

A Cross-Sectional Study on the Relationship of Job Stress with Natural Killer Cell Activity and Natural Killer Cell Subsets among Healthy Nurses

Yuko MORIKAWA¹, Kazuyo KITAOKA-HIGASHIGUCHI², Chie TANIMOTO², Midori HAYASHI², Reiko OKETANI³, Katsuyuki MIURA¹, Muneko NISHIJO¹ and Hideaki NAKAGAWA¹

¹Department of Public Health, Kanazawa Medical University, ²Department of Nursing, Ishikawa Prefectural Nursing University and ³Department of Nursing, Ishikawa Prefectural Takamatsu Hospital, Japan

Abstract: A Cross-Sectional Study on the Relationship of Job Stress with Natural Killer Cell Activity and Natural Killer Cell Subsets among Healthy Nurses: Yuko MORIKAWA, *et al.* Department of Public Health, Kanazawa Medical University—

The present study investigated the effects of job stress on cellular immune function, such as NK cell activity and NK cell subsets. The participants were 61 female nurses aged 23–59, who worked in a public psychiatric hospital in Ishikawa, Japan. Each subject completed the Nursing Job Stressor Scale (NJSS) and their NK cell activity and lymphocyte surface antigens (CD16⁺56⁺) were evaluated as immune system parameters. The NJSS has seven subscales: conflict with other nursing staff, nursing role conflict, conflict with physicians or autonomy, conflict with death or dying, quantitative work load, qualitative work load and conflict with patients. Factors influencing NK cell activity, and the proportion and cell counts of CD16⁺56⁺ lymphocytes were evaluated. Increase in quantitative work load significantly decreased NK cell activity. Conversely, no linear relationship was observed between qualitative work load and immunological variables, with the highest percentage of CD16⁺56⁺ lymphocytes observed among participants in the medium work load group. The other five NJSS subscales did not relate to immune parameters. In conclusion, the results suggest that perceived job strains, particularly quantitative work load, decreased NK cell function.

(J Occup Health 2005; 47: 378–383)

Key words: Immune function, Job stress, Lymphocyte

subset, Natural killer cell, Nurse, CD16, CD56

Psychological stress is thought to increase vulnerability to illness. The hypothalamic-pituitary-adrenal (HPA) axis has been considered as a pathway along which psychological stresses are transposed into impaired immune function^{1–3}. Among many indices of immune function, natural killer (NK) cell activity and NK cell subsets have been of interest to researchers because NK cells are known to be important in host defense against viral disease and they appear to play a significant role in protection against neoplastic growth⁴. Studies concerning the effects of psychological stress on immune function have mainly dealt with depression or life events. Human studies have indicated that long lasting life stress is associated with a decrement of NK cell activity^{5–7}. Meta-analysis has also demonstrated a decrease in NK activity with chronic life stress^{8, 9}. In the field of occupational health, cell activity and the proportion or numbers of NK cell subsets (CD16⁺57⁺) were shown to decrease with depersonalization (DP) scores of the Maslach Burnout Inventory (MBI) among office workers¹⁰. While the decrements of these indices associated with the level of job stress^{11, 12} or overwork¹³, were also reported, further findings are necessary to fully understand this issue. In addition, few studies have evaluated the differences among sorts of job stressors in effects on NK cell function.

The aims of this study were to investigate the effects of chronic job stress on cellular immune function, such as NK cell activity and NK cell subset, and to investigate what sorts of job stress would affect cellular immune function. Nurses were selected for this investigation as they are considered to be under particularly high psychological stress.

Received Nov 11, 2004; Accepted May 23, 2005

Correspondence to: Y. Morikawa, Department of Public Health, Kanazawa Medical University, 1–1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan
(e-mail: ymjr@kanazawa-med.ac.jp)

Table 1. Demographic features by age class

	Age class			Total
	23–38	39–47	48–59	
Numbers	20	21	20	61
Managerial class (number, %)	0 (0.0)	1 (4.8)	15 (75.0)	16 (26.2)
Married (number (%))	13 (65.0)	20 (95.2)	17 (85.0)	50 (82.0)
Children at home (number, %)	14 (70.0)	20 (95.2)	13 (65.0)	47 (77.0)
Working years as nurse (mean, SD)	11.8 (5.7)	22.9 (3.5)	30.4 (4.2)	21.7 (8.9)
Sleeping hours before sampling (mean, SD)	6.8 (0.9)	6.8 (1.2)	6.6 (1.0)	6.7 (1.0)

Materials and Methods

Subjects

The study population was 115 women aged 23–59, who worked in a public hospital for the mentally disturbed in Ishikawa, Japan. The research protocol was approved by the ethical review committee of Kanazawa Medical University. All participants were fully informed of the procedure of this study and 70 women (60.9%) provided informed consent for participation. Nine women were excluded because they were taking medication, they smoked or they had a cold, all of which are factors known to affect the immune system. The remaining 61 participants had a mean age of 42.9 ± 8.5 yr. Table 1 shows the demographic features and occupational characteristics of the subjects by tertile of age. Sixteen nurses (26.2%) held managerial positions in the hospital and 15 nurses belonged to the highest age group. Forty-eight nurses (78.7%) were shift workers. Most subjects in all age categories except the highest age group were shift workers. Twenty of 21 nurses belonging to the medium age group were married and living with their children, therefore, job position, shift work and family structure were found to depend on age.

Questionnaires

Each subject completed 24-h pre-sampling sleep diaries. They also completed the Nursing Job Stressor Scale (NJSS)^{14, 15}. The NJSS consists of 33 items that describe potentially stressful situations for nurses in the performance of their duties. Nurses were asked how intensely they experienced these situations in their present workplace. The four allowed responses were: (1) rarely noticeable, (2) mild, (3) quite strong, and (4) very strong. A value of zero was given if the respondent indicated that she never experienced strain. There are seven subscales: conflict with other nursing staff, nursing role conflict, conflict with physicians or autonomy, conflict with death or dying, quantitative work load, qualitative work load and conflict with patients.

Blood sampling and immunological analysis

Heparinized venous blood was taken between 08.00 and 09.00 before the start of the daytime shift. Participants were not allowed to eat or move vigorously for 30 min before blood sampling. The immune parameters that were evaluated were NK cell activity and lymphocyte surface antigens (assayed by BML Inc., Tokyo, Japan). NK cell activity was measured by a standard chromium release assay¹⁶. The effector/target (E/T) ratios were 20:1. Percent cytotoxicity was calculated. Lymphocyte populations were analyzed with dual color direct immunofluorescence on a flowcytometer (Cytoron-Absolute, Ortho, USA). Available commercial fluorescein isothiocyanate (FITC) or phyco-erythrin (PE) monoclonal antibodies that recognize NK cell surface antigens were CD16-FITC and CD56-PE (BD Bioscience Pharmingen, USA). Estimates of absolute numbers of the NK cell populations were determined by multiplying peripheral lymphocyte counts by the percentage of surface marker.

Statistical analysis

Statistical analyses were performed using SPSS software version 11.0. One way analysis of variance (ANOVA) was applied to test the effects of age on immune parameters and job stress level. The evaluations of factors related to immune parameters were carried out by a simple regression model and analysis of covariance (ANCOVA) using the scores of NJSS subscales classified into low, medium and high according to the tertile distribution. If an overall ANCOVA test showed a significant difference, then Tukey's multiple comparison test was carried out as well.

Results

Table 2 shows mean NJSS subscale scores, NK cell activity, and NK cell subset by age group according to the tertile distribution. Among the NJSS subscales, the qualitative work load was found to be significantly higher among the medium age group. No significant differences were observed for immunological variables, such as NK

Table 2. Mean (SE) score of Nursing Job Stressor subscales and mean value of NK cell activity and NK cell subsets by age group

	Age class						Total	
	23–38		39–47		48–59			
NJSS								
Conflict with nursing staff	2.68	(0.9)	2.82	(0.57)	2.27	(0.74)	2.59	(0.77)
Nursing role conflict	2.78	(0.6)	2.77	(0.57)	2.69	(0.6)	2.75	(0.58)
Conflict with physicians	2.15	(1.15)	2.53	(0.81)	2.01	(0.77)	2.24	(0.94)
Conflict with dying	1.73	(1.16)	1.80	(0.9)	1.78	(1.1)	1.77	(1.04)
Qualitative work load	2.86	(0.67)	3.31	(0.46)	2.79	(0.65)	2.99	(0.63)*
Quantitative work load	3.08	(0.56)	3.16	(0.67)	2.71	(0.65)	2.99	(0.65)
Conflict with patients	2.83	(0.61)	2.95	(0.71)	2.45	(0.76)	2.75	(0.72)
Immunological parameters								
NK cell activity	18.9	(10.9)	16.7	(12.3)	20.1	(9)	18.6	(10.8)
CD16+56+ (%)	15.4	(7.8)	17.6	(7.5)	15.7	(7.7)	16.2	(7.6)
CD16+56+ (counts/mm ³)	249	(146)	311	(131)	305	(263)	289	(187)

*: $p < 0.05$ by one way ANOVA

Table 3. Pearson's correlation coefficient between variables and NK cell activity and NK cell subset

Variables	NK cell activity	CD16+56+	
		%	counts (/mm ³)
Age (yr)	0.00	0.00	0.14
Sleep hours (h)	-0.16	0.02	0.10
Quantitative work load	-0.35*	-0.20	-0.16
Qualitative work load	-0.16	0.10	0.15
Nursing role conflict	0.02	-0.25	-0.15
Conflict with nursing staff	-0.12	0.01	0.08
Conflict with physicians	-0.26*	-0.07	-0.05
Conflict with patients	-0.15	0.02	0.05
Conflict with dying	-0.08	0.00	-0.07

*: $p < 0.05$

cell activity and the proportion and cell counts of CD16+56+ lymphocytes between age groups.

Table 3 shows Pearson's correlation between immunological variables and NJSS subscale scores, age and sleeping hours. Age and sleeping hours did not relate to immunological variables. The score of quantitative work load and conflict with physicians correlated negatively with NK cell activity with statistical significance. None of the factors correlated with the proportion or cell counts of CD16+56+ lymphocytes.

The factors influencing NK cell activity, and the proportion and cell counts of CD16+56+ lymphocytes were evaluated by analysis covariance (ANCOVA) using age as a covariate. Figure 1 shows age adjusted mean values of NK cell activity, and the proportion and cell counts of CD16+56+ lymphocytes by the tertile groups of

quantitative work load. Quantitative work load related with immunological variables linearly, indicating that the group with the highest score of quantitative workload showed the lowest of NK cell activity and the lowest proportion and cell counts of CD16+56+ lymphocytes. There was a statistically significant difference in NK cell activity between the low and high quantitative work load groups. Figure 2 shows estimated mean values of NK cell activity, and the proportion and cell counts of CD16+56+ lymphocytes by the groups of qualitative work load. The relations between qualitative work load and immunological variables were not linear. Each immunological variable was the highest in the medium qualitative group of work load. There was a statistically significant difference between the medium and low qualitative work load groups in the proportion of

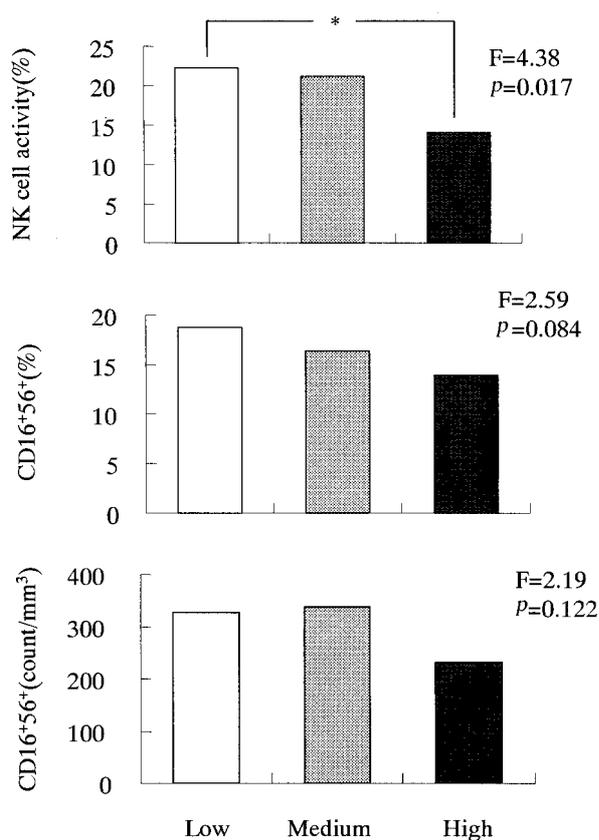


Fig. 1. Age adjusted mean value of NK cell activity and proportion and cell numbers of CD16⁺56⁺ lymphocyte in the subjects grouped according to the tertile distribution of quantitative work load (ANCOVA) *: $p < 0.05$

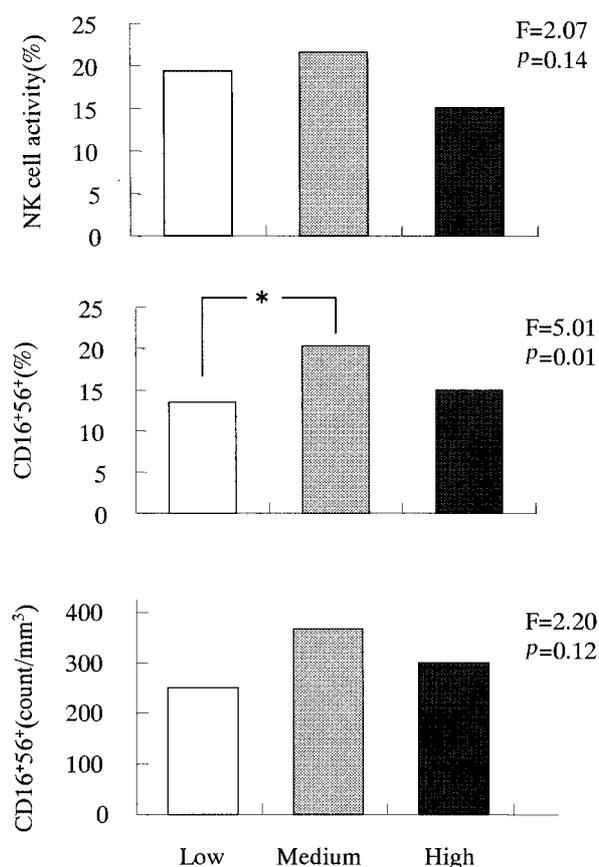


Fig. 2. Age adjusted mean value of NK cell activity and proportion and cell numbers of CD16⁺56⁺ lymphocyte in the subjects grouped according to the tertile distribution of qualitative work load (ANCOVA) *: $p < 0.05$

CD16⁺56⁺ lymphocytes. The other scores of NJSS, conflict with other nursing staff, physicians, patients and dying and nursing role conflict did not relate to immunological variables significantly.

Discussion

The aim of this study was to investigate the effects of job stress on cellular immune function. Nurses who worked in a public psychiatric hospital participated by allowing the evaluation of their NK cell activity and the proportion and cell numbers of the NK cell subset: CD16⁺56⁺ lymphocytes. We used the Nursing Job Stressor scale as a measure of chronic professional stress^{14, 15}.

It has been reported that immunological function is influenced by lifestyle habits such as smoking and sleep deprivation¹⁷⁻²¹. Since the numbers of smokers were quite small among the subjects, we excluded them from the analysis. The analysis was adjusted for confounding factors, such as age and sleeping hours in the night before sampling. Other occupational factors, such as employment position and working style (shift work or

fixed daytime work) depended on age. Therefore, these occupational factors were not included in the analytic model to avoid a multi-collinearity.

Two of the seven subscales of the NJSS, quantitative work load and qualitative work load, were significantly related to the immune variables. Quantitative work load was inversely related to NK cell activity with statistical significance, and the proportion and cell counts of CD16⁺56⁺ lymphocyte also tended to decrease with quantitative work load. Therefore, we considered that the decrement of NK cell activity with quantitative work load was due to a decrease in the number of NK cells. The qualitative work load of NJSS increased the proportion of CD16⁺56⁺ lymphocyte to a certain extent, but NK cell activity was not increased with the increase of the proportion of CD16⁺56⁺ lymphocyte. Under the highest qualitative work load, the NK cell activity was the lowest. Therefore, we considered that qualitative work load had a negative effect on NK cell function. These results are compatible with the other reports finding decrement of NK cell activity and NK cell subsets with

chronic stress. Meta-analysis demonstrated a decrease in NK cell activity with chronic life stress^{8,9}. For job stress, the decrements of NK cell activity and proportion or number of NK cell subsets were reported in relation with the Maslach Burnout Inventory (MBI)¹⁰, job stress^{11,12} and overwork¹³. Other reports dealing with the effects of job stress on T cell function or T cell subpopulations showed inverse relationships between the two^{11,22,23}.

Our study revealed that quantitative work load was the strongest predictor for NK cell function among seven subscales of job stress for nurses. Quantitative work load was found to predict the syndrome of burnout as measured by the Maslach Burnout Inventory (MBI) and was considered to be a primary factor for the burnout syndrome in a cross sectional study¹⁵. Further study is needed to investigate whether quantitative work load influenced NK cell function directly or via psychological response. In either case, controlling quantitative work load would reduce its negative effects on cellular immune function.

In the present study, we did not evaluate the stress outside the workplace, such as difficulties of daily life. Perceived job stress would be influenced by the stress level from outside the workplace. However, a more comprehensive study evaluating stress level from both sides of workplace and daily life should be carried out. Also, our study population had special characteristics, since they were nurses in a public hospital for the mentally disturbed. However, the subscales detected as significant predictors of cellular immune function were quantitative work load and qualitative work load which are common characteristics for all jobs. Therefore, the results of our study would bear generalization to all jobs.

In conclusion, we investigated the effects of job stress on NK cell activity and NK cell subset and what sorts of job stress would affect cellular immune function. Our study revealed that the perceived job stress, particularly quantitative work load, reduced NK cell activity through a decrease in NK cell numbers. The data suggest that psychological mechanisms in stress are associated with cellular immune function. More research is needed to investigate whether these immunological findings are associated with vulnerability or not.

Acknowledgments: This study was supported by a grant-in-aid for scientific research (14570362) from the Ministry of Education, Culture, Science and Technology of Japan.

References

- 1) EM Friedman and DA Lawrence: Environmental stress mediates changes in neuroimmunological interactions. *Toxicol Sci* 67, 4–10 (2002)
- 2) MR Larson, R Ader and JA Moynihan: Heart rate, neuroendocrine, and immunological reactivity in response to an acute laboratory stressor. *Psychosom Med* 63, 493–501 (2001)
- 3) JL Pike, TL Smith, RL Hauger, PM Nicassio, TL Peterson, J McClintick, C Costlow and MR Irwin: Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. *Psychosom Med* 59, 447–457 (1997)
- 4) RB Herberman and JR Ortaldo: Natural killer cells: their role in defenses against disease. *Science* 214, 24–30 (1981)
- 5) N Kawamura, Y Kim and N Asukai: Suppression of cellular immunity in men with past history of posttraumatic stress disorders. *Am J Psychiatry* 158, 484–486 (2001).
- 6) JA Boscarino and J Chang: Higher abnormal leukocyte and lymphocyte counts 20 years after exposure to severe stress, research and clinical implications. *Psychosom Med* 61, 378–386 (1999)
- 7) SE Locke, L Kraus, J Leserman, MW Hurst, JS Heisel and RM Williams: Life change stress, psychiatric symptoms, and natural killer cell activity. *Psychosom Med* 46, 441–453 (1984)
- 8) TB Herbert and S Cohen: Stress and immunity in humans: a meta-analytic review. *Psychosom Med* 55, 364–379 (1993)
- 9) EP Zolllia, L Luborsky, JR McKay, R Rosenthal, A Houldin, R McCorkle, DA Seligman and K Schmidt: The relationship of depression and stressors to immunological assays; a meta analytic review. *Brain Behav Immun* 15, 199–226 (2001)
- 10) H Nakamura, H Nagase, M Yoshida and K Ogino: Natural killer cell activity and NK cell subsets in workers with a tendency of burnout. *J Psychosom Res* 46, 569–578 (1999)
- 11) A Nakata, S Araki, T Tanigawa, A Miki, S Sakurai, N Kawakami, K Yokoyama and M Yokoyama: Decrease of suppressor-inducer (CD4+CD45RA) T lymphocytes and increase of serum immunoglobulin G due to perceived job stress in Japanese nuclear electric power plant workers. *JOEM* 42, 143–150 (2000)
- 12) VD Gucht, B Fischler and C Demanet: Immune dysfunction associated with chronic professional stress in nurses. *Psychiatry Res* 85, 105–111 (1999)
- 13) A Yasuda, K Iwasaki, T Sasaki, T Oka and N Hisanaga: Lower percentage of CD56+ cells associated with long working hours. *Ind Health* 29, 221–223 (2001)
- 14) K Higashiguchi, Y Morikawa, K Miura, M Nishijo, M Tabata and H Nakagawa: The job stressor experienced by hospital nurses; Development of the Nursing Job Stressor Scale and examination of psychometric properties. *Jap J Health Psychology* 11, 64–72 (1998) (in Japanese)
- 15) K Kitaoka-Higashiguchi and H Nakagawa: Job strain, coping, and burnout among Japanese nurses. *Jpn J Health & Human Ecology* 69, 66–79 (2003)
- 16) HF Pross, MG Baines, P Rubin, P Shragge and MS Patterson: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. *J Clin*

- Immunol 1, 51–63 (1981)
- 17) LC Ginns, JH Ryu, PR Rogol, NL Sprince, LC Oliver and CJ Larsson: Natural killer cell activity in cigarette smokers and asbestos workers. *Am Rev Respir Dis* 131, 831–834 (1985)
 - 18) DA Hughes, PL Haslam, PJ Townsend and M Turner-Warwick: Numerical and functional alterations in circulatory lymphocytes in cigarette smokers. *Clin Exp Immunol* 61, 459–466 (1985)
 - 19) M Ferson, A Edwards, A Lind, GW Milton and P Hersey: Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. *Int J Cancer* 23, 603–609 (1979)
 - 20) J Born, T Lange, K Hansen, M Molle and HL Fehm: Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol* 158, 4454–4464 (1997)
 - 21) K Morimoto, T Takeshita, C Inoue-Sakurai and S Maruyama: Lifestyle and mental health status are associated with natural killer cell and lymphokine-activated killer cell activities. *Sci Total Environ* 270, 3–11 (2001)
 - 22) N Kawakami, T Tanigawa, S Araki, A Nakata, S Sakurai, K Yokoyama and Y Morita: Effects of job strain on helper-inducer (CD4+CD29+) and suppressor inducer (CD4+CD45RA+) T cells in Japanese blue-collar workers. *Psychother Psychosom* 66, 192–198 (1997)
 - 23) A Bargellini, A Barbieri, S Rovesti, R Vivoli, R Roncaglia and P Bprella: Relation between immune variables and burnout in a sample of physicians. *Occup Environ Med* 57, 453–457 (2000)