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Article *in* Bone marrow transplantation · March 2009

DOI: 10.1038/bmt.2009.1 · Source: PubMed

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Bone Marrow Transplantation (2009) 44, 175–183

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ORIGINAL ARTICLE

Characterization of hemopoietic engraftment kinetics and development of secondary cytopenia in AML post auto-SCT and its correlation with survival outcome

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We performed a single center retrospective analysis of 84 autologous hemopoietic stem cell transplants done for AML to characterize the pattern of hemopoietic engraftment, post-transplant cytopenia and their impact on survival outcome. Following autologous transplant and engraftment, 30 patients (35.7%) had a transient secondary decline in their plt counts, which was not associated with graft rejection, relapse or infection. The median time to onset of thrombocytopenia was 59 days post transplant, with spontaneous recovery after a median period of 41 days. A secondary decline in ANC also occurred in eight patients. Patients with secondary plt decline had a significantly earlier primary plt engraftment (median 15 days) and a trend towards earlier neutrophil engraftment compared with patients who maintained steady plt counts (median 21 days). There was a trend towards a lower incidence of secondary plt decline in patients who received BM stem cells compared with those who received PBSC. No cause was evident for the occurrence of a secondary cytopenia, and it did not adversely affect survival. We conclude that secondary cytopenia is a common and harmless occurrence after autologous transplant especially from PBSC graft.
Bone Marrow Transplantation (2009) **44,** 175–183; doi:10.1038/bmt.2009.1; published online 9 February 2009
Keywords: acute myeloid leukemia; autologous hemopoietic stem cell transplant; engraftment kinetics; secondary platelet decline; secondary cytopenia

Introduction

AML refers to a group of clonal hemopoietic stem cell disorders, which are characterized by failure of differentiation and excessive proliferation of the stem cells leading to accumulation of non-functional immature myeloblasts, both in peripheral blood and BM. Anthracycline-based chemotherapeutic regimens, which are conventionally given for induction of remission, are known to produce CR in about 60–80% of patients.[1–3] However, despite the initial good response by the myeloblasts to these agents, they are usually associated with a high rate of resistance and relapse, and it has been reported that majority of AML patients who achieve CR following induction will relapse within 12–24 months, and only about 7–34% will be alive and disease free by 5 years after diagnosis.[4–6] Autologous hemopoietic SCT (AHSCT) has therefore been used in AML either as a component of consolidation following the initial CR or as part of salvage therapy for patients in second remission to improve the poor prognosis associated with chemotherapy alone.[7–9] AHSCT has also been used for AML patients who do not immediately have matched allogeneic donors and in elderly patients who are above 60 years of age.[10]

The pattern of engraftment and hemopoietic recovery following AHSCT has been extensively studied in patients with AML.[11,12] Following the myeloablative conditioning, which is used in autologous transplantation in AML patients, the time taken for the restoration of hemopoietic activity as measured by the presence of adequate number of blood cells is known to follow a predictable pattern within the first month post transplantation, usually first with an increase in the number of granulocytes followed by plts and lymphocytes, and finally the RBCs.[13] Several factors have been associated with the delay in the recovery time of the cell lines, such as the source of stem cells, dose of stem cells infused, disease status at transplant and infections, especially by CMV, and many studies have reported a poorer outcome in the OS of patients with delayed engraftment.[14–16]

Several authors have studied the pattern of granulocyte recovery following AHSCT, and this has led to the recognition that a minimum number of $CD34^+$ stem cells

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Received 1 October 2008; revised 19 December 2008; accepted 19 December 2008; published online 9 February 2009

in the graft is required for re-establishment of hemopoiesis.[17–19] Although a rapid and consistent engraftment of all the cell lines has been reported to occur when a $CD34^+$ cell dose of above 5 million per kg body weight is infused, lower dose could result in comparable rate of granulocyte engraftment with a slower plt engraftment.[19]

Early lymphocyte recovery following hemopoietic SCT has also been used as a predictive factor for prolonged survival in patients with many hematological malignancies.[20–24] Porrata *et al*.[25] in their study reported that, in case of AML, a recovery of the absolute lymphocyte count (ALC) $\geqslant 0.5 \times 10^9$ cells/l on day 15 post-autologous transplantation was associated with improved survival in their patients.

Thrombocytopenia following myeloablative AHSCT is a common occurrence and poses a particular clinical problem due to increased resource utilization in the form of supportive plt transfusions until plt engraftment occurs. In addition, the phenomenon of 'secondary failure of plt recovery' or 'Idiopathic secondary post-transplant thrombocytopenia' described as a delayed decline in plt counts following an initial plt recovery, which is unassociated with either graft rejection, relapse or infection, has been reported to occur in patients who underwent hemopoietic SCT by many authors.[26–28] Thrombocytopenia typically occurs within the first 100 days post transplantation. Although there is availability of specific commercially prepared myeloid and erythroid growth factors for tackling post-transplant neutropenia and anemia, no such agent is yet available for the management of post-transplant thrombocytopenia. Assessment of the pattern of plt recovery may therefore provide a true reflection of hemopoietic engraftment following auto-SCT.

Most previous studies, which investigated the pattern of hemopoietic engraftment, secondary plt failure and ALC recovery in AHSCT setting, were carried out in patients with various hematological malignancies grouped together or comprising autologous and allogeneic transplantations with paucity of data specifically for AHSCT in AML patients. Furthermore, many of the reported data were from transplant centers across Europe and America with scant data on the Asian patients with AML who had AHSCT. Hence, we carried out this single center retrospective analysis of all our AML patients who underwent AHSCT at the Singapore General Hospital, which so far may be the single largest homogenous group of AML patients to be studied to assess the pattern of hemopoietic engraftment, post-transplant secondary cytopenia and survival outcome of patients in relation to these parameters.

Patients and methods

The case records of all cases of AML that underwent autologous hemopoietic SCT at the Singapore General Hospital from January 1988 to December 2007 were retrospectively reviewed and followed up till June 2008. There were a total of 104 AML patients who underwent AHSCT within the study period. Twenty patients were excluded from our study, including eight who were involved in an IL2 trial, 10 patients (transplanted before 1994) who had incomplete records of plt engraftment, one patient who died early before engraftment and another patient who was lost to follow-up before plt engraftment; leaving 84 patients, who formed the cohort of our study group.

All the patients were given chemotherapy with the standard anthracycline (DNR or idarubicin) with cytarabine for induction of remission and were in either first CR (CR1) or second remission (CR2) before transplantation. All the patients were evaluated from the day of transplant, which was defined as day 0, and were followed up until the day of relapse, death or day last seen on routine follow-up.

Survival was documented at post transplantation follow-up visits; or the day of death confirmed from patients' case records and the computer database (iSoft Clinical Manager Software) of the hospital. Causes of death were available for all the patients who died within the study period.

Prognostic factors

To identify the prognostic factors associated with survival and outcome of patients post AHSCT, the following variables, which were selected from earlier studies,[26,27] were evaluated: age of patient at transplant, patient's sex, cytogenetic abnormalities at diagnosis, sources of stem cells (BM vs peripheral blood) and total $CD34^+$ stem cells infused. Particular emphasis was put on analysis of engraftment kinetics of neutrophils, lymphocytes and plts, as well as any subsequent secondary decline of plt or neutrophil counts and their impact on survival outcome.

Definitions

The day of transplant was designated as day 0 and the patients were evaluated for hemopoietic engraftment or survival from this day.

The day of neutrophil engraftment was defined as the first day when the patient had an ANC $\geqslant 0.5 \times 10^9$ cells/l for at least 3 consecutive days from the transplant date.

The incidence of a secondary decline of neutrophil count was defined as ANC $< 0.5 \times 10^9$ cells/l on 2 different days after initial neutrophil engraftment.

The day of plt engraftment was defined as the first of 3 consecutive days when patients had plt counts of $\geqslant 20 \times 10^9$ cells/l post transplantation without plt transfusion support in the previous 72 h. To study subsequent fall of plt counts, we set the following definitions for secondary plt decline. Plt peak day was defined as the day when the highest plt count was achieved following initial plt engraftment before any noticeable plt count drop. The day of secondary plt decline was defined as the day from transplantation when there is a fall in plt count to $\leqslant 20 \times 10^9$ cells/l or to $\leqslant 50\%$ of the peak plt count following initial plt engraftment in the absence of relapse. Plt nadir count was defined as the lowest plt count recorded following the secondary decline in plt count. The day of plt recovery after secondary plt decline was defined as the day when the plt count rose to twice the nadir count or $\geqslant 50\%$ of the plt peak count without plt transfusion in the previous week. The percentage of plt decline is calculated by the following formula: (peak plt−nadir plt)/peak plt $\times$ 100%.

Lymphocyte engraftment was defined as an ALC $\geqslant 0.5 \times 10^9$ cells/l post transplantation based on earlier reports.[23–25] Further evaluation of the ALCs on day 15, day 30 and day 60, as well as the presence of any lymphocytosis (defined as ALC $\geqslant 4 \times 10^9$ cells/l) were carried out in our patients to determine their impact on patients' survival.

CD34 quantitation

R phycoerythrin-conjugated CD34 (clone 581), Fluorescein isothiocynate (FITC)-conjugated CD45 (clone J33), R phycoerytrin-IgG1 isotype control antibodies (Beckman Coulter, Marseille, France) were used for staining of an aliquot of peripheral blood or leukapheresis product, followed by NH_4Cl lysis and addition of 7-Amino-actinomycin (7AAD). The sample was analyzed in a flow cytometer for CD34 staining on gated $CD45^+$ $7AAD^-$ cells. Cells that were $CD34^+$ SSC^{low} $CD45^{dim}$ represent true hemopoietic cells. At least 80 000 $CD45^+$ events and 100 $CD34^+$ events were acquired. The percentage of $CD34^+$ hemopoietic progenitor cells (HPC) was calculated as ($CD34^+$ HPC events)/($CD45^+$ Leukocytes events) $\times$ 100%. The total number of WBC in the samples was counted using automatic cell counter, the Sysmex SE900 (Tokyo, Japan). The absolute $CD34^+$ cells/μl was therefore calculated as %$CD34^+$ cells $\times$ WBC $\times 10^3$.

Statistical analysis of data

The patients' demographic data, diagnosis, date of transplantation, type of conditioning regimen, status of disease at transplant, stem cell source and dose, blood counts and the engraftment characteristics were entered into the study database using the SPSS for Windows Version 10.1 Software (SPSS Inc., Chicago, IL, USA).

Comparisons between the mean values were carried out using Student's *t*-test, whereas comparisons of frequency between the two groups were performed with the Fisher's exact test. OS and the relapse-free survival (RFS) estimations were done using the Kaplan–Meier method, with statistical significance analyzed by log-rank test.[29] Statistical significance of data was taken as P-value <0.05. The various statistical analyses were done with the SPSS v. 10.1 software.

Results

Patients' characteristics

The characteristics of the 84 patients are shown in Table 1.

The median age of the patients at transplantation was 44 years (range, 13–68 years) and there were 54 (64.3%) males and 30 (35.7%) females giving a male:female ratio of 1.8:1.

81 patients (96.4%) were transplanted in CR1 and only three patients (3.6%) were transplanted in CR2. The source of stem cells was BM in six patients (7.1%), PBSC in 60 patients (71.4%) and a combination of BM and PBSC in 18 patients (21.4%) due to inadequate PBSC collection. PBSC were harvested over a median of 4 days (range, 1–9 days).

For PBSC mobilization before harvesting, 74 patients (88.1%) had a combination regimen consisting Cytarabine (Ara-C) at 1 g/m^2 over 4 h twice daily for 4 days and Etoposide at 10 mg/kg daily by continuous infusion for 4 days. Four patients (4.7%) were given a combination of CY and Etoposide. All the patients received G-CSF at 600 mcg per day after chemotherapy as a part of the mobilization protocol. PBSC harvesting was started when peripheral blood $CD34^+$ count recovered to $\geqslant 10$ cells/μl upon marrow recovery after chemotherapy. This was continued daily until a minimum target dose of 2.5×10^6 $CD34^+$/kg was achieved.

For the high dose chemotherapy, 74 patients (88.1%) received CY at 60 mg/kg per day for 2 days and BU 4 mg/kg per day for 4 days, whereas 10 patients (11.9%) had Etoposide at 30 mg/kg for 2 days instead of CY for conditioning.

The median cell dose of $CD34^+$ PBSC infused was 6.2 (range, 2.5–22.8) $\times 10^6$/kg, whereas the median cell dose for BM mononuclear cells was 2.6 (range, 1.7–4.2) $\times 10^8$/kg.

Table 1 Characteristics of 84 patients with AML at transplantation

Variables	*No. (percentage)*
Gender	
Male	54 (64.3%)
Female	30 (35.7%)
Age at transplant (median, years)	44.0 (range, 13–68)
Ethnicity	
Chinese	74 (88.1%)
Indian	2 (2.4%)
Malay	5 (6.0%)
Pakistani	1 (1.2%)
Sri Lankan	1 (1.2%)
Caucasian	1 (1.2%)
FAB Classification	
M0	3 (3.6%)
M1	32 (38.1%)
M2	31 (36.9%)
M3	2 (2.4%)
M4	3 (3.6%)
M5a	6 (7.1%)
M5b	2 (2.4%)
M6	1 (1.2%)
Acute biphenotypic	1 (1.2%)
AML from MDS	3 (3.6%)
Cytogenetics	
Good prognosis	12 (14.3%)
Intermediate prognosis	62 (73.8%)
Poor prognosis	10 (11.9%)
Disease status at transplant	
CR1	81 (96.4%)
CR2	3 (3.6%)
Source of stem cell	
BM	6 (7.1%)
PBSC	60 (71.4%)
Both	18 (21.4%)
Conditioning regimen	
Bu/Cy	74 (88.1%)
Bu/VP-16	10 (11.9%)

Abbreviations: Bu/VP-16 = busulphan + etoposide; FAB = French American British classification for AML; MDS = myelodysplastic syndrome.

Table 2 Pattern of hematopoietic recovery

Variable cells/μl	*Median time (range)*	*Median count (range) cells/μl*
ANC >500	Day+11 (Day +8–25)	650 (350–2240)
Plt count >20 000	Day+17 (Day +10–72)	35×10^3 ($10–101\times10^3$)
ALC >500	Day+15	910 (506–2743)

Abbreviation: ALC = absolute lymphocyte count.
Median time and count indicates median engraftment day and median engraftment count.

Hemopoietic recovery following AHSCT

The median time taken for engraftment of neutrophil, plt and lymphocytes is listed on Table 2.

Neutrophil engraftment with ANC $\geqslant0.5\times10^9$ cells/l occurred at a median of day 11 (range, 8–25 days). The median ANC at day 11 was 0.65×10^9 cells/l (range, $0.35–2.240\times10^9$ cells/l).

Plt engraftment with plt counts $\geqslant20\times10^9$ cells/l was achieved in all the patients studied at a median of day 17 (range, 10–72 days). 35 patients (41.7%) achieved plt counts $\geqslant20\times10^9$ cells/l by day 15 post transplantation. 54 patients (64.3%) maintained plt counts $\geqslant20\times10^9$ cells/l throughout the observation period, whereas a secondary decline in plt counts was observed in 30 patients (35.7%) following the initial engraftment of plt counts.

ALC recovery of $\geqslant0.5\times10^9$ cells/l by day 15 post-autologous transplantation occurred in 51 patients (60.7%) with a median ALC of 0.91×10^9 cells/l (range, 0.506–2.743×10^9 cells/l) and 33 patients (39.3%) had ALC $<0.5\times10^9$ cells/l at day 15 with a median ALC of 310/μl (range, 10–493/μl). Seventy seven patients (91.7%) had ALC $\geqslant$500/μl by day 30 with a median ALC of 1691/μl (range, 518–9307/μl), and 82 patients (97.6%) had ALC $\geqslant$500/μl by day 60 post transplant with a median ALC of 2120/μl (range, 559–9307/μl). Absolute lymphocytosis with ALC $\geqslant$4000/μl was observed in 12 patients at a median of day 35 (range, 18–132 days) with a median count of 5078/μl (range, 4057–9307/μl), and ALC normalized to counts <4000/μl at a median of day 68 (range, 20–142 days).

Secondary decline of plt counts following initial plt engraftment

Out of the 84 patients who had AHSCT, 30 patients (35.7%) had a secondary decline in their plt counts following primary plt engraftment post transplantation. The median day of onset of secondary plt decline was day 59 (range, 34–94 days) with a median plt nadir count of 35 000/μl (range, 10 000–101 000/μl). All the patients who had a secondary decline in plt counts recovered their counts at a median of day 90 (range, 38–109 days). The median duration of secondary plt decline was 41 days (range, 3–275 days). Those without a secondary plt decline maintained their plt counts well above 20 000/μl throughout the observation period. Within this group of 30 patients with a transient secondary plt decline, two patients had plts falling to a nadir below 10×10^9/l, five patients had a nadir between $11–15\times10^9$/l and three other patients between $16–20\times10^9$/l. The mean absolute degree of plt decline from peak to nadir was 78 000/μl (range 23 000–182 000/μl). The percentage of plt decline in these 30 patients from their respective peaks was 67 ± 28% (mean ± 2s.d.). This shows that all the cases with secondary plt decline were indeed real and were of a clinically significant degree.

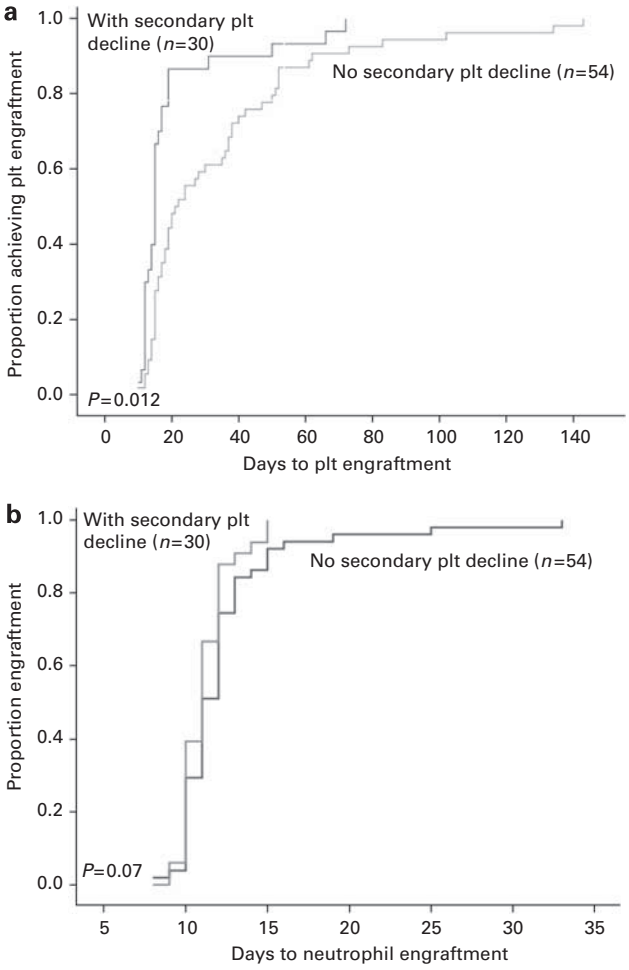

Figure 1 (**a**) Cumulative plt engraftment for the groups without and with secondary plt decline. The group with secondary plt decline had a significantly faster plt engraftment than the group without any secondary plt decline. (**b**) Cumulative neutrophil engraftment for the groups without and with secondary plt decline. The group with secondary plt decline showed a trend towards faster neutrophil engraftment than the group without any secondary plt decline.

The median time for primary plt engraftment in the group with secondary plt decline was 15 days (range, 10–72 days) as compared with a median time of 21 days (range, 10–92 days) for the 54 patients who did not develop any secondary decline in plt count ($P=0.012$). Figure 1a shows the cumulative plt engraftment of the two groups. Similarly, this group also showed a trend ($P=0.07$) towards earlier neutrophil engraftment as compared with the group without secondary plt decline (Figure 1b).

Further investigations were done in some patients with secondary plt decline. BM aspirations and trephine biopsies were performed in 14 (46.7%) of the 30 patients at the onset of the thrombocytopenia. Cellularity was reported as hypocellular (10–20% cellularity) with reduced mega-

karyocytes in six patients, normocellular (35–70% cellularity) with normal megakaryocytes and trilineage engraftment in six patients and hypercellular (>75% cellularity) with trilineage engraftment in two patients. Immune markers, including anti-nuclear antibodies, anti-double stranded DNA antibodies and anti-cardiolipin antibodies, were carried out in only four patients with one patient being positive for anti-nuclear antibodies and another for anti-cardiolipin antibodies. Viral markers, including immunofluorescence for CMV Ag, serologies for EBV Ag and Parvovirus, were also done in four patients, of whom none were positive. One patient had clinically evident development of Hepatitis (Non A, Non B, Non C) infection that might have accounted for the transient fall in plt count, whereas the majority of patients could not be explained by any concomitant clinical events. Bactrim prophylaxis at 960 mg twice daily two times a week, which was started upon engraftment, was continued for most patients and withheld for a few patients without any cause–effect relationship observed in terms of plt recovery. There was no form of treatment given during the period of the thrombocytopenia until the plt counts recovered.

When analyzed by the dose of $CD34^+$ cells in the autograft, there was no difference between two groups (median $CD34^+ = 6.11$ vs 5.27 million $CD34^+$/kg, $P = 0.64$). When analyzed by the source of stem cells, it was found that of the six patients who received BM alone, none of them developed a secondary plt decline, whereas 24 (40%) of the 60 patients who received PBSC alone developed a secondary plt decline ($P = 0.057$).

Secondary decline of neutrophils following initial neutrophil engraftment

Similarly, we looked at the sustainability of neutrophil engraftment in these patients. Eight patients had a fall in ANC to severely neutropenic level (ANC <500/μl) after initial neutrophil engraftment. The median day at secondary neutrophil decline was day 59 (range, 27–80 days), lasting a median of nine days (range, 5–46 days) and recovering spontaneously to above 500/μl at a median of 72 days (range, 43–90 days) post transplantation. Interestingly, a concomitant decline in the ANC to counts <500/μl occurred in 5 of the 30 patients with secondary decline in plt count at the median time of day 58 (range, 27–80 days) and recovered their neutrophil counts to >500/μl at a median of day 72 (range, 43–90 days). There was no difference in the initial neutrophil engraftment rate between the group without or with subsequent neutrophil decline, $P = 0.485$. There was also no difference in the infused $CD34^+$ cell dose between the two groups (5.72 vs 5.78 million $CD34^+$/kg, $P = 0.42$). Of note, none of the patients with secondary neutrophil decline developed any neutropenic sepsis.

Analysis of survival and outcome

In this study, analysis of the prognostic factors for improved survival and outcome in our patients did not identify age, gender and the cytogenetic abnormalities at diagnosis as statistically significant prognostic factors for OS and RFS (data not shown).

In particular, we analyzed for any association between the various engraftment characteristics and survival outcomes. There was no significant difference in RFS between the groups with ALC <500/μl ($n = 33$) or >500/μl ($n = 51$) at day 15 ($P = 0.15$) or between the groups with ($n = 35$) and without ($n = 49$) plt engraftment by day 15 (RFS = 61 vs 52%, $P = 0.84$), or between the groups with ($n = 81$) or without ($n = 3$) ANC engraftment by day 15 (RFS = 67 vs 52%, $P = 0.85$). We further compared the groups with or without secondary plt decline and found no difference in RFS between these two groups (Figure 2a). Similarly, there was no difference in RFS between groups with or without secondary neutrophil decline (Figure 2b). In addition, the

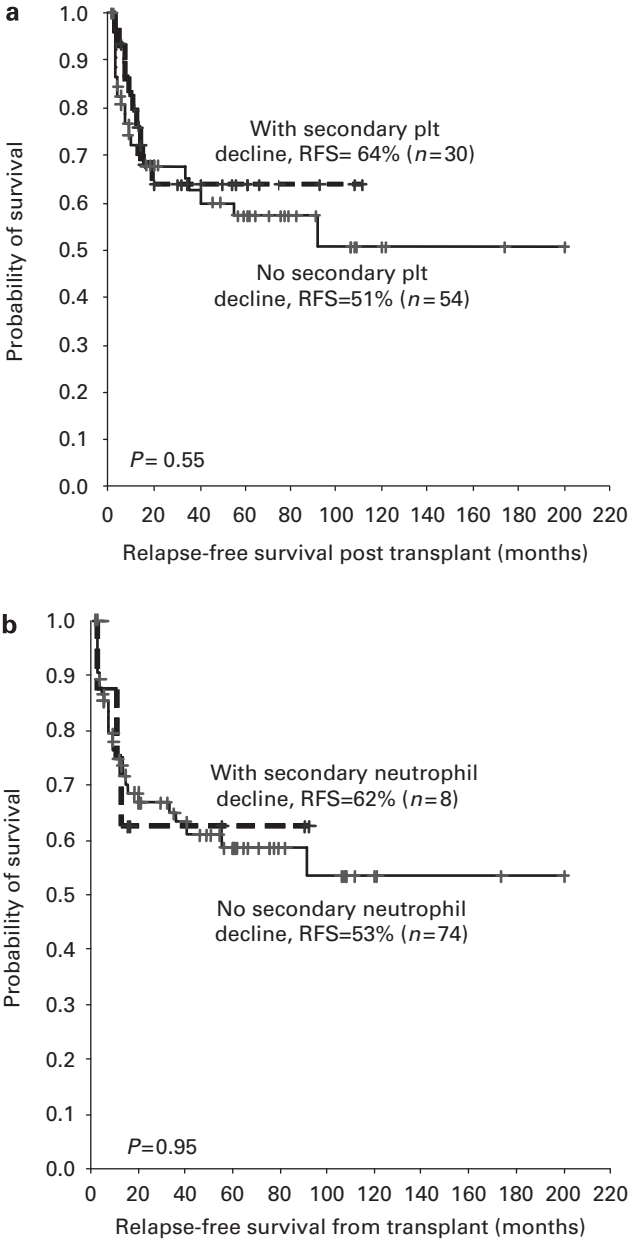

Figure 2 (**a**) Relapse-free survival (RFS) for patients who developed secondary plt decline vs patients who did not. (**b**) Relapse-free survival (RFS) of patients who developed secondary neutrophil decline vs patients who did not.

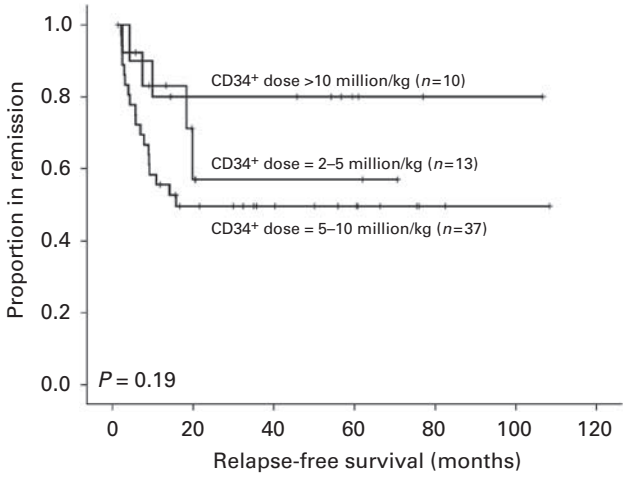

Figure 3 Relapse-free survival (RFS) of patients post-autologous transplant when the PBSC cell doses were classified into 3 categories (2–5, 5–10, >10 million $CD34^+$ cells per kg body weight).

development of lymphocytosis anytime after transplant, which occurred in 12 patients, did not confer any survival benefit (RFS = 56% with lymphocytosis vs 50% without lymphocytosis, $P = 0.16$). We also analyzed the impact of $CD34^+$ cell dose in the 60 patients who received PBSC only, stratified into dose levels of 2–5, 5–10 and >10 million $CD34^+$ cells/kg, and did not find any significant difference amongst the groups, with overall *P*-value or *P*-trend of 0.19, although there was a trend towards a longer RFS on pair-wise comparison between the groups with $CD34^+$ counts of >10 million/kg and 5–10 million/kg ($P = 0.1$) (Figure 3).

The median follow-up period was 45 months (range, 1.3–200 months). Of the 84 patients, 45 (53.6%) are still alive and disease free post transplantation, 31 (36.9%) had died from various causes, consisting of relapse and disease progression ($n = 28$, 33%), intracranial hemorrhage ($n = 1$, 1.2%) and second malignancy ($n = 2$, 2.4%). Eight patients (9.5%) were lost to follow-up, either because they defaulted from follow-up clinics ($n = 6$, 7.1%) or went back to their country of origin ($n = 2$, 2.4%). Of note, two of the patients who relapsed post AHSCT and had further courses of chemotherapy were back to CR, and still alive and disease free.

The 10-year probability for RFS in our patients was 54%.

Discussion

This study is a large, possibly the largest so far, single center report from an Asian population on the kinetics of hemopoietic engraftment post AHSCT for AML patients and its impact on the survival outcome.

The results obtained in our study on the pattern of hemopoietic engraftment following transplantation are in agreement with earlier observations which showed that AHSCT is associated with rapid hemopoietic engraftment within 2 weeks post transplantation.[13,30,31] The median times to reach ANC $\geqslant 500/\mu l$ and plt counts $\geqslant 20\,000/\mu l$ in all our patients were 11 and 17 days, respectively, which is similar to that of Rubia *et al*.[32] which was 13 and 14 days, respectively. The rapid recovery of neutrophils and plts after AHSCT has been extensively studied and many reports have associated this with the total number of $CD34^+$ hemopoietic progenitor cells present in the infused autograft.[17–19,33] All our patients met the minimum recommended stem cell dose of 2.5×10^6 CD34/kg, and this likely explains the early neutrophil and plt engraftment observed.

We are interested to explore whether the kinetics of engraftment of the various cell lines has an impact on survival outcomes. In AML patients, Porrata *et al*.[25] in their study of early lymphocyte recovery showed that ALC recovery to $\geqslant 500/\mu l$ on day 15 post AHSCT was associated with improved long-term outcome in disease-free survival and OS. In our study, 51 patients (60.7%) achieved ALC $\geqslant 500/\mu l$ on day 15, post transplantation. However, we did not observe any significant difference in the RFS ($P = 0.15$) and OS ($P = 0.20$) in this group of patients when compared with the patients who had ALC $< 500/\mu l$ on day 15 (Figure 3a). The reason that had been given for the improved RFS and OS observed in patients with early lymphocyte recovery following AHSCT is the possibility that the early immune reconstitution may have a protective effect against disease progression in a way that is analogous to the GVL effect that is seen in patients with Allo-SCT, in which the donor immune system is considered to be responsible for the eradication of residual disease in the recipient.[25,34,35] Also, it has been suggested that the repopulating lymphocytes may have been derived from other sources other than from the transplanted progenitor stem cells, such as the mature natural killer lymphocytes, present in the transplant. The presence of an increased number of natural killer cells in the peripheral blood post AHSCT was reported by Porrata *et al*.[25,36] to be associated with recovery of ALC within two weeks and a better long-term survival in their study. The lymphocyte subsets were not known due to the retrospective nature of our study, and this may have accounted for the insignificant difference in the RFS and OS recorded in our patients with early lymphocyte recovery and those without.

To further explore the effect of ALC recovery on the survival of our AML patients, we analyzed the presence of lymphocytosis, which we defined albeit arbitrarily as ALC $\geqslant 4000/\mu l$, on two different occasions within 100 days post transplantation. There was no statistically significant difference in RFS ($P = 0.16$) and OS ($P = 0.16$) between the groups of patients with lymphocytosis and without.

A transient secondary decline in the plt count following primary plt engraftment has been observed frequently post AHSCT, the clinical and prognostic relevance of which is not known and is still under investigation.[26,28] Although the phenomenon has been well described among allogeneic transplant patients, very few reports are available in the literature for autologous transplant patients, more so in AML patients.[37,38] The few studies that have reported their observations on the effect of secondary thrombocytopenia on patients' survival and outcome in AML post-autologous transplantation had small numbers of AML patients in their study groups. A significant

proportion of our patients ($n = 30$, 35.7%) developed a secondary decline in plt counts post transplantation. A similar observation was made by Ninan *et al*.[26] in their study, in which the incidence of secondary thrombocytopenia was put at 17%, but their study was carried out in different groups of patients with various hematological malignancies who had AHSCT (18 cases of acute leukemias out of 62 patients). Also, the study by Bruno *et al*.[27] on various malignancies reported an incidence of 19% among their autologous transplant patients. Narimatsu *et al*.[28] from Japan reported a high incidence of secondary thrombocytopenia (64%) in their study of patients with acute promyelocytic leukemia who had autologous and syngeneic SCT. The higher incidence of secondary thrombocytopenia observed in their study might be due to the smaller sample size (7 out of 11 patients). Although Bruno *et al*. and Ninan *et al*. have reported poor outcome in their patients with secondary thrombocytopenia post-autologous transplantation, we report to the contrary in our patients as there was no significant difference in the RFS ($P = 0.55$) and OS ($P = 0.62$) in the group of patients with secondary decline in plt counts and the group without. We believe that our current observation is reflective of the truth as it was based on a homogenous group of AML patients, whereas the earlier studies were carried out on a diverse group of patients with various types of malignancies and with fewer AML patients, or included patients who had allogeneic transplantations which involved many more confounding factors.

In this retrospective analysis, we made an interesting and apparently paradoxical observation that the group without secondary plt decline, in fact, took longer (median 21 days) to engraft plt and possibly also neutrophil, compared with the group with secondary plt decline (median 15 days). Moreover, we observed in our study that patients who were transplanted with BM alone had a reduced incidence approaching statistical significance of secondary plt decline when compared with patients who received PBSC only ($P = 0.057$). Although the number was small and therefore not reaching statistical significance, we think this is real. It could possibly be explained by the fact that BM stem cells are richer in the long-term repopulating cells that are able to maintain the engraftment and sustain their proliferative functions in the microenvironment of the BM stroma.

The etiology of the secondary thrombocytopenia following primary plt engraftment in the absence of relapse or infections in AML patients is still unknown.[26,27,39–41] The immunological mechanism was explored by Ninan *et al*.[26] in their study, but it did not show any autoimmune basis in their patients, neither was there any response in the plt counts with i.v. Ig administration. In our study, secondary thrombocytopenia observed in our patients was found to be self-limiting. Having excluded the possibility of relapse, no form of treatment was given and all the patients recovered their plt counts. Within this cohort there were nevertheless a few patients with such profound thrombocytopenia that plt transfusion was necessary, adding significantly to the cost of post-transplant management. Further laboratory studies are still needed to fully characterize this clinical entity so as to assist the clinicians in its prevention and appropriate management of their patients.

The advancement of AHSCT over the past 20–30 years has made it a widely applied clinical practice and a standard of care for several hematological malignancies. In this retrospective review we have described the pattern of hemopoietic engraftment post AHSCT for AML patients and characterized the benign, but nevertheless, sometimes troublesome phenomenon of transient secondary plt and neutrophil decline after initial engraftment. Continued research and enrichment in the knowledge of the biology of BM and PBSC will definitely lead to the identification of more refined selection of the best AHSCT options and post-transplant management for patients with AML and other malignancies.

Acknowledgements

Dr Babatunde acknowledges the support provided by the University of Ilorin Teaching Hospital, Ilorin, Nigeria and the Postgraduate Medical Institute (PGMI), Singapore General Hospital, for the Clinical Fellowship in Bone Marrow Transplantation at the Department of Haematology, Singapore General Hospital. We would like to thank the research coordinators Ms Valerie Wee and Ms Perumal Premalatha for their assistance in providing the data, and the nursing and paramedical staff in the Department of Haematology for their excellent work in patient care. This study is supported by the Singapore Cancer Syndicate Grant for Bone Marrow Transplant Consortium Registry.

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