF assessment of residual bone lesions after ASCT enables the identification of a subset of patients with an extremely poor prognosis, reflected by a post-ASCT PFS of less than one year. In our study, the evaluation of rFF was performed as a double read, ensuring greater reliability in the measurement process. Interobserver agreement was not specifically assessed, based on the assumption that the reproducibility of the MY-RADS framework has already been validated. The MY-RADS guidelines provide standardized criteria for the acquisition, interpretation, and reporting of whole-body MRI in MM, reducing the variability between readers. Furthermore, previous studies have already confirmed a high interobserver agreement in the measurement of rFF,[5,19] supporting its reliability as a reproducible imaging biomarker. When assessing response among multiple target lesions with DW-MRI, the predominant one (the higher category) was reported for the assignment of the final RAC score after transplant. In particular, the increase in ADC above the cut-off value of 1,400 $\mu m^2/s$ has been adopted for the assignment of RAC1, according to MY-RADS guidelines. However, ADC values can be influenced by the relative proportion of plasma cells, fat, and myeloid cells within the BM, potentially leading to false-positive results when misinterpreted as residual disease in cases of post-therapy BM reconversion with restricted diffusion. To address this limitation, the DIXON FF sequence provides a reliable method to evaluate the degree of adipose repopulation in responding lesions post treatment. This approach enhances specificity, reduces false-positive interpretations, and allows for better discrimination between responding and therapy-resistant lesions. The observation that a similar proportion of patients exhibited RAC ≥2 and unfavorable

rFF further supports this concept. This finding suggests that when MY-RADS recommendations are rigorously applied, integrating rFF alongside ADC for lesion characterization, the likelihood of false-positive RAC classifications is minimized, thereby improving the accuracy of residual disease assessment. This underscores the importance of strict adherence to MY-RADS criteria in optimizing the diagnostic performance of DW-MRI and ensuring the most precise RAC classification possible. Notably, the use of rFF is of particular importance for radiologists in cases where baseline pre-treatment DW-MRI is not available. This scenario is not uncommon in clinical practice, particularly in situations where the patient's clinical condition requires treatment to be initiated as soon as possible, making it challenging to obtain a pre-treatment DW-MRI.

Of note, according to MY-RADS criteria, it is possible to assign a RAC1 score when the ADC is <1,400 $\mu m^2/s$ but shows an absolute increase of >40% compared to pre-treatment MRI. In cases where the post-therapy ADC value is elevated but remains <1,400 $\mu m^2/s$ and no baseline pre-treatment MRI is available, assigning a RAC1 score after ASCT is formally unfeasible. In this context, the use of the rFF becomes even more essential for characterizing residual bone lesions after ASCT. The rFF provides valuable information to identify whether the diffusion restriction after treatment is due to residual cellularity of the clonal plasma cell population or to increased cellularity resulting from adipose repopulation. In the latter case, rFF values will indicate non-pathological findings, thus helping the accurate characterization of the lesion.

It is important to emphasize, however, that the predictive power of the rFF does not replace the application of the RAC score, which constitutes the first crucial step in the assessment of treatment response. Instead, the rFF improves outcomes stratification, enabling the early identification of patients at high risk of relapse. The results of our study showed an intermediate trend in 8 patients with RAC1 and low rFF. Notably, 5 of these patients presented ADC values in the main target lesion significantly exceeding the 1,400 $\mu m^2/s$ threshold required for RAC1 assignment, while rFF values remained below the 17.2% cut-off. In these cases, post-transplant bone lesions appeared devoid of internal cellularity due to post-therapy necrosis, which consequently resulted in markedly reduced rFF values. Importantly, none of these 5 patients experienced an early relapse, with ADC values remaining persistently elevated over time, even during subsequent follow-ups. For this reason, we propose that the prognostic value of rFF should be applied to residual bone lesions with ADC values <1,500 $\mu m^2/s$, in line with findings reported in the literature for patients at the diagnostic stage.[5]

Our results revealed that neither high-risk FISH abnormalities, nor R-ISS stage, nor R2-ISS stage, nor the presence of EMD were significantly correlated with post-treatment imaging parameters. However, we acknowledge that the limited sample size of EMD cases may have compromised detection of potential correlations. This finding highlights that the prognostic information provided by post-ASCT imaging biomarkers such as RAC and rFF may be independent of conventional risk stratification at diagnosis.

The present study has some limitations, primarily due to its retrospective nature, and it requires validation in prospective studies to confirm its findings. One limitation of our study is the relatively small sample size, which may impact the generalizability of our findings. Additionally, the heterogeneity of induction regimens used before ASCT represents a potential confounding factor. However, despite sample size constraints, our data showed a strong statistical association between rFF and patient outcomes, with robust performance in identifying FHR patients. To address the issue of treatment heterogeneity, we recognize the need for larger, prospective validation in a more homogeneous population treated with the same induction therapy prior to transplantation. In this regard, the 17.2% cut-off should be further investigated, or potentially refined, within the framework of a multicentric data collection effort, which will allow for standardized validation and enhance the clinical applicability of rFF in post-ASCT risk stratification.

In conclusion, our single-center experience highlights rFF as a powerful prognostic biomarker that complements the RAC score in stratifying patients post ASCT, providing a practical tool for refining post-transplant management and paving the way for tailored strategies in high-risk MM patients. These high-risk patients may benefit from intensified maintenance therapy or novel therapeutic interventions to mitigate the risk of early relapse.

Based on these findings, it could be hypothesized that the persistence of imaging residual disease, as assessed by the RAC score and low rFF, may represent a high-risk feature to be incorporated into dedicated clinical trials for high-risk MM patients with suboptimal responses after first-line treatment.

Disclosures

AB has served on the advisory boards for Amgen, Jannsen, GSK, Pfizer, Sanofi, and Menarini Stemline. AT has served on the advisory boards for Janssen, Gentili, Sanofi, Abbvie, Incyte, Takeda, Kiowa Kyrin, and Lilly. AR received research funding from Associazione Italiana per la Ricerca sul Cancro (AIRC), Associazione Italiana contro Leucemie-linfomi e mieloma (AIL) Brescia, and has served on the advisory boards for Abbvie, Beigene, Janssen, and Roche. All of the other authors have no conflicts of interest to disclose.

Contributions

AB designed the study, collected data, analyzed data, created tables and figures, and wrote and revised the article. Data preparation and collection was performed by AB, ST, RR, ClCr, SF, NB, CB, ChCa. BF, CS, CaCa, AG and LG analyzed MRI images and contributed to the study design. MC and

VG performed marrow MRD evaluations and contributed to the study design. AR participated in content planning and contributed to the study design. AT participated in content planning, contributed to the study design, reviewed data, and commented on drafts.

Data-sharing statement

Individual patient data from this study cannot be publicly shared due to ethical committee restrictions. However, data may be available upon reasonable request to the corresponding author, subject to ethical and institutional approval.

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