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ORIGINAL ARTICLE

Circulatory miR-155 correlation with platelet and neutrophil recovery after autologous hematopoietic stem cell transplantation, a multivariate analysis

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Abstract

The involvement of microRNAs in the regulation of hematopoietic stem cells paves the way for their use in the management of autologous HSC transplantation (AHSCT). We aimed to evaluate the predictive value of circulatory microRNAs in extracellular vesicles (EVs) and plasma in platelet and neutrophil engraftment. Circulatory miR-125b, mir-126, miR-150, and miR-155 expression was assessed in isolated EVs and plasma in samples collected from AHSCT candidates. Multivariate analysis, COX models, and ROC assessment were performed to evaluate the predictive values of these microRNAs in platelet and neutrophil engraftment. miR-155 expression following conditioning with other clinical factors such as chemotherapy courses after diagnosis was the most significant predictors of platelet/neutrophil engraftment. A $CD34^+$ cell count $\geq 3.5 \times 10^6$/kg combined with miR-155 could be used as an engraftment predictor; however, in cases where the $CD34^+$ cell count was $< 3.5 \times 10^6$/kg, this parameter lost its predictive value for engraftment and could be replaced by miR-155. The correlation between miR-155 and platelet/neutrophil engraftment even with lower numbers of $CD34^+$ cells suggests the importance of this microRNA in the prediction of AHSCT outcome. Moreover, miR-155 could be utilized in therapeutic approaches to provide a better outcome for patients undergoing AHSCT.

Keywords MicroRNAs · Hematopoietic stem cell transplantation · Platelet · Neutrophils · Engraftment · Prediction

Introduction

It is about a half-century that autologous hematopoietic stem cell transplantation (AHSCT) or rescue therapy is considered as the main as well as the effective treatment approach for multiple myeloma (MM), lymphomas, and other neoplasia [1, 2]. It should be noted that the success of AHSCT is related to rapid hematologic recovery following the procedure [3] and this would not meet unless the adequate number of $CD34^+$ hematopoietic stem cells (HSCs) has been collected from apheresis. However, the poor mobilization of $CD34^+$ cells that occurs in 5–40% of patients leads to engraftment failure [4]. Even when the number of HSCs seems to be adequate, appropriate engraftment does not achieve in some patients due to unknown factors [3]; suggesting the necessity of identification of new biomarkers of engraftment that could predict the change of successful AHSCT in hematologic patients.

It is well established that HSCs microenvironment and paracrine signals determine the fate of transplanted HSCs, such as quiescence, proliferation, and survival [5]. In addition to these extrinsic factors, the residential microRNAs in the bone marrow (BM) niche have also fundamental effects on the regulation of HSCs [6]. These groups of regulatory RNAs have been shown to could participate in BM recovery after high-dose chemotherapy and HSCT [7]. It has been indicated that cells could wrap miRNAs in membranous enclosed structures, called extracellular vesicles (EVs), and release them toward definite cellular targets. While the circulating miRNAs are protected from environmental factors, they could readily communicate between the residential cells

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in the BM niche and change their intracellular events in a way that enhances their survival [8, 9]. Given these, in the present study, we sought to evaluate the possible correlation between circulatory microRNAs either in plasma or encapsulated in EVs and the success of platelet and neutrophil engraftment in AHSCT candidates.

Patients and methods

Patients and sample collection

Candidates of AHSCT including MM and lymphomas patients that were referred to the Department of Bone Marrow Transplantation, Taleghani Hospital (Tehran, Iran), between October 2019 and May 2020 were subjects of the study. Exclusion criteria included age under 18, infections, bleeding, splenomegaly, and the use of myelosuppressive drugs during the transplantation process. Granulocyte colony-stimulating factor (G-CSF) was used in steady state for mobilization and Melphalan/Velcade and CEAM (Lomustine, Etoposide, Cytarabine, Melphalan) or BEAM (Carmustine, Etoposide, Cytarabine, Melpha) were conditioning regimens for MM and lymphoma patients, respectively. Data from diagnosis to recovery (early engraftment) after AHSCT were also collected. Platelet and neutrophil engraftment day was defined as the first day of three consecutive days with a platelet count of $\geq 20{,}000/\mu l$ and neutrophil count of $\geq 500/\mu l$ [10]. To introduce reliable predictive factors and evaluate the possible effects of mobilization and conditioning on microRNAs expression, peripheral blood samples were obtained before mobilization and post-conditioning and instantly, plasma fraction was isolated through centrifugation at 1000*g* for 10 min.

EV isolation and confirmation

Ultra-centrifugation was performed to isolate EVs. To do so, plasma was first diluted with PBS with the ratio of 1/1 and then was centrifuged at 2000*g* for 10 min for precipitation of dead cells. Then, the supernatant was re-centrifuged at 10000*g* for 30 min to discard cell debris. Afterward, ultra-centrifugation was done on supernatant at 100000*g* for 80 min for deposition of EVs. The resulting supernatant was discharged, the precipitated EVs were diluted by filtered PBS, and then the mixture was filtered with a 0.2 µm filter to remove apoptotic bodies. The step of ultra-centrifugation was done twice. To confirm the isolation of EVs, flow cytometry (AttuneTM NXT Flow Cytometer) was done for CD9 and CD63 markers (Dako, Denmark), size of the EVs was assessed by dynamic light scatter (DLS) (Zeta-sizer, Malvern, UK) and morphologic analysis was done by transmission electron microscopy (TEM) technique.

Circulatory microRNA extraction and expression analysis

300–500 µl of EVs and/or plasma was used for total RNA extraction by the GeneAll RiboEx™ LS Kit (GeneAll Biotech, Korea) according to the manufacturer's instruction. Aiming to better precipitation of RNAs, coupled with the instruction, incubation in -80° C after addition of isopropanol to the aqueous phase was done for 1 h. Extracted microRNAs were converted to complementary DNA (cDNA) using stem-loop primers and the ExcelRT ™ Reverse Transcriptase kit according to the manufacturer's instruction. The expression of the circulatory miR-125b, miR-126, miR-150, and miR-155 was analyzed based on SYBR-Green assay using real-time PCR technique. Selected primers and stem-loop sequence (synthesized by Metabion, Germany) are shown in Table 1. Real-time PCR reactions were performed in the total volume of 15 µl containing 7.5 µl of Master mix 2× (Real Q Plus Master Mix), 0.5 µl of each primer, 3 µl of the synthesized cDNA, and 3.5 µl of nuclease-free water in Corbett Rotor-Gene 600 thermocycler. Condition of PCR reaction included denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 10 s, 53–54 °C for 15 s, 72 °C for 20 s, and 10 min for the melting curve. SNORD47 and miR-16 were used as housekeeping genes, and their geometric mean was used to calculate $2^{-\Delta CT}$ or basal expression.

Table 1 Primers sequence of microRNA expression analysis and stem-loop primer for cDNA synthesis

miR-125b (5P)	5-GATGTCCCTGAGACCCTAA-3
miR-126 (3P)	5-GACAGTCGTACCGTGAGTA-3
miR-150 (5P)	5-GTATGTCTCCCAACCCTTG-3
miR-155 (5P)	5-GCTCAGTTAATGCTAATCGTG-3
miR-16 (5P)	5-GACAGTAGCAGCACGTAAAT-3
SNORD47	5-CCAATGATGTAATGATTCTGCC-3
Reverse	5-GAGGAAGAAGACGGAAGAAT-3
Stem loop	5-GAAAGAAGGCGAGGAGCAGATCGAGGAAGA AGACGGAAGAATGTGCGTCTCGCCTTCTTT CHVMHNN-3

Statistical analysis

The time (day) of platelet and neutrophil engraftment was the outcome of the study. The multivariate analysis was carried out using COX hazard proportional models. The stepwise multivariate analysis was performed to identify the variables with the highest predictive values. A significant level of 5% was considered to exclude variables from the model. The statistical analysis was conducted using STATA software (College Station, Texas, USA, V14). In addition, receiver-operating characteristic (ROC) analysis was used for the evaluation of the predictive value of variables for platelet and neutrophil engraftment. Cumulative incidence was estimated by Kaplan–Meier for platelet and neutrophil engraftment according to effective microRNA. Spearman test was performed to evaluate the correlation between microRNAs and platelet/neutrophil count at engraftment day. Moreover, the correlation between age, body mass index, height, the total dose of infused G-CSF (TDIG) for mobilization, and the number of days of G-CSF infusion for mobilization and microRNA expression was assessed in the present study.

Results

50 AHSCT candidates including 50% of each gender were incorporated in the study with a mean age of 42.4 ± 14.6 years. The median time for both platelet and neutrophil engraftment was 11 days and the median number of platelet and neutrophil count at engraftment day were 28×10^3/µl and 0.9×10^3/µl, respectively. The demographic characteristics of AHSCT candidates together with the median number of $CD34^+$ cell count per kilogram ($CD34^+$ CPK), mononuclear cell (MNC) count, and other AHSCT related processes are summarized in Table 2. It should be noted that the transfusion of platelet was necessary for a group of patients; however, stable and sufficient platelet count 4–5 days after the transfusion was indicated as the patients' platelet recovery. The platelet transfusion as a variable did not include in the statistical analysis.

Confirmation of EV isolation

To confirm the isolation of EVs, flow cytometry, DLS, and TEM techniques were used. The results are shown in Fig. 1.

Circulatory microRNA expression

Basal expression analysis of circulatory microRNAs revealed that the expression level of miR-125b was higher in EVs as compared to plasma before mobilization (p value < 0.001). Similarly, we found that the expression levels of miR-150 and miR-155 were also greater in EVs than in plasma post-conditioning (p value < 0.05). There was no significant difference in microRNA expression before mobilization and post-conditioning, except for miR-150 that its expression was higher before mobilization in both plasma and EVs (Fig. 2). Moreover, the expression of miR-126 BP, miR-150 BP, miR-150 PE, and miR-150 PP in the lymphoma patients were more than MM patients with a p value < 0.005 for all. Although we failed to identify any difference between microRNA expression in each gender group, we have evaluated correlation between microRNA expression and clinical features and significant results are shown in supplementary file.

Factors predicting the platelet and neutrophil engraftment

Multivariate analysis and COX model were performed to evaluate the effect of independent variables on platelet and neutrophil engraftment. miR-155 post-conditioning in plasma (PP), along with chemotherapy courses after diagnosis, G-CSF dose after HSCs infusion, and TDIG for mobilization were significant predictors of platelet engraftment. mir-155 PP, along with the disease type, conditioning days, and TDIG for mobilization were significant predictors of neutrophil engraftment (Table 3). ROC analyses were performed to evaluate the predictive value of variables (Fig. 3). We evaluated the $CD34^+$ CPK in the model for correlation and prediction of platelet/neutrophil engraftment, but only $CD34^+$ CPK $\geq 3.5\times10^6$/kg has shown to be a promising predictor of platelet/neutrophil engraftment in multivariate analysis (Table 3). Multivariate analysis also showed that although the number of $CD34^+$ CPK $\geq 3.5\times10^6$/kg could be a strong predictor of platelet and neutrophil engraftment, the numbers less than this value could not predict the successful engraftment of AHSCT (Table 3). This finding was also confirmed by ROC analysis, as it shows that while $CD34^+$ CPK $\geq 3.5\times10^6$/kg could be an outstanding predictor for platelet/neutrophil engraftment, the counts less than 3.5×10^6/kg was suggestive of poor engraftment predictor (ROC curves are shown in supplementary file). Moreover, as we have been shown the significant influence of miR-155 PP on platelet/neutrophil engraftment by multivariate analysis in Table 3, ROC analysis has also shown the acceptable predictive value of miR-155 PP in both groups of $CD34^+$ CPK $\geq 3.5\times10^6$/kg and $< 3.5\times10^6$/kg (Fig. 4), thus this microRNA can be a replacement for $CD34^+$ CPK in count of less than 3.5×10^6/kg for platelet/neutrophil engraftment prediction. Based on the cutoff obtained from ROC curve for miR-155 PP predicted platelet/neutrophil, patients were categorized into two groups of miR-155 PP expression ≥ 2.1 and < 2.1. Then cumulative incidence of engraftment according to miR-155 PP expression groups was evaluated by

Table 2 Participants characteristic ($N = 50$)

Features	Median (range)/frequency (%)
Age (year)	40.5 (18–70)
Gender	
Male	25 (50)
Female	25 (50)
Diagnosis	
Multiple myeloma	24 (48)
Hodgkin disease	15 (30)
Non-Hodgkin lymphoma	11(22)
BMI before transplantation	27.2 (15.2–43.7)
BMI after transplantation	27.1 (14.8–42.1)
Number of days of G-CSF infusion (day)	6 (5–9)
Total dose of infused G-CSF for mobilization (µg)	3750 (1500–7200)
WBC count at day of start of mobilization ($\times 10^3$/µl)	6.05 (2.4–22.1)
Last injection of G-CSF to apheresis (hour)	6 (1–22)
WBC count at apheresis day ($\times 10^3$/µl)	39.55 (11–97.3)
Neutrophil count on apheresis day ($\times 10^3$/µl)	35.13 (10.7–94.5)
Platelet count on apheresis day ($\times 10^3$/µl)	172.5 (66–281)
Apheresis duration (min)	377.5 (270–520)
ACD volume for apheresis (ml)	1507 (950–3000)
Total processed blood in apheresis (l)	17.97 (10.4–25.5)
Apheresis bag volume (ml)	400 (250–600)
WBC count in apheresis product ($\times 10^8$/kg)	10.9 (4.2–26.2)
MNC count in apheresis product ($\times 10^8$/kg)	6.20 (3.3–17.5)
CD45$^+$ in apheresis product (%)	98.05 (95–99.5)
CD45$^{+\text{dim}}$ in apheresis product (%)	1.20 (0.2–9.2)
CD34$^+$ CPK ($\times 10^6$/kg)	3.5 (1–15.4)
CD34$^+$ in PAPBS (/µl)	35 (4.1–116)
G-CSF dose after HSCs infusion (µg)	3900 (900–12,600)
Platelet engraftment day	11 (9–19)
Platelet count at engraftment day ($\times 10^3$/µl)	28 (20–110)

BMI body mass index, *WBC* white blood cell, *ACD* acid citrate dextrose, *MNC* mono-nuclear cell, *CPK* Count per kilogram, *PAPBS* pre-apheresis peripheral blood sample

Kaplan–Meier test (Fig. 5). Incidence of platelet engraftment was 87% and 48% in patients whose miR-155 PP expression were higher and lower than 2.1 as threshold, respectively (*p* value < 0.001). In addition, incidence of neutrophil engraftment was 81% and 47% in patients whose miR-155 PP expression were higher and lower than 2.1, respectively (*p* value: 0.012). The results of the Spearman correlation test also revealed that there was no correlation between microRNAs expression and platelet/neutrophil count at engraftment day (data not shown).

Discussion

AHSCT is a complex process that numerous factors could affect its success or failure and thereby no single factor is sufficiently powerful for predicting its outcome. Thus, a combination of tissue-specific and systemic biomarkers may be necessary to increase the predictive value of tests [4, 11]. MicroRNAs play a vital role in the regulation of HSCs functions and numerous studies, thus far, have studied the biomarker potential of EVs containing microRNAs in different diseases [12, 13]. It should be noted that while there is no significant difference in the expression of microRNAs between plasma and EVs in normal conditions, the results of the previous studies declared that the expression of some microRNAs could be increased in EVs in pathologic conditions such as the formation of malignancies or inflammations [14]. Given these, in the present study, we decided to evaluate the predictive value of microRNAs for the prediction of platelet and neutrophil engraftment, two parameters that could determine the success of AHSCT. Due to protection of microRNAs against RNases, comprehensive evaluation of physiological and pathological changes will be obtained

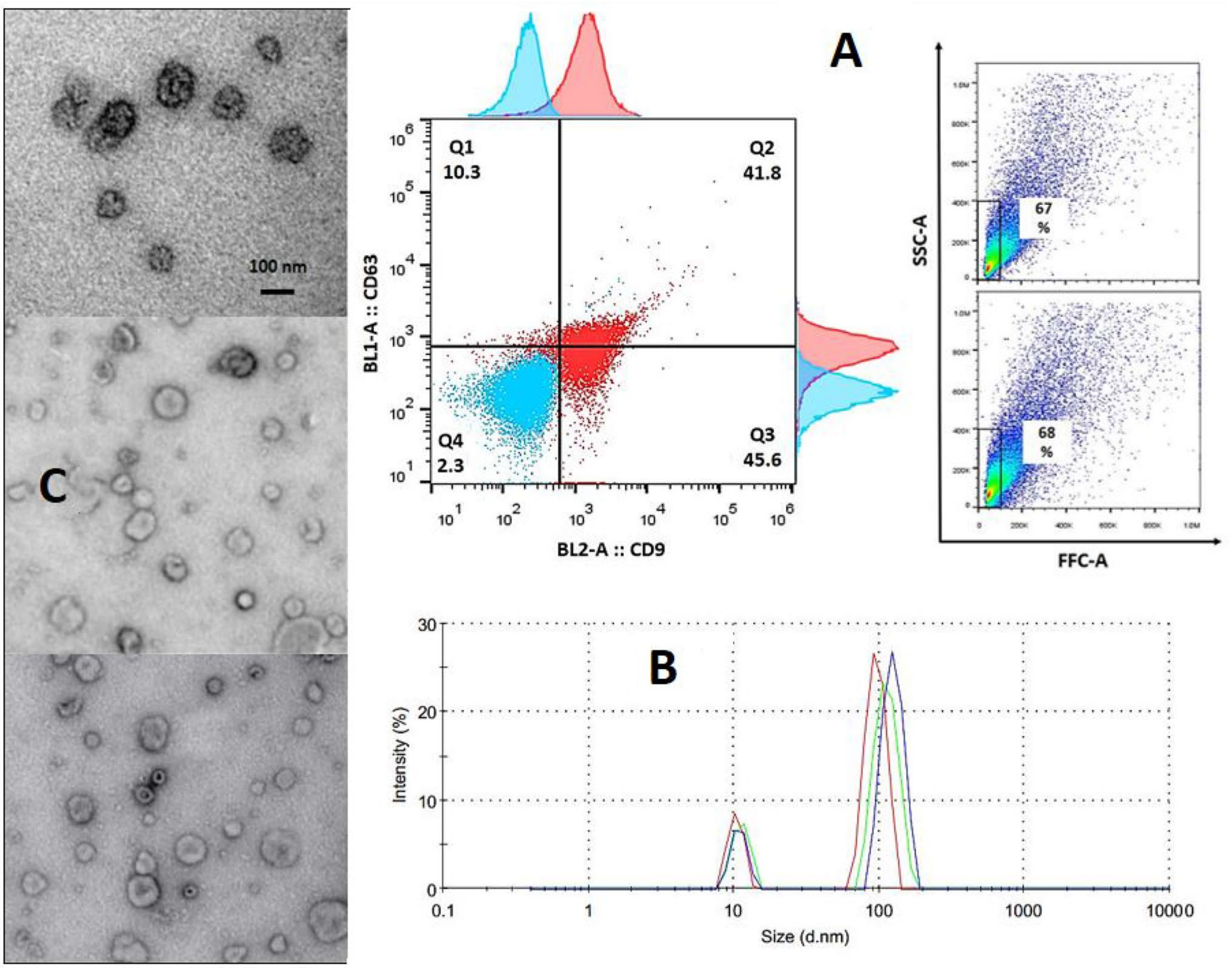


Fig. 1 Confirmation test's results of EVs. **a** Flow cytometry of EVs shows 52.1% $CD63^+$ and 87.4% $CD9^+$. Here, the gates of EVs are shown given to their size. **b** Dynamic light scatter (DLS) result that was done as triplicate. **c** Transmission electron microscopy images that are shown the EVs size about 100 nm

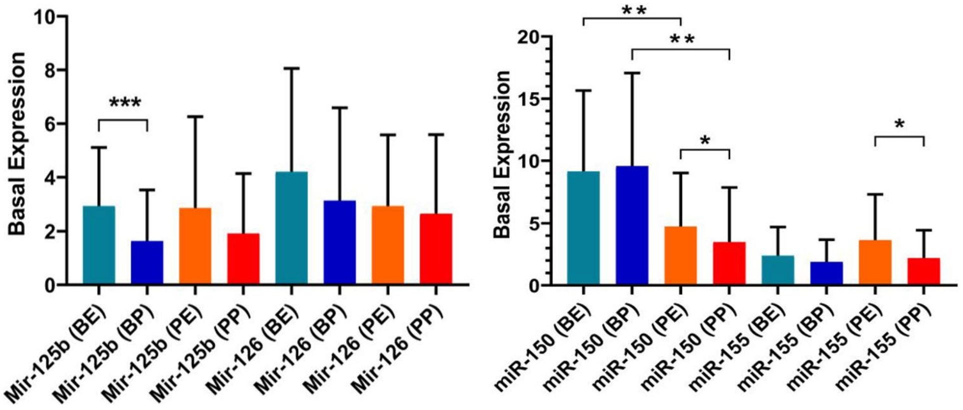


Fig. 2 B: Basal expression of microRNAs in EVs and plasma. Significant differences are shown by *, ** and *** for *p* value of <0.5, <0.01 and <0.001, respectively. *BE* Before $_{\text{(mobilization)}}$ EVs, *BP* before $_{\text{(mobilization)}}$ plasma, *PE* post $_{\text{(conditioning)}}$ EVs, *PP* post $_{\text{(conditioning)}}$ plasma

by analysis of EV-surrounded microRNAs [13]. Our results showed that the expression of microRNAs was higher in EVs as compared to plasma. Montagnana et al. have reported randomly different expression of microRNAs in EVs and plasma [15] and since we have analyzed the relative expression of microRNAs in comparison with the housekeeping

Table 3 Multivariate analysis of microRNAs influencing the platelet and neutrophil engraftment with other clinical variables by Cox Hazard model

	Platelet engraftment		Neutrophil engraftment	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Age	1.09 (0.77–1.53)	0.67	0.98 (0.84–1.2)	0.48
Gender				
Male	1.25 (0.66–2.36)	0.56	1.01 (0.89–1.14)	0.76
Female	1		1	
BMI before HSCT	1.06 (1.01–1.13)	0.1	0.97 (0.93–1.03)	0.28
BMI after HSCT	1.06 (1.005–1.15)	0.12	1.02 (0.99–1.01)	0.35
Height	1.01 (0.92–1.05	0.52	0.98 (0.94–1.02)	0.48
Disease type	1.1 (0.75–1.3)	0.1	**0.52 (0.28–0.94)**	**0.031***
Chemotherapy courses after diagnosis	**1.15 (1.04–1.27)**	**0.005***	0.94 (0.85–1.03)	0.22
TDIG for mobilization	**0.99 (0.998–0.999)**	**0.004***	**0.99 (0.998–0.999)**	**0.03***
Number of days of G-CSF infusion	0.72 (0.52–1.007)	0.1	0.92 (0.82–1.03)	0.17
WBC count at day of start of mobilization	1.06 (0.99–1.13)	0.11	0.94 (0.85–1.03)	0.22
Last injection of G-CSF to apheresis	0.97 (0.89–1.05)	0.54	0.99 (0.99–1)	0.76
WBC count at apheresis day	0.98 (0.95–1.004)	0.16	1.07 (0.92–1.2)	0.36
Neutrophil count on apheresis day	0.47 (0.22–0.99)	0.1	0.61 (0.48–0.95)	0.23
Platelet count on apheresis day	0.43 (0.3–0.95)	0.2	0.55 (0.45–0.91)	0.26
Apheresis duration	1.004 (0.99–1.01)	0.33	0.96 (0.81–1.15)	0.67
ACD volume for apheresis	1.89 (0.31–11.51)	0.56	1.95 (0.46–9.2)	0.48
Total processed blood in apheresis	4.97 (0.9–18.4)	0.12	5.3 (2.1–15.1)	0.1
Apheresis bag volume	1.001 (0.99–1.005)	0.52	1.2 (1.01–1.09)	0.1
WBC count in apheresis product	0.93 (0.86–1.01)	0.17	0.93 (0.84–1.03)	0.18
MNC count in apheresis product	0.92 (0.82–1.04)	0.28	1.04 (0.94–1.16)	0.36
CD45$^+$ in apheresis product	1.09 (0.77–1.53)	0.67	1.03 (0.87–1.21)	0.18
CD45^{+dim} in apheresis product	0.97 (0.84–1.12)	0.77	0.94 (0.85–1.03)	0.22
CD34$^+$ CPK				
$\geq 3.5 \times 10^6$	**2.2 (1.4–2.1)**	**0.001***	**1.9 (1.1–2.6)**	**0.001***
$< 3.5 \times 10^6$	1.005 (0.99–1.01)	0.52	1.02 (0.91–1.15)	0.47
CD34$^+$ in PAPBS	1.02 (0.75–1.005)	0.25	1.05 (0.93–1.18)	0.36
G-CSF dose after HSC infusion	**0.99 (0.998–0.999)**	**0.003***	0.89 (0.78–1.02)	0.11
Conditioning days	1.2 (0.7–1.05)	0.26	**0.7 (0.53–0.91)**	**0.008***
miR-125b BE	1.04 (0.89–1.22)	0.56	0.94 (0.85–1.03)	0.24
miR-125b BP	0.79 (0.58–1.09)	0.16	0.9 (0.84–1.1)	0.2
miR-125b PE	1.03 (0.89–1.2)	0.62	1.005 (0.9–1.11)	0.8
miR-125b PP	0.98 (0.96–1.01)	0.42	1.04 (0.93–1.18)	0.35
miR-126 BE	1.001 (0.93–1.07)	0.1	1.03 (87–1.16)	0.33
miR-126 BP	0.94 (0.88–1.02)	0.18	1.01 (0.9–1.13)	0.65
miR-126 PE	1.06 (0.9–1.23)	0.45	0.98 (0.87–1.05)	0.41
miR-126 PP	0.86 (0.69–1.06)	0.19	0.95 (0.87–1.03)	0.26
miR-150 BE	1.05 (0.92–1.2)	0.39	1.02 (0.91–1.14)	0.5
miR-150 BP	0.94 (0.87–1.01)	0.1	0.95 (0.91–1.1)	0.3
miR-150 PE	1.01 (0.89–1.14)	0.86	1.02 (0.89–1.12)	0.72
miR-150 PP	0.88 (0.77–1.02)	0.1	0.9 (0.82–1)	0.37
miR-155 BE	0.97 (0.93–1.02)	0.27	1.05 (0.96–1.23)	0.19
miR-155 BP	0.93 (84–1.03)	0.18	0.9 (0.73–1.1)	0.33
miR-155 PE	0.98 (0.94–1.1)	0.47	0.95 (0.77–1.2)	0.58
miR-155 PP				
With CD34$^+$ CPK $\geq 3.5 \times 10^6$	**1.35 (1.1–1.7)**	**0.005***	**1.23 (1.04–1.4)**	**0.015***
With CD34$^+$ CPK $< 3.5 \times 10^6$	**1.3 (1.08–1.63)**	**0.006***	**1.28 (1.7–1.53)**	**0.01***

*Statisticaly significant results or p value less than 0.05

BMI Body mass index, *TDIG* Total dose of infused G-CSF, *ACD* Acid citrate dextrose, *MNC* Mono-nuclear cell, *CD34$^+$ CPK* CD34$^+$ count per kilogram, *PAPBS* Pre-apheresis peripheral blood sample, *BE* Before $_{\text{mobilization}}$-extra-cellular vesicles (EVs), *BP* Before-plasma, *PE* Post

Table 3 (continued)

$_{\text{conditioning}}$-EVs, *PP* Post-plasma

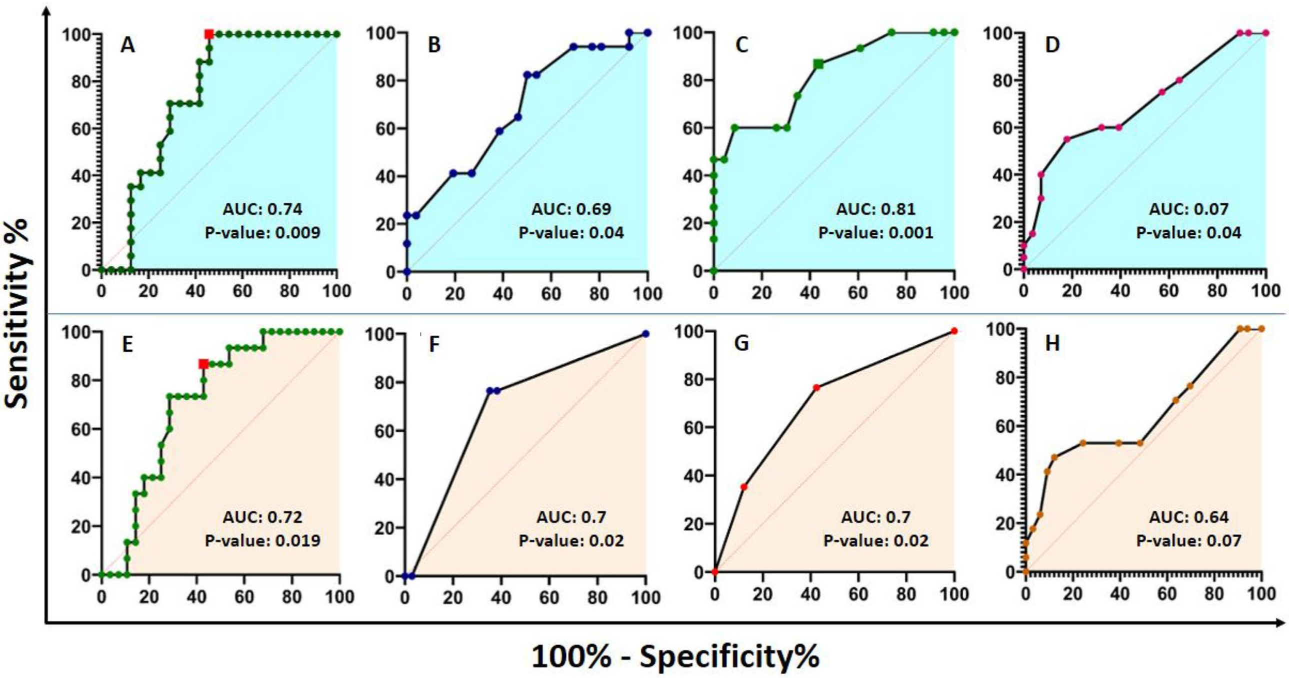


Fig. 3 ROC curves for variables predicting the platelet and neutrophil engraftment. **a–d** ROC curves for miR-155 PP, chemotherapy courses after diagnosis, G-CSF after HSC infusion, and TDIG, respectively, for platelet engraftment. E–H: ROC curves for miR-155 PP, disease type, conditioning days and TDIG, respectively, for neutrophil engraftment

microRNA, it is assumed that degradation of microRNAs by RNases is a random event and it is important which of target or housekeeping degrade more.

Mobilization of a sufficient number of CD34$^+$ HSCs could improve platelet and neutrophil engraftment. A large body of evidence has shown that microRNAs profoundly influence the mobilization and engraftment of HSCs. In this regard, a study demonstrated that EVs containing miR-126 increases the mobilization through the reduction of vascular cell adhesion molecule 1 on HSCs [16]. Moreover, in another study, it has been claimed that miR-155 responds to stromal-derived factor (SDF) via controlling SH-2 containing inositol 5′ polyphosphatase [17]. miR-150 downregulation in mouse models also showed to increase the mobilization of HSCs [18]. Apart from maintaining the survival and metabolization of HSCs, microRNAs also could induce the reconstruction of the BM niche after high-dose chemotherapy and AHSCT. In this regard, it has been indicated that the reduced expression of miR-126 and miR-146a through conditioning regimen results in enhanced engraftment and bone marrow regeneration after AHSCT [7]. In addition, miR-125b prompts the serial transplantation times of HSCs in mice by inhibition of apoptosis [19]. In contrast, miR-150 overexpression could prolong the hematologic recovery time after transplantation [20]. Multivariate analyses in our study revealed that increased expression of circulatory miR-155 was associated with the faster platelet and neutrophil engraftment. In agreement with our study, it has been indicated that miR-155 induces the proliferation of HSCs and inhibits the quiescence [6, 17]. Likewise, other studies also reported the protective role for miR-155 in HSC maintenance in the BM [21]. In other studies, using the multivariate analysis, it has been reported the CD34$^+$ CPK, lymphocytes count in the pre-apheresis peripheral blood sample, and the number of days of mobilizer drug injection are the factors that could predict the delay in engraftment [22]. It could be assumed that probably the difference in the sample size, number of variables, and data used in multivariate analysis are the main reasons that could lead to the discrepancy in the results of the different studies. Interestingly, we found that CD34$^+$ CPK value greater than 3.5×10^6/kg is a strong predictor of the engraftment and, in contrast, as the number of CD34$^+$ CPK dropped from the indicated value the predictive value of CD34$^+$ CPK about platelet and neutrophil engraftment reduces. In the cases of lower CD34$^+$ CPK value, this is miR-155 that serves as a promising indicator

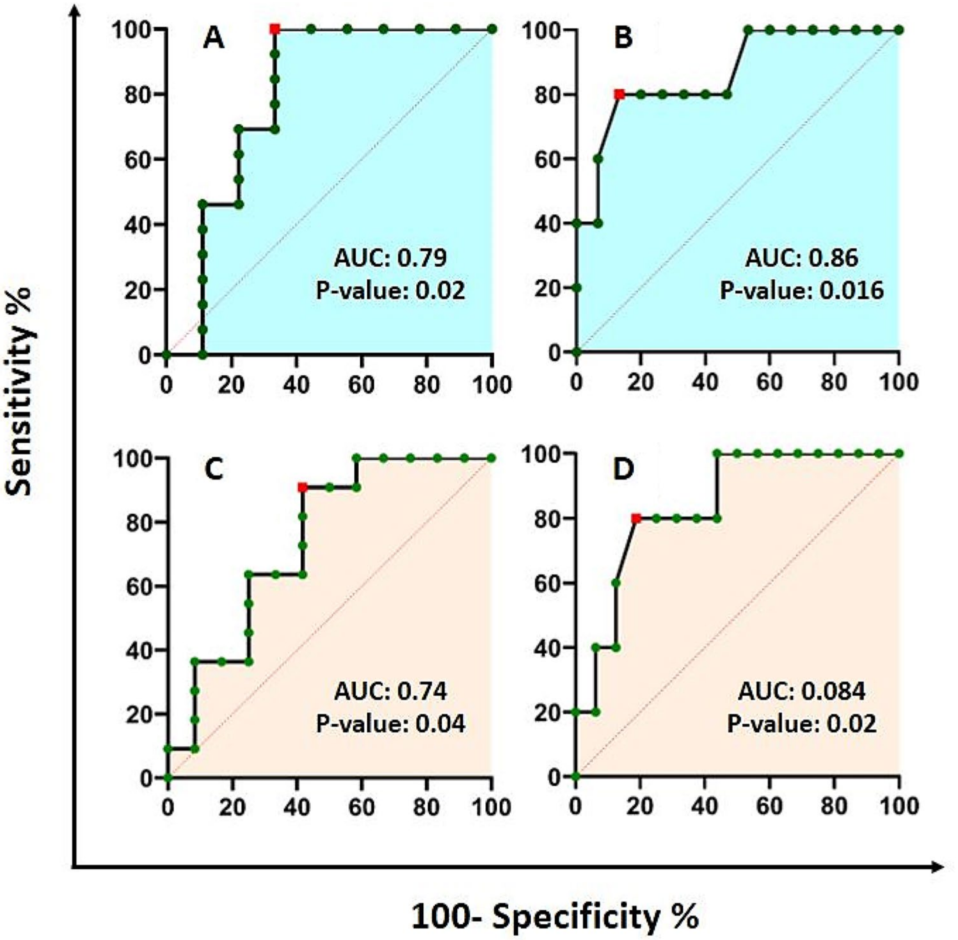


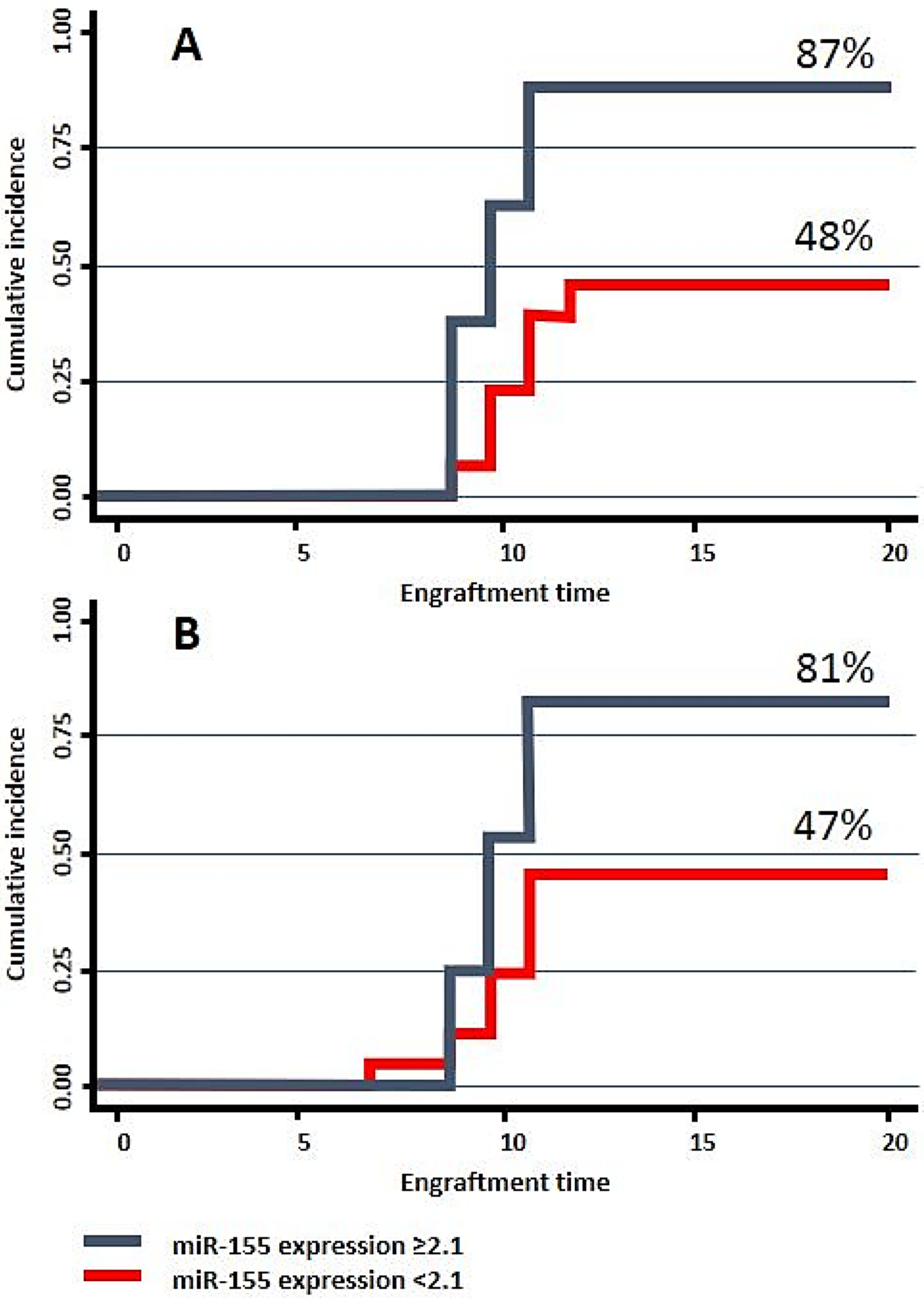
Fig. 4 ROC curves for miR-155 PP in both groups of CD34$^+$ CPK $\geq 3.5\times10^6$/kg and $<3.5\times10^6$/kg for prediction of platelet and neutrophil engraftment. **a**, **b** In $\geq 3.5\times10^6$/kg and CPK $<3.5\times10^6$/kg, respectively, for platelet engraftment. **c**, **d** In $\geq 3.5\times10^6$/kg and CPK $<3.5\times10^6$/kg, respectively, for neutrophil engraftment

and can be compensatory for the prediction via CD34$^+$ cells. In accordance with our study, Weaver et al. have introduced CD34$^+$ CPK value higher than 5×10^6/kg as a reliable indicator of successful platelet and neutrophil engraftment [23]. To the best of our knowledge, although several studies have shed light on the significance of CD34$^+$ CPK as a predictor of engraftment, our study indicated that the lower value of this factor could not be a good candidate for the prediction of engraftment. It seems that there are some hematopoiesis repressors, such as receptor-type protein tyrosine phosphatase-sigma (PTPσ) and intracellular adapter protein, Lnk, that probably weaken the positive correlation of CD34$^+$ cells in lower counts with platelet and neutrophil engraftment [24, 25].

Our study revealed that chemotherapy or treatment courses after diagnosis correlate positively with platelet engraftment time, but it has been shown in murine models that chemotherapy in addition to its adverse effects on HSCs, could reduce the hematologic reconstitution post-transplantation [26] and postpone the engraftment by damaging the nervous system in BM [27]. Maybe the difference in the samples of our study (human sapience), chemotherapy regimen, or the type of studied malignancies in the present study seems to be different from other studies and this was the reason for some contradictory results, but in similarity with the previous studies, it has been indicated that those patients without complete remission before AHSCT, have a poorer outcome in engraftment [28]. Our analyses showed that high doses of G-CSF for mobilization (or TDIG) can lead to longer engraftment. Some reports indicated that long-term and high-dose G-CSF could result in hyperplasia of the BM cells and mobilization of mature leukocytes to the blood, events that lead to weakened engraftment [29]. In addition, engraftment after transplantation of 2.5×10^6/kg of CD34$^+$ cells resulted from a short mobilization period is faster than transplantation of 5×10^6/kg CD34$^+$ cells, resulted from the long mobilization period [3]. Thus, based on other studies, we suggest the injection of G-CSF in 40/μg/kg/day up to 4 days for better engraftment [3, 30]. Various conditioning regimens show the different effects on engraftment [31, 32]. The present study showed the number of conditioning days result in the delay of neutrophil engraftment. In accordance, Reshef et al. have shown the low intensity of conditioning results in faster engraftment [33].

Overall, the results of the present study introduced miR-155 as a valuable predictor of early platelet and neutrophil

Fig. 5 Cumulative incidence of platelet (**a**) and neutrophil (**b**) engraftment based on miR-155 PP expression categorized by threshold of 2.1



engraftment in MM and lymphoma patients undergoing AHSCT. The CD34$^+$ CPK in the population of more than 3.5×10^6/kg is a predictor of the engraftment, and if a patient failed to collect this amount of CD34$^+$ cell, this variable lost its value. However, even in these cases, miR-155 expression level could be used as an appropriate predictor of AHSCT success.

Conclusion

Early prediction of the engraftment outcome is a desire for both physicians and patients, as it not only could expand the means of intervention but also can guide in prioritizing therapies. For complex and multifactorial outcomes like engraftment, it is better to implicate a combination of biomarkers to achieve the best results in most patients. Given the direct regulatory role of microRNAs on hematopoiesis and the correlation of miR-155 with the engraftment, we suggest the utilization of this microRNA for prediction of AHSCT outcome.

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Declarations

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

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