

## COMPARISON OF COLONY FORMING UNIT-ASSAY RESULTS OF DIFFERENT HEMATOPOIETIC STEM CELL SOURCES

### *FARKLI HEMATOPOETİK KÖK HÜCRE KAYNAKLARININ KOLONİ OLUŞTURMA COLONY FORMING UNIT-ASSAY KAPASİTELERİNİN KARŞILAŞTIRILMASI*

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#### ABSTRACT

**Aim:** Hematopoietic stem cell transplantation (HSCT) is practiced successfully especially for hematologic malignancies. The source of hematopoietic stem cells for HSCT is bone marrow in allogeneic transplantation or peripheral blood stem cells after mobilization in autologous transplantation. The aim of this study is to investigate CFU-ASSAY results of two different hematopoietic stem cell sources.

**Method:** Hematopoietic stem cells collected from bone marrow in 7 patients and peripheral blood in 7 patients were included in the study. Hematopoietic stem cells were cultured in semi-solid agar culture medium (Methocult, H 4434 Classic, Stem Cell Technologies, Canada) and CFU-ASSAY was performed after 14-18 day of culture period for each group.

**Results:** CFU-Assay results of peripheral blood stem cells for autologous donors were as follows; CFU-GM:28.44x104/kg, BFU-E:40.7x104/kg, CFU-E:0.79x104/kg, CFU-GEMM :1.95x104/kg. CFU-Assay results of bone marrow stem cells for allogeneic donors were as follows; CFU-GM:7.03x104/kg, BFU-E:10.46x104/kg, CFU-E:0.16x104/kg, CFU-GEMM:0.51x104/kg.

**Conclusion:** CFU-Assay results revealed that colony forming capacity of peripheral blood stem cells are higher than that of bone marrow stem cells. Peripheral blood stem cells transplantation is becoming more prevalent especially in adults. In pediatric groups, to identify the correlation between invitro results and clinical observations may help to increase preference of peripheral blood stem cell transplantation.

**Key words:** Stem Cell, CFU-Assay, hematopoietic stem cell transplantation

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**ÖZET:**

**Giriş ve Amaç:** Günümüzde Hematopoetik Kök Hücre Nakli (HKHN) özellikle hematolojik malignitelerin tedavisinde başarıyla uygulanmaktadır. Hematopoetik kök hücre (HKH) kaynağı olarak allojenik nakillerde genellikle direkt kemik iliğinden, otolog nakillerde ise genellikle kemik iliğinden mobilize edilip periferik kandan elde edilen hematopoetik kök hücreler kullanılmaktadır. Bu çalışmada farklı kaynaklardan elde edilen hematopoetik kök hücrelerin koloni oluşturma kapasitelerinin karşılaştırılması amaçlandı.

**Gereç ve Yöntemler:** Hematopoetik kök hücre örnekleri 7 olguda direk kemik iliğinden, 7 olguda da periferik kandan elde edildi. Elde edilen hematopoetik kök hücrelerle invitro hücre kültür ortamlarına alındı. Periferik kan ve kemik iliği kaynaklı HKH örnekleri uygun Semi-Solid Agar besiyerinde (Methocult, H 4434 Classic, Stem Cell Technologies, Canada) kültüre edilerek 14-18 gün sonra koloni sayıları belirlenerek Colony Forming Unit Assay (CFU-Assay) hesaplandı.

**Bulgular:** Otolog 7 vericiden alınan periferik kan HKH örneklerinin koloni sayımlarının ortalamaları CFU-GM:28.44x104/kg, BFU-E:40.7x104/kg, CFU-E:0.79x104/kg, CFU-GEMM :1.95x104/kg. Allojenik 7 vericiden alınan kemik iliği HKH örneklerinin Koloni sayımlarının ortalamaları CFU-GM:7.03x104/kg, BFU-E:10.46x104/kg, CFU-E:0.16x104/kg, CFU-GEMM :0.51x104/kg.

**Sonuç:** CFU-Assay ile değerlendirilen periferik kan kaynaklı HKH kolonilerinin sayısı kemik iliği kaynaklı HKH kolonilere göre daha yüksek bulundu. Hematopoetik kök hücre nakillerinde özellikle erişkinlerde periferik kan kaynaklı hematopoetik kök hücrelerin kullanımı giderek yaygınlaşmaktadır. Pediatrik grupta da klinik verilerle invitro hücre kültür bulgularının desteklenmesi periferik kan kaynaklı HKH kullanımı için tercih sebebi olabilir.

**Anahtar Sözcükler:** Kök hücre, CFU-Assay, hematopoetik kök hücre nakli

**INTRODUCTION**

The early studies of Hematopoietic Stem Cell Transplantation (HSCT) began in 1950s until 1970s by E. Donald Thomas and colleagues from Fred Hutchinson Cancer Research Center, University of Washington. Thereafter, these studies brought him a Nobel price in 1990 (1-6). In 1968, Dr. Robert Good and his colleagues from University of Minnesota, carried out the first bone marrow transplantation in humans from a matched sibling donor for an infant with primary immuno-deficiency (7-8). Since then, HSCT utilizing bone marrow as a stem cell source has become an accepted treatment modality for a variety of metabolic, immunologic, and hematologic malign and non-malign disorders (9-11). Mature hematopoietic cells arise from pluripotent hematopoietic stem cells in bone

marrow. Most pluripotent stem cells can be identified by expression of CD34 antigen and these cells have a high engraftment capacity. The discovery and clinical application of granulocyte colony-stimulating growth factor (G-CSF) and granulocyte –macrophage colony-stimulating growth factor (GM-CSF) led to the observation that CD34+ bone marrow stem cells can be mobilized in to the peripheral blood in large numbers. After this observation, transplantation of peripheral blood stem cells (PBSCs) from G-CSF mobilized healthy donors from related or unrelated donors have become available during the last years. Collection of PBSCs became practical with the development of centrifuge technology named apheresis. The major advantages of this technique are no need for general anesthesia; no need for post-harvest hospital-

ization; less traumatic; less need for pre-harvest transfusion; more rapid engraftment, and decreased infection.

This study aimed to evaluate and compare CFU-assays of bone marrow (BM) derived or peripheral blood (PB) derived hematopoietic stem cells of allogenic and autologous transplantation donors.

### MATERIAL AND METHOD

HSCT of 50 (fifty) patients were performed between April 2010 and August 2011 in Bone Marrow Transplantation Unit of Ankara Children's Hematology Oncology Education and Research Hospital. 7 (14%) of 50 allogenic/autologous transplantations were performed from PBSC donors. In this study, we included 7 donors for each group in order to evaluate the same number of CFU-assay in each group.

**CFU-ASSAY:** Hematopoietic stem cells derived from either bone marrow or peripheral blood were cultured in Semi-solid agar culture medium (Methocult, H 4434 Classic, Stem Cell Technologies, Canada) and after 14-18 days of culture period Colony Forming Unit Assay (CFU-ASSAY) was performed for each group. Each colony was classified according to the cell type and number of mature cells as follows: Burst Forming Unit-Erythroid (BFU-E); Colony Forming Unit-Granulocyte, Macrophage (CFU-GM); Colony Forming Unit-Granulocyte,

Erythroid, Macrophage, Megakaryocyte (CFU-GEMM); Colony Forming Unit-Erythroid (CFU-E).

### RESULTS

BFU-E, CFU-GM, CFU-GEMM, CFU-E forming capacity of BM derived HSCs was lower than colony forming capacity of autologous/allogenic PBSCs after 14 days of cell culture (Table 1 and 2).

PBSCs collected by apheresis were cultured and formed colonies at day 14 are photographed under inverted microscope (Figure-1 a,b,c, and d).

CFU-GEMM registered as colony forming unit-granulocyte, erythroid, macrophage, megakaryocyte which produces 20 or more cells including some from at least the first 2 types named (Figure 1a). CFU-GM and CFU-E were observed together (Figure 1b). BFU-E produces 3 or more clusters of erythroblasts or an equivalent number of erythroblasts (Figure 1c). CFU-GEMM colonies including all hematopoietic series are viewed in cell culture (Figure 1d).

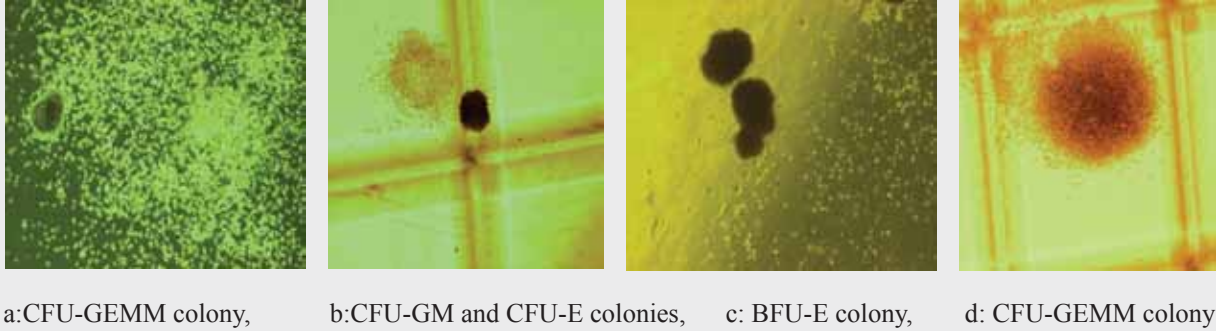
Invitro BM derived HSCs colonies are also registered after 14-18 day culture (Figure 2 a,b,c, and d). CFU-GEMM, CFU-GM, CFU-E, BFU-E colonies of BM derived HSCs are also observed in cell culture, however the number of colonies are lower than PBSC colonies (Figure 2a,b,c, and d).

**Table 1. The average number of BM derived HSC colonies**

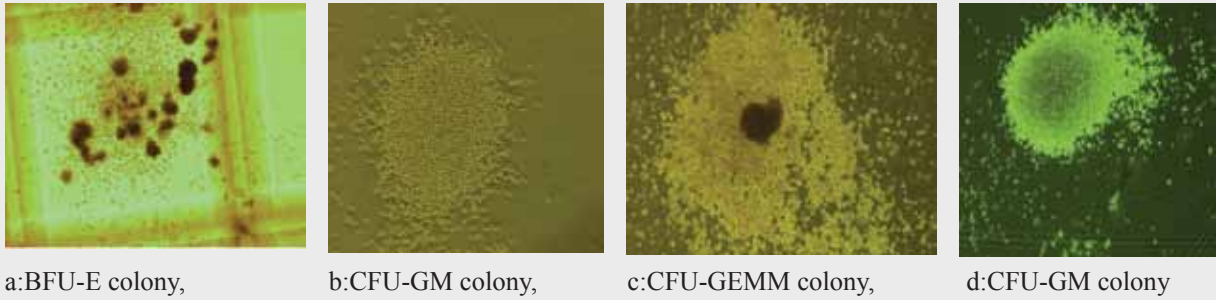
BFU-E X10 <sup>4</sup> /KG	CFU-GM X10 <sup>4</sup> /KG	CFU-GEMM X10 <sup>4</sup> /KG	CFU-E X10 <sup>4</sup> /KG
10,46	7,03	0,51	0,16

**Table 2. The average number of PB derived HSC colonies**

BFU-E X10 <sup>4</sup> /KG	CFU-GM X10 <sup>4</sup> /KG	CFU-GEMM X10 <sup>4</sup> /KG	CFU-E X10 <sup>4</sup> /KG
10,46	7,03	0,51	0,79



**FIGURE-1 PB derived HSC colonies**



**FIGURE-2 BM derived HSC colonies**

## DISCUSSION AND CONCLUSION

Most early studies of HSCT used marrow as the source of stem cells. Later, CD34<sup>+</sup> cells separated from the marrow were shown to be effective (12). The use of HSCs from different sources has changed the terminology from bone marrow transplantation (BMT) to haemopoietic stem cell transplantation.

Transplantation with peripheral blood stem cells (PBSCs) rather than marrow began with the demonstration of these cells in the blood of mice, dogs, and non-human primates (13-15). PBSCs were used for transplantation for patients whose marrow could not be collected because of disease or previous irradiation therapy (16).

It was reported that the number of PBSCs in circulation could be increased by chemotherapy and by the administration of haemopoietic growth factors (17). Stem

cells can be separated and purified based on their expression of CD34. Although PBSCs has been increasingly used for autologous transplantation, they can also be used for allogeneic transplantation but an increased risk of chronic graft-versus host disease (GVHD) has been reported (18). Even though, it was suggested that the presence of mature T cells in peripheral blood increases the risk of GVHD when compared with BM grafts, whereas an enhanced graft-versus leukemia (GVT/L) effect may result in a higher cure rate for malignancies. The potential advantages of PBSC vs BM as a stem cell source include more rapid hematologic and immunologic engraftment leading to a decrement in infection and a potential for diminished morbidity and mortality. When pediatric PBSC donors are compared with pediatric bone marrow donors, the potential risks are as follows; 1-The need for proper vascular access, 2-The early, and as yet unknown,

late side effects of G-CSF administration, 3-The risks of apheresis. The potential benefits of PBSC harvesting include the use of little or no general anesthesia; no need for post harvest hospitalization; less physical difficulty and emotional stress; and less inconvenience to the donor and family (19).

All hematopoietic and progenitor cell assays measure two main parameters: cell proliferation (measured by the number of cells produced) and differentiative potential (estimated by the number of different lineages represented in its progeny) (20). In order to evaluate cell proliferation and differentiation capacity of HSCs, we performed short term in vitro CFU-assay in our laboratory. The number of CFU colonies proliferate from PBSCs found to be higher than the number of BM derived from HSCs. This result can be explained with large number of CD34+ cells mobilized after G-CSF administration for PBSC apheresis. Although, the results of the in-vitro assay need to be elucidated with in-vivo clinical correlation.

PBSC mobilized by G-CSF began to replace BM as the preferred stem cell source for autologous transplantation as a result of rapid engraftment and decreased infection rates in adults. During the last decade, PBSC transplantation became a serious alternative to BM HSC transplantation also in pediatric patients and the result of this study favours this approach. Therefore, an analysis of the risk to benefit ratio which addressed to effectivity and safety of the procedure should be defined in pediatric groups.

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