Original

Effects of moderate intensity aerobic exercise on T and NK cells in patients with hematological malignancies who have low physical activity

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Although moderate intensity aerobic exercise (MIAE) is known to improve physical function and quality of life in patients with hematological malignancies, the effects on T cells and NK cells have not been fully studied. We sought to clarify the effects of MIAE on T cells and NK cells of patients with hematological malignancies in sterile rooms. We measured the population of T cells and NK cells at two points before and after MIAE in patients and healthy controls and evaluated physical activity using a triaxial accelerometer. In both groups, we observed a significant decrease in the CD4/8 ratio and $CD4^+/CD8^-$ cells as % of total lymphocytes as well as increased $CD4^-/8^+$ cells after MIAE. In the patient group, % $CD56^+/CD16^+$ cells did not change between pre- and post-MIAE, although the percentage of $CD4^+/CD25^+/Foxp3^+$ cells and the $CD4^+$ fraction significantly decreased, unlike the control group. Patients with low physical activity were particularly associated with a decrease in the percentage of $CD4^+/CD25^+/Foxp3^+$ cells after MIAE. Our data suggest that MIAE may not be an optimal physical therapy for frail patients with low physical activity. However, further investigations are required to clarify the mechanisms by which MIAE alters T cells and NK cells in patients with hematological malignancies. Furthermore, we also need to examine whether MIAE affects their clinical outcomes.

J Saitama Medical University 2021; 48(1): 1-11
(Received October 28, 2020/Accepted December 24, 2020)

Keywords: hematological malignancies, T cells, NK cells, moderate intensity aerobic exercise, physical activity

Introduction

Patients with hematological malignancies such as leukemia, malignant lymphoma, and multiple myeloma have compromised immune function due to hematopoietic abnormalities and high dose chemotherapy, and their recovery takes months to years after treatment[1,2]. In addition, patients with hematological malignancies are potentially prone to a variety of infections, with fever occurring in 80% of cases after chemotherapy[3]. Thus, the impaired immune function of patients with hematological malignancies causes a variety of complications, interferes with treatment, and increases mortality, and preventive measures should be considered.

The utility of cancer rehabilitation has been investigated as a preventive measure for immune function decline. Physical exercise is known to alter the nervous and endocrine systems and to affect the immune function of the organism. A 20% reduction in the incidence of upper respiratory tract infections has been reported in moderately active adults compared to those who are predominately nonathletic[4]. In contrast, it has also been reported that the incidence of upper respiratory tract infections in marathon runners was twice as frequent in individuals who undertook excessive amounts of training compared to those with less training[5]. Thus, the available evidence suggests that moderate exercise improves immune function and reduces the incidence of upper respiratory tract infections, whereas vigorous exercise reduces immune function and increases the incidence of upper respiratory tract infections. A possible *in vivo* explanation for this phenomenon is that exercise has been shown to affect lymphocyte fractions and the

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◯ The authors declare that there are no conflicts of interest associated with the present study.

numbers of T and NK cells[6].

The association between exercise and T and NK cells is often examined using clusters of differentiation (CD) by monoclonal antibodies that bind to cell surface antigens. Total T cell and NK cell counts, as well as $CD4^+$ counts and the CD4/8 ratio, are considered indicators of protective mechanisms against infection because decreases in $CD4^+$ counts and in the CD4/8 ratio are associated with an increase in infectious morbidity[7,8]. $CD8^+$ cells are cytotoxic cells that recognize and destroy cells expressing non-self-antigens, and are associated with increased exercise-induced inflammation and pro-inflammatory cytokines[9]. NK cells are innate immune cells that destroy virus-infected cells and tumor cells, and are altered by a variety of physical and psychological stress[8]. Regulatory T cells (Tregs) transcribe the Fork-head box protein 3 (Foxp3) and account for 5–10% of $CD4^+$ T cells. They are responsible for the suppression of autoimmune diseases, modulation of the transplant immune response, control of infections, and anti-inflammatory effects[10]. In addition, Tregs have recently received attention not only in terms of immune regulation but also in relation to exercise[6, 11-14]. Therefore, T cells and NK cells are important cells at the core of immune function and have been shown to increase or decrease depending on the exercise intensity and patients' physical activities.

Exercise intensity associated with T cells and NK cells is categorized as low, moderate, or vigorous intensity, and is defined based on the subject's physical ability from indicators such as maximal oxygen uptake, heart rate, and metabolic equivalents (METs)[15]. Cancer rehabilitation with moderate intensity aerobic exercise (MIAE) is recommended for patients with hematological malignancies in the early stages of treatment for the primary disease to improve physical function and quality of life[16,17], and the safety and feasibility of exercise have been verified in patients with severe pancytopenia[18]. The relationship between T cells and NK cells and exercise has previously been reported in healthy subjects and mice[6,11-14], however, there is no available evidence for patients with hematological malignancies that are potentially immunocompromised. It has not yet been adequately investigated that MIAE is effective for the prevention of infection in this patient population. In addition, it has been reported that the changes in T cells and NK cells pre- and post-exercise were different between those accustomed and those unaccustomed to exercise[19]; therefore, patients with hematological malignancies whose physical activity levels are limited to the sterile room may have different transient changes in T cells and NK cells due to MIAE compared to healthy subjects.

The purpose of this study was to determine the effect of MIAE on the percentage of T cells and NK cells in patients with hematological malignancies in a sterile room. In addition, we sought to predict the changes in the percentage of T and NK cells according to MIAE from the physical activity of patients with hematological malignancies.

Materials and methods

Study design

This was a prospective cohort study. After measuring physical activity for more than one week, participants underwent one dose of cancer rehabilitation with MIAE between 9 a.m. and 10 a.m. on the days that their leukocyte count improved to 2000/μL or more from treatment-induced myelosuppression. Blood samples were collected pre- and post-MIAE. Post-MIAE blood samples were collected within 10 minutes of finishing the protocol for the MIAE. This recovery period is usually the time when the exercise intensity of cancer rehabilitation is increased during the treatment process. Healthy volunteers were enrolled as controls. Large variation of the numbers of T cells and NK cells in each patient was expected, so we evaluated not the absolute numbers but the changes of ratios of T cells and NK cells in this study. This study was approved by the ethics committee of Saitama Medical Center, Saitama Medical University (approval number: 1622-II), and written informed consent was obtained from all participants.

Subjects

Twenty-one patients with hematological malignancies (mean age ± SD: 47.1 ± 11.8 years, 11 men and 10 women) and 15 healthy subjects (mean age ± SD: 26.7 ± 3.2 years, 11 men and 4 women) participated in this study. The eligibility criteria for patients included: 1) patients diagnosed with hematological malignancies and those who provided written informed consent to participate in the study, 2) patients admitted to the sterile care unit at Saitama Medical Center, Saitama Medical University between June 2017 and December 2018 for physical therapy, 3) patients aged 18 years or older, and 4) patients with an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 or 1. The exclusion criteria included 1) patients with limited gait due to a past history of orthopedic or central nervous system disease, and 2) patients with grade 2 or higher on the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE 4.0). Patient eligibility according to ECOG-PS and CTCAE were assessed by both physicians and physical therapists. Eligibility criteria for healthy subjects was staff at Saitama Medical Center, Saitama Medical University between May 2020 and June 2020 who provided written informed consent to participate in the study. The exclusion criteria for healthy controls were: 1) those performing regular exercise at least once a week and 2) those having a history of orthopedic or central nervous system disease.

The ECOG-PS scoring was defined as follows: 0, fully active, able to carry on all pre-disease performance without restriction; 1, restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, (e.g., light house work, office work) ; 2, ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours; 3, capable of only limited selfcare, confined to bed or chair more than 50% of waking hours; 4, completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair; and 5, dead[20].

Moderate intensity aerobic exercise

The definition of MIAE was exercise that raised the heart rate to between 40% and 60% of the predicted maximum heart rate calculated using the Karvonen formula (target heart rate = (220 - age - resting heart rate) × 0.4 to 0.6 + resting heart rate)[15] and from 11 (fairly light) to 13 (somewhat hard) on the Borg scale[21]. The protocol for the MIAE was composed of a bicycle ergometer (cordlessbikeV67i, OG Wellness Co., Ltd., Okayama, Japan) with 3 min of warm up, 30 min of MIAE, and 3 min of cool down.

Physical activity

Physical activity was measured using a triaxial accelerometer (Active Stile pro HJA-750C, Omron Healthcare, Kyoto, Japan) that recorded the number of steps, the amount of exercise (Ex) , and the metabolic equivalents (METs). In principle, it was explained to participants that the triaxial accelerometer should be worn on the lower back from the time of waking up to bedtime, except for bathing.

Measurements of physical activity were calculated by averaging the values for the past week from the date of MIAE, and the values for days below 200 steps/day were excluded because the participants were considered not to be wearing a triaxial accelerometer. METs were classified by intensity levels with 1.0 to 2.9 METs as light intensity physical activity (LPA) , 3.0 to 5.9 METs as moderate intensity physical activity (MPA) , and 6.0 or more as vigorous intensity physical activity (VPA)[22] , and the total time for each intensity physical activity was calculated.

Analyses of lymphocyte subsets of T and NK cells

The percentage of T cells and NK cells in the lymphocyte fraction were analyzed by flow cytometry using venous blood samples collected pre- and post-MIAE. After isolating peripheral blood mononuclear cells, the mononuclear cells were stained with monoclonal antibodies. Lymphocyte fractions were measured by two-color and multi-color analyses using BD FACSVerse™ Flow Cytometer (BD Biosciences, San Diego, CA, USA). Monoclonal antibodies were used fluorescein isothiocyanate (FITC) -CD4 monoclonal antibody, phycoerythrin (PE) -CD8a monoclonal antibody, FITC-CD16 monoclonal antibody, PE-CD56 monoclonal antibody, PE-CD25 monoclonal antibody, and allophycocyanin-Foxp3 monoclonal antibody (all from eBioscience; Thermo Fisher Scientific, Inc., Tokyo, Japan).

We evaluated the percentage of $CD4^+/CD8^-$ cells in the lymphocyte fraction (CD4%) , the percentage of $CD4^-/CD8^+$ cells in the lymphocyte fraction (CD8%) , the CD4/8ratio, the percentage of $CD56^+/CD16^+$ cells in the lymphocyte fraction (NK%) , the percentage of $CD4^+/CD25^+/Foxp3^+$ cells in the lymphocyte fraction (Treg%) , and the percentage of $CD4^+/CD25^+/Foxp3^+$ cells in the $CD4^+$ cell fraction (Treg% CD4) of samples.

Statistical analysis

IBM SPSS Statistics for Windows software version 25.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Participants' characteristics were presented as mean ± standard deviation, and the percentage of T and NK cells in lymphocyte fractions and physical activity are presented as median values with interquartile range (IQR). The Wilcoxon signed rank test was used to assess differences between the pre- and post-MIAE for the percentage of T and NK cells in the lymphocyte fraction. The Mann-Whitney U test was used to compare physical activity between groups. We used a stepwise multiple linear regression analysis in which the dependent variable was the change in the percentage of T cells and NK cells in the lymphocyte fraction according to MIAE and the independent variable was the amount of physical activity, and investigated the relationship between the change in the percentages of T cells and NK cells and physical activity. The significance level was set at 5%. The effect size (ES) was calculated using Excel as described in Cohen's report[23].

Results

Thirty-six participants were enrolled in the study. One patient was excluded due to withdrawal of consent. We studied 20 patients (mean age ± SD: 47.5 ± 12.1 years, 11 males and 9 females) and 15 healthy individuals (mean age ± SD: 26.7 ± 3.2 years, 11 males and 4 females) (Table 1). All subjects completed MIAE protocols. There were no treatment-related adverse events and the saturation of percutaneous oxygen was above 95% during MIAE.

Patients were diagnosed with acute leukemia (AL) in 10 cases, and 6 cases were malignant lymphoma (ML). As the clinical stages of the patients with ML according to Ann Arbor classification, 2 patients were Stage III and 4 patients were stage IV. Six patients of ML and 2 patients with multiple myeloma (MM) were received autologous stem cell transplantation (auto-SCT). Two patients with AL and 1 patient with myelodysplastic syndrome (MDS) received allogeneic stem

Table 1. Characteristics of participants

	Patients with hematological malignancies (n = 20)	Healthy subjects (n = 15)
Age (years)	47.5 ± 12.1	26.7 ± 3.2
Male/Female (n)	11/9	11/4
Body mass index (kg/m2)	22.9 ± 3.2	21.1 ± 2.3
ECOG-PS (score)	0.6 ± 0.5	–
Disease (n)		
AL		
Acute lymphocytic leukemia	6	–
Acute myeloid leukemia	4	–
ML		
Diffuse large B-cell lymphoma	3	–
Mantle cell lymphoma	2	–
Hodgkin lymphoma	1	–
MM	2	–
MDS	1	–
CML-BC	1	–
Treatment (n)		
Auto-SCT	8	–
Allo-SCT	3	–
Hyper-CVAD	3	–
IDR-AraC	2	–
Hyper-CVAD+Dasa	1	–
Dasa+PSL	1	–
GRAALL2003 (Induction therapy)	1	–
JALSG ALL202-O (Induction therapy)	1	–
Number of treatments until MIAE (n)		
Primary Treatment	7	–
Second cycles <	13	–
Recurrence (n)		
Yes	3	–
No	17	–
Treatment response at MIAE (n)		
Complete remission	4	–
Hematological complete remission	6	–
Molecular complete remission	2	–
Near complete remission	1	–
Stringent complete remission	1	–
Undecided	6	–
Treatment response at 2 months after MIAE (n)		
Complete remission	9	–
Hematological complete remission	6	–
Molecular complete remission	2	–
Near complete remission	2	–
Stringent complete remission	1	–
White blood cell count (/μl)	3215 ± 1313	–
Lymphocyte count (/μl)	612 ± 339	–
Red blood cell count (10^6/μl)	3.2 ± 0.6	–
Hemoglobin (g/dl)	10.0 ± 1.8	–
Platelet count (10^3/μl)	127 ± 132	–
Albumin (g/dl)	3.6 ± 0.4	–
C-reactive protein (mg/dl)	0.8 ± 1.4	–
From the start of treatment to the date of measurement of immune function (days)	31.3 ± 18.2	–

Presented as number of subjects or mean ± standard values. AL: Acute leukemia, ML: Malignant lymphoma, MM: Multiple myeloma, MDS: Myelodysplastic syndromes, CML-BC: Chronic myeloid leukemia in blast crisis, Auto-SCT: Autologous stem cell transplant, Allo-SCT: Allogeneic stem cell transplant, Hyper-CVAD: Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and prednisolone, IDR-AraC: Idarubicin and high dose cytarabine, Hyper-CVAD+Dasa: Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and prednisolone plus dasatinib, Dasa+PSL: Dasatinib plus prednisolone, GRAALL: Group for research on adult acute lymphoblastic leukemia, JALSG: Japan adult leukemia study group.

cell transplantation (allo-SCT). Further 9 patients were received chemotherapy. The auto-SCT patients had received 5.9 ± 1.4 cycles of chemotherapy prior to transplantation. As conditioning regimens of auto-SCT, 6 patients with ML received MCVAC (Ranimustine; 450 mg/m2, Cytarabine 16 g/m2, Etoposide; 1600 mg/m2, Cyclophosphamide (CY) ; 100 mg/kg) and 2 patients with MM received high dose melpharan (200 mg/m2). As conditioning regimens of allo-SCT, 2 patients received iv busulfan (BU) /CY (iv BU 12.8 mg/kg, CY 120 mg/kg) , 1 patient received CY/total body irradiation (TBI) (CY 120 mg/kg, TBI 12 Gy). Allo-SCTs were performed in 2 patients at 1 st CR and 1 patient at non-remission status. Two patients associated grade 2 of acute graft versus host disease (aGVHD). Nine of 20 patients received steroids before MIAE. The average of total dose of prednisolone was 2179 ± 393 mg, and that of dexamethasone was 306 ± 28 mg. There were 8 patients with ECOG-PS scores of 0 and 12 patients with ECOG-PS of 1. We measured MIAE and the percentage of T cells and NK cells in the lymphocyte fraction of patients at a median of 31.3 days after starting treatment in the sterile room. Until the point of 2 months after MIAE, no patients had experienced relapse nor progression of the disease.

The percentages of T cells and NK cells in lymphocyte fractions pre- and post-MIAE are shown in Table 2 and Figure 1. The healthy subjects had a significant increase in CD8% (ES = 0.68) , NK% (ES = 0.70) , Treg% (ES = 0.78) , and Treg% CD4 (ES = 0.88) post-MIAE, and a significant decrease in CD4% (ES = 0.57) and the CD4/8 ratio (ES = 0.62) post-MIAE. The patients had a significant increase in CD8% (ES = 0.70) post-MIAE and a significant decrease in CD4% (ES = 0.62) , the CD4/8 ratio (ES = 0.65) , Treg% (ES = 0.73) , and Treg% CD4 (ES = 0.44) post-MIAE.

The results of physical activity are shown in Table 3. The median physical activity was 8549 steps/day in healthy subjects and 2648 steps/day in patients. The daily Ex was 5.40 and 1.55 Ex/day, respectively. MPA was 80.4 and 24.7 min/day, and VPA was 2.3 and 0.5 min/day, respectively, revealing a significant reduction in physical activity in patients compared with healthy subjects. LPA in healthy subjects was 512.3 minutes/day while it was 619.1 minutes/day in patients, indicating a significantly longer LPA in patients than in healthy subjects.

The effects of physical activity on the change in the percentage of T cells and NK cells in the lymphocyte fraction are shown in Table 4. The results of a stepwise multiple linear regression analysis showed that for healthy subjects, LPA was the factor affecting the amount of change in Treg% ($\beta = -0.526$, $R^2 = 0.277$) , and the change in the other T cell and NK cell compartments in the lymphocyte fraction was not significantly associated with physical activity. In patients with hematological malignancies, LPA was similarly identified as the factor affecting the amount of change in Treg% ($\beta = -0.56$, $R^2 = 0.314$) , and the change in other T and NK cell compartments in the lymphocyte fraction was not significantly

Table 2. Comparison of the percentage of T and NK cells in lymphocyte fractions pre- and post-moderate intensity aerobic exercise

	Participants	Pre	Post	Absolute median change	p value	Effect size
CD4% (%)	Patients with hematological malignancies (n = 20)	32.10 (15.88, 53.88)	27.05 (11.58, 41.90)	-3.05 (-7.53, 0.10)	0.006 *	0.62
	Healthy subjects (n = 15)	39.36 (35.39, 43.17)	37.32 (32.44, 41.97)	-4.82 (-6.72, 0.29)	0.027 *	0.57
CD8% (%)	Patients with hematological malignancies (n = 20)	33.90 (27.65, 56.40)	43.35 (28.78, 61.33)	3.40 (0.08, 4.58)	0.002 *	0.70
	Healthy subjects (n = 15)	37.29 (31.79, 40.4)	38.56 (32.27, 43.53)	1.31 (0.48, 2.94)	0.009 *	0.68
CD4/8 ratio	Patients with hematological malignancies (n = 20)	0.89 (0.32, 1.78)	0.78 (0.22, 1.59)	-0.08 (-0.42, -0.03)	0.004 *	0.65
	Healthy subjects (n = 15)	1.08 (0.92, 1.38)	1.02 (0.75, 1.32)	-0.16 (-0.28, 0.01)	0.016 *	0.62
NK% (%)	Patients with hematological malignancies (n = 20)	8.50 (5.05, 30.85)	16.85 (6.00, 31.10)	1.60 (-1.08, 5.63)	0.140	0.33
	Healthy subjects (n = 15)	11.34 (9.55, 20.41)	15.92 (13.27, 22.38)	4.20 (-0.03, 6.55)	0.006 *	0.70
Treg% (%)	Patients with hematological malignancies (n = 20)	2.86 (1.68, 4.49)	2.31 (1.20, 3.55)	-0.56 (-1.28, -0.13)	0.001 *	0.73
	Healthy subjects (n = 15)	1.93 (1.50, 2.31)	2.13 (1.68, 2.53)	0.25 (0.05, 0.31)	0.003 *	0.78
Treg%CD4 (%)	Patients with hematological malignancies (n = 20)	10.06 (7.66, 17.31)	9.48 (6.81, 17.29)	-0.55 (-1.75, -0.07)	0.048 *	0.44
	Healthy subjects (n = 15)	5.05 (4.78, 7.33)	6.38 (5.9, 8.36)	1.11 (0.95, 1.60)	0.001 *	0.88

Presented as median (interquartile range) values. *Significant difference between pre- and post-moderate intensity exercise (p<0.05).

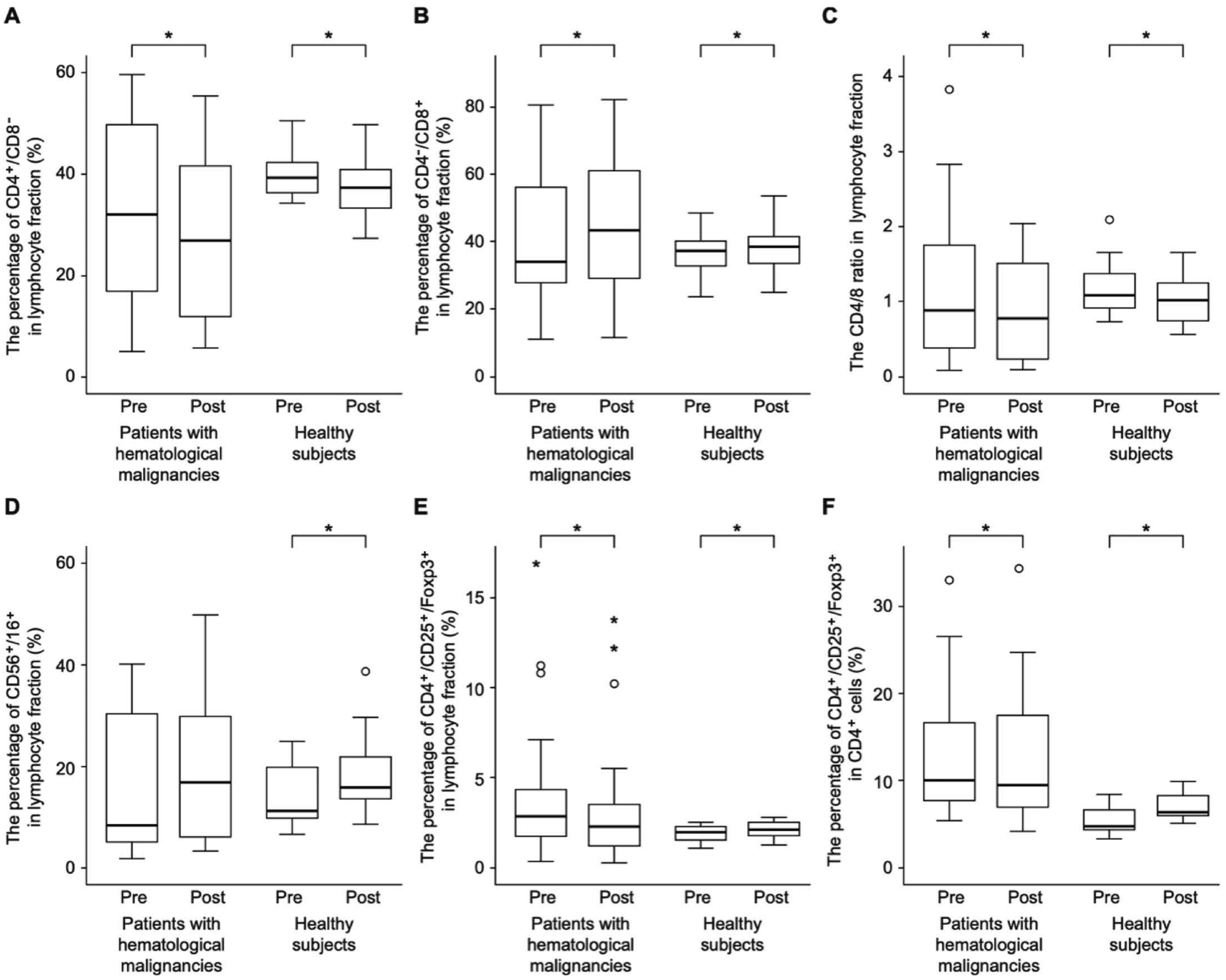

Fig. 1. Pre- and post-moderate intensity aerobic exercise and T and NK cells as a percentage of total lymphocytes
(A) Pre - and post-moderate intensity aerobic exercise and the percentage of CD4+/CD8- cells in the lymphocyte fraction; (B) Pre- and post-moderate intensity aerobic exercise and the percentage of CD4-/CD8+ cells in the lymphocyte fraction; (C) Pre- and post-moderate intensity aerobic exercise and the CD4/8 ratio in the lymphocyte fraction; (D) Pre- and post-moderate intensity aerobic exercise and the percentage of CD56+/CD16+ cells in the lymphocyte fraction; (E) Pre - and post-moderate intensity aerobic exercise and the percentage of CD4+/CD25+/Foxp3+ cells in the lymphocyte fraction; (F) Pre- and post-moderate intensity aerobic exercise and the percentage of CD4+/CD25+/Foxp3+ cells in the CD4+ compartment.
The asterisks denote a significant difference between pre- and post-moderate intensity aerobic exercise within each group ($p<0.05$) using the Wilcoxon signed-rank test.

Table 3. Physical activity in participants

	Patients with hematological malignancies (n = 20)	Healthy subjects (n = 15)	p value	Effect size
Step count (steps)	2648 (1537, 4656)	8549 (8007, 9570)	0.000 *	0.77
Ex (Ex)	1.55 (1.21, 2.72)	5.40 (4.82, 5.76)	0.000 *	0.83
LPA (minute)	619.1 (538.1, 925.6)	512.3 (466.4, 537.6)	0.001 *	0.54
MPA (minute)	24.7 (18.6, 46.8)	80.4 (73.7, 88.0)	0.000 *	0.79
VPA (minute)	0.5 (0.2, 1.1)	2.3 (1.7, 5.4)	0.000 *	0.73

Presented as median (interquartile range) values. Ex: amount of exercise, LPA: low physical activity, MPA: Moderate intensity physical activity, VPA: Vigorous physical activity.
*Significant difference between healthy subjects and patients with hematological malignancies ($p<0.05$).

Table 4. Stepwise multiple linear regression analysis for the amount of change in the percentage of T and NK cells in lymphocyte fraction as an independent variable

	Dependent variable	Independent variable	Unstandardized coefficients B	Standardized coefficients β	t	p value	95% confidence interval Lower	Upper	R^2
Patients with hematological malignancies (n = 20)	Treg%	LPA	-0.002	-0.56	-2.87	0.01	-0.003	-0.001	0.314
Healthy subjects (n = 15)	Treg%	LPA	-0.002	-0.526	-2.229	0.044	-0.005	0	0.277

associated with physical activity.

Discussion

In this study, we found that patients with hematological malignancies showed a decrease in CD4% and the CD4/8 ratio and an increase in CD8% after MIAE similar to healthy subjects. In addition, patients with hematological malignancies were characterized by unchanged NK% and decreased Treg% and Treg% CD4 after MIAE, which we did not observe in healthy subjects. Furthermore, we found that patients with hematological malignancies who had a higher percentage of LPA in daily activity had decreased Treg% after MIAE.

Rhind et al. previously reported that MIAE (bicycle ergometer, 60 minutes, 60% of maximum oxygen uptake) decreased the CD4/8 ratio and increased $CD56^+/CD16^+$ NK cells in healthy subjects, which suggested homeostasis of the defense mechanism against infection[24]. Clifford et al. reported that the percentage of $CD3^+/CD4^+/Foxp3^+/CD25^{++}/CD127^-$ Tregs in the lymphocyte fraction and in the $CD4^+$ cell fraction decreased immediately after running a marathon and increased the next day in healthy subjects, suggesting a switch from a pro-inflammatory environment that induces cell damage to an anti-inflammatory environment that protects against excessive cell damage[13]. Our results in healthy subjects were consistent with previous studies in which the CD4% and CD4/8 ratio decreased after MIAE, but the NK% increased after MIAE, suggesting homeostasis of the defense mechanism against infection. In addition, healthy subjects showed increased CD8% after MIAE, as well as increased Treg% and Treg% CD4, suggesting promotion of the anti-inflammatory environment.

Patients with hematological malignancies showed a decrease in CD4% and the CD4/8 ratio and an increase in CD8% after MIAE, similar to healthy subjects. However, unlike our results in healthy subjects, the NK% did not change and the Treg% and Treg% CD4 were reduced. Previous studies have shown that the percentage of Leu7/CD16 positive NK cells were reduced after completing a triathlon[25] and that the percentage of $CD3^+/CD4^+/Foxp3^+/CD25^{++}/CD127^-$ Tregs in the lymphocyte fraction and in the $CD4^+$ cell fraction were reduced after completion of a marathon[13] , indicating that vigorous intensity exercise decreases NK%, Treg%, and Treg% CD4. Our results suggest that MIAE in patients was associated with a decrease in NK%, Treg%, and Treg% CD4, similar to vigorous intensity exercise in healthy subjects. This is likely because patients with hematological malignancies were vulnerable and in a situation of physical and mental stress. In summary, we suggest that the percentage of T cells and NK cells in the lymphocyte fraction of patients with hematological malignancies after MIAE may indicate that the defense mechanism against infection was reduced and a pro-inflammatory environment was promoted.

As a reason for different patterns of changing T cells ratio and NK cells ratio by MIAE between patients and healthy volunteers, we thought the association of catecholamine and glucocorticoid to changing these cells ratios. Exercises with equal or more intensity of moderate increased catecholamine which recruited cells with high-dense of adrenalin receptors (NK cell > CD8 > CD4) from spleen and lymph node to peripheral blood[26,27]. Cortisol is increased by vigorous intensity exercise, which promotes lymphocytes migration and decreases interleukin-2 (IL-2) that associates proliferation and differentiation of T cells and NK cells[25]. These mechanisms suggest that T cells and NK cell fractionation was altered in the subjects in this study after MIAE. In addition, we suggest that MIAE in patients with hematological malignancies released catecholamine and cortisol similar to vigorous intensity exercise in healthy subjects, and that NK% and Treg% and Treg% CD4 were inhibited in their differentiation and altered lymphocyte fractionation. In the biophylactic mechanism against viral infections and malignant tumors, NK cell activity is thought to be important as well as NK cells count. NK cells count and NK cell activity are increased by MIAE in contrast to being decreased by vigorous intensity exercise through increasement of prostaglandin E2 (PGE2)[6,28]. Changes of NK% were not observed after MIAE in the patients with hematological malignancies in this study. This finding suggested that PGE2 is increased in these patients as well as healthy subject in vigorous intensity exercise. We thought the possibility that activities of NK cells of these patients may decrease or remain unchanged through the PGE2 after MIAE. Though we did not examine hormones, cytokines, NK cell activity and PGE2 in this study. We think that further examinations are required.

LeVoy et al. revealed that the exercise of ascending and descending of the stairs increased tumor associated antigens such as melanoma-associated antigen 4 (MAGE-A4) and preferentially expressed antigen in melanoma (PRAME) as well as cytotoxic T cells which is specific for hematological malignant cell related antigens i.e. MAGE-A4 and PRAME[29]. Bigley et al. reported that exercise with the lactate threshold within −5% lactate threshold both killer immunoglobulin-like receptor $(KIR)^{+}$/ NK group 2 member A $(NKG2A)^{-}$ and KIR^{+}/ $NKG2A^{+}$ on NK cells, which might lead to conduct apoptosis of tumor cells[30]. These reports suggested that physical exercise may be a useful treatment for patients with hematological malignancies. Although we didn't measure those tumor antigens in our study, the continuation of CR at 2 month after MIAE in all patients who received MIAE suggested that physical exercise brings no disadvantage for the tumor treatment.

We further examined whether the change in the percentage of T cells and NK cells in lymphocyte fractions could be predicted from an individuals' physical activity. Our results show that higher levels of LPA were a predictive factor for a decrease in Treg% after MIAE in patients with hematological malignancies. Yonekura et al. reported that levels of C-reactive protein, creatine kinase MB, and interleukin-6 were elevated after vigorous aerobic exercise in those without exercise habits, indicating the occurrence of exercise-induced inflammation[31]. In addition, Suzuki reported that the increase in serum myoglobin levels and neutrophils, which are markers of muscle damage, were attenuated in healthy subjects after repeating the same intensity of exercise on a bicycle ergometer[32]. Taken together, these findings demonstrate that patients who are unaccustomed to intensive exercise in their daily life may develop increased levels of cytokines, neutrophils, and lymphocytes, which are indicators of exercise-induced inflammation, while patients who are accustomed to a comparable level of daily activity are likely to attenuate this acute inflammatory response. We opine that patients with hematological malignancies who have LPA tend to have decreased Treg% after MIAE, promoting a pro-inflammatory environment due to the unaccustomed intensity of MIAE.

However, our study had several limitations. First, it is unclear whether changes in the percentage of T and NK cells in the lymphocyte fraction pre- and post-MIAE can be considered to be values that affect clinical outcomes, such as infectious morbidity. Therefore, it is necessary to clarify the long-term changes in the percentage of T cells and NK cells in the lymphocyte fraction after MIAE and to investigate their relationship with clinical outcomes. Second, the association between the percentage of T cells and NK cells in the lymphocyte fraction and physical activity is difficult to generalize to the larger population because of the small sample size and the possibility of beta error, so caution should be exercised in interpreting the results of this study. Third, the healthy subjects in the control group were younger than the patients with hematological malignancies. Previous studies have reported that T cells and NK cells did not differ between young (18−40 years old) and intermediate age groups (41−60 years old)[33] , but it is generally known that T and NK cells change with age[34]. We have not evaluated the change of ratios of T cells and NK cells in the patients with hematological malignancies who have not received MIAE. Furthermore, the establishment of age-adjusted and non-MIAE control groups may be necessary to characterize the relationship between T cells and NK cells and MIAE in patients with hematological malignancies.

To the best of our knowledge this is the first study to clarify the effect of MIAE on the percentage of T cells and NK cells in the lymphocyte fraction of patients with hematological malignancies in a sterile room. In recent years, the immunomodulatory effects of exercise have received much attention as a means of adjunctive therapy and improved survival in patients with hematological malignancies[35] ; therefore, the results of this study provide valuable clinical information on exercise prescriptions and their effect on immune function in this patient population. Patients with hematological malignancies are fragile subjects; thus, in the future, it should be investigated if cancer rehabilitation with low intensity aerobic exercise rather than MIAE can improve their protective mechanisms against infection and help promote an anti-inflammatory environment. In addition, we would like to clarify the mechanism by which exercise alters T cells and NK cells in patients with hematological malignancies, based on the changes in cytokines and hormones pre- and post-exercise, and to investigate the effect of exercise on clinical outcomes, such as infectious morbidity.

Conclusion

MIAE resulted in a decrease in CD4%, the CD4/8 ratio, Treg%, and Treg% CD4, and an increase in CD8% in the lymphocyte fraction of patients with hematological malignancies. In addition, it may be possible to predict a decrease in Treg% after MIAE in patients with higher LPA. We hypothesize that patients with hematological malignancies in a sterile room have reduced protective mechanisms against infection and a more pro-inflammatory environment after MIAE. However, we are not suggesting that patients with hematological malignancies not perform MIAE. MIAE is important for improving physical and mental function in patients with hematological malignancies, and a prescription of MIAE should be considered in accordance with the existing levels of physical activity.

Acknowledgments

The authors thank all of the patients and staff members involved in this study, especially the Hematology Medicine staff in our hospitals. We thank Chika Nakabayashi for her excellent technical support. We also thank Michio Shiibashi for his support with statistical analysis. This work was supported by a Kamoda grant (01F111, 2019).

Conflicts of interest

The authors have no conflicts of interest directly relevant to the content of this article.

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無菌治療室の造血器腫瘍患者における T，NK 細胞に中強度運動が与える変化および身体活動の関連

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【目的】中強度運動は，無菌治療室の造血器腫瘍患者の身体機能や生活の質を改善させるが，T 細胞と NK 細胞に与える効果が明らかとなっていない．そこで，本研究は，無菌治療室の造血器腫瘍患者の T 細胞と NK 細胞に中強度運動が与える効果および身体活動の関連を明らかにすることを目的とした．
【方法】対象は，無菌治療室にて加療された造血器腫瘍患者 20 人，健常者 15 人とした．対象者は，身体活動を測定した後に中強度運動前後の T 細胞と NK 細胞を測定し，その変化を比較した．また，中強度運動による T 細胞と NK 細胞の変化量と日常の身体活動との関連について分析した．
【結果】造血器腫瘍患者は，健常者と同様に，中強度運動後で $CD4^+/CD8^-$ と CD4/8 比が有意に減少し，$CD4^-/CD8^+$ が有意に増加した．また，造血器腫瘍患者は，健常者と異なり，中強度運動後で $CD56^+/CD16^+$ が変化せず，リンパ球分画および $CD4^+$ 分画における $CD4^+/CD25^+/Foxp3^+$（Treg）が有意に減少した．更に，造血器腫瘍患者では，中強度運動におけるリンパ球分画における Treg の変化量に影響を与える要因として 1.0～2.9 Mets の活動時間が抽出された．
【結論】中強度運動は，無菌治療室の造血器腫瘍患者に対して，CD4/8 比と Treg を減少させ，リンパ球分画における Treg の変化量に 1.0～2.9 Mets の活動時間が関連していることを明らかにした．中強度運動は，造血器腫瘍患者の感染に対する防御機構を低下させ，Pro-inflammatory environment を促進させる可能性が示唆されたが，そのメカニズムや臨床成績との関連について更なる検討が必要である．

