New Proposed Guidelines for Early Identification of Successful Myeloid and Erythroid Engraftment in Hematopoietic Stem Cell Transplantation

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Background: After hematopoietic stem cell transplantation (HSCT), early detection of engraftment would be of critical value for clinicians. The aim of this study was to identify faster parameters for engraftment. **Methods:** We evaluated blood cell parameters including complete blood count (CBC), differential counts, and various reticulocyte parameters in 115 patients who received HSCT (allogeneic, $n = 93$; autologous, $n = 22$) in the purpose of identifying possible improved laboratory guidelines for engraftment prediction. **Results and Conclusion:** Days to white blood cell (WBC) count over 100 cells/ μ l with more than two-fold increase from nadir after transplantation (proposed new WBC guideline) preceded absolute neutrophil count (ANC) >500 cells/ μ l by 1.7 days. Among ery-

throid parameters, the earliest marker for erythroid engraftment was high light scattering reticulocytes (HLR) >0.1 (proposed new red blood cell guideline), which preceded reticulocyte counts (RET) >1% and immature reticulocyte fraction >0.5 by 3.9 and 1.6 days, respectively. Among the clinical parameters compared, those with statistically significant influence on myeloid engraftment were donor type ($P = 0.009$) and conditioning intensity ($P = 0.009$). As for erythroid recovery, ABO incompatibility was the only significant factor. In conclusion, the new guidelines may ensure engraftment several days earlier than the conventional parameters, which may help clinicians for decision-making on rescue therapy earlier. *J. Clin. Lab. Anal.* 28:469–477, 2014. © 2014 Wiley Periodicals, Inc.

Key words: erythroid engraftment; hematopoietic stem cell transplantation; HLR; myeloid engraftment; reticulocytes

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is currently an important curative option in the management of various medical conditions, predominantly hematologic malignancy. During HSCT, native hematopoietic cells of recipients are replaced by donor stem cells, which then populate the marrow and become responsible for hematopoiesis. Consequently, in the immediate period posttransplantation manifested by profound and prolonged myelosuppression, patients are exposed to high risk of opportunistic infections as well as other complications from the lack of properly functioning blood elements. Various types of HSCT, based on donor-recipient HLA compatibility, the origin of donor stem cells, and the type of conditioning regimen, can all affect the time

for immune reconstitution. Therefore, prediction of the individual time required to recover from this absence of hematopoiesis that demands extensive support would be of critical value for clinicians. For these reasons, one of the key roles played by laboratory tests in the immediate posttransplant period would be an early detection of adequate engraftment.

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After a successful transplantation procedure, infused stem cells are known to populate the marrow and reinitiate the hematopoietic process in a predictable pattern. Typically, the first cells to engraft are monocytes followed by granulocytes, platelets, and natural killer cells (1). The conventionally used marker for myeloid engraftment is the first day that the number of absolute neutrophil count (ANC) is $>500/\mu\text{l}$. While this approach is widely used nowadays, it does come with some significant limitations. It takes approximately 2 weeks for the ANC to rise above the accepted cut off, and in some cases, this delay may be even longer, although the use of lower cut-off for myeloid recovery, $\text{ANC} >100/\mu\text{l}$, had also been validated (2). Moreover, reports have shown cases dependent on red blood cell (RBC) transfusion with delayed erythropoiesis following successful myeloid engraftment after transplants from ABO-incompatible donors (3–5). According to Lee et al., 10 out of 35 patients receiving ABO-incompatible graft developed pure red cell aplasia (PRCA) (6). This phenomenon seems to affect the recipients of blood type O receiving grafts from other blood types, in particular (7). Of some biological parameters tested for early predictors of hematopoietic recovery, peripheral blood (PB) reticulocyte count (RET) $>1.0\%$ has been the most attractive surrogate marker (8, 9), but it is frequently too late to consider as an early engraft marker. For megakaryocyte engraftment, the conventional marker is platelet count $>20,000/\mu\text{l}$ for three successive days independent of transfusion although in some patients receiving preemptive platelet supplements, the time for megakaryocytic engraftment may be underestimated.

Over the past decade, the technology available in automated hematology cell analyzers has evolved significantly with both the creation of several new cellular parameters and improvements in the accuracy of the traditional parameters. An improvement of modern instruments is extended counting time and data fusion software, specifically targeting severe leukopenic samples like in the immediate post-HSCT period. However, to the best of our knowledge, no studies have yet been published in the literature focusing on how this improved accuracy has impacted the value of the complete blood count with differential (CBC-diff) in the early identification of bone marrow engraftment.

Another improvement of modern instruments is the creation of new parameters as the result of recent improvements in instrument hardware (better lasers and improved flow cells) and algorithms, allowing diminished impact of interfering substances, such as cryoglobulins, and better resolution of various cell populations in the blood. Since these new parameters are directly correlated to the presence of immature elements in the blood, their main proposed application has been evaluating the level

of hematopoiesis. Previous studies have demonstrated earlier increases in both immature reticulocyte fraction (IRF) and immature platelet fraction (IPF) by 48 hr on average prior to days required for ANC to reach the cut-off value of 500 per microliter in patients with successful engraftment (2, 9, 10). However, these studies did not address the question of possible false-positive results in the cases without engraftment using that specific cut-off value and did not identify lineage-specific engraftment.

The objective of this study is to identify possible improved laboratory guidelines for confirming engraftment post-HSCT and identify lineage-specific parameters that could predict myeloid and erythroid engraftments, separately. Another objective of this study is to analyze the effect of various clinical characteristics to the myeloid and erythroid engraftment day.

MATERIALS AND METHODS

Patient Selection and Data Collection

One-hundred and fifteen patients who received HSCT at Seoul St. Mary's Hospital, Seoul, Korea between December 2011 and April 2012 were included for this study. Data on all the CBC-diff studies performed every work days were collected. Basic characteristics of patients and transplant-related factors are described in Table 1. Briefly, 80% of all patients underwent allogeneic HSCT from conventional ($n = 64$) or alternative ($n = 29$) donors at a median age of 32.5 years (range, 1–65), while the rest 22 patients with a median age of 45.5 years (range, 1–62) received high-dose chemotherapy followed by autologous stem cell reinfusion. Majority of the patients used PB as stem cell source and the median $\text{CD}34^+$ cells infused was $4.23 \times 10^6/\text{kg}$ (range 0.78–42.02) for allogeneic HSCT and $4.72 \times 10^6/\text{kg}$ (range 0.31–29.87) for autologous HSCT, respectively. After graft infusion, patients received granulocyte colony-stimulating factor (Lenograstim) at a dose of $5 \mu\text{g}/\text{kg}/\text{day}$ from D7 until the day of $\text{ANC} >3,000/\text{mm}^3 \times 10^6$. The CBC-diff as well as the “research use only” parameters such as the percentage and absolute number of high light scattering reticulocytes (HLR) results were obtained using automatic hematology analyzer (DxH 800, Beckman Coulter Inc., Miami, FL). HLR, the ratio of HLR (in region 3–10) count to the total RBC count, indicates reticulocytes containing much amount of nucleic acid, therefore, very young reticulocytes (11–12). We organized the data in an ascending sequence based on the posttransplant date in which the test was performed. In this way, we could easily visualize trends in each patient counts as they evolved posttransplant. This study was fully approved by our institutional review board.

TABLE 1. Characteristics of Patients ($n = 115$)

Variables	Allogeneic HSCT ($n = 93$)	Autologous HSCT ($n = 22$)
Median age, years (range)	32.5 (1–65)	45.5 (1–62)
Sex: male/female	41/52	14/8
Diagnosis		
ALL	20	2
AML	36	6
MDS/CMML ($n = 1$)/JMML ($n = 2$)	17	–
MM	1	10
SAA/PRCA ($n = 1$)/FA ($n = 1$)	16	–
Others ^a	3	4
Conditioning regimen		
Nonmyeloablative	44	–
Myeloablative, non-TBI	18	–
Myeloablative, TBI $\leq$ 1200 cGy	12	–
Myeloablative, TBI >1200 cGy	19	–
High-dose radio/chemotherapy ^b	–	22
Graft source		
BM/PB/cord blood	22/68/3	22
Stem cell type		
Conventional: MSD/MUD	39/29	–
Alternative:	9/13/3	–
MMUD/haploidentical/cord blood		
Autologous	–	22
ABO incompatibility		
Match	49	–
Major mismatch	20	–
Donor/recipient: A or B/O	8	–
Minor mismatch	24	–
ATG		
Yes	65	–
No	28	22
Median MNC $\times 10^8$ /kg (range)	7.60 (0.28–46.38)	7.62 (0.03–21.26)
Median CD34 + cells $\times$ 10^8 /kg (range)	4.23 (0.78–42.04)	4.72 (0.31–29.87)

^aNon-Hodgkin lymphoma ($n = 5$); neuroblastoma ($n = 1$); primary neuroectodermal tumor ($n = 1$).

^bConditioning regimen used for autologous HSCT included high-dose melphalan ($n = 10$), busulfan+melphalan+thiotepa ($n = 2$), total body irradiation+ cytarabine+ melphalan ($n = 8$), and carboplatin+VP+thiotepa ($n = 2$).

HSCT, hematopoietic stem cell transplantation; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; MM, multiple myeloma; SAA, severe aplastic anemia; PRCA, pure red cell aplasia; FA, Fanconi's anemia; BM, bone marrow; PB, peripheral blood; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; ATG, antithymocyte globulin; MNC, mononuclear cell.

Identification of a New Myeloid Engraftment Parameter

Using the excel sheet with all patient data, we identified the patients that had a successful myeloid engraftment, based on achievement of an ANC >500 cells/ μ l, and highlighted in the file the posttransplant day when that value was achieved. Out of all 115 cases in the study, three cases had a failed engraftment and never achieved ANC >500 cells/ μ l and two other cases did not reach a nadir with the ANC counts maintained above 500/ μ l throughout the recovery period after infusion day due to donor granulocytes infusions (DGI). The mean white blood cell (WBC) counts and ANC from D0 among 112 cases, excluding the patients with graft rejection ($n = 3$), are displayed in Figure 1. We then manually reviewed other daily CBC parameters performed from D0, seeking for earlier WBC parameter after nadir. All successful HSCT patients should reach that count earlier or at the same day to the day of ANC >500 cells/ μ l, and those destined for primary engraftment failure never reach that level. The value of the parameter within the next few days, at least on the next day of engraftment, should be increased than that of the engraftment day. Based on this review, we developed a new myeloid engraftment parameter.

Identification of a New Erythroid Engraftment Guideline

Using the excel sheet with all patient data, we identified all patients who achieved a successful erythroid engraftment based on RET >1%, and highlighted in the file the posttransplant day when that value was achieved. Of all cases in the study ($n = 115$), three cases failed to achieve erythroid engraftment and never achieved RET >1%, within the observation study period.

We then manually reviewed other RBC parameters performed before the day of RET >1% achievement, searching for new erythroid parameter showing earlier increase after nadir. RET, mean reticulocyte volume (MRV), IRF, HLR, and absolute HLR count (HLR#) after HSCT were examined. The MRV greater than 110 fL was excluded from the candidate new erythroid engraftment parameters because it was greater than 110 fL from D0 in 37 out of 112 cases (33.0%) showing engraftment. All successful HSCT patients should reach that count earlier or at the same day to the day of RET >1%, and the failed HSCT patients never reached that level. The parameter's value and RET or immature reticulocyte count of next few days, at least next day of engraftment, should be increased than that of the engraftment day. Based on this review, we developed a new successful erythroid engraftment guideline.

Analysis of the performance of the new myeloid and erythroid engraftment parameters in predicting engraftment.

Fig. 1. Mean values of total white blood cell (WBC, black line) and absolute neutrophil count (ANC, gray line) after HSCT ($n = 112$): cases with donor granulocyte infusion excluded ($n = 3$). The mean nadir period begins to recover from D9. X-axis and Y-axis represent days from transplant and cell counts ($\times 10^2/\mu\text{l}$), respectively.

The engraftment days of all HSCT patients using the new myeloid and erythroid engraftment parameter were compared to the days the ANC reached currently used cut-off level ($>500/\mu\text{l}$) and RET $>1\%$, respectively. Using the cases of sustained engraftment, we calculated the average differences of posttransplant days required to reach each of the two new guidelines for predicting engraftment. We then compared the time to newly identified guidelines according to transplant-related clinical variables.

Statistical Analysis

We used Student's t -test or ANOVA to test for the effect of transplant-related clinical variables on the engraftment day using our new engraftment guidelines. All analyses were performed with MedCalc v11.2 (Mariakerke, Belgium).

RESULTS

New WBC Guideline for Early Prediction of Successful Myeloid Engraftment

The mean values of WBC and ANC of 112 patients with primary engraftment after HSCT revealed that the

mean nadir period was until D8 and began to recover from D9 (Fig. 1) with variation from patient to patient. The mean time of ANC $>500/\mu\text{l}$ was 12.1 ± 2.2 days after infusion and the recovery began a few days before. The new successful myeloid engraftment guideline was:

- WBC >100 cells/ μl **and**
- WBC $>$ two-fold increase from the lowest value after transplant (nadir)

In the remainder of this article, we will refer to this rule as “new WBC guideline”. The performance of the new WBC guideline in predicting myeloid engraftment after HSCT was as followings:

- Sensitivity and positive predictive value: All of the patients (100%) recovered to ANC $>500/\mu\text{l}$ level and showed sustained myeloid engraftment.
- Specificity and negative predictive value: None of the three cases (100%) with myeloid engraftment failure achieved the new WBC guideline.
- The mean time to achievement of new WBC guideline after HSCT in 110 patients (excluding three patients with engraftment failure and two patients without nadir

TABLE 2. Time to Engraftment Using Different Parameters ($n = 110$)^a

Clinical variables	Days after HSCT (mean $\pm$ SD)									
	WBC > 100	ANC > 500	MRV $\geq$ 110fL	Platelet ^b > 20,000	RET > 1%	IRF $\geq$ 0.5	HLR > 0.1	HLR# > 0.003	HLR# > 0.005	
All patients	10.4 $\pm$ 1.8	12.1 $\pm$ 2.2	11.4 $\pm$ 3.5	16.3 $\pm$ 15.4	18.1 $\pm$ 14.4	15.3 $\pm$ 12.6	13.6 $\pm$ 8.4	14.5 $\pm$ 12.6	15.2 $\pm$ 12.7	
Disease										
ALL	10.0 $\pm$ 1.4	11.9 $\pm$ 1.9	12.1 $\pm$ 4.8	18.4 $\pm$ 11.0	18.3 $\pm$ 11.9	14.0 $\pm$ 4.1	15.8 $\pm$ 11.7	15.6 $\pm$ 11.7	16.3 $\pm$ 11.6	
AML	10.7 $\pm$ 2.0	12.6 $\pm$ 1.9	11.4 $\pm$ 2.3	13.9 $\pm$ 6.3	18.6 $\pm$ 12.4	15.9 $\pm$ 11.3	13.1 $\pm$ 4.5	13.1 $\pm$ 4.5	14.4 $\pm$ 5.7	
MDS/CMML ($n = 1$)/JMML ($n = 2$)	11.0 $\pm$ 1.8	12.5 $\pm$ 3.1	10.3 $\pm$ 2.0	22.4 $\pm$ 34.9	15.0 $\pm$ 5.6	15.4 $\pm$ 8.0	11.6 $\pm$ 5.7	12.2 $\pm$ 5.6	13.1 $\pm$ 5.4	
MM	9.5 $\pm$ 1.4	11.0 $\pm$ 1.6	10.0 $\pm$ 1.9	10.5 $\pm$ 1.0	11.6 $\pm$ 1.8	10.6 $\pm$ 1.8	11.1 $\pm$ 2.0	11.1 $\pm$ 2.0	11.1 $\pm$ 2.0	
AA/PRCA ($n = 1$)/FA ($n = 1$)	10.6 $\pm$ 2.0	12.0 $\pm$ 2.4	12.7 $\pm$ 7.6	20.0 $\pm$ 9.9	25.7 $\pm$ 29.3	21.5 $\pm$ 27.9	17.4 $\pm$ 15.4	23.6 $\pm$ 30.2	23.8 $\pm$ 30.1	
Others ^c	9.3 $\pm$ 1.4	10.9 $\pm$ 1.6	10.2 $\pm$ 0.8	8.5 $\pm$ 0.6	15.4 $\pm$ 4.9	9.7 $\pm$ 1.9	10.7 $\pm$ 2.2	10.7 $\pm$ 2.2	10.7 $\pm$ 2.2	
Donor type										
Conventional	10.7 $\pm$ 1.9	12.1 $\pm$ 2.0	11.1 $\pm$ 3.7	19.0 $\pm$ 19.3	18.4 $\pm$ 16.1	16.4 $\pm$ 15.0	14.2 $\pm$ 10.4	15.7 $\pm$ 16.3	16.3 $\pm$ 16.2	
Alternative	10.5 $\pm$ 1.6	12.7 $\pm$ 2.3	12.4 $\pm$ 4.2	13.7 $\pm$ 7.5	18.1 $\pm$ 14.8	15.9 $\pm$ 11.0	13.7 $\pm$ 6.1	13.7 $\pm$ 6.1	14.5 $\pm$ 6.5	
Autologous	9.3 $\pm$ 1.7	11.3 $\pm$ 2.2	10.5 $\pm$ 2.1	10.9 $\pm$ 2.2	17.1 $\pm$ 7.9	11.0 $\pm$ 2.7	11.7 $\pm$ 2.5	11.7 $\pm$ 2.5	12.9 $\pm$ 5.1	
Stem cell source										
BM	10.9 $\pm$ 1.8	12.7 $\pm$ 2.0	11.6 $\pm$ 2.6	23.4 $\pm$ 31.1	26.0 $\pm$ 24.4	21.9 $\pm$ 24.5	16.4 $\pm$ 12.7	20.6 $\pm$ 25.3	21.6 $\pm$ 25.1	
PB	10.2 $\pm$ 1.8	11.9 $\pm$ 2.1	11.2 $\pm$ 3.8	14.3 $\pm$ 7.6	16.0 $\pm$ 10.3	13.4 $\pm$ 7.0	12.8 $\pm$ 6.8	12.9 $\pm$ 6.8	13.5 $\pm$ 7.1	
Cord blood	12.0 $\pm$ 0	16.5 $\pm$ 2.1	12.5 $\pm$ 0.7	29.5 $\pm$ 17.7	29.5 $\pm$ 16.2	27.0 $\pm$ 19.8	23.5 $\pm$ 14.8	23.5 $\pm$ 14.8	24.0 $\pm$ 14.1	
Conditioning intensity										
Myeloablative	10.7 $\pm$ 1.7	12.5 $\pm$ 1.9	11.6 $\pm$ 3.9	19.9 $\pm$ 22.1	19.2 $\pm$ 17.8	17.3 $\pm$ 17.2	14.6 $\pm$ 9.5	16.4 $\pm$ 17.6	17.2 $\pm$ 17.4	
Nonmyeloablative	10.5 $\pm$ 1.8	12.0 $\pm$ 2.3	11.6 $\pm$ 4.0	14.8 $\pm$ 7.1	17.4 $\pm$ 13.2	15.2 $\pm$ 9.4	13.6 $\pm$ 9.0	13.8 $\pm$ 9.0	14.2 $\pm$ 9.2	
High-dose radio/chemotherapy ^d	9.4 $\pm$ 1.7	11.3 $\pm$ 2.2	10.5 $\pm$ 2.1	10.9 $\pm$ 2.2	17.1 $\pm$ 7.9	11.0 $\pm$ 2.7	11.7 $\pm$ 2.5	11.7 $\pm$ 2.5	12.9 $\pm$ 5.2	
Use of ATG										
Yes	10.4 $\pm$ 1.8	12.1 $\pm$ 2.1	11.8 $\pm$ 4.5	16.3 $\pm$ 9.2	16.5 $\pm$ 11.7	14.6 $\pm$ 8.1	13.2 $\pm$ 8.1	13.5 $\pm$ 8.0	14.0 $\pm$ 8.1	
No	10.3 $\pm$ 1.9	12.1 $\pm$ 2.2	10.8 $\pm$ 2.1	16.3 $\pm$ 20.8	20.0 $\pm$ 17.0	16.1 $\pm$ 16.8	14.1 $\pm$ 8.9	15.7 $\pm$ 16.8	16.6 $\pm$ 16.9	
ABO incompatibility										
Compatible	11.1 $\pm$ 1.6	12.8 $\pm$ 2.2	10.7 $\pm$ 1.5	18.7 $\pm$ 21.7	15.5 $\pm$ 5.2	15.3 $\pm$ 8.8	12.4 $\pm$ 4.0	12.3 $\pm$ 3.9	13.0 $\pm$ 3.9	
Minor incompatibility	9.9 $\pm$ 1.6	11.7 $\pm$ 1.7	12.5 $\pm$ 5.8	16.1 $\pm$ 8.0	14.3 $\pm$ 5.4	12.7 $\pm$ 4.3	12.0 $\pm$ 4.5	12.5 $\pm$ 4.3	12.8 $\pm$ 4.2	
Major incompatibility	10.2 $\pm$ 2.1	11.6 $\pm$ 2.2	13.0 $\pm$ 5.7	16.0 $\pm$ 7.1	29.6 $\pm$ 30.0	22.5 $\pm$ 25.6	20.7 $\pm$ 17.1	25.2 $\pm$ 27.5	25.9 $\pm$ 27.4	
Autologous	9.4 $\pm$ 1.7	11.3 $\pm$ 2.2	10.5 $\pm$ 2.1	10.9 $\pm$ 2.2	17.1 $\pm$ 7.9	11.0 $\pm$ 2.7	11.7 $\pm$ 2.5	11.6 $\pm$ 2.5	12.9 $\pm$ 5.2	

^aThe cases with engraftment failure ($n = 3$) and with high value from D0 without nadir period ($n = 2$) were excluded.^bFour patients with missing value.^cNon-Hodgkin lymphoma ($n = 4$); neuroblastoma ($n = 1$); primary neuroectodermal tumor ($n = 1$).^dConditioning regimen used for autologous HSCT included high-dose melphalan ($n = 10$), busulfan+melfalan+thiotepa ($n = 2$), total body irradiation+cytarabine+melfalan ($n = 8$), and carboplatin+VP+thiotepa ($n = 2$).

HSCT, hematopoietic stem cell transplantation; SD, standard deviation; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; MM, multiple myeloma; SAA, severe aplastic anemia; PRCA, pure red cell aplasia; FA, Fanconi's anemia; BM, bone marrow; PB, peripheral blood; ATG, antithymocyte globulin

Fig. 2. Results of immature reticulocyte fraction (IRF) and HLR after HSCT in two cases who showed successful granulocytic engraftment but never achieved reticulocyte (RET) >1%. Case 77 showed no increase in any erythroid engraftment parameters until relapse of acute myeloid leukemia at D37. Case 26 showed successful erythroid engraftment using the new RBC guideline, HLR >0.1 on D19 and IRF >0.5 and HLR# >0.005 on D20. Although RET did not reach to 1%, it began to increase from 0.27% on D19, 0.51% on D20, and 0.78% on D21. The patient died on D21 due to pulmonary aspergillosis.

period) was 10.4 ± 1.8 day, while that of $\text{ANC} > 500/\mu\text{l}$ was 12.1 ± 2.2 days (Table 2). Seventy six cases (67.3%) of 113 cases without receiving DGI showed earlier granulocytic engraftment using new WBC guideline than using $\text{ANC} > 500/\mu\text{l}$ from 1 to 9 days. The new WBC guideline could confirm successful engraftment as early as on day 8 in 20 cases, but the $\text{ANC} > 500/\mu\text{l}$ could confirm that in only two cases. The mean difference between engraftment day using new WBC guideline and the platelet engraftment day was 6.0 ± 15.1 days.

New RBC Guideline for Early Prediction of Successful Erythroid Engraftment

RET >1% was not achieved in five cases of all 115 cases (4.3%) including three myeloid graft failure cases. The other two cases showed successful granulocytic engraftment but never achieved RET >1% after HSCT. The kinetics of RET and other erythroid parameters in these two patients from D0 is shown in Figure 2. Erythroid parameters other than RET in case number 26 showed increases on D19 to D20 but further follow-up was prohibited by death on D21. In case number 77, all erythroid parameters failed to achieve the cut-off values before the date of relapse of acute myeloid leukemia (AML; D37). Two cases showed high RET value from D0. The mean values of all the erythroid parameters in the rest of the 107 cases showing RET >1% after HSCT is described in Table 3.

The earliest marker for erythroid engraftment was HLR >0.1 with the mean value of 13.6 ± 8.4 days after graft infusion. The mean engraftment days for other parameters were 18.1 ± 14.4 days for RET >1%, 15.3 ± 12.6 days for IRF ≥ 0.5 , 14.5 ± 12.6 days for HLR# >0.003, and 15.2 ± 12.7 days for HLR# >0.005. The HLR >0.1 preceded RET >1% and IRF >0.5 by 3.9 ± 9.9 and 1.6 ± 11.7 days, respectively (Table 3). Of note, IRF on the next day of IRF >0.5 achievement dropped to less than 0.5 in 19 cases (16.5%) while decrease of HLR to less than 0.1 did not occur in any cases, which suggests that HLR >0.1 is not only a earliest predictor for erythroid engraftment but also provides a stable guideline. Therefore, we decided to use HLR >0.1 as a new erythroid engraftment guideline. The performance of the new RBC guideline in predicting erythroid engraftment after HSCT was as follows:

- Sensitivity and positive predictive value: All cases ($n = 110$) who achieved successful erythroid engraftment using RET >1% showed earlier HLR >0.1, which preceded 3.9 ± 9.9 days.
- Specificity and negative predictive value: All of the three cases who had failed to granulocytic engraftment did not show successful erythroid engraftment using both RET >1% and HLR >0.1. One of the two cases (50%) who had successful granulocytic engraftment but did not show successful erythroid engraftment using RET >1% showed successful erythroid engraftment

TABLE 3. The Mean Value of Erythroid Parameters and the Mean Differences between Each Parameter in 107 Cases Showing RET >1% after HSCT. The Earliest Marker of Erythroid Engraftment was HLR >0.1. The Difference between the Day of HLR >0.1 and RET >1% was 3.9 ± 9.9 day, and between the Day of HLR >0.1 and IRF >0.5 was 1.6 ± 11.7 Day

Erythroid parameters	Days posttransplantation (mean $\pm$ SD)	Differences between parameters	Days posttransplantation (mean $\pm$ SD)
RET > 1%	18.1 $\pm$ 14.4	RET – IRF	3.1 $\pm$ 8.8
IRF $\geq$ 0.5	15.3 $\pm$ 12.6	RET – HLR > 0.1	3.9 $\pm$ 9.9
HLR > 0.1	13.6 $\pm$ 8.4	RET – HLR# > 0.003	3.5 $\pm$ 6.5
HLR# > 0.003	14.5 $\pm$ 12.6	RET – HLR# > 0.005	2.8 $\pm$ 5.7
HLR# > 0.005	15.2 $\pm$ 12.7	IRF – HLR > 0.1	1.6 $\pm$ 11.7
		IRF – HLR# > 0.003	0.8 $\pm$ 8.9
		IRF – HLR# > 0.005	0.0 $\pm$ 8.7

SD, standard deviation; RET, reticulocyte count; IRF, immature reticulocyte fraction; HLR, high light scattering reticulocytes (the ratio of high light scattering reticulocyte count to the total RBC count); HLR#, absolute HLR count.

using HLR >0.1 (Fig. 2). This case was regarded as a false-negative case using RET >1%.

- The mean difference between engraftment day using the ANC >500/ μ l and the RET >1% was 6.0 ± 14.2 days, but that between the new WBC guideline and HLR >0.1 was 3.3 ± 8.2 days (Table 2). These results indicate that HLR >0.1 allows us to confirm erythroid engraftment at much similar time after HSCT to granulocytic engraftment. The mean difference between engraftment day using HLR >0.1 and the platelet engraftment day was 2.7 ± 16.0 days.

Analysis of Clinical Factors Influencing Engraftment Indicated by Different Parameters

Table 4 illustrates the mean differences in the days required to reach the each cut off of various parameters indicating engraftment according to transplant-related clinical factors. The cases of engraftment failure ($n = 3$) and cases with DGI ($n = 2$) were excluded. Disease, stem cell type, conditioning intensity, and ABO incompatibility had significant influence on the time to engraftment according to the new WBC guideline. Specifically, the pairs with statistical significance ($P < 0.05$) by multiple comparisons (t -test) were myeloid vs. lymphoid malignancy ($P = 0.048$), HSCT with HLA-matched donor vs. autologous HSCT ($P = 0.007$), myeloablative vs. reduced intensity conditioning ($P = 0.007$), and ABO-matched vs. autologous HSCT ($P = 0.001$) for granulocytic engraftment. Whereas, the only significant factor affecting the time to achieve erythroid engraftment by the new RBC guideline was ABO incompatibility. Although significance was not shown, the time to erythroid engraftment from conventional donor was 14.2 ± 10.4 days and that after autologous HSCT, while the time to erythroid engraftment after myeloablative conditioning was 14.6 ± 9.5 days and that after autologous HSCT was 11.7 ± 2.5 days. HSCT

from ABO-mismatched donor showed delayed granulocytic and erythroid engraftment than autologous HSCT ($P = 0.003$). By multiple comparisons, significant differences in erythroid engraftment days (mean $\pm$ SD) were shown between ABO major incompatibility (20.7 ± 17.1) and ABO minor incompatibility (12.0 ± 4.5 ; $P = 0.004$), ABO compatibility (12.4 ± 4.0) and ABO major incompatibility ($P = 0.001$), and ABO major incompatibility and autologous HSCT (11.7 ± 2.5 ; $P = 0.004$). Of note, the erythroid engraftment days from ABO major mismatched donors to blood type O recipients were much more delayed to 45.9 ± 37.8 days and variable from 11 to 116 days.

DISCUSSION

Recent research efforts have been made to identify better laboratory tests for the monitoring of post-HSCT patients, with the goal of allowing a faster confirmation of successful engraftment. The quality of supportive care provided to patients undergoing HSCT transplantation is constantly evolving, and the best results are achieved with targeted support to specific components of posttransplant care. This approach is critical to optimize results by decreasing patient's exposure to the risks and side effects associated with supportive care (13–15). Therefore, a clear understanding of the engraftment status of a post-HSCT patient, specific to each of the hematopoietic elements (myelopoiesis, erythropoiesis, and thrombopoiesis) can be extremely helpful in allowing clinicians to deploy each of the supportive care options only when they are necessary.

Our study successfully described a new WBC guideline that would allow clinicians to confirm successful myeloid engraftment on average 1.7 days sooner than the current standard of care, using the ANC value. For myeloid engraftment detection, we propose a simple guideline based on one of the most widely used parameters in medicine,

TABLE 4. Effect of Clinical Factors on the Time to Achieve the New WBC ($>100/\mu\text{l}$ Plus $>$ Two-Fold Increase from Nadir after D0) and RBC Guidelines (HLR >0.1) ($n = 110$)^a

Clinical factors	N	New WBC guideline	ANOVA	New RBC guideline	ANOVA
		Days, mean $\pm$ SD (range)	P	Days, mean $\pm$ SD (range)	P
Disease type					
Myeloid malignancy	57	10.6 $\pm$ 1.9 (8–15)	0.046	12.9 $\pm$ 4.9 (6–34)	0.328
Lymphoid malignancy	37	9.8 $\pm$ 1.4 (8–13)		13.8 $\pm$ 9.1 (7–64)	
Others	2	10.5 $\pm$ 1.9 (8–16)		16.4 $\pm$ 14.7	
Stem cell type					
Conventional	61	10.7 $\pm$ 1.9 (8–16)	0.009	14.2 $\pm$ 10.4 (6–64)	0.564
Alternative	27	10.5 $\pm$ 1.6 (8–13)		13.7 $\pm$ 6.1 (6–34)	
Autologous	22	9.3 $\pm$ 1.7 (8–15)		11.7 $\pm$ 2.5 (7–19)	
Graft source					
BM	20	10.9 $\pm$ 1.8	0.140	16.4 $\pm$ 12.7	0.053
PB	89	10.2 $\pm$ 1.8		12.8 $\pm$ 6.8	
Cord blood	2	12.0 $\pm$ 0		23.5 $\pm$ 14.8	
Conditioning intensity					
Myeloablative	46	10.7 $\pm$ 1.7 (8–15)	0.009	14.6 $\pm$ 9.5 (6–64)	0.498
Nonmyeloablative	42	10.5 $\pm$ 1.8 (8–16)		13.6 $\pm$ 9.0 (6–63)	
High-dose radio/chemotherapy	22	9.4 $\pm$ 1.7 (8–15)		11.7 $\pm$ 2.5 (7–19)	
Use of ATG			0.490		0.548
Yes	60	10.4 $\pm$ 1.8 (8–16)	0.001	13.2 $\pm$ 8.1 (6–63)	0.001
No	50	10.3 $\pm$ 1.9 (8–15)		14.1 $\pm$ 8.9 (7–64)	
ABO incompatibility					
Compatible	49	11.1 $\pm$ 1.6 (8–15)	0.001	12.4 $\pm$ 4.0 (7–19)	0.001
Minor incompatibility	21	9.9 $\pm$ 1.6 (8–13)		12.0 $\pm$ 4.5 (7–29)	
Major incompatibility	19	10.2 $\pm$ 2.1 (8–16)		20.7 $\pm$ 17.1 (9–64)	
autologous	22	9.4 $\pm$ 1.7 (8–15)		11.7 $\pm$ 2.5 (6–34)	

^aThe cases with engraftment failure ($n = 3$) and with high value from D0 without nadir period ($n = 2$) were excluded.

WBC, white blood cell; ANOVA, analysis of variance; RBC, red blood cell; SD, standard deviation; BM; bone marrow; PB, peripheral blood; ATG, antithymocyte globulin

the WBC count. After HSCT, these counts are monitored continuously, usually drop for the first three to four days, reach a lowest value called nadir, and start to rise again as the engrafted bone marrow starts to produce new WBCs. We demonstrated once these values rise above twice the nadir, and above a cut-off point of 100 cells/ μl , this was a clear indication that a successful engraftment had occurred. Since the WBC counts are part of the CBC differential in all hematology analyzers available in the market, it is intriguing that early attempts to establish engraftment markers did not focus on this parameter. One possible explanation is that these counts were less accurate in previously available instruments, especially in severe leukopenic samples, rendering the results of WBC counts less useful clinically. Using this new WBC guideline, 76 out of (67.3%) 113 cases without receiving DGI showed earlier granulocytic engraftment using new WBC guideline than using $\text{ANC} > 500/\mu\text{l}$ from 1 to 9 days. These results indicate that the new WBC guideline allows clinicians to reassure patients that myeloid engraftment has occurred approximately 1 to 9 days earlier than $\text{ANC} > 500/\mu\text{l}$ in these patients. The new WBC guideline could have confirmed successful engraftment as early as on day 8 in 20 cases, but the $\text{ANC} > 500/\mu\text{l}$ could have

confirmed successful engraftment on day 8 in only two cases. It is important to confirm granulocytic engraftment because absence of hematopoiesis demands extensive support to the patients.

The two cases showed successful granulocytic engraftment but never achieved $\text{RET} > 1\%$ after HSCT. But erythroid parameters other than RET in one case showed increases on D19 to D20 and the other case did not show any results suggesting erythroid engraftment. It means we need another erythroid engraftment parameter than $\text{RET} > 1\%$ and we have to monitor erythroid engraftment separate from granulocytic engraftment after HSCT.

In this study, the earliest marker for erythroid engraftment was $\text{HLR} > 0.1$ with the mean value of 13.6 ± 8.4 days after graft infusion. HLR is a ratio of HLR (in region 3–10) count to the total RBC count. IRF is a ratio of same HLR (in region 3–10) count to the total reticulocyte count. Therefore, HLR indicates the proportion of immature reticulocytes among RBC which is not influenced by total reticulocyte count. The $\text{HLR} > 0.1$ preceded $\text{RET} > 1\%$ and $\text{IRF} > 0.5$ by 3.9 ± 9.9 and 1.6 ± 11.7 days, respectively. Of note, IRF on the next day of $\text{IRF} > 0.5$ achievement dropped to less than 0.5 in 19 cases (16.5%) while decrease of HLR to less than 0.1 did not

occur in any cases, which suggests HLR >0.1 is not only a earliest predictor for erythroid engraftment but also provides a stable guideline. The performance of the new RBC guideline in predicting erythroid engraftment after HSCT was very good with 100% specificity.

Allogeneic HSCT from HLA-matched donor (conventional) showed delayed granulocytic and erythroid engraftment than autologous HSCT. The conditioning intensity also affects granulocytic engraftment day, most delayed after myeloablative conditioning. However, the erythroid engraftment days were not statistically different according to the donor types or conditioning types. It may have been due to significant delay of erythroid engraftment in HSCT from ABO-mismatched donor regardless of donor type or conditioning type.

Erythroid engraftment of HSCT with ABO-mismatched donor was significantly delayed compared to that of ABO-matched transplant. The time to erythroid engraftment in ABO major mismatched HSCT was much delayed than that in minor mismatched HSCT. Especially, the erythroid engraftment from ABO major mismatched donors to blood type O recipients were much more delayed by 45.9 ± 37.8 days and varied from 11 to 116 days. This suggests that although ABO matched or mismatched donor is important for engraftment, the exact time for engraftment is very variable. Therefore, daily monitoring of erythroid engraftment marker after HSCT is recommended.

In summary, in this paper we proposed new laboratory guidelines to be used by clinicians to determine whether proper bone marrow engraftment has occurred. The guidelines seem to be capable of confirming successful engraftment a few days prior to the conventional parameters and are lineage specific, so that it would be useful for clinicians to separately determine whether myeloid or erythroid engraftment has occurred. Further studies are needed to fully validate these results, ideally including more cases of transplant failure and evaluating the performance of these guidelines using the same parameters obtained with different hematology analyzers.

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