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Circulating tumor plasma cells and peripheral blood measurable residual disease assessment in multiple myeloma patients not planned for upfront transplant

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Abstract

Circulating tumor plasma cells (CTPCs) provide a noninvasive alternative for measuring tumor burden in newly diagnosed multiple myeloma (NDMM). Moreover, measurable residual disease (MRD) assessment in peripheral blood (PBMRD) can provide an ideal alternative to bone marrow MRD, which is limited by its painful nature and technical challenges. However, the clinical significance of PBMRD in NDMM still remains uncertain. Additionally, data on CTPC in NDMM patients not treated with transplant are scarce. We prospectively studied CTPC and PBMRD in 141 NDMM patients using highly sensitive multicolor flow cytometry (HS-MFC). PBMRD was monitored at the end of three cycles (PBMRD1) and six cycles (PBMRD2) of chemotherapy in patients with detectable baseline CTPC. Patients received bortezomib-based triplet therapy and were not planned for an upfront transplant. Among baseline risk factors, CTPC ≥ 0.01% was independently associated with poor progression-free survival (PFS) (hazard ratio [HR] = 2.77; $p = 0.0047$) and overall survival (OS) (HR = 2.9; $p = 0.023$) on multivariate analysis. In patients with detectable baseline CTPC, undetectable PBMRD at both subsequent time points was associated with longer PFS (HR = 0.46; $p = 0.0037$), whereas detectable PBMRD at any time point was associated with short OS (HR = 3.25; $p = 0.004$). Undetectable combined PBMRD (PBMRD1 and PBMRD2) outperformed the serum-immunofixation-based response. On multivariate analysis, detectable PBMRD at any time point was independently associated with poor PFS (HR = 2.0; $p = 0.025$) and OS (HR = 3.97; $p = 0.013$). Thus, our findings showed that CTPC and PBMRD assessment using HS-MFC provides a robust, noninvasive biomarker for NDMM patients not planned for an upfront transplant. Sequential PBMRD monitoring has great potential to improve the impact of the existing risk stratification and response assessment models.

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INTRODUCTION

Several attempts have been made to devise a risk-stratification strategy for patients with multiple myeloma (MM) that can be uniformly applicable and provide a basis to modify the treatment; however, such a risk-stratification model providing the basis for response-adapted therapy remains elusive.[1–3] The currently used Revised International Scoring System (RISS) and recently proposed second revision (R2ISS) predominantly depend on cytogenetics and serum-based biomarkers and lack incorporation of biomarkers reflecting direct tumor burden.[1,2,4,5] Plasma cell percentage in bone marrow (BM) directly measures tumor burden in plasma cell neoplasms (PCNs).[6] It differentiates monoclonal gammopathy of undetermined significance from smoldering myeloma and is now incorporated as a myeloma-defining event in the diagnostic criteria of MM.[7–10] Additionally, BM measurable residual disease (MRD) status has been proven to be a powerful predictor of outcomes in MM.[9,11–13] Thus, bone marrow assessment for tumor plasma cell (TPC) burden at diagnosis and response monitoring has become an integral part of laboratory work-up in PCNs. However, BM-TPC enumeration has several challenges that limit accurate and reproducible results, including patchy involvement, hemodilution, TPC adherence to lipid-enriched spicules, and so forth.[4,14–17] In addition, BM aspiration is a painful procedure and may not be feasible to perform multiple times for sequential response monitoring.[17–19] It also has limited use in assessing extramedullary disease. Notably, current International Myeloma Working Group (IMWG) guidelines indicate BMMRD assessment only in patients achieving complete remission (CR) and restrict its applicability to patients who could not achieve CR.[9] Thus, accurate BMMRD assessment can be challenging to implement outside clinical trial settings.

Contrarily, circulating tumor plasma cell (CTPC) assessment provides an easily accessible, noninvasive, painless, reproducible, and devoid-of-hemodilution alternative for determining myeloma burden in peripheral blood (PB).[4,6,20–22] Additionally, circulating tumor cells represent neoplastic plasma cells with an ability to disseminate and possibly support the disease progression independent of the site and extent of primary involvement.[16,18,23] Studies have demonstrated that CTPC possesses stem cell-like features and altered genetic characteristics with high clonogenic potential.[18,19,24–28] The utility of periodic MRD assessment is increasing in myeloma with emerging MRD-related treatment decisions.[15,17,29–32] The ability to detect MRD in the PB (PBMRD) can improve ease of testing and holds the potential to decrease the number of painful BM procedures.[18] Therefore, assessing PBMRD can be ideal for monitoring sequential therapeutic response.[4,6,19,21,33–36] However, systematic studies demonstrating the independent prognostic value of PBMRD are lacking. Further, recent reports have strongly indicated the role of baseline circulating tumor cell measurement for risk stratification in MM.[4,16,18,23,33,37] These studies mainly included MM patients who received autologous stem cell transplants (ASCTs). However, the data on the clinical value of circulating tumor cell assessment in MM patients treated without ASCT are extremely scarce.

We investigated the prognostic value of CTPC levels in newly diagnosed MM (NDMM) patients not planned for upfront ASCT due to financial/resource constraints or transplant ineligibility. We prospectively validated the feasibility and evaluated the clinical relevance of PBMRD assessment in the prediction of progression-free survival (PFS) and overall survival (OS) in these patients.

PATIENTS AND METHODS

Patients and samples

We prospectively enrolled NDMM patients intended to be treated with a bortezomib-based triplet regimen and not planned for upfront ASCT from June 2016 to October 2019. The diagnosis of MM was performed as per IMWG criteria.[7] The detailed clinical features and laboratory/radiological findings were noted. Response assessment was performed at the end of three and six cycles of chemotherapy as per IMWG response criteria.[9] This study was approved by the Institutional Ethical Committee (IEC), written informed consents were taken, and conducted as per the Declaration of Helsinki.

CTPC and PBMRD assessment

Approximately 3.0–7.5 mL (median, 5 mL) of PB was processed for CTPC quantitation using a single-tube 10-color highly sensitive multicolor flow cytometry (HS-MFC) assay (Supporting Information S1: Table S1). CTPC levels were studied at diagnosis, and PBMRD was assessed at the end of three cycles (PBMRD1) and six cycles (PBMRD2) of chemotherapy. As described earlier, a suspension of 10–50 million cells was prepared using a bulk-lyse method.[15,38] Briefly, the cell suspension was prepared after red cell lysing with ammonium chloride-based lysing reagent, and intracellular staining was performed using the FIX & PERM™ Cell Permeabilization Kit (Thermo Fisher Scientific). The antibody panel for MFC included antihuman antibodies against CD20 (AH7, BV510), CD14 (63D3, BV421), kappa (Polyclonal, FITC), lambda (Polyclonal, PE), CD19 (HIB19, PE-CF594), CD27 (1A4CD27, PerCP-Cy5.5), CD56 (N901 (NKH-1), PE-Cy7), CD138 (B-A38, APC), CD45 (J.33, APC-A-700), and CD38 (LS198-4-3, APC-A-750) (Supporting Information S1: Table S1). The cells were acquired on a DxFLEX flow cytometer (Beckman Coulter), and data were analyzed with Kaluza software using the recommended approach (shown in Supporting Information S2: Figure S1).[12,31] The median of total CD45+ number of cells acquired was 5,040,812 (range: 1,685,708–22,050,701). Percentages of CTPC were calculated in total CD45+ white blood cells (WBCs). Absolute CTPC counts were determined using a dual-platform method.[39] The limit of blank was established at six events using four normal age-matched PB samples on the predefined template and gating strategy (Supporting Information S1: Table S2 and Supporting Information S3: Datasheet 2). The limit of detection (LOD) and a lower limit of quantitation (LLOQ) of the assay were determined and validated with dilution and spiking experiments as described in Supporting Information S1: Datasheet 1 (Tables S2, S3, and S4 and Supporting Information S2: Figure S2). LOD of 10 CTPC events was established with an assay sensitivity of 0.0001%, and LLOQ of 20 CTPC events with a sensitivity of 0.0002%. Eighty-four percent of samples achieved the sensitivity with LOD of 0.0005%, 56% of 0.0002%, and LOD between 0.0002% and 0.0001% was achieved in 11 samples. Twenty-three samples could not reach LOD of 0.0005% either due to lower WBC counts or low sample quantity. The proportion of baseline BM-TPC in all BM plasma cells (%BM-TPC/PC) was also evaluated using HS-MFC. PBMRD(+) (detectable) status was defined with CTPC ≥ 0.0001%.

BMMRD was not evaluated as CR was not achieved in most patients (as indicated by IMWG response criteria[9]).

Cytogenetic study

Cytogenetic studies were performed using interphase fluorescence in situ hybridization (FISH) on plasma cells enriched using CD138-coated magnetic beads (Miltenyi Biotec). FISH analysis was performed in 200 CD138+ plasma cells in samples with ≥1.0% plasma cells (detected by flow cytometry). In samples with <1.0% plasma cells, it was performed on directly harvested BM aspirate samples as the plasma cell sorting protocol for FISH was standardized for

samples with ≥1.0% plasma cells. The details of probes used for FISH analysis are given in Supporting Information S1: Datasheet 1.

Statistics

The sample size was calculated for the presence of CTPC to achieve a sensitivity of 85% and specificity of 70% with a 95% confidence interval (CI). Based on this assumption, the sample size of 141 patients was determined with the precision for "Specificity" = 0.094 and the precision for "Sensitivity" = 0.1058. The correlation between the CTPC and BM-TPC levels was studied using Spearman's rank correlation. CTPC levels between baseline risk groups were studied using the Kruskal–Wallis test. Cut-off values for CTPC and BM-TPC were determined using ROC analysis against PFS. PFS was calculated from the date of induction phase initiation until the date of progression, relapse, or death due to any cause. OS was calculated from the induction phase initiation date until death due to any cause or, if alive, till the date of the last follow-up. The association of risk factors with PFS and OS was studied by using the Kaplan–Meier estimation method. Multivariate analysis was performed using a Cox proportional hazards model. Covariables with $p \le 0.1$ on univariate analysis were included in multivariate analysis. For Kaplan–Meier landmark analysis of the combined effect of PBMRD1 and PBMRD2, the landmark was set at the time of PBMRD1 assessment as only two patients died between these two time points, and both were PBMRD1(+). Statistical analysis of the data was performed using MedCalc Statistical Software version 14.8.1 (MedCalc Software) and Stata version 13 (StataCorp LP).

RESULTS

A total of 141 NDMM patients were enrolled during the study period. The median age of the study population was 55 years (range: 27–82 years) and two-thirds of patients were male (male:female = 94:47). Majority of patients were R-ISS stage II 83 (61.9%) with R-ISS-I in 20 (14.9%) and R-ISS III in 31 (23.1%) patients. The patients' other demographic and laboratory details are described in Table 1 (additional details are given in Supporting Information S1: Table S5). Of 141, 45 patients were transplant-ineligible as per standard criteria,[40,41] and the remaining 96 patients were planned for therapy without ASCT due to patient preference or resource constraints, or logistic issues. Ninety-eight of 141 patients received VCd (bortezomib, cyclophosphamide, dexamethasone)-based therapy and 43 of 141 received VRd (bortezomib, lenalidomide, dexamethasone)-based therapy depending on patients' clinical status and financial conditions. The median number of cycles received by patients was 12 cycles (range: 2–12 cycles). Among 98 patients, 11 received less than six cycles, 57 received 6–9 cycles, and 30 received 12 cycles of VCd. Among 43 patients receiving VRd, three received less than six cycles, 22 received 6–9 cycles, and 18 received 12 cycles. Patients received maintenance therapy for 2 years or were kept under observation at the treating physician's discretion; 46 patients received bortezomib maintenance therapy, and 41 received lenalidomide maintenance therapy. Twenty-six patients were kept under observation. Of the remaining 28 patients, 14 died within the first 6 months of therapy, and 14 were lost to the follow-up before initiation of maintenance therapy.

The response was categorized based on the best response achieved in the first 6 cycles of chemotherapy as per IMWG response criteria.[9] Complete response (CR) was achieved in 7.1%, very good partial response (VGPR) in 43.97%, partial response (PrR) in 33.33%, stable disease in 2.84%, progressive disease in 2.84%, and 14 (9.9%) patients died during initial therapy (12 within three cycles and two between three and six cycles of therapy). Patients who achieved CR or VGPR were classified into the good response (GR) category, and patients who achieved a PrR or less were classified into the poor response (PR) category. The median follow-up duration was 37 months

TABLE 1 Demographic and laboratory findings of patients (n = 141).

Characteristics	Number of patients (%)
Age (years), median (range)	55 (27–82)
≥55 years	69/141
<55 years	72/141
Bone marrow plasma cells	
BM PC on morphology, median (range)	20% (1%–84%)
BM PC on MFC, median (range)	5.70% (0.01%–92.70%)
BM TPC in Total PC on MFC, median (range)	97.8% (1.5%–100%)
>90% BM-TPC	103/136 (75.7%)
>95% BM-TPC	91/136 (66.9%)
Cytogenetic abnormalities (n = 132)	
Trisomy 3, 5, 7, 9, 11, 15, 19, and/or 21	50/132 (37.88%)
Monosomy Ch13/del13q	30/132 (22.73)
IgH translocations	
t(4;14)	11/132 (8.33%)
t(14;16)	02/132 (1.52%)
t(14;20)	-
Del(17p)	06/132 (4.55%)
Gain or amplification of chromosome 1q	19/132 (14.39%)
NOS	04/132 (3.03%)
LDH levels (n = 134)	
High	40/134 (29.85%)
Normal	94/134 (70.15%)
CTPC	
CTPC detectable at diagnosis	108/141 (76.6%)
Percentage, median (range)	0.024% (0.00012%–4.1%)
Absolute, median (range)	1.56/μL (0.01–1151/μL)
PBMRD1—detectable	43/98 (44%)
Median (range) (%)	0.002% (0.0001%–2.5%)
PBMRD2—detectable	33/96 (34.4%)
Median (range) (%)	0.001% (0.0001%–1.45%)
Revised International Scoring System	
RISS (n = 134)	
I	20 (14.9%)
II	83 (61.9%)
III	31 (23.1%)
R2ISS (n = 128)	
Low risk	21 (16.4%)
Low-intermediate risk	25 (19.5%)
Intermediate-high risk	69 (53.9%)
High risk	13 (10.1%)

Abbreviations: BM, bone marrow; CTPC, circulating tumor plasma cells; LDH, lactate dehydrogenase low; MRD, measurable residual disease; NOS, not specified; PB, peripheral blood; Sr, serum; TPC, tumor plasma cells.

(1–80 months), with the median PFS of 22 months (1–79 months). Sixty-six patients showed disease progression, and 43 patients died, including 14 deaths within 6 months of initiation of therapy; 22 deaths were due to disease progression, and the cause of death in seven patients was unknown.

Association of CTPC with survival outcomes

Plasma cells (including normal and tumor PCs) were detected in PB of all MM patients (median, 0.098%; range: 0.0013%–4.1%), and CTPCs were detected in 108/141 (76.6%) patients. The median (range) of total CD45+ events acquired in patients with detectable and undetectable CTPC were 4,913,368 (1,685,708–22,050,701) and 4,694,850 (1,702,538–12,574,567), respectively. No statistically significant difference was observed between the number of events acquired between samples with detectable and nondetectable CTPC ($p = 0.85$). This finding indicated that undetectable CTPC was not significantly affected by the number of events acquired in most samples in our study.

The median (range) of CTPC percentages and absolute counts were 0.024% (0.00012%–3.96%) and 1.56 CTPC/μL (0.01–1151 CTPC/μL), respectively. ROC-based cut-offs against PFS for the percentages and absolute count of CTPC were ≥0.01% and ≥1 cell/μL in CD45+ WBCs. Of 141, 78 (55.3%) patients had ≥0.01% and 67 (47.5%) had absolute counts ≥1/μL of CTPC. Median PFS was significantly shorter in patients with CTPC ≥ 0.01% compared to patients with <0.01% or undetectable CTPC (21 vs. 55 months; hazard ratio [HR]: 2.67; $p < 0.0001$) (Figure 1A). The 3-year PFS rate in patients with CTPC < 0.01% was 63.8% against 29.2% of patients with CTPC ≥ 0.01%. The median OS was also shorter in patients with CTPC ≥ 0.01% (52 months vs. not reached; HR = 2.28; $p = 0.01$) (Figure 1B). Similarly, the absolute CTPC ≥ 1/μL showed an association with shorter median PFS (21 vs. 40 months; HR = 1.9; $p = 0.0027$) (Figure 1C). The 3-year PFS rate was 55.4% versus 34.2% in patients with CTPC < 1/μL versus CTPC ≥ 1/μL, respectively. The median OS was also shorter in patients with CTPC ≥ 1/μL (HR = 1.8; $p = 0.05$) (Figure 1D).

The details of BM tumor plasma cell (BM-TPC) findings and their relation with CTPC are described in Supporting Information S1: Datasheet 1 (Figures S3, S4, and S5).

Relation between CTPC and other baseline risk factors

BM-TPC levels, high-risk cytogenetics, RISS, and R2ISS were also found to be associated with shorter PFS and age ≥55 years, and high

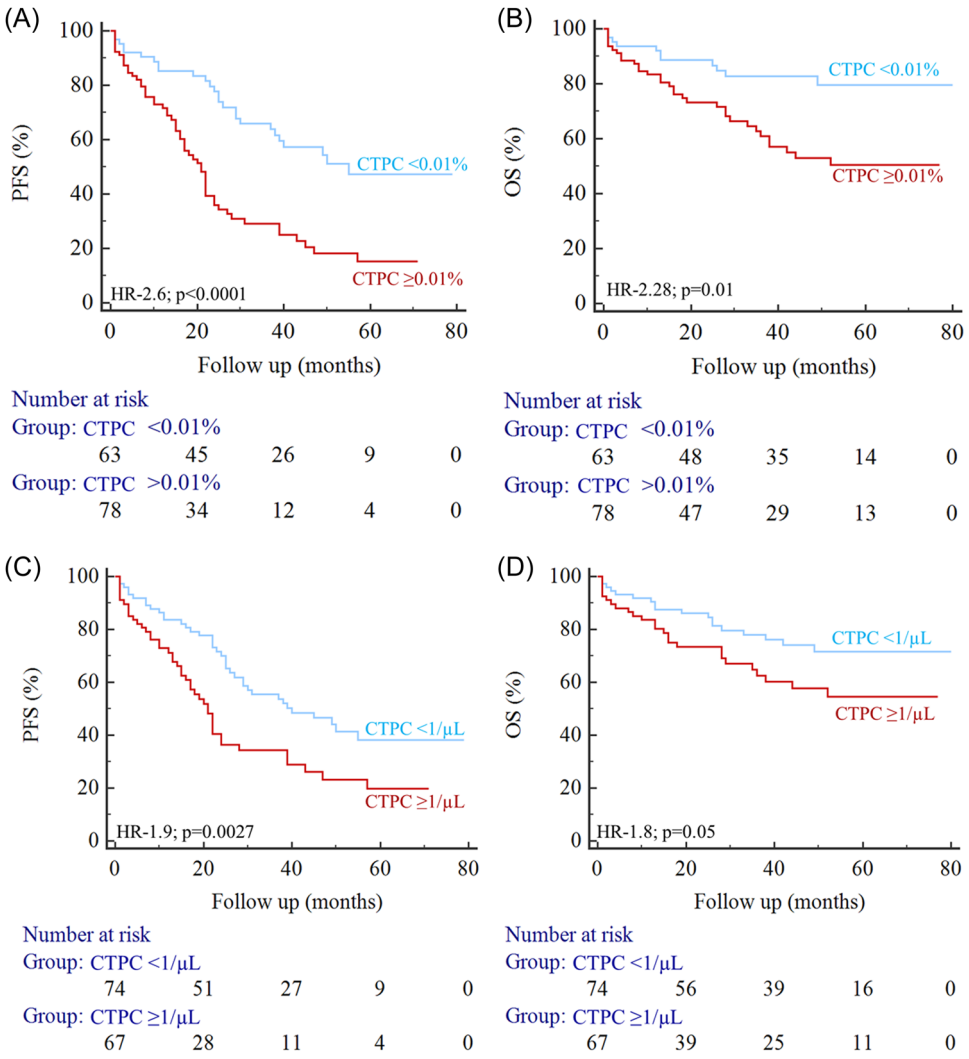

FIGURE 1 The Kaplan–Meier estimates of progression-free survival (PFS) and overall survival (OS) in newly diagnosed multiple myeloma patients categorized according to the percentage of CTPC (≥0.01% vs. <0.01%) (A and B) and absolute levels of CTPC (≥1 vs. <1/μL) (C and D), respectively.

lactate dehydrogenase (LDH) levels and R-ISS were significantly associated with poor OS on univariate analysis (Figure 2 and Supporting Information S2: Figure S6). Of note, the treatment protocol, that is, VCd versus VRd, did not show a significant association with PFS ($p = 0.23$) or OS ($p = 0.31$) on univariate analysis. However, we noticed a trend with relatively better outcomes in patients receiving VRd. On the evaluation of the distribution of CTPC levels among various risk groups, patients belonging to high-risk cytogenetics, RISS-II/III, and R2ISS of intermediate-high and high-risk categories had significantly higher levels of CTPC (see Supplementary Datasheet 1 and Supporting
Information S2: Figure S7). On multivariate analysis, CTPC ≥ 0.01% was independently associated with poor PFS (HR = 2.77; 95% CI: 1.37–5.59; $p = 0.0047$) and OS (HR = 2.9; 95% CI: 1.16–7.26; $p = 0.023$) (refer to Supporting Information S1: Table S7A,B). We also performed a similar analysis in a subgroup of transplant-ineligible patients ($n = 45$) defined as per standard criteria. The CTPC ≥ 0.01% showed a strong association with PFS (HR = 3.1; $p = 0.0018$) (but not with OS) (Supporting Information S2: Figure S8). None of the remaining parameters showed any significant association with PFS or OS on univariate analysis in this subgroup of patients due to the small sample size.

PBMRD

PBMRD was performed in patients with detectable CTPC at diagnosis ($n = 108/141$). Of these 108 patients, 10 patients died before the PBMRD1 time point. Hence, PBMRD1 was monitored in 98 of these 108 patients. PBMRD1 was detectable in 43/98 (44%) patients (median: 0.002%; range: 0.0001%–2.5%). Of these 98 patients, two patients with detectable PBMRD1 died in the next 3 months, that is, before PBMRD2. So, PBMRD2 was monitored in 96 patients, including 41 PBMRD1(+) patients. Among these 96 patients, PBMRD2 was detectable in 33/96 (34.4%) (median: 0.001%; range: 0.0001%–1.45%). Hence, statistical analysis of combined PBMRD at both time points could be assessed in only 96 patients, and 41/96 patients had detectable PBMRD at any (PBMRD1 or PBMRD2 or both) time point that included 25 with detectable PBMRD at both time points, 16 at only PBMRD1, and eight at only PBMRD2 time points

Detectable PBMRD1 status was strongly associated with short PFS (14 vs. 39 months; HR = 2.12; $p = 0.0035$). The 3-year PFS rates were 52% versus 24.5% in patients with undetectable PBMRD1 versus detectable status. The median OS was also shorter in patients with detectable PBMRD1 (HR = 2.01; $p = 0.05$) (Figure 3A,B). Similarly, detectable PBMRD2 was associated with short PFS (17 vs. 35 months; HR = 1.9; $p = 0.018$). The persistence of PBMRD at the second time point (PBMRD2) was strongly associated with poor OS (41 months vs. not reached; HR = 3.4; $p = 0.0011$) (Figure 3C,D). The 3-year PFS rates were 48.1% versus 27.1% in patients with undetectable PBMRD2 versus detectable status. These results indicate that undetectable PBMRD1 status at the end of three cycles of chemotherapy predicted better PFS and the persistence of PBMRD2 MRD until the end of six cycles of chemotherapy was predictive of poor OS.

We further evaluated the combined results of PBMRD at both time points using Kaplan–Meier landmark analysis in patients with follow-up from the time of PBMRD1 assessment as only two patients died between these two time points, and both were PBMRD1(+) (Figure 3E,F). PBMRD-negative (PBMRD(−)) status at both time points (PBMRD1 and PBMRD2) was predictive of longer PFS from the time of PBMRD1 assessment (45 versus 22 months; HR = 0.46; $p = 0.0037$). The 3-year PFS rate was 55.3% versus 33.3% in patients with undetectable PBMRD at both time points versus detectable status at any time point. Time to MRD negativity (PBMRD1(−) versus PBMRD2(−)) did not affect the PFS significantly. The median OS was also longer (not reached) in patients with undetectable PBMRD at both time points (not reached versus 52 months; HR = 3.25; $p = 0.004$) compared with detectable PBMRD at any time point. Patients with PBMRD detectable at any time point had

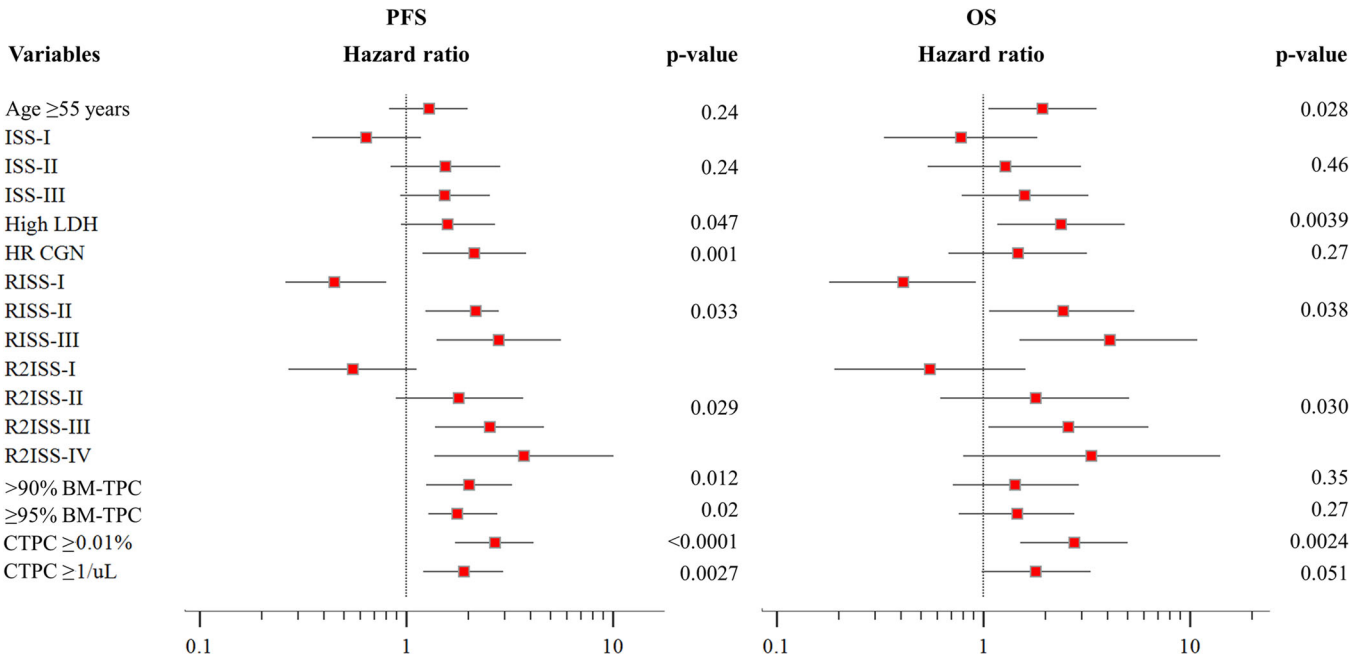

FIGURE 2 Association of baseline parameters with progression-free survival (PFS) and overall survival (OS) outcomes in newly diagnosed multiple myeloma patients. BM, bone marrow; CGN, cytogenetics; CTPC, circulating tumor plasma cells; HR, high risk; ISS, International Scoring System; LDH, lactate dehydrogenase; OS, overall survival; PC, plasma cells PFS; progression-free survival; RISS, Revised International Scoring System; R2ISS, second revision of ISS; TPC, tumor plasma cells.

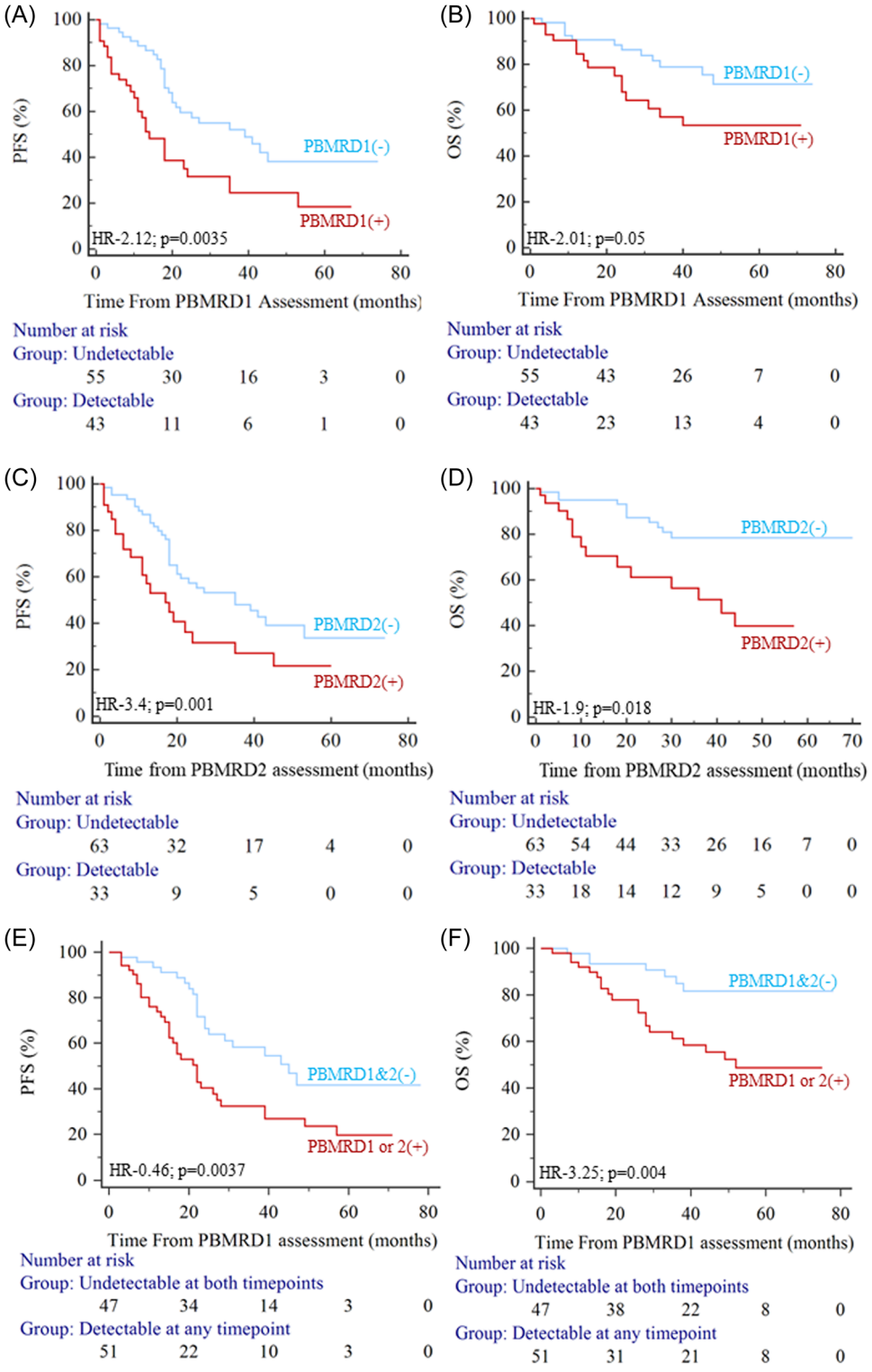

FIGURE 3 Kaplan–Meier estimates of progression-free survival (PFS) and overall survival (OS) in newly diagnosed multiple myeloma patients grouped according to the peripheral blood measurable residual disease (PBMRD) status at the first time point, that is, PBMRD1 (A and B), at the second time point, that is, PBMRD2 (C and D), and combined results of both (PBMRD1 and 2) time points (E and F), respectively. The landmark for PBMRD1 was set at the time from the PBMRD1 assessment and for PBMRD2 time from the PBMRD2 assessment. For the effect of combined PBMRD1 and PBMRD2 results, the landmark was set at the time of PBMRD1 assessment as only two patients died between these two time points and were PBMRD1 positive.

intermediate PFS; however, patients with detectable PBMRD1 but undetectable PBMRD2 had relatively better OS compared to patients with undetectable PBMRD1 but detectable PBMRD2, who had OS similar to that of detectable PBMRD at both time points (Supporting Information S2: Figure S9).

PBMRD versus serological response and baseline risk factors

There was no association between the frequency of PBMRD detection rate and treatment protocol, that is, VCD versus VRD ($p = 0.79$). The relation between serum immunofixation (sIF) and PBMRD is given in Supporting Information S1: Table S8. The prognostic value of combined (sequential) PBMRD status across the known baseline risk factors, including age, ISS, cytogenetics, LDH levels, RISS, R2ISS, and BM-TPC was studied (Figure 4). Undetectable sequential PBMRD was associated with a reduction in risk of disease progression or death, leading to improved PFS across all baseline risk factors. Notably, patients with GR had inferior PFS and OS if PBMRD was detectable at any time point compared to undetectable PBMRD (Figure 5). Inversely, PFS and OS were longer in patients with a poor response if PBMRD was undetectable at both time points. The PFS and OS of patients with GR were not significantly different from patients with poor response if they had detectable PBMRD at any time point (Figure 5). PBMRD detectable at any time point was independently associated with shorter PFS (HR = 2.0; $p = 0.025$) and poor OS (HR = 3.97; $p = 0.013$) on multivariate analysis (refer to Supporting Information S1: Table S9A,B).

DISCUSSION

In this study, we showed the prognostic impact of circulating tumor plasma cells and longitudinal PBMRD assessment in NDMM patients not planned for upfront ASCT. Circulating tumor cells have emerged as a strong prognostic marker in MM receiving ASCT.[4,21,22,33] Although transplant remains an important treatment modality, with the advent of novel agents and the availability of treatment options at relapse, the role of upfront ASCT is being debated.[42] NDMM patients are increasingly treated without upfront ASCT as a deferred-ASCT approach due to the availability of newer therapies achieving deeper responses.[41,43] Even fewer patients undergo ASCT due to transplant ineligibility or resource/financial constraints in lower-and-middle-income countries (LMIC).[41,44,45] Our study was focused on patients not upfront planned for ASCT as less than 20% of our patients undergo ASCT.[45,46]

In this study, circulating tumor plasma cells were detected in approximately 77% of patients, which is in the range reported by previous studies, that is, 67%–92% of NDMM patients.[4,22,25,33,47] However, the CTPC detection rate in our study was slightly lower compared to the recent few reports even though the median LOD of

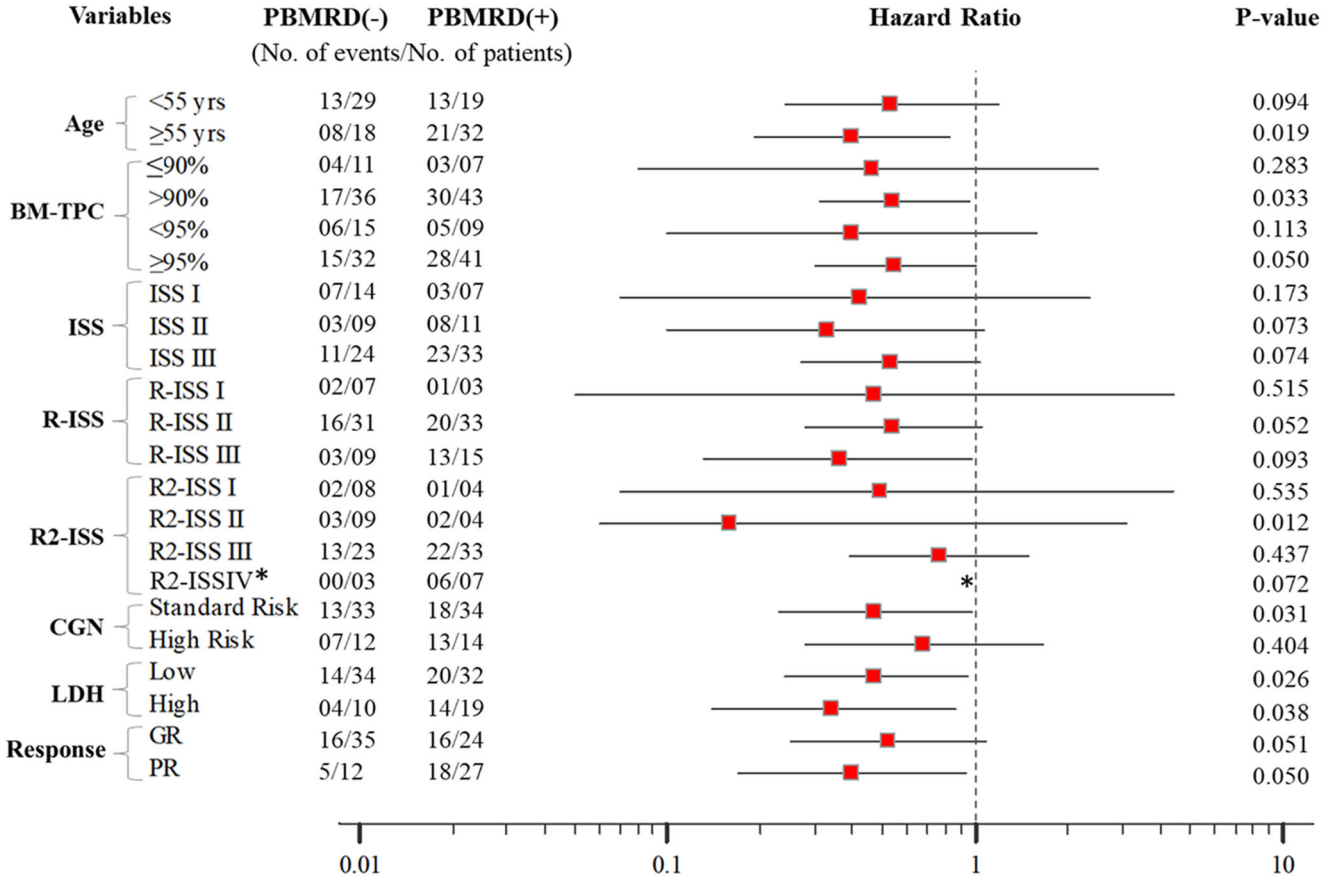

FIGURE 4 Association of the negative status of peripheral blood measurable residual disease at both time points with progression-free survival in different baseline risk groups and serological response (*insufficient patient numbers for calculation). BM, bone marrow; CGN, cytogenetics; CTPC, circulating tumor plasma cells; HR, high risk; ISS, International Scoring System; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; RISS, Revised International Scoring System; R2ISS, second revision of ISS; TPC, tumor plasma cells.

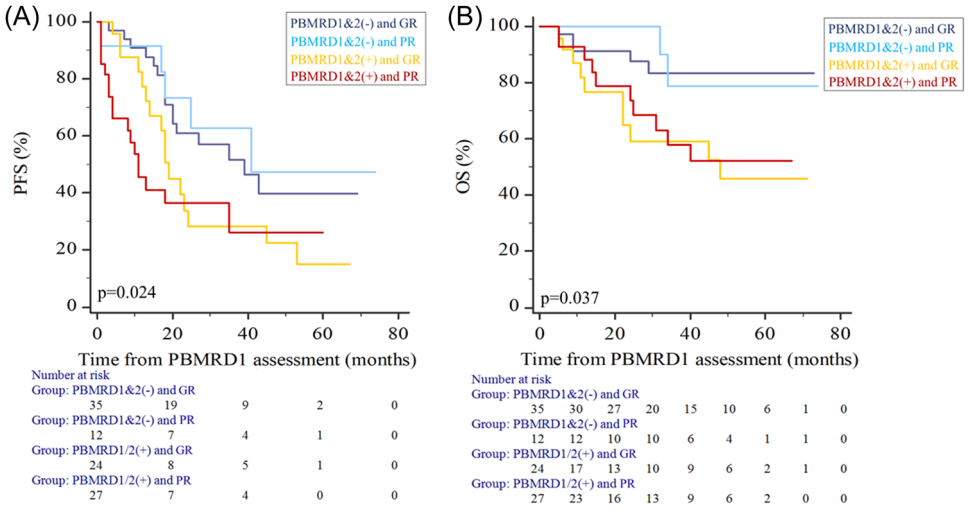

FIGURE 5 Kaplan–Meier estimates of progression-free survival (A) and overall survival (B) in newly diagnosed multiple myeloma patients grouped according to the combined results (negative at both time points against positive at any time point) of both, that is, peripheral blood measurable residual disease 1 (PBMRD1) and PBMRD2 time points and good response (complete response and very good partial response) against poor response (partial response and less).

0.0002% was similar to the study reported by Kostopoulos and colleagues.[4,22] No statistically significant difference was observed between the number of events acquired between samples with detectable and nondetectable CTPC in our study. Nevertheless, the samples from 23 patients who could not reach LOD of 0.0005% might have partly influenced our CTPC detection rate. Despite these real-world challenges, the available data suggest that CTPC can be detected in almost 80% of NDMM patients using high-sensitivity techniques. Like previous reports, we also found a strong similarity in immunophenotypic profile between the BM-TPC and CTPC and a modest correlation between their levels.[4,22,33]

A reproducible cut-off for circulating tumor cell levels can provide broadly acceptable criteria for risk stratification, and hence, attempts are being made in that direction.[4,20–22,33] Our data revealed the cut-off of CTPC ≥ 0.01% using HS-MFC, which was identical to the results reported by Garcés et al. using next-generation flow cytometry.[4] Bertamini et al. suggested a cut-off of ≥0.07% (eight-color flow cytometry), whereas reports by Bae et al. (five-color MFC) and Kostopoulos et al. (high-sensitivity eight-color MFC) suggested ≥0.02%. Thus, a reproducible cut-off can be achieved using high-sensitivity techniques.

Our data showed a strong association of increased CTPC levels with shorter PFS and OS. It emerged as a superior high-risk baseline factor in multivariate analysis after comparison with current prognostication parameters, including age, RISS, LDH, BM-TPC, cytogenetics, and treatment protocol. It was also independent of R2ISS, which was not incorporated in earlier studies. Our results in non-transplant setting are similar to data reported in patients treated with ASCT, suggesting a prognostic relevance of CTPC levels independent of treatment modalities such as ASCT.[4,21,22,33,47,48]

Further, we studied the impact of sequential PBMRD as a minimally invasive MRD monitoring method. We observed a higher PBMRD detection rate (PBMRD1+, 44% and PBMRD2+, 33%) compared to 28% reported by Sanoja-Flores et al.[18] This difference can be attributed to the difference in the treatment protocols as their data documented stringent CR/CR in 60% (71/118) of patients as opposed to 7.1% in our study. Sanoja-Flores et al. reported PBMRD positivity in 40% of all BMMRD-positive and BMMRD-negative PBMRD in all BMMRD-negative patients. Similar results were reported using allele-specific oligonucleotide polymerase chain reaction by Huhn et al.[21] These results suggest that a staged approach with PBMRD assessment followed by BMMRD in patients with undetectable PBMRD can be applicable to one-third of patients and these patients can be spared of painful BM procedure. Conversely, the study by Huhn et al. also reported PBMRD negativity in 34% sIF-positive and 20% sIF-negative patients.[18] Our study also showed PBMRD negativity despite detectable M-protein by sIF (Supporting Information S1: Table S8). We demonstrated that the predictive value of PBMRD is independent of sIF-based response monitoring. Patients with detectable PBMRD at any time point had poor PFS and OS even if they achieved a GR. Inversely, we observed that patients with undetectable PBMRD at both time points had better PFS and OS even though the response was PR or less. This finding was limited to a small cohort of patients. However, it was significant despite a small number, indicating a higher impact and substantial clinical implications; hence, its validation in a larger cohort of patients is needed. Interestingly, the PFS and OS of patients with GR were not significantly different from patients with poor response if they had detectable PBMRD at any time point. Thus, the inclusion of PBMRD monitoring substantially improves the clinical impact of serum-based monitoring in NDMM patients. This finding is particularly valuable as the management of NDMM patients in real-world practice depends on serum-based response monitoring, and BMMRD is still not part of standard practice outside clinical trials due to reasons such as high cost, low feasibility in older patients, and limited applicability in patients not achieving CR.

We also studied the value of PBMRD status at each time point. Patients with undetectable PBMRD at both time points achieved the best PFS and OS. Patients who were PBMRD1(+)PBMRD2(−), PBMRD1(−)PBMRD2(+), and PBMRD1(+)PBMRD2(+) showed poor PFS, whereas patients who were PBMRD1(+)PBMRD2(−) had intermediate OS compared to that of PBMRD1(−)PBMRD2(+) and PBMRD1(+)PBMRD2(+) patients who showed poor OS. Thus, early clearance of CTPC from circulation was strongly associated with longer PFS and its persistence or reappearance at a later time point was associated with significantly short OS. Moreover, PBMRD(−) status at both time points improved the positive impact of baseline standard-risk parameters and reduced the negative impact of high-

risk parameters. The prognostic impact of the sequential PBMRD status superseded the baseline prognostic parameters and serological response. These findings are similar to BMMRD results published earlier.[11,13,30]

BMMRD and M-protein/FLC secreted by myeloma cells represent the presence of residual disease surviving the given therapy, whereas residual circulating tumor cells provide information on the ability of residual tumor cells to disseminate and support the disease progression.[19,24] This could explain the better clinical outcome in patients with undetectable PBMRD compared to detectable PBMRD, independent of the treatment given. A few recent studies from clinical trial settings have shown a positive correlation between CTPC reduction and survival outcomes using molecular methods.[21,23,35,49,50] However, many were limited to a small cohort of patients treated with ASCT, and none proved the independent prognostic value of PBMRD assessment.

The current study has a few limitations, such as data on parallel BMMRD assessment was unavailable due to low CR rate. This could have added to the limited literature on the relationship between PBMRD and BMMRD. Our study included 141 patients (as enrollment of patients significantly slowed down due to the coronavirus disease 2019 pandemic) and the inclusion of a larger number of patients would have strengthened our findings further. Our cohort included patients treated with VCd or VRd and the number of chemotherapy cycles varied based on response and toxicity. CR rate was relatively lower than data from patients treated with ASCT and other clinical trials. This heterogeneity in the treatment and response rate could influence our results. The absence of such heterogeneity would have improved our results further. Nevertheless, our cohort represents real-life practice, especially in LMIC. Despite these limitations, the present study is the first to demonstrate the independent prognostic relevance of PBMRD in NDMM patients not planned for upfront ASCT.

Our findings showed that baseline circulating tumor cell quantification and PBMRD assessment using HS-MFC is feasible in the majority of NDMM patients and provides the most relevant non-invasive biomarker. Negative PBMRD status strongly indicates better survival and provides additional biomarkers to the existing prognostic models and response monitoring method. Thus, assessing tumor burden and response monitoring using HS-MFC in PB is more convenient for standard clinical practice and can be included in future risk stratification models.

AUTHOR CONTRIBUTIONS

Prashant R. Tembhare designed and performed the study, performed the data analysis, interpreted the data, statistical analysis, and wrote the paper. Harshini Sriram, Twinkle Khanka, and Sanghamitra Gawai performed sample collection, processing, and data collection. Gaurav Chatterjee, Sweta Rajpal, Nikhil V. Patkar, Papagudi G. Subramanian, and Sumeet Gujral interpreted the data and performed the diagnosis. Sitaram G. Ghogale, Nilesh Deshpande, Karishma Girase, and Jagruti Patil performed the quality control and sample processing. Dhanalaxmi Shetty performed the cytogenetic studies. Kinjalka Ghosh performed biochemical data analysis. Syed Khaizer Hasan performed the data analysis and interpreted the data. Bhausaheb Bagal, Manju Sengar, Hasmukh Jain, Lingaraj Nayak, Sumeet Mirgh, Nishant Jindal, Navin Khattry, Sachin Punatar, and Anant Gokarn recruited patients, and performed management of patients and clinical data analyses. All authors contributed to the manuscript writing and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available within the paper and its Supplementary Information. The patient-related data generated in this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was approved by the Institutional Ethical Committee (IEC), written informed consent was taken, and the study was conducted as per the Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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