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CD4 T-cell function assay using Cylex ImmuKnow and lymphocyte subset recovery following allogeneic hematopoietic stem cell transplantation

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ABSTRACT

We analyzed CD4 T-lymphocytes ATP levels along with lymphocyte subsets in 160 samples from 111 post-allogeneic hematopoietic stem cell transplantation (alloHSCT) patients. In patients with stable status, ImmuKnow levels changed over time and the 6-month post-alloHSCT levels were significantly higher than those tested within 6 months post-alloHSCT ($P < 0.001$). Although, ImmuKnow levels for acute graft-versus-host disease (GVHD) or infection episodes were not significantly different compared to those for stable alloHSCT, the levels were correlated with specific lymphocyte subpopulations at different times; the results within 6 months post-alloHSCT showed low positive correlation with natural killer cell count ($r = 0.328$) ($P < 0.05$) and the values tested later than 6 months post-alloHSCT were positively correlated with CD4 T cell count ($r = 0.425$) ($P < 0.05$). Two patients who developed acute GVHD and two who experienced an infection episode showed increased ImmuKnow levels in sequential tests. The combined test of ImmuKnow levels and lymphocyte subsets may be helpful for immune monitoring following alloHSCT.

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1. Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) induces severe and prolonged immunodeficiency after transplantation. It can take months for a new hematopoietic graft to achieve normal immune cell function [1]. Immature immune function and delayed reconstitution of lymphocytes cause opportunistic infection, relapse of underlying diseases and acute or chronic graft versus host disease (GVHD) [2]. Although, the assessment of an individual's immune status is important to optimize immunosuppression therapy and to avoid adverse effects, there are no validated routine laboratory tests for cellular immune function measurement.

Immune status after alloHSCT is determined using complete blood count and flow cytometry examination of lymphocyte subsets [3–5]. The ImmuKnow assay (Cyclex, Columbia, MD, USA) quantifies the ATP produced from activated T-helper cells responding to in vitro mitogenic stimulation by phytohemagglutinin-L. The assay has been approved by the United States Food and Drug Administration as a measure of the global immune responses of CD4 + T cell function from whole blood. Immune responses are categorized as strong (ATP ≥ 525 ng/mL), moderate (226–524 ng/mL) or low (≤225 ng/mL), which were previously determined for solid organ recipients rather than HSCT recipients. Because T cells are crucial in alloimmune responses and ATP is essential for the immune effector functions, measurement of ATP levels from activated CD4 + cells may be a useful assessment of the immune status in post-alloHSCT patients [2,6–12].

The ImmuKnow test might be clinically useful in the management of immunocompromised patients and in predicting the risks of graft failure or infection in organ transplantation [6,13]. However, two studies in HSCT recipients have reported conflicting results [1,9] and the clinical values of ImmuKnow assay after HSCT are not conclusive [2,9].

The aims of this study were to explore the potential of the ImmuKnow assay as a useful tool for predicting clinical events after alloHSCT, and to investigate the laboratory parameters affecting CD4 + ATP levels prior to and post-alloHSCT.

2. Materials and methods

2.1. Study population

This retrospective cohort study was approved by the Institutional Review Board of Seoul St. Mary's hospital. A total of 160 blood samples were collected from 111 patients from 1 month to 2 years following allogeneic peripheral blood stem cell transplantation between April 2010 and February 2011. The patients received transplants with conditioning regimens according to our institution's transplantation protocol

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplantation; CBC, complete blood count; GVHD, graft-versus-host disease; WBC, white blood cell.

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[13–15]. Conditioning regimens were classified as myeloablative conditioning (MAC), nonmyeloablative conditioning (NMA) and reduced intensity conditioning (RIC), using the previously described criteria by Bacigalupo et al. [16] (Table 1). All samples were requested for complete blood count (CBC) and lymphocyte subset tests to assess the immunologic status or monitor the therapeutic response, and were simultaneously tested using the ImmuKnow assay. ImmuKnow levels were not used for clinical diagnosis and treatment plan processes. A retrospective chart review was performed to evaluate the clinical and laboratory immune status including CBC, acute GVHD, alloHSCT source, allele matching and infection episodes. Engraftment was defined as the first of three consecutive days with an absolute neutrophil count $>0.5 \times 10^9$/L [4]. Chimerism status was determined using short-tandem repeat polymerase chain reaction, with mixed chimerism defined as greater than 1% recipient cells. Demographic characteristics of the patient population are detailed in Table 1. From 39 patients, two or more blood samples were collected with a 3-month interval. Of 160 samples, 138 samples were from 91 patients during immunological stability without acute GVHD or infection episodes.

2.2. Cellular immune function test

Cellular immune function was measured using the ImmuKnow assay according to the manufacturer's instructions. Briefly, sodium heparin anti-coagulated whole blood diluted 1:4 (100 μL) was incubated alone or with phytohemagglutinin for 15–18 h in an atmosphere of 5% CO_2 at 37 °C. CD4+ cells were positively selected using anti-CD4 monoclonal antibody-coated magnetic beads (Dynal, Oslow, Norway). After washing, the cells were lysed and the released intracelluar ATP was measured by luminometry using the luciferin/luciferase mixture (Cylex). The concentration of ATP was calculated from a calibration curve generated with calibrators (0, 1, 10, 100 and 1000 ng/mL). Results were categorized as low (ATP ≤ 225 ng/mL), normal (225–525 ng/mL) or high (≥525 ng/mL) according to the previous studies [2,6].

Table 1
Demographic characteristics of patient population.

Characteristics of patients	Number of patients (%) or median (range)
Number of patients	111
Median age, years (range)	25 (2–67)
Men:women	66:45 (59.5:40.5)
Diagnosis	
Acute myeloid leukemia	27 (24.3)
Acute lymphoblastic leukemia	23 (20.7)
Myelodysplastic syndrome	36 (32.4)
Aplastic anemia	16 (14.4)
Other	9 (8.1)
HLA matched:mismatched	89:22 (80.2:19.8)
PBMC source	
Related donor	63 (56.8)
Unrelated donor	48 (43.2)
Conditioning regimen	
Myeloablative conditioning	70 (63.1)
Nonmyeloablative conditioning	21 (18.9)
Reduced intensity conditioning	20 (18.0)
Median day of engraftment (range)	12 (9–33)
Number of ImmuKnow samples per patient	
1	72 (64.9)
2	29 (26.1)
3	10 (9.0)
Sampling before 6 months post-SCT[a]	72 (45.0)
Sampling after 6 months post-SCT[a]	88 (55.0)
Median day post-PBSCT for ImmuKnow test (range)	193 (21–727)

[a] Number of samples for ImmuKnow levels.

2.3. Immunophenotyping of peripheral blood lymphocytes

Immunophenotypic characterization of peripheral blood lymphocytes was determined using BD Multitest 6-color TBNK reagent (BD Biosciences, San Jose, CA, USA) and a FACS Canto II flowcytometer (BD Biosciences). Absolute cell counts of T-cells (CD3+CD19−), B-cells (CD3−CD19+), natural killer (NK) cells (CD3−CD56+), NKT cells (CD3+CD56+), T4NK cells (CD3−CD4+CD56+), T8NK cells (CD3−CD8+CD56+), T4NKT cells (CD3+CD4+CD56+) and T8NKT cells (CD3+CD8+CD56+) were measured using TruCount (BD Bioscience).

2.4. Statistical analyses

Statistical analyses were performed with SPSS version 12.0 (SPSS, Chicago, IL, USA). Comparisons were made using the Chi-square test for categorical data and the Mann–Whitney *U* test for non-normally distributed variables. Between-group differences of each parameter were compared by Student's *t*-test. Pearson's correlation test was used to investigate the correlation between ATP levels and laboratory parameters. The strength of positive or negative correlation was assessed according to the Pearson's *r* value ($r < 0.2$ insignificant, 0.2–0.4 low, 0.4–0.7 moderate, >0.7 strong correlation). All *P*-values were two-tailed and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. ImmuKnow level in stable patients after alloHSCT

In 91 patients with stable status, 138 ImmuKnow tests were available. The ImmuKnow levels (mean ± standard deviation) at post-alloHSCT 1 week to 3 months, 3–6 months, 6–12 months and thereafter were 181 ± 173, 146 ± 76, 266 ± 162 and 216 ± 100 ng/mL, respectively. ImmuKnow levels changed over time after alloHSCT, and the levels were significantly increased more than 6 months post-alloHSCT compared to prior to 6 months post-alloHSCT (254 ± 150 versus 162 ± 129 ng/mL, $P < 0.001$) (Fig. 1). The frequency of normal or high ImmuKnow results (>225 ng/mL) was also higher in samples later than 6 months post-alloHSCT compared with those within 6 months post-alloHSCT (50.6% vs. 15.8%; $P < 0.001$).

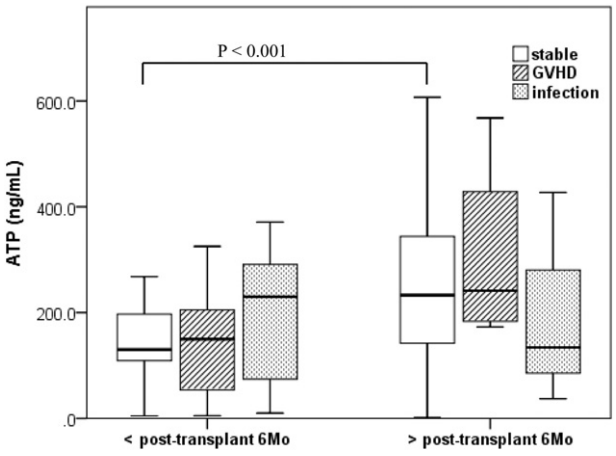

Fig. 1. Comparision of ATP levels from patients with stable, acute GVHD or infection at early (6 months before) and late (later than 6 months) post-alloHSCT. Box plots indicate median, interquartile range and 95% confidence intervals.

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