# Immune Reconstitution After Allogeneic Bone Marrow Transplantation in Early Posttransplant Period

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**ABSTRACT**

In this prospective study, the immune reconstitution of the T, B and NK cells, immunoglobulin and complements were investigated in the early posttransplant period of BMT.

Twenty patients (16 male, 4 female; mean age 27.55) were followed up for six months. Peripheral blood samples were obtained on the 14th, 28th, 100th and 180th days.

CD4+ cell count was below the normal till the 180th day. CD8+ cell count showed a rapid increase after the first month and reached to normal at six months. CD4/CD8 ratio was below 1. NK cell count was above the normal during the first 3 months. B cell count was normal. IgA levels were below the normal till the 100th day. IgM levels were normal. IgG levels showed a significant decline after first month. C3c level were above the normal and C4 level were normal.

In conclusion, this study indicates that NK cells show more rapid recovery than the CD8+ and CD19+ cells, while CD4+ cells are still below normal, six months after BMT.

**Key Words:** Bone marrow transplantation, Immune reconstitution

**ÖZET**

**Allojeneik Kemik İliği Transplantasyonu Uygulanan Olgularda Posttransplant Erken Dönemde İmmün Yeniden Yapılanma**

Bu prospektif çalışmada allojeneik kemik iliği transplantasyonundan sonra erken dönemde; T, B ve NK hücreleri, immunglobulinler ve kompleman düzeyleri ile immun yeniden yapılanma paterni incelendi.

Toplam 20 hasta (16 erkek, 4 kadın; ortanca yaş 27.55) transplantasyondan sonra altı ay boyunca izlendi. 14., 28., 100. ve 180. günlerde periferik kan örnekleri alındı.

CD4+ hücre sayısı 180. güne kadar normal düzeyin altında idi. CD8+ hücre sayısı birinci aydan sonra hızlı bir artış gösterdi ve altıncı ayda normal düzeye ulaştı. CD4/CD8 oranı 1'in altında idi. NK hücre sayısı ilk 3 ay boyunca normalin üzerinde idi. B hücre sayısı normal idi. IgA düzeyleri 100. güne kadar normalin altında idi. IgM düzeyleri ise normaldi. IgG düzeylerinde birinci aydan sonra belirgin bir azalma gözlendi. C3c ve C4 düzeyleri çalışma süresince normaldi.

Sonuç olarak bu çalışmada; NK hücreler, CD8+ ve CD19+ hücrelerden daha hızlı bir düzelme göstermiştir. Buna karşın CD4+ hücreler posttransplant altıncı ayda bile normal düzeyin altında kalmıştır.

**Anahtar Kelimeler:** Kemik iliği transplantasyonu, İmmun yeniden yapılanma

## INTRODUCTION

The success of the high dose chemotherapy and bone marrow transplantation (BMT) in the treatment of the malign diseases are limited by transplantation related morbidity and mortality besides of the disease persistence and relapse. The causes of the allogeneic BMT related mortalities are infections and acute graft versus host disease (GVHD). Both of these complications are caused by immune system anomalies which develop during the hematopoietic recovery.

Immune reconstitution after BMT was investigated in many studies (1-4). The main characteristics of the immune reconstitution after allogeneic BMT are the rapid recovery of the CD8+ cells and slow recovery of the CD4+ cells. This situation is more significant in the early posttransplant period, in patients who develop acute or chronic GVHD and viral infections (5, 6). Subnormal CD4/CD8 ratio has been ascertained in 1-2 years after allogeneic BMT (7-10).

While IgG and IgM levels return to normal levels 3-4 months after BMT, low IgA levels may persist for years (11). On the other side, C3c and C4 levels return to normal during the first 3 months of BMT (12).

In this prospective trial, the kinetics of immunologic reconstitution of T and B lymphocytes and Natural Killer (NK) cells, and the immunoglobulin and complement levels in the early posttransplant period (0-180 days) were investigated in patients who underwent allogenic BMT.

## PATIENTS AND METHODS

### Patients

Twenty patients who underwent allogeneic BMT in our BMT center have been studied prospectively regarding immunological reconstitution in early posttransplant period (0-180 days). Patient characteristics are shown in Table 1. Written consent to participate in this study program was obtained from all patients prior to initiation of the study.

### Collection Bone Marrow

Allogeneic bone marrow cells were collected by using standart technics and infused by intravenous route in same day.

### Preparative Regimens

Total body irradiation (TBI) (2 Gy/day and total 12 Gy; Co-60 Teletheraphy Unit) + Cyclophosphamide (Cy) (60 mg/kg) to 17 patients and only Cy (60 mg/kg) to 3 patients, regimens were administered.

### Posttransplant Hematopoietic Growth Factor

In order to accelerate the engraftment in the posttransplant period, 5 $\mu$g/kg/day G-CSF (Neupogen, Roche) by 2-hour infusion have been given to all patients. The hematopoietic growth factor was started on day +1 and continued during three consecutive days till the leukocyte count reached $\geq 1x10^9$/l.

### Posttransplant Supportive Treatment

After infusion, patients were isolated in conventional rooms with ultraviolet. Low bacterial diet, nonabsorbable oral antibiotics and total parenteral nutrition were given to all patients. In case of fever above 38°C that lasted more than two hours or if clinically infection was suspected, blood samples were taken and wide spectrum antibiotics were initiated. Afterwards, according to microbiological culture results antibiotic treatment were readjusted. To keep the hemoglobin level above 8 g/dl and the platelet count above 20 x $10^9$/l, erythrocyte and platelet transfusions were administered. In all cases blood products were irradiated with 25 Gy.

### Evaluation of Lymphocyte Count, Lymphocyte Subsets, Immunoglobulin and Complement Levels

In the posttransplant period CD3+, CD4+, CD8+, CD19+ and CD56+ cells were determined by flow cytometry in the peripheral blood samples collected on the 14th, 28th, 100th and 180th days. CD3 FITC (Leu-4), CD4 FITC (Leu-3a), CD8 PE (Leu-2a), CD19 PE (Leu-12), CD56 PE (Leu-19) monoclonal antibodies (BD Immunocytometry Systems, San Jose, CA 95131 USA) were used for analysis. One million cells in the sample were mixed with 20 $\mu$l monoclonal antibody and incubated at room temperature (20-25$^0$C) 15- 30 minutes. At the end of the incubation, the erythrocytes in the medium

**Table 1.** Patient characteristics

| | n | % |
|---|---|---|
| Number (n) | 20 | |
| Gender (M/F) | 16/4 | 80/20 |
| Age (mean, year) | 27.55 (21-50) | |
| Diagnosis | | |
| ALL | 5 | 25 |
| AML | 6 | 30 |
| KML | 4 | 20 |
| Aplastic anemia | 4 | 20 |
| Multiple myeloma | 1 | 5 |
| Preparative regimen | | |
| Cy * | 3 | 15 |
| TBI ** + Cy | 17 | 85 |
| TNC *** count ($x10^8$/kg) | 3.25 (0.61-7.84) | |
| CD34+ cell count ($x10^6$/kg) | 4.84 (1.64-5.54) | |
| Posttransplant growth factor | | |
| G-CSF **** | 20 | 100 |
| Posttransplant fever (>38$^0$C) | 7 | 35 |
| Dx-Tx interval (months) ***** | 7.75 (2-29) | |
| GVHD ****** | 10 | 50 |
| Acute, Grade-I | 4 | 20 |
| Acute, Grade-II | 3 | 15 |
| Acute, Grade-III | 1 | 5 |
| Chronic | 2 | 10 |
| GVHD prophylaxis | | |
| Methotrexate + Cyclosporine-A | 20 | 100 |

* Cy: Cyclophosphamide
** TBI: Total Body Irradiation
*** TNC: Total Nucleated Cells
**** G-CSF: Granulocyte Colony Stimulating Factor
***** Dx: Diagnosis Tx: Transplantation
******GVHD: Graft Versus Host Disease

were incubated in the dark with FACS lysing solution (BD San Jose, CA 95131, USA). Counting at least 10.000 cells was made with using the cellquest program of FACSCalibur (BD San Jose, USA) model flow cytometry. The absolute counts of CD3+, CD4+, CD8+, CD19+, and CD56+ cells were calculated by multiplying the percent values obtained by flow cytometry with the total number of cells obtained at the same time by the automatic blood counter (Coulter MD18, USA). 1-4X$10^9$/l for total lymphocyte count, 0.66-3.22 X$10^9$/l for CD3, 0.405-2.205 X$10^9$/l for CD4, 0.165-1.33 X$10^9$/l for CD8, 0.6-2.8 for CD4/CD8 ratio, 0.03-0.525 X$10^9$/l for CD19, 0.015-0.455 X$10^9$/l for CD56 were accepted as normal range (13).

Immunoglobulin and complement levels were determined by the nephelometric method (Nephelometry analyzer, Behring, Germany). 8-16 g/l for IgG, 1-4 g/l for IgA, 0.7-4 g/l for IgM, 0.5-0.9 g/l for C3c and 0.1-0.4 g/l for C4 were accepted as normal range (14).

**Table 2.** The mean values of the parameters during the follow-up period.

| Parameter (normal range) | Day 14 | Day 28 | Day 100 | Day 180 |
|---|---|---|---|---|
| Lymphocyte number (1-4x$10^9$/L) | 981,82 | 1357,89 | 1508,33 | 2063,64 |
| CD3 (0.66-3.22x$10^9$/L) | 536,35 | 635,56 | 1078,83 | 1580,7 |
| CD4 (0.405-2.205 x$10^9$/L) | 325,35 | 326,11 | 257,75 | 408,3 |
| CD8 (0.165-1.33 x$10^9$/L) | 380,70 | 557,44 | 885,92 | 1320,2 |
| CD4/CD8 (0.6-2.8) | 2,17 | 0,73 | 0,28 | 0,38 |
| CD19 (0.03-0.525 x$10^9$/L) | 38,35 | 52,02 | 54 | 73,8 |
| CD56 (0.015-0.455 x$10^9$/L) | 507 | 613,5 | 532,73 | 373,8 |
| IgG (8-16 g/L) | 13,73 | 13,87 | 11,36 | 8,73 |
| IgA (1-4 g/L) | 1,17 | 1,15 | 1,11 | 0,81 |
| IgM (0.7-4 g/L) | 0,98 | 0,95 | 1,09 | 1,17 |
| C3 (0.5-0.9 g/L) | 1,03 | 1,02 | 0,93 | 0,91 |
| C4 (0.1-0.4 g/L) | 1,21 | 0,24 | 0,42 | 0,24 |

### Statistical Analysis

Mean values of the study parameters were determined (Table 2). The distribution characteristics of the variables were analysed with Kolmogorov-Smirnov and Shapiro-Wilks tests. Nonparametric tests were used as the distribution was not normal.

The associations between immunological reconstitution parameters and the CD34+ cell count were analyzed with Pearson (for numeric variables) and Spearmen (for nominal variables) correlation tests. Multivariate analysis was used to establish the effects of certain parameters (presence of GVHD, TBI, total number of nuclear cells, time interval between the diagnosis and transplantation, age, gender) on the period of immune reconstitution.

## RESULTS

### TNC and CD34+ Cell Counts

Patients were given a median of 3.25 x $10^8$ /kg TNC (0.61-7.84) and 4.84 x $10^6$ /kg CD34 cells (1.64-15.54).

### Total Lymphocyte Count

Total lymphocyte count was below the 1x$10^9$/l on the 14th day, then gradually increased and reached to normal level at 180th day (Figure 1). Any significant difference was not observed between the total lymphocyte count and CD34+ cell count infused.

### T cell Counts

#### CD3+ cells

CD3+ cells were below 1x$10^9$/l on the 14th day. After 28th day, it showed a rapid increase and approached to normal level at 180th day (Figure 1). Any statistically significant difference was not observed between the CD3+ cell count and CD34+ cell count infused.

#### CD4+ cells

CD4+ cells were below the normal range during the study period (<0.405 x $10^9$/l) (Figure-1). Any statistically significant difference was not observed between the CD4+ cell count and CD34+ cell count infused.

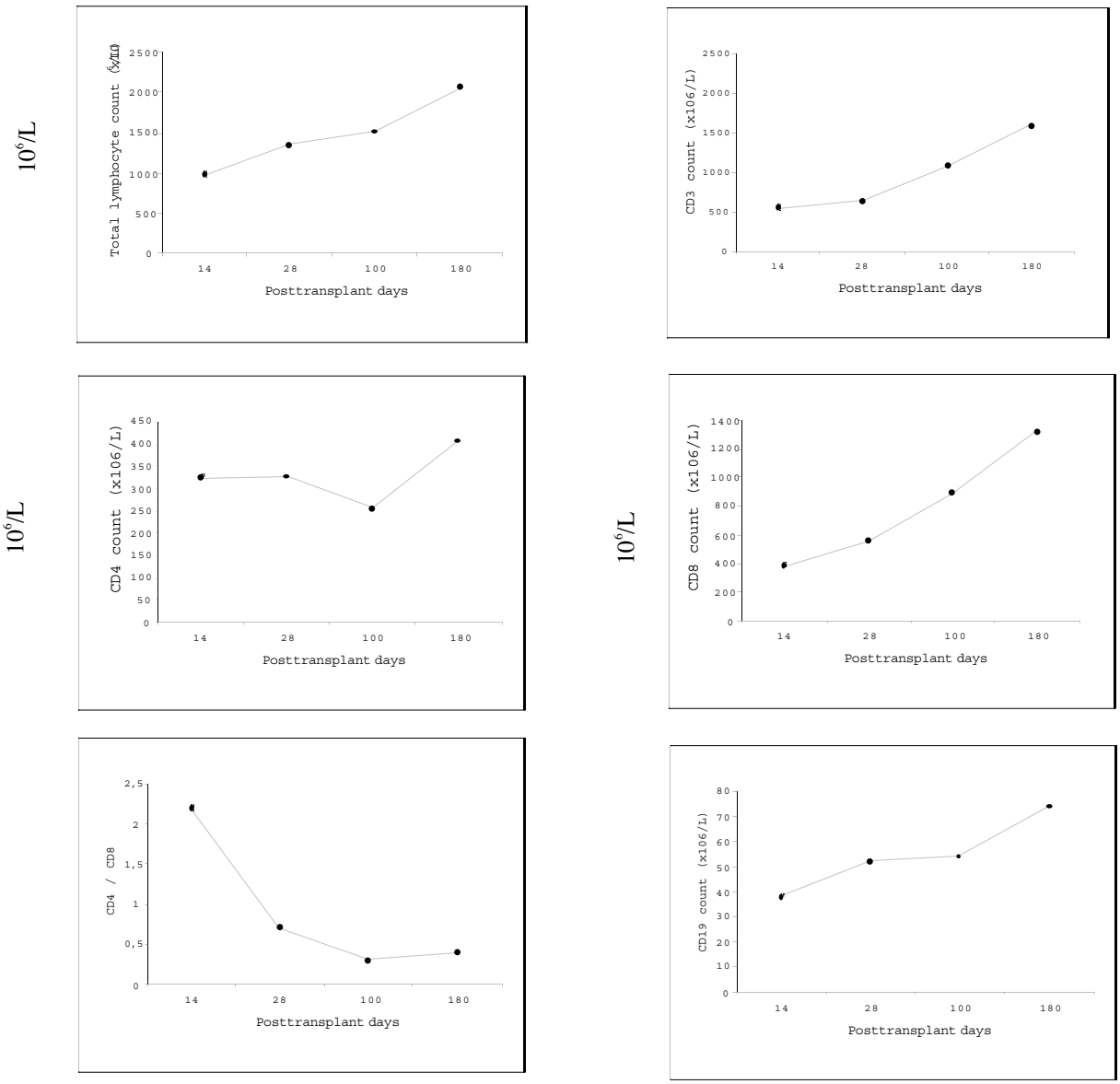


**Figure 1.** Immune reconstitution pattern of total lymphocyte count, CD3 cells, CD4 cells, CD8 cells, CD4/CD8 ratio and CD19 cells in posttransplant period.

### CD8+ cells

CD8+ cells were at the lowest limit on the 14th day. Then, it showed a rapid increase till the 100th day and reached the normal level (Figure-1). Any statistically significant difference was not observed between the CD8+ cell count and CD34+ cell count infused.

### CD4/CD8 ratio

The CD4/CD8 ratio was normal at day 14, but then it started to decrease gradually and remained at the lowest limit of the normal range (<0.6) until the 180th day (Figure 1). Any significant difference was not observed between the CD4/CD8 ratio and CD34+ cell count infused.

$10^6$/L

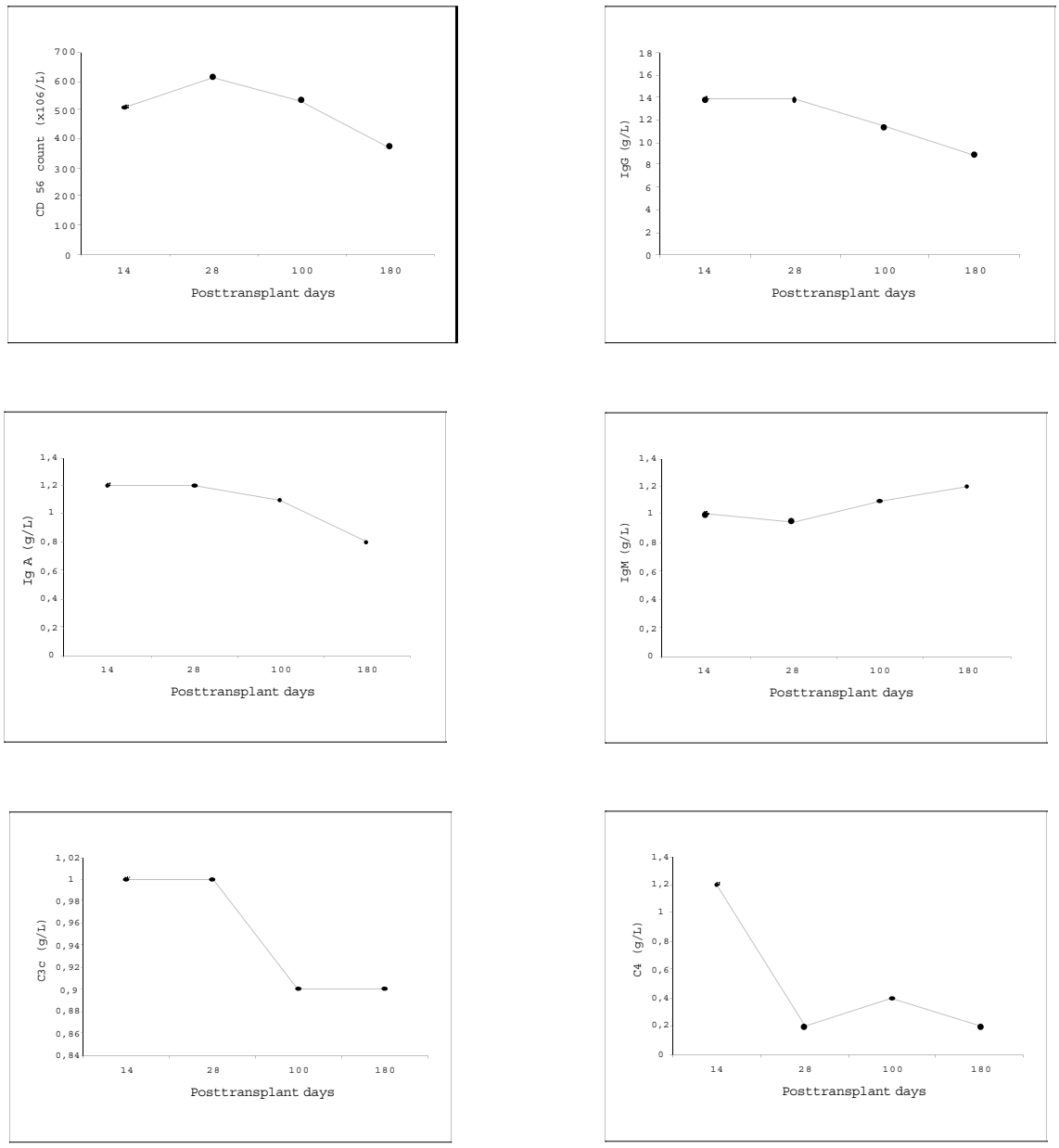


**Figure 2.** Immune reconstitution pattern of CD56 cells, IgG level, IgA level, IgM level, C3c level and C4 level in posttransplant period.

### B cell counts

CD19+ cells were at the lowest limit of the normal range till the 180th day (Figure-1). There was a statistically significant difference between the CD19+ cell count and CD34+ cell count infused at day 28 (p=0.022 r=0.535).

### NK cell counts

CD56+ cells were above the normal range till the 28th day, then it showed a gradual decline to normal range at the 180th day (Figure 2). Any statistically significant difference was not observed between the CD56+ cell count and CD34+ cell count infused.

**Immunoglobulin Levels**

***IgG level***

The IgG level was remained at the upper limit of the normal range till the 28th day, then it showed a gradual decline to lower limit of the normal range at the 180th day (Figure-2). Any statistically significant difference was not observed between the IgG level and CD34+ cell count infused.

***IgA level***

IgA level was normal till the 100th day then it showed a gradual decrease to lower limit of the normal range (Figure 2). There was a statistically significant difference between the IgA level and CD34+ cell count infused at day 14 (p=0.026 r=0.555).

***IgM level***

IgM level was normal till the 180th day (Figure 2). Any statistically significant difference was not observed between the IgM level and CD34+ cell count infused.

**Complement Levels**

***C3c level***

C3c levels were above the normal range (> 0.9 g/l) till the 180th day (Figure 2). There was a statistically significant difference between the C3c level and CD34+ cell count infused at 28th day (p=0.04, r= -0.501).

***C4 level***

The C4 levels were above normal range at the 14th day, then it showed a rapid decline and returned to normal range at the 28th day (Figure 2). Any statistically significant difference was not observed between the C4 level and CD34+ cell count infused.

**In multivariate analysis;**

– TNC count infused affected the C3c, C4 and IgG levels (p=0.018, p=0.029, p=0.03 respectively).

– CD34+ cell count infused and total body irradiation affected the C56+ cell count (p=0.026, p=0.023 respectively).

– Time between the diagnosis and transplantation affected the IgG level (p=0.03).

**DISCUSSION**

Reconstitution of the functional immune system is a critical event after BMT. Complete maturation of the immune system after BMT occurs in 1-2 years (15). Maturation process involves lots of important events including the repair of local defense mechanism; reconstitution of bone marrow; maturation of special lymphocytes responsible for the production of the lymphokine, immunoglobulin and cell membrane receptors. Many factors like GVHD, antibiotics and corticosteroids affect the recovery period (16,17).

The issues affecting the immune reconstitution are not well described. A successful and complete immune reconstitution depends on the improvement of many different factors of the immune system. However the key factor on immune reconstitution is the time after transplantation. Significant depressions on the immune functions of the recipients occur in posttransplant 4th or 5th months (18-20).

The previous studies reported that the main characteristics of the immune reconstitution after BMT are the rapid recovery of the CD8+ cells and the slower recovery of the CD4+ cells (21, 22). The functions of the mature T lymphocytes have been improved in one year. The absolute count and ratio of the CD2+ and CD3+ cells are normal or near normal in posttransplant 3-4 months. In most patients however, CD4+ cells were lower and CD8+ cell counts were higher than the normal ranges in posttransplant 3th or 6th months (21-23). As a matter of fact in our study, CD4+ lymphocytes were below the normal range till the 180th day and CD8+ lymphocytes showed a rapid increase after the first month and reached the normal level at six months. CD4/CD8 ratio remained below 1 during the study period. These findings are consistent with literature.

In most bone marrow recipients, NK cell activity returns to normal levels rapidly in first 30-50 days of BMT, but insufficient cytotoxic functions persist in 20% of patients for one year or more (8,24). In our study, NK cell count were above the normal range in first 3 months.

Abnormal B lymphocyte counts and functions (in regard of immunoglobulin production and proliferation) have been reported. B lymphocyte counts improve more rapidly than the T lymphocytes in first 6 moths (3). In our study, B lymphocyte counts were below the normal range till the 6 months.

In allogeneic bone marrow recipients, IgG and IgM levels return to normal after 4 months. Sometimes, the return of the IgG and IgM levels to normal range may last more than 9 months. Also, IgA level may remain below the normal after 1 year posttransplant (11,17). In our study, IgA levels were below the normal range and IgM levels were normal during the follow-up period. IgG level showed a rapid decline after the 3th month of BMT. The cause of the normal IgG level in first 3 months can be explained by the administration of the intravenous immunoglobulin.

Total hemolytic complement, C3c and C4 level are reported to be normal in 3 months after BMT (12). In our study, C3c levels were above the normal range but C4 levels were normal during the study period.

In conclusion, in this prospective study, NK cells showed the most rapid recovery after posttransplant period. It was followed by the recovery of the CD8+ cells and CD19+ cells. However, CD4+ cells were not reached the normal level at six months.

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