

## Initial white blood cell kinetics for assessment of individual response to the conditioning regimen in pediatric hematopoietic stem cell transplantation patients

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**SUMMARY:** Kuşkonmaz B, Yalçın SS, Azkur D, Aytaç-Eyüpoğlu S, Özdemir P, Çetin M, Uçkan-Çetinkaya D. Initial white blood cell kinetics for assessment of individual response to the conditioning regimen in pediatric hematopoietic stem cell transplantation patients. Turk J Pediatr 2014; 56: 23-30.

We hypothesized that the individual hematological response to chemo/radiotherapy may be used as a parameter to assess the degree of myeloablation and probability of transplant-related events. This study included 58 pediatric patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT). White blood cell (WBC) ratio (pre-conditioning WBC: day 0 WBC), day 0 WBC count, and WBC nadir day were used as potential indicators of myeloablation. The association between WBC kinetics and clinical result of HSCT was investigated. There was a positive correlation between WBC ratio and the date of engraftment. A positive correlation was noted between day 0 WBC count and engraftment day. There was a negative correlation between WBC nadir day and engraftment day. WBC nadir day was lower in patients with acute graft-versus-host disease (GVHD) than in cases without acute GVHD. Among patients who had fever during the conditioning regimen, the WBC ratio was higher, day 0 WBC count was lower, and WBC nadir day was lower in patients who developed >5 days of fever between day 0 and day +30. The present preliminary study suggests that WBC kinetics may be used as a measure of initial hematological response to the conditioning regimen and perhaps in determining the degree of myeloablation.

**Key words:** white blood cell kinetics, children, hematopoietic stem cell transplantation.

The hematopoietic stem cell transplantation (HSCT) period is associated with increased morbidity and mortality, particularly in the allogeneic setting. Pancytopenia, immune-compromised state, tissue/endothelial injury induced by the toxic conditioning regimens, resulting in hypercytokinemia, inflammatory state, and immune interactions between the patient and donor are all contributory factors for development of life-threatening events<sup>1-9</sup>.

Transplant-related events and outcome vary among patients with similar diagnoses who receive similar conditioning regimens. The transplant course is very complex and is influenced by a large number of patient/donor-dependent and -independent factors; therefore, development of an objective and

useful risk scoring system is difficult in HSCT patients. Several polymorphism studies have been performed in transplant patients in order to pre-determine the patient's potential for transplant complications<sup>10-12</sup>. Genes involved in inflammation, coagulation and enzyme and drug metabolisms are among those that are considered to be highly relevant<sup>10,12</sup>. However, due to the complexity of the HSCT system, including patient- and donor-related genetic factors, dual cellular interactions, patient microenvironment, prior exposure of the patient and donor to infectious and other environmental agents, and the enormous numbers of drugs used, determination of polymorphisms even in several genes has not been useful in clinical practice<sup>10-18</sup>.

Some patients may experience unfavorable outcome as a consequence of an increased inflammatory response. Engraftment syndrome, graft-versus-host disease (GVHD), veno-occlusive disease (VOD) (or sinusoidal obstruction syndrome, as recently named), and lymphoproliferative disease are among those life-threatening conditions associated with cytokine excess and inflammation<sup>5,8,19,20</sup>. On the other hand, engraftment failure is attributed to inadequate conditioning of the host or inability of donor immune-inflammatory cells to eradicate host lymphohematopoiesis<sup>21</sup>. Although some of the studies have shown an association between certain genotypes of the patient and/or donor with morbidity/mortality or outcome, it has been difficult to assess the propensity for inflammation or other transplant-related events as a whole<sup>22,23</sup>.

We hypothesized whether the individual hematological response to the conditioning regimen can be used as a parameter to assess the probability of transplant-related complications such as acute GVHD (aGVHD), infections, and timing of engraftment. Individual patient response to a conditioning therapy, namely, the conditioning challenge, may then be used as a measure of the degree of myeloablation, which may correlate with toxicity and complications. In this preliminary study, white blood cell (WBC) kinetics, including WBC ratio, used to define the ratio of initial WBC count (at the beginning of the conditioning regimen) to “day 0” WBC, day 0 WBC counts, and the WBC nadir day, were used as measures to test hematological response and to estimate the degree of myeloablation.

### Material and Methods

This study included 58 pediatric patients who underwent allogeneic HSCT at Hacettepe University İhsan Doğramacı Children’s Hospital, Bone Marrow Transplantation (BMT) Unit, for malignant and non-malignant disorders. The median age of the patients was  $7.8 \pm 4.6$  years (range: 0.25-17.0 years); 42 of 58 (72.4%) were males (Table I). Twenty-seven patients were transplanted for malignant diseases [chronic myeloid leukemia (CML): 9, acute myeloid leukemia (AML): 9, myelodysplastic syndrome (MDS): 5, acute lymphoblastic leukemia (ALL): 3, neuroblastoma: 1], and 31 had non-malignant diseases [thalassemia major:

7, aplastic anemia: 5, Fanconi aplastic anemia: 4, adrenoleukodystrophy: 4, osteopetrosis: 3, metachromatic leukodystrophy: 2, Griscelli syndrome: 2, congenital neutropenia: 1, leukocyte adhesion defect: 1, mannosidosis: 1, Wiskott-Aldrich syndrome: 1]. Transplants were performed from human leukocyte antigen (HLA) 6/6 matched (n=49) or mismatched (n=9) family donors.

Infection prophylaxis included fluconazole, acyclovir, trimethoprim-sulfamethoxazole, and ciprofloxacin. Weekly intravenous immunoglobulin was administered to all patients until discharge. Cyclosporin A  $\pm$  short-term methotrexate were used for GVHD prophylaxis.

Mucositis was graded according to the World Health Organization (WHO) classification<sup>24</sup>. aGVHD and VOD were diagnosed and graded according to conventional criteria<sup>25,26</sup>.

“Engraftment day” was used to define WBC engraftment day, and was defined as the first of three consecutive days when the neutrophil count was higher than  $0.5 \times 10^9/L$ . Patients who did not engraft as well as those with transient engraftment of donor cells were considered to have graft failure.

White blood cell (WBC) ratio was used to define the ratio of initial WBC count (at the beginning of the conditioning regimen) to “day 0” WBC count. Day 0 WBC was used to define the WBC count at day 0. WBC nadir day was used to define the date of the lowest WBC achieved after completion of the conditioning regimen.

Patients at risk for graft failure in the pre-transplant evaluation were described as those with “engraftment resistance”. Engraftment resistance included patients with HLA incompatibility greater than 1/6, transfusion of blood products of more than 50 units, pre-transplant history of allo-immunization, splenomegaly, and second transplantation.

Forty-seven patients received intensive conditioning regimens that included a classical myeloablative transplant consisting of at least 1200 cGy total body irradiation (TBI), or busulfan at a dose of  $\geq 14$  mg/kg orally, or intravenous busulfan at a dose of  $\geq 12.8$  mg/kg (44 patients received busulfan-based regimen, 3 received TBI). Among the 47

patients, 10 received additional melphalan, etoposide or thiotepea. Eleven patients received reduced intensity regimens consisting of cyclophosphamide  $\pm$  antithymocyte globulin (ATG)  $\pm$  fludarabine, or busulfan ( $<8$  mg/kg) + ATG + fludarabine, or TBI less than 750 cGy + ATG  $\pm$  fludarabine.

Patients with high risk of disease recurrence or progression were defined as those with advanced malignancy and included (1) advanced or resistant AML or ALL, (2) MDS–refractory anemia with excess of blasts (RAEB) or MDS–AML, and (3) CML in blast phase.

Drug-related adverse effects included clinical descriptive features attributed to drug use such as toxic hepatitis, cardiotoxicity, neurotoxicity, hemorrhagic cystitis, parotitis, drug eruption, urticarial rash, serum sickness, and anaphylaxis, after exclusion of other causes including infection.

Documented infection was defined as a microbiologically (blood culture received from peripheral blood and/or central catheter or urine culture) and/or clinically demonstrated infection.

#### Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL). Kolmogorov-Smirnov test was used to analyze the distribution of variables, and logarithmic transformation was done for variables with skewed distribution. Student's t-test (or Mann-Whitney U test) was used for statistical comparisons where appropriate. The relationships between the date of engraftment and WBC kinetics (WBC ratio, day 0 WBC, WBC nadir day) were analyzed with Pearson correlation for univariate comparison and multiple linear regression analysis after adjusting for HLA compatibility, female to male transplantation, intensity of the conditioning regimen, use of granulocyte colony-stimulating factor (G-CSF), malignant or non-malignant disease, and engraftment resistance. Values were given as mean (or geometric mean)  $\pm$  standard error of mean. A value of  $p < 0.05$  was accepted as statistically significant.

#### Results

Clinical features of the patients are shown in Table I.

#### *The Effect of Initial WBC Kinetics on Engraftment Day*

White blood cell (WBC) engraftment was achieved in all except four patients. The median engraftment day was 15 days (range: 9-29 days).

**WBC ratio:** There was a statistically significant positive correlation between the logarithmic value of the WBC ratio and the date of engraftment in univariate analyses ( $r=0.281$ ,  $p=0.039$ ). The correlation remained significant in multivariate analysis controlling for variables including HLA match or mismatch, female to male transplantation, intensity of the conditioning regimen, use of G-CSF, disease diagnosis (malignant or non-malignant), and engraftment resistance ( $\beta=0.104$ ,  $p=0.001$ ). When the patients were analyzed according to diagnosis, the correlation was significant for patients with malignant disease ( $r=0.649$ ,  $p < 0.001$ ). This correlation was still significant for malignant disease in multivariate analysis controlling for HLA match or mismatch, female to male transplantation, intensity of the conditioning regimen, use of G-CSF, engraftment resistance, and advanced disease ( $p=0.001$ ).

**Day 0 WBC:** A positive correlation in multivariate analysis was noted between logarithmic value of day 0 WBC count and engraftment day controlling for the above-mentioned parameters ( $p=0.005$ ) (for univariate analysis  $p > 0.05$ ).

**WBC nadir day:** There was a negative correlation between WBC nadir day and engraftment day ( $r=-0.243$ ,  $p=0.076$ ). This relation was significant in multivariate analysis ( $p=0.002$ ). A negative correlation was still present for malignant disease in univariate ( $r=-0.385$ ,  $p=0.052$ ) but not in multivariate analysis. On the other hand, multivariate analysis including HLA match or mismatch, female to male transplantation, intensity of the conditioning regimen, use of G-CSF, and engraftment resistance showed a negative correlation ( $p=0.023$ ) for non-malignant disease without a significance in univariate analysis.

#### *The Effect of Initial WBC Kinetics on aGVHD*

Nineteen of 58 patients (32.8%) developed aGVHD  $\geq$  grade II. Geometric mean titer of WBC ratio was higher in patients with aGVHD

Table I. Clinical Characteristics of the Patients

Age (mean±SD)	7.8±4.6
Gender (Male)*	42 (72.4)
Malignant disease	27 (46.6)
Advanced malignancy	18/27 (66.7)
Non-malignant disease	31 (53.4)
HLA mismatch ≥1/6	9 (15.5)
Female to male transplant	21 (36.2)
Engraftment resistance	29 (50.0)
Conditioning regimen	
Intensive conditioning regimen	47 (81.0)
Reduced-intensity conditioning regimen	11 (19.0)
G-CSF usage	43 (74.1)
Neutrophil engraftment	54 (93.1)
Acute GVHD (≥Grade II)	19 (32.8)
VOD	10 (17.2)
Mucositis (grades 3-4)	20 (34.5)
Documented infection	32 (55.2)
Drug-related adverse effects	15 (25.9)
Overall survival	39 (67.2)
TRM	10 (17.2)

\*values was given as n (%)

G-CSF: Granulocyte colony-stimulating factor. GVHD: Graft-versus-host disease. HLA: Human leukocyte antigen. TRM: Transplant-related mortality. VOD: Veno-occlusive disease.

but not statistically significant ( $10.28 \pm 1.09$  vs  $6.11 \pm 0.44$ ,  $p=0.079$ ) (Table II). Day 0 WBC count was lower in patients who developed aGVHD but not statistically significant ( $837 \pm 816$  vs  $1728 \pm 2014$ ,  $p=0.070$ ) (Table II). Day 0 WBC count was not different in patients with and without aGVHD when malignant and non-malignant diseases were analyzed separately.

White blood cell (WBC) nadir day was lower in patients with aGVHD than in cases without aGVHD in all cases ( $3.11 \pm 0.80$  vs  $5.13 \pm 0.44$ ,  $p=0.035$ ) (Table II). When cases with malignant disease were analyzed separately, lower WBC nadir day was noted in patients with aGVHD ( $3.67 \pm 2.78$  vs  $5.83 \pm 1.72$ ,  $p=0.055$ ). However, no difference was observed in cases with non-malignant diseases.

#### *The Effect of Initial WBC Kinetics on Other Transplant-Related Events*

Sinusoidal obstruction syndrome developed in 10 (17.2%), grades 3-4 mucositis in 20 (34.5%), fever during the conditioning regimen in 20 (34.5%), fever ≥5 days after day 0 in 31 (54.5%), and drug-related adverse effects in 15 (25.9%) patients, and documented infection on 32 occasions (55.2%) (Table I). Nineteen patients died (32.8%), 10 of them (17.2%) due to transplant-related events.

In patients who had fever during the conditioning regimen, the geometric mean titer of WBC ratio was higher and geometric mean titer of day 0 WBC count was lower ( $12.88 \pm 1.12$  vs  $5.25 \pm 0.39$ ,  $p=0.001$  for WBC ratio;  $426.58 \pm 47.69$  vs  $1071.52 \pm 88.08$ ,  $p=0.006$  for day 0 WBC). WBC nadir day was lower in patients who developed fever after day 0 for >5 days between day 0 and day 30

(3.55±0.59 vs 5.35±0.58, p=0.038) (Table II).

There was no relationship between WBC kinetics and the other transplant-related events

mentioned above (Table II).

Since the diagnoses of the patients were heterogeneous and the number of relapsed

**Table II.** Association between WBC Kinetics and Post-Transplant Events

	n	WBC ratio	Day 0 WBC	WBC nadir day
<b>aGVHD</b>				
No	39	6.11±0.44	1728±322.5	5.13±0.44
Yes	19	10.28±1.09	837±187.2	3.11±0.80
P		NS	NS	0.035
<b>VOD</b>				
No	48	7.33±0.49	630.96±51	4.33±0.48
Yes	10	6.93±1.04	831.76±99.95	5.1±0.57
P		NS	NS	NS
<b>Mucositis (grades 3-4)</b>				
No	38	8.13±0.57	1287±201.48	4.16±0.52
Yes	20	5.89±0.68	1720±554.32	5.05±0.58
P		NS	NS	NS
<b>Fever during the conditioning regimen</b>				
No	37	5.25±0.39	1071.52±88.08	4.51±0.49
Yes	20	12.88±1.12	426.58±47.69	4.2±0.77
P		0.001	0.006	NS
<b>Fever after day 0 (&gt;5 days)</b>				
No	23	10.52±2.5	870.96±76.28	5.35±0.58
Yes	31	12.83±2.77	676.08±74.07	3.55±0.59
P		NS	NS	0.038
<b>Drug-related toxicity</b>				
No	43	12.16±1.85	794.33±64.20	4.49±0.49
Yes	15	12.69±4.92	707.95±98.71	4.4±0.8
P		NS	NS	NS
<b>Outcome (ex)</b>				
No	39	13.39±2.47	724.44±62.64	3.45±0.09
Yes	19	10.06±2.48	851.14±99.58	2.46±0.13
P		NS	NS	NS
<b>Documented infection</b>				
No	26	11.22±2.61	933.25±87.85	2.75±0.11
Yes	32	13.18±2.61	660.69±66.57	3.47±0.11
P		NS	NS	NS

aGVHD: Acute graft-versus-host disease. NS: Nonsignificant. VOD: Veno-occlusive disease.

leukemia cases was small, the effect of WBC parameters on relapse was not investigated.

### Discussion

Genetic polymorphism in the patient and/or donor has been suggested to contribute to transplant-related events in HSCT patients<sup>2,27</sup>. The final inflammatory, hematological, enzymatic, or systemic response would then reflect the contribution of several genetic factors in different patients with variable microenvironmental conditions. Due to this complexity, it is difficult in clinical practice to make a reasonable risk assessment in transplant patients.

An immediate response to induction chemotherapy is suggested as a favorable prognostic marker in patients with acute leukemia, and is considered more important than other variables<sup>28-30</sup>. The present study was performed to determine the effects of the conditioning regimen on initial WBC depletion in HSCT patients. We hypothesized that the individual hematological response to chemo/radiotherapy may be used as a parameter to assess the degree of myeloablation and probability of transplant-related events in pediatric BMT patients. The response of the patient to a chemo/radiotherapy challenge would then reflect the end result of genetic polymorphisms and other environmental factors that may contribute to engraftment and other transplant-related events.

Day 0 WBC count, WBC nadir day, and the WBC ratio were used as potential indicators of myeloablation. There was a statistically significant positive correlation between the WBC ratio and the date of neutrophil engraftment. When the patients were separated according to diagnosis, the correlation remained significant in patients with malignant disease. Higher WBC ratio suggested higher cell kill, which may be consistent with more efficient myeloablation. The statistical significance was independent from WBC values at the beginning of the conditioning regimen; thus, it was not attributed to peripheral blast kill in malignant patients, and all patients were free of peripheral blasts. The correlation remained significant in multivariate analysis, including factors with potential effect on engraftment. It has been shown in the present study that WBC engraftment occurred later in patients with

higher WBC ratio. This finding may suggest more efficient myeloablation and perhaps additional microenvironmental damage that possibly interfered with earlier engraftment. In fact, engraftment was achieved in all patients except four in this patient group; therefore, the probability of engraftment according to WBC ratio was not determined. The correlation between WBC ratio and engraftment date was more pronounced in patients with malignant disease. Thus, larger scale studies in patients with hematological malignancies may be useful to study the post-transplant relapse rate according to WBC ratio values. However, the patient diagnoses were heterogeneous and the patient numbers with relapse were not sufficient for comparison in this study.

Most importantly, the effect of WBC ratio on the date of engraftment was not affected by the other variables listed, including advanced disease status and intensity of the conditioning regimen used. If supported in future studies, this finding may suggest that WBC ratio can be used as a simple but valuable tool to determine individual hematological response to the conditioning regimen and perhaps the degree of myeloablation. Furthermore, a hypothetical chemotherapy challenge before admission to the BMT Unit may perhaps be used in the future to assess the patient's potential response to conditioning. Thus, such a strategy may contribute to selection of the regimen accordingly.

Apart from WBC ratio, day 0 WBC and WBC nadir day had an effect on the engraftment day. A positive correlation in multivariate analysis was noted between logarithmic value of day 0 WBC count and engraftment day. There was a negative correlation between WBC nadir day and engraftment day.

Previous studies have suggested that myeloablative regimens are associated with engraftment with full donor chimerism when compared to non-myeloablative regimens in which full donor chimerism is achieved at later stages in spite of very early recovery of WBC<sup>31,32</sup>. In our center, routine chimerism studies are performed after day 30, but not in the immediate post-transplant period; therefore, the donor or recipient origin of the initial engrafting WBCs remains unclear.

Several variables were used in this study for

multivariate analyses. The association of WBC ratio on engraftment date was independent of variables including the number of cells infused, HLA compatibility, advanced disease status, and intensity of conditioning.

Besides engraftment, the association of WBC kinetics with other transplant-related events was investigated. A positive correlation was evident between WBC ratio and aGVHD and a negative correlation between day 0 WBC count, WBC nadir day and aGVHD. This finding may be reasonable and may suggest that in patients with effective myeloablation, engraftment is more likely at the expense of GVHD. If the probability of engraftment and the degree of myeloablation achieved are foreseen, then appropriate measures can be taken to prevent GVHD and perhaps toxicity.

A positive correlation was present between WBC ratio and fever during the conditioning regimen and a negative correlation between day 0 WBC count and fever during the conditioning regimen. WBC nadir day was lower in patients who developed fever after day 0 for more than 5 days between day 0 and day 30. Thus, more liberal use of anti-infective prophylaxis may be suggested in patients whose WBC kinetics indicate efficient cell kill. However, more studies are needed.

The limitation of this study was the considerable diversity in both the diagnoses of the patients and in the conditioning regimens used. Additional studies in this area that include patients with the same diagnosis and same conditioning regimen are needed.

In summary, the present preliminary study suggests that WBC kinetics, particularly the WBC ratio (pre-conditioning: day 0 WBC) may be used as a measure of initial hematological response to the conditioning regimen and perhaps in determining the degree of myeloablation achieved in BMT patients with malignant disease. Since the positive correlation between the WBC ratio and the date of engraftment was not influenced by the intensity of the conditioning regimen or the other variables, it is suggested that the WBC ratio may be used as a simple test to indicate individual sensitivity to the conditioning regimen. However, larger scale studies in more homogeneous patient groups are needed before definitive conclusions can be drawn.

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