Predictors of Mononuclear Cell Yield in Patients Undergoing Autologous Mononuclear Stem Cell Therapy in Non-haemopoietic Degenerative Disorders

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Abstract Cellular therapy outcomes are influenced by cellular composition of the product. We analyzed the cellular profiles (TNC, MNC and CD34+ cells) of patients receiving mononuclear cell therapy in terms of age, gender, BMI, pre-harvest haematological counts and clinical conditions. Cellular profiles of 262 patients were analyzed in terms of age (age < 40 year, age 41–60 year and age > 60 year), gender, BMI (BMI < 22 kg/m², BMI 22–25 kg/m² and BMI > 25 kg/m²), pre-harvest haematological profile and clinical conditions (chronic disorders, group A, acute vascular group B and traumatic events, group C). A steady decline was observed in TNC and MNC counts with increasing age and BMI. In clinical conditions, group C showed a highest cellular yield followed by group A and group B respectively. Amongst the three age groups, group I (age < 40 year) showed a better cellular profiles than group II (age 41–60 year) and group III (age > 60 year). Patients with Higher TLC (>7000/μl) and platelet count (>200 × 10³/μl) yielded better cellular profile in the harvest. Patient age, BMI, haematological counts and clinical condition significantly affect the bone marrow cellular profile.

Keywords Autologous bone marrow mononuclear cell therapy · Bone marrow cellular profiles · Advanced medicinal therapeutic products

Introduction

Cellular therapy has evolved rapidly over the last few decades and significant insights have been gained in pre-clinical research and clinical trials. Adult as well as embryonic stem cells have been explored as potential therapeutic strategies for various diseases. Adult stem cells, especially haemopoietic stem cells (HSCs), have generated huge interest in the fields of regenerative medicine due to their exclusive biological properties and ease of harvest. There are several clinical trials and case reports in literature, with autologous bone marrow stem cell therapy for the treatment of various degenerative disorders like diabetic mellitus, spinal cord injury, Alzheimer's disease, Parkinson's disease, motor neuron disease, peripheral vascular disease, stroke and myocardial infarction. Quantity of CD34 positive stem cells as marker for hematopoietic stem cells (HSC) is considered as a predictor for the success of bone marrow stem cell therapy.

The successful clinical outcome of bone marrow stem cell therapy depends on the cellular composition of the product; hence it is important to analyze the composition of these products. Bone marrow cellular profiles and cellular characteristics from patients might be altered when compared with healthy donors, due to various factors such as sedentary life style, body mass index and alteration in mechanism controlling haematopoiesis after tissue injury. In the study conducted by Chernykh et al., the functional and phenotypical profile of bone marrow stem cells were compared in healthy adults and spinal cord injury patients. The authors observed a higher percentage of CD34+/CD38– stem cells in the bone marrow aspirates from patients than in healthy subjects [1].

In this study, we tried to assess the predictors of MNC yield by a comparative analysis of bone marrow aspirates

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Fig. 1 Bone marrow collection

cellular profile i.e. total nucleated cells (TNC), mononuclear cells (MNC) and CD34+ cells of 262 patients admitted for autologous stem cell therapy under different clinical specialities in terms of patients variables i.e. age, gender and Body Mass Index (BMI), haematological profile {haemoglobin (Hb), total leukocyte count (TLC) and platelet count} and underlying clinical conditions.

Materials and methods

Patient Population

The data was collected from 262 patients with clinical conditions such as spinal cord injury, myocardial infarction, avascular necrosis of hip joint, Type 2 diabetes mellitus, stroke, motor neuron disease, optic nerve injury, post traumatic facial nerve palsy and peripheral vascular

disease. These patients were admitted in the institute for autologous mononuclear stem cell therapy under various clinical research trials. Approval from the Institutional stem cell ethics committee was obtained for all the clinical trials and informed consent was also taken from the patients. The data was analysed over a period of seven and half years from January 2007 to June 2014. The patients were included only if they fulfilled the inclusion criteria as per the concerned research proposal and were non reactive for HIV 1/2, HBsAg, HCV and VDRL and they did not have any underlying bone marrow disorder.

Bone Marrow Collection

All the bone marrow collection procedures were performed in operation theatre under the local (2% lignocaine/xylocaine)/epidural anaesthesia from posterior superior iliac spine by following all aseptic techniques by the trained staff (Fig. 1). Bone marrow needle was inserted into the selected area on the posterior superior iliac spine of the patient and multiple bone marrow aspirates of 3–5 ml were done with the help of 20 ml syringe (previously rinsed with heparinised normal saline) and on an average 143.6 ml marrow was collected into the 350 ml blood collection bag (Make: Terumo Penpol, Trivandrum, India) under sterile conditions with gentle mixing to prevent clot formation [2–4].

Cell Counts

Total nucleated cell counts and mononuclear cell counts in the harvested products were analysed on fully automated haematology analyser (Make: Orion 60, Pacific

Table 1 Age wise distribution of Bone Marrow cellular profile (TNC, MNC and CD34+ cell counts) in all patients

Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
I. Age < 40 year (n = 110)	25 $\pm$ 10	24 (9–59)	9.1 $\pm$ 3.8	8.3 (2.6–22)	0.97 $\pm$ 0.42	1 (0.1–2.1)
II. Age 41–60 year (n = 125)	23.7 $\pm$ 8.9	22 (7–51)	8.9 $\pm$ 3.4	8.5 (2–18.6)	0.96 $\pm$ 0.54	1 (0.2–5)
III. Age > 60 year (n = 27)	20 $\pm$ 9.1	18 (6.1–40)	8 $\pm$ 4.6	7 (2–21)	1.1 $\pm$ 1	0.8 (0.1–3.9)
P value	TNC		MNC		CD34+ cell	
I versus II	0.485		0.835		0.426	
I versus III	0.015		0.155		0.551	
II versus III	0.031		0.229		0.823	

Bold values indicate $P < 0.05$

Fig. 2 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) between different age groups

Table 2 Gender wise distribution of Bone Marrow cellular profile (TNC, MNC and CD34+ cell counts) in all patients

Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
Male (n = 203)	23.8 $\pm$ 9.6	22 (6.1–59)	8 $\pm$ 3.6	8.3 (1.9–21.6)	1 $\pm$ 0.6	1 (0.1–5)
Female (n = 59)	24.3 $\pm$ 9.1	25 (7–47)	9 $\pm$ 4.1	8.5 (2–22)	0.9 $\pm$ 0.4	1 (0.1–1.8)
P value	0.491		0.327		0.943	

Fig. 3 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in male and female patients

Diagnostics, India). CD34+ cell counts were done using a monoclonal anti CD45 antibody (FITC-conjugated) and anti CD34 antibody (PE-conjugated) on FACS Aria II (BD, Biosciences, San Jose, CA). CD34+ cells analysis was done in accordance with ISHAGE guidelines [5, 6].

Statistical Analysis

Data was expressed as mean $\pm$ SD and median (min.–max.). Standard data analysis and graphics were performed with Excel, Microsoft, Redmond, WA. Significance test for

Table 3 BMI wise distribution of Bone Marrow cellular profile (TNC, MNC and CD34+ cell counts) in all patients

Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
i. BMI $< 22 \text{ kg/m}^2$ (n = 45)	24.46 $\pm$ 8.48	23 (10–61)	9.47 $\pm$ 3.48	9.1 (3.9–22)	0.98 $\pm$ 0.41	1.1 (0.2–2.1)
ii. BMI 22–25 kg/m^2 (n = 76)	25.67 $\pm$ 10.04	25 (8–59)	9.47 $\pm$ 3.65	8.9 (3–21)	0.94 $\pm$ 0.42	1 (0.1–1.9)
iii. BMI $> 25 \text{ kg/m}^2$ (n = 85)	23.32 $\pm$ 9.77	21 (9–55)	8.07 $\pm$ 3.40	7 (2.6–20.3)	0.96 $\pm$ 0.50	0.9 (0.2–3.3)
<i>P</i> value	TNC		MNC		CD34+ cell	
i versus ii	0.648		0.793		0.461	
i versus iii	0.301		0.008		0.372	
ii versus iii	0.074		0.006		0.875	

Bold values indicate $P < 0.05$

comparisons between 2 groups were conducted with Mann–Whitney U-tests, non-parametric test. Values between more than 2 groups were compared using Kruskal–Wallis test, using a commercial statistics program (SPSS version 21, USA). P value less than 0.05 was considered significant.

Results

A detailed comparative data analysis was carried out to understand the effect on yield of the harvested product by categorising patient population on the basis of patient variables i.e. age, gender and body mass index (BMI), haematological profile (Hb, TLC and platelets) and clinical conditions as follows:

1. Age wise—three groups were defined (age < 40 year, age 41–60 year and age > 60 year).
2. Gender wise—two groups were defined (male and female).
3. BMI—three groups were defined (BMI $< 22 \text{ kg/m}^2$, BMI 22–25 kg/m^2 and BMI $> 25 \text{ kg/m}^2$).
4. Haematological Profile (Hb, TLC and platelets)-
 - a. Hb wise—two groups were defined (Hb 9–12 gm/dl and Hb 12–15 gm/dl).
 - b. TLC wise—three groups were defined (TLC $< 7000/\mu\text{l}$, TLC 7000–10,000/ μl and TLC $> 10,000/\mu\text{l}$).
 - c. Platelet count wise—two groups were defined (platelets $< 200 \times 10^3/\mu\text{l}$ and platelets $> 200 \times 10^3/\mu\text{l}$).
5. Clinical disorder wise—three groups were defined:
 - a. Chronic disorders (Type 2 diabetes mellitus, motor neuron disease, avascular necrosis of hip joint and peripheral vascular disease).
 - b. Acute vascular events (myocardial infarction and stroke).

- c. Acute traumatic events (spinal cord injury, optic nerve injury and post traumatic facial nerve palsy).

The age wise distribution of bone marrow cellular profile (TNC, MNC and CD34+ cells counts) are presented in Table 1 and Fig. 2. There was a steady decline in bone marrow TNC and MNC profiles with increasing age and it was found to be statistically significant for TNC ($P = 0.045$). In addition the progressive decline in TNC with age was also found to be statistically significant between the patient age group > 60 years when compared with the age group < 40 year and 41–60 years with P values 0.015 and 0.031 respectively. However, the mean CD34+ cell count was found to be similar across all the three age groups with slightly unpredictable rise in patients over 60 years of age, but was not statistically significant.

The gender wise distribution of bone marrow cellular profile (TNC, MNC and CD34+ cell counts) are presented in Table 2 and Fig. 3. An average bone marrow TNC and MNC profiles were slightly better in female patients than male patients, whereas male patients showed slightly higher CD34+ cell count as compared to female patients. However, these differences in cell numbers in both the sexes were not statistically significant.

The BMI wise distribution of bone marrow cellular profile (TNC, MNC and CD34+ cells counts) are presented in Table 3 and Fig. 4. There was a steady decline in average TNC count as the BMI increased, but was not found to be statistically significant ($P = 0.193$). However average MNC count decreased as the BMI increased from 25 kg/m^2 and was also statistically significant ($P = 0.005$). In addition CD34+ cells count was slightly higher in BMI $< 22 \text{ kg/m}^2$ as compared to BMI 22–25 kg/m^2 and BMI $> 25 \text{ kg/m}^2$, but was not statistically significant ($P = 0.657$).

The clinical condition wise distribution of bone marrow cellular profile (TNC, MNC and CD34+ cell

Fig. 4 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in different clinical groups **Table 4** Clinical disorders wise distribution of Bone Marrow cellular profile (TNC, MNC and CD34+ cell counts) in all patients

Groups	Age (Mean $\pm$ SD)	TNC $\times 10^6$ /ml		MNC $\times 10^6$ /ml		CD34+ cell (%)	
		Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
A. Chronic disorders (n = 166)	47 $\pm$ 11.8	22.6 $\pm$ 8.3	20.5 (6.1–54)	9.1 $\pm$ 3.8	8 (2–22)	0.9 $\pm$ 0.4	1.0 (0.1–3.3)
B. Acute vascular events (n = 25)	51.2 $\pm$ 10.8	22.5 $\pm$ 8.4	22 (9–52)	7.1 $\pm$ 2.7	6.4 (4–16)	1.2 $\pm$ 0.4	1.2 (0.2–2.1)
C. Acute traumatic events (n = 71)	34 $\pm$ 13.7	27.7 $\pm$ 11.4	26 (12–59)	9.3 $\pm$ 3.8	9 (2.6–21.6)	1.1 $\pm$ 0.8	1.0 (0.1–5)
P value		TNC		MNC		CD34+ cell	
A versus B		1.00		0.003		0.002	
A versus C		0.002		0.716		0.374	
B versus C		0.049		0.003		0.036	

Bold values indicate $P < 0.05$

Fig. 5 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in different BMI groups

counts) are presented in Table 4 and Fig. 5. For clinical condition wise distribution of the patient population, the mean age of patients group A (chronic disorders, $n = 166$) was 47 ± 11.8 years and group B (acute vascular events, $n = 25$) was 51.2 ± 10.8 years and group C (acute traumatic events, $n = 71$) was 34 ± 13.7 years. The TNC yields were highest in bone marrow aspirate from patients with acute traumatic events (group C) followed by chronic disorders (group A) and acute

vascular events (group B) and this difference was found to be statistically significant with P value of 0.002 (group A vs C) and P value of 0.049 (group B vs C), whereas the MNC yields in group A and C were comparable and statistical higher than group B with P value of 0.003 (group A vs B and group C vs B). In addition, CD34+ yields were comparable in group A and C and significantly higher than group B with P value of 0.002 (group A vs B) and 0.036 (group C vs B).

Table 5 Pre-Harvest Haematological profile (Hb, TLC and Platelets count) wise distribution of Bone Marrow cellular profile (TNC, MNC and CD34+ cell counts) in all patients

Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
(A)						
Hb (9–12 gm %) (n = 75)	24.45 $\pm$ 9.5	23 (8–55)	8.4 $\pm$ 3.09	7.5 (2.6–15.3)	0.94 $\pm$ 0.5	0.9 (0.1–2.1)
Hb (12–15 gm %) (n = 126)	24.19 $\pm$ 9.71	23 (7–59)	9.12 $\pm$ 3.88	8.5 (2–22)	0.97 $\pm$ 0.44	1 (0.1–3.3)
P value	0.705		0.402		0.628	
Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
(B)						
1. TLC < 7000/ μl (n = 93)	22.35 $\pm$ 7.78	21 (7–50)	8.58 $\pm$ 3.09	7.9 (2–18.3)	0.96 $\pm$ 0.47	1.0 (0.1–2.1)
2. TLC 7000–10,000/ μl (n = 92)	25.18 $\pm$ 10.47	24.5 (8–55)	9 $\pm$ 4.15	9 (3–22)	1 $\pm$ 0.47	1 (0.1–3.3)
3. TLC > 10,000 (n = 23)	27.69 $\pm$ 10.61	27 (15–59)	9 $\pm$ 2.89	8 (5–16)	1 $\pm$ 0.36	1.0 (0.4–1.9)
P value	TNC		MNC		CD34+ cell	
1 versus 2	0.078		0.569		0.826	
1 versus 3	0.015		0.675		0.997	
2 versus 3	0.342		0.978		0.953	
Groups	TNC $\times 10^6/\text{ml}$		MNC $\times 10^6/\text{ml}$		CD34+ cell (%)	
	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)	Mean $\pm$ SD	Median (min.–max.)
(C)						
Platelets < $200 \times 10^3/\mu\text{l}$ (n = 112)	22.98 $\pm$ 9.13	21 (7–54)	8.16 $\pm$ 3.29	7.45 (2–21)	0.98 $\pm$ 0.42	1.0 (0.2–3.3)
Platelets > $200 \times 10^3/\mu\text{l}$ (n = 94)	25.80 $\pm$ 9.82	26 (11–59)	9.69 $\pm$ 3.77	9.35 (2.6–22)	0.94 $\pm$ 0.50	0.9 (0.1–3.3)
P value	0.021		0.003		0.149	

Bold value indicate $P < 0.05$

Thus, the overall cellular profile (TNC, MNC and CD34+ cell) yield was higher in group C followed by group A and group B respectively. This could be due to the fact that majority of the patients in group C (acute traumatic events) belonged to a younger age group (age < 40 years) whereas in group A and B majority of the patients were above 40 years of age (age 41–60 years) and this difference was found to be statistically significant ($P = 0.00$). However the age difference in patient population of group A and B was not statistically significant.

The haematological profile was also correlated with bone marrow cellular profile (TNC, MNC and CD34+ cells counts) as presented in Table 5 (Hb wise—A, TLC wise—B, and platelets wise—C) and Figs. 6, 7 and 8. Pre harvest haemoglobin value did not appear to significantly affect the bone marrow cellular profile except for the higher concentration of MNC and CD34+ cells in the product from patients with a haemoglobin value more than 12gm/dl. Similarly, the pre harvest TLC count did not

significantly affect bone marrow cellular profile except for TNC counts which were significantly higher in harvest when the peripheral TLC count exceeded 10,000/ μl in the patients ($P = 0.038$). However, patients with platelets count $>200 \times 10^3/\mu\text{l}$ showed significantly higher TNC and MNC in the harvested products as compared to those patients with a platelet count $<200 \times 10^3/\mu\text{l}$ ($P = 0.021$ and $P = 0.003$). However CD34+ cells were comparable in the two groups.

Discussion

This study presents the results of analysis of bone marrow cellular profile (TNC, MNC and CD34+ cells) of patients in different age groups, gender, BMI, haematological profile (Hb, TLC and platelets) and clinical disorders, who have presented themselves for bone marrow mononuclear cell therapy as a regeneration medicine option. We tried to

Fig. 6 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in different pre-harvest Hb groups

study the effect of patient variables (age, sex and BMI), haematological profile (Hb, TLC and platelets) and clinical disorders in the cellular yield. This study found that the patient's age, BMI, haematological profile and disease state significantly affect the bone marrow cellular profile.

A lot of literature exists on the correlation between MNC counts and CD34+ yields in bone marrow aspirates however such correlation with age and disease state is

scarce. In 2012 Vidyasagar et al. has reported a study of 332 patients for MNC therapy in spinal cord injury, wherein they observed that MNC and CD34+ cells in bone marrow decreased with age in spinal cord injury patients [7].

We did not observe any significant difference in bone marrow cellular profiles (TNC, MNC and CD34+ cells) between male and female patients implying thereby that

Fig. 7 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in different pre-harvest TLC groups

gender does not have any kind of influence on bone marrow cellular profiles (TNC, MNC and CD34+ cells). There was a steady decline in TNC and MNC counts with age but CD34+ cells showed an unpredictable rise above 60 years of age. Similar observations have been reported by Klaudia Kuranda et al., in 2011 where in, the authors observed that higher CD34+ CD38- cells in the bone marrow of aged (>70 year) individuals, but these cells had a less differentiation capability to produce B-cell progenitors and myeloid lineage cells [8].

A study published in 2007 by Stuart M. Chambers et al., had also revealed similar trend of rise in haemopoietic stem

cells with advancing age but with decline in function in case of mice. Further, they also showed that haemopoietic stem cells also undergo age related changes in the form of loss of epigenetic regulations leading to decreased cellular and metabolic function and increased tendency for neoplastic transformation [9].

In our study, we found that there were significant variations in TNC, MNC and CD34+ cell counts amongst the three different clinical groups. The patients with acute traumatic events, who were otherwise healthy, had better cellular profiles as compared to the patients of chronic disorders and acute vascular events. This could be due to

Fig. 8 Average bone marrow TNC and MNC counts (a) and CD34+ cells % (b) in different pre-harvest platelets groups

the fact that the patients were younger in age (age < 40 - years) without any underlying metabolic condition which can have an overall systemic suppressive effect on the rapidly regenerating tissues such as bone marrow due to release of various cytokines.

Our patients with chronic disorders such as type 2 diabetes mellitus and peripheral vascular disease had certain life style related factors such as obesity, sedentary life style

and history of chronic smoking which could have been responsible for lower cellular yield in the bone marrow aspirates from these patients.

In this study, we also observed that the pre-harvest haematological profile i.e. TLC and platelet count significantly affect the bone marrow cellular profiles, but these statistically significant differences can be unpredictable due to the blood loss in acute traumatic patients

and anti-platelet drugs in ischemic stroke and cardiovascular patients.

The impact of donor demographics and other life style related factors on the bone marrow cellular profiles (TNC, MNC and CD34+ cell) has been studied in the healthy subjects previously wherein the variables such as genetics, body mass index, sedentary life style and obesity has been found to influence the quantity and other characteristics of HSCs in the marrow. In 2011, Bellows et al., studied the effect on bone marrow cellular profiles and observed that the healthy subjects with high BMI had increased numbers of circulating progenitor cells especially hematopoietic and endothelial precursors than low BMI subjects [10]. In addition, Huertas et al., in 2010 has also described that individuals with low BMI had a significantly lower CD34+ cell counts in bone marrow as compared to the high BMI such patients of chronic obstructive pulmonary disease [11]. However in our present study, statistically significant difference was found in MNC counts only, but not in TNC and CD34+ cell counts across the different BMI groups.

In our study, we enumerated the CD34+ cell population by flow cytometry but could not include the functional assay such as Colony Forming Unit (CFU) which shows a strong correlation with genetic factors influencing the stem cell profile through aging. Hence, it would have been ideal to evaluate CFU across different age groups and clinical conditions rather than estimating CD34+ cells yields only. Thus, it would have given us the comprehensive (numerical and functional) understanding of the variations in bone marrow cellular profile in our patients. Such analysis would certainly go a long way in predicting the outcome of stem cell therapies in various clinical conditions amongst different age groups. However, the bone marrow cellular indices i.e. quantity of TNC and MNC per millilitres of bone marrow and CD34+ cells (%) can be used as a tool to guide the bone marrow collection in patients for bone marrow stem cell therapy and the CD34+ cell number in the harvest has been accepted as a predictor of engraftment. A recent study on 435 unrelated cord blood transplants by Page et al. suggested that the CFU quantification assay is strong and independent predictor of neutrophil and platelet engraftment than CD34 enumeration [12].

Thus, further studies are recommended on bone marrow cellular profile analysis and CFU assay across different patient age groups and healthy individuals to provide a better understanding into the biology of these cellular elements used for therapeutic applications in regenerative medicine.

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