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An Occupational Health Study of Emergency Physicians in Japan: Health Assessment by Immune Variables (CD4, CD8, CD56, and NK Cell Activity) at the Beginning of Work

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Abstract: An Occupational Health Study of Emergency Physicians in Japan: Health Assessment by Immune Variables (CD4, CD8, CD56, and NK cell activity) at the Beginning of Work: Hiroteru OKAMOTO, *et al*. Department of Preventive Medicine and Public Health, School of Medicine, Kyorin University—This study was conducted to evaluate the occupational health of Japanese physicians in emergency medicine. Subjects participating in this study were eighty-nine physicians working at 12 medical facilities (10 critical care emergency centers) in Japan. Participants were asked to complete a questionnaire of work conditions and to provide blood samples for immune variable measurements (CD4, CD8, CD56 and natural killer cell (NK cell) activity) before commencing their work. The data collected from seventy-four of 89 participating physicians were analyzed. The traditional work group comprised of 39 emergency physicians, who were significantly overworked compared to other two groups: the shift work group and the day work group. Among these three groups, no immune variable was significantly different except lymphocyte, number of CD4, and NK cell activity; and the NK cell activity of the shift work group was significantly lower than those of the traditional work group ($p<0.01$) and the day work group ($p<0.01$) in terms of Bonferroni's multiple comparison, probably due to circadian rhythm. It was indicated that NK cell activity was significantly lower in samples collected at night versus in the morning (OR=8.34, 95%CI: 1.95–35.6, $p<0.01$) through multiple logistic regression analyses. NK cell activity was significantly lower in individuals taking 0–3 days off per month, as compared to those taking 4 or more days off (OR=4.65, 95%CI: 1.27–17.0, $p=0.02$), according to multiple logistic regression analyses. Therefore, the low NK cell activity appears to have reflected the extent of fatigue arising from physicians' overwork. Overwork would have been a potential risk for the physicians' health, resulting in a lower quality of Japanese emergency medical services than that which could have been achieved otherwise. This study suggests that it would be better for the Japanese emergency physicians to take 4 or more days off per month for their health and the quality of their services.
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Key words: Occupational health, Emergency physicians (EPs), Workload of chronic overwork, Shift work, Natural killer cell (NK cell), CD56, Quality of emergency medical services

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In Japan, overwork is a current occupational health hazard among pediatricians, gynecologists, anesthesiologists and emergency physicians (EPs), due to medical doctors' inadequate regional distribution and poor working conditions. Japanese EPs work long hours due to frequent emergency duties at night, sometimes working longer than 24 h continuously. Few occupational health reports regarding Japanese physicians, including EPs, have been published in English, and there are only a few Japanese articles with English abstracts[1, 2]. One such article described that emergency medicine residents had significantly lower arousal levels than staff EPs at a tertiary critical care emergency center, and the low arousal levels might indicate a high risk of residents committing medical mistakes[2].

There are many overseas publications, mostly from the United Kingdom, United States, Canada and Australia,

Table 1. Tertiary critical care emergency centers and hospitals

No.	Prefecture	Region	Work style	Medical services employed(N)	Physicians (N)	Participants
1	Fukui	rural	12 h shift	primaryEM-tertiary EM	19	
2	Fukui	urban	8 h shift	primaryEM-tertiary EM	6	34
3	Tokyo	rural	12 h shift	primaryEM-tertiary EM	7	
4	Kumamoto	urban	12 h shift	primaryEM-tertiary EM	13	
5	Osaka	urban	no shift	tertiary EM	8	
6	Saitama	suburban	no shift	tertiary EM	32	
7	Tokyo	urban	no shift	tertiary EM	38	44
8	Tokyo	urban	no shift	tertiary EM	23	
9	Tokyo	urban	no shift	tertiary EM	20	
10	Tokyo	suburban	no shift	tertiary EM	36	
11	Kanagawa	rural	fixed day work	rehabilitation medicine	13	
12	Tokyo	rural	fixed day work	community medical services	18	11

EM: emergency medicine
Japanese emergency medical services were divided into primary EM (for patients who did not need hospitalization), secondary EM (for patients who needed hospitalization), and tertiary EM (for patients who needed intensive care).
No.1–4 were tertiary critical care emergency centers of the shift work group.
No.5–10 were tertiary critical care emergency centers of the traditional work group.
No.11 &12 were hospitals of the day work group.

which have investigated EPs' occupational stress and mental health[3–14]. Some have reported that EPs tend to have a higher rate of burnout than other medical professionals[3, 4], and that emergency medicine is perceived as a stressful specialty[5–9]. Other studies have shown that shift work, night shift and weekend work are associated with increased stress[10, 11], that emergency medicine is a high stress profession[12], and that women among emergency medicine residents experience more stress and depression than men[13].

Several researchers have reported workers' immune function in terms of workload[15–17], and mental health[18–25]. Kobayashi *et al*. reported that nurses' NK cell activity was low during the night shift[15], while Xu *et al*. reported that truck drivers' NK cell activity significantly decreased after work[16]. Furthermore, Yasuda *et al*. reported first that a low CD56 (NK cell subset) percentage had a significant correlation with both long work and short sleep among Japanese male engineers[17]. No study has reported on physicians' immune function in Japan thus far.

In the present study, our hypothesis was that the overwork among Japanese EPs would result in a poor state of immune variables. We utilized CD4, CD8, CD56, and NK cell activity at the start of work as indicators, hypothesizing they would reflect job fatigue resulting from chronic overwork among EPs. The workload of chronic overwork was also indicated by weekly working hours, frequency of monthly night duties, and frequency of monthly days off.

The purpose of the present study was to examine our hypothesis and to investigate the current state of work conditions for Japanese EPs and the effects on their health.

Subjects and Method

Subjects

The study population consisted of 233 physicians working in 10 tertiary critical care emergency centers and 2 hospitals (including a community hospital and a rehabilitation hospital) in Japan. Table 1 outlines the occupational characteristics of these 10 tertiary emergency centers and 2 hospitals.

Subjects were selected according to the following criteria:

(i) Exclusion of individuals older than 50 yr of age. Most physicians older than 50 yr of age are not engaged in EPs' duties due to lack of physical endurance;

(ii) The investigation took place on a weekday (from Monday to Friday); and

(iii) There was no compulsion to participate in this study.

Eighty-nine healthy physicians aged 24–49 participated in this study. All the participating physicians were informed of the study procedures and gave their written informed consent.

Participants were classified into three groups; the shift work group, the traditional work group, and the day work group, according to their work styles (Table 1). The shift work group consisted of 8- or 12-h rotating shift work

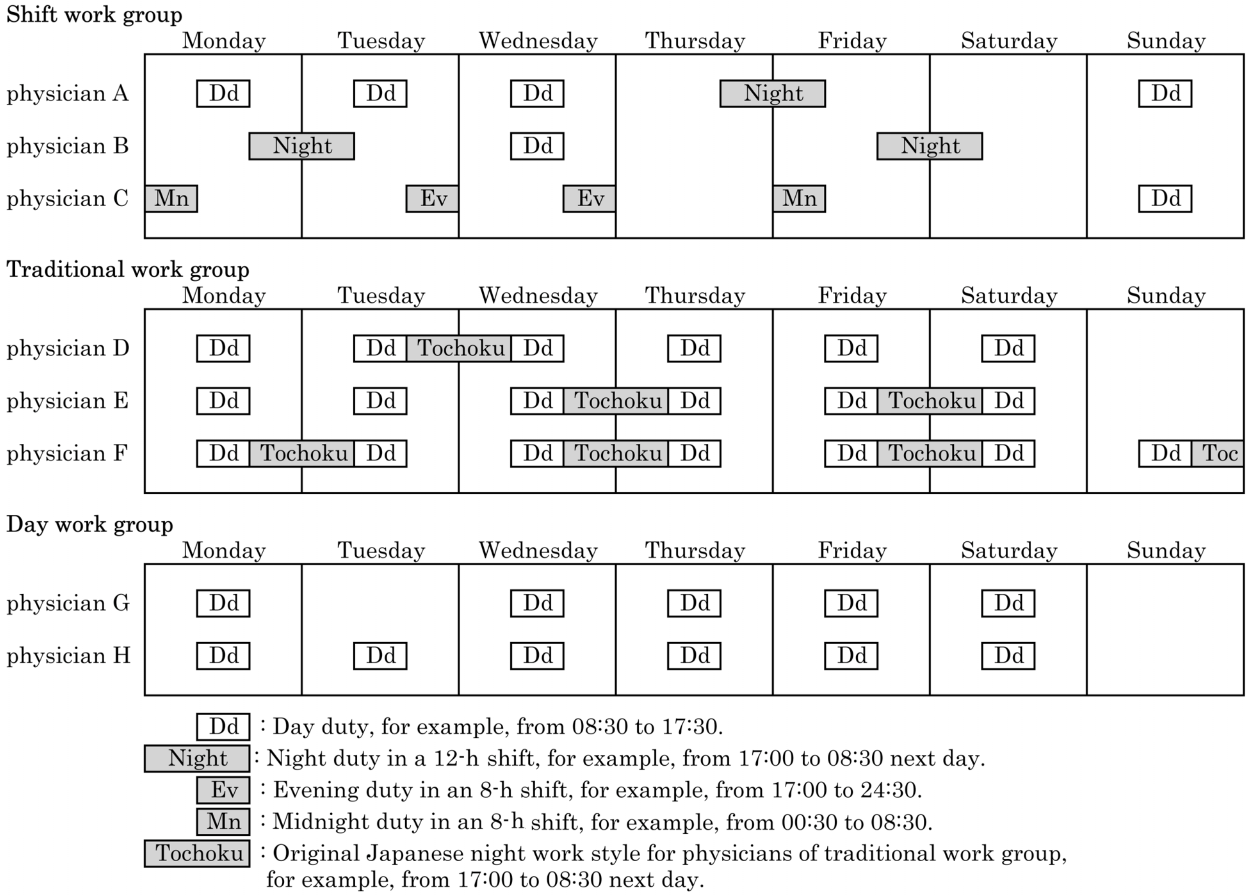

Fig. 1. Representative samples of Japanese physicians' work schedules during a typical week.

physicians in 4 critical care emergency centers (No. 1–4 in Table 1). The traditional work group included non-shift work physicians in 6 critical care emergency centers (No. 5–10 in Table 1), and the day work group consisted of day work physicians in 2 hospitals (No. 11 and 12 in Table 1).

Figure 1 illustrates the representative work schedule of Japanese physicians during a week. Representative samples of the shift work group are shown for physicians A–C. The work schedule of physician A showed that he or she worked 4 day duties and 1 night duty in 12-h shifts. The work schedule of physician B consisted of 1 day duty and 2 night duties in 12-h shifts. Physician C worked 1 day duty, 2 evening duties, and 2 midnight duties in 8-h shifts. The shifts used in these hospitals are not necessarily typical in Japan.

Generally, the traditional work styles of Japanese EPs are not shift workers. Physicians D–F represented the traditional work group. Physicians D and E were representative samples of Japanese EPs. Physician D worked 4 day duties and 1 day-*tochoku*-day duty (a combination of day duty and "tochoku", as explained below, and another day duty of the next day). Physician E worked 2 day duties and 2 day-*tochoku*-day duties. Physician F worked more than 3 day-*tochoku*-day duties, which was the heaviest work schedule. The day-*tochoku*-day duty of 32 continuous working hours is a long and arduous workload and is a schedule peculiar to the Japanese emergency medical services as far as we know. This is illustrated as | Dd | Tochoku | Dd | (Fig. 1).

"Tochoku" is a unique Japanese night work style for physicians of the traditional work group, and is different from night duty. Originally, "tochoku" was called "shukuchoku", and the main tasks of "tochoku/shukuchoku" were management or engagement of keeping watch in hospitals (i.e., ward rounds, examination of inpatients with emergency events, and responding to telephone inquires of outpatients without examination) at night, with sufficient time for sleep. On the other hand, the main task of night duty was to examine outpatients at night. Although "tochoku/shukuchoku" is distinguished from night duty in Japan's Labour Standards Law (article 41), "tochoku" has gradually changed its character and now is nearly the same as night duty.

The work schedules of physicians G and H (day work group) consisted of 5 or 6 day duties, but no night duty.

In this study, data sampling schedules were as follows: at the beginning of night duty for the shift work group, at the beginning of the day-*tochoku*-day duty for the traditional work group, and at the beginning of day duty for the day work group.

Questionnaire

An original self-administered questionnaire on Japanese EPs' work conditions and their workload was used for this study, since no questionnaire for this purpose had previously been devised. To evaluate Japanese EPs' overwork three items were selected: weekly working hours, frequency of monthly night duties, and frequency of monthly days off. The questionnaire also included demographic characteristics of subjects (age, gender, position, regularity of meals, smoking, and sleeping hours at home). Each participant was asked to fill in this self-administered questionnaire at the beginning of their duty.

Blood sampling and immune variables

Blood samples were collected from 89 healthy Japanese physicians at the start of their duties from January to June in 2005. The samples were taken in the morning for the traditional work group and the day work group, and in the evening or midnight for the shift work group.

Immune variables were investigated, including NK cell activity and lymphocyte subpopulations, specifically CD4 for T helper cells, CD8 for T suppressor cells, and CD56 as it is the most frequent NK cell surface antigen. We had both NK cell activity and lymphocyte subpopulations measured by an independent laboratory, SRL Inc. (Tokyo, Japan). The NK cell activity was determined using the ^{51}Cr (Chromium-51) release method[26] at an effector:target (E/T) ratio of 20:1. The ^{51}Cr release method was performed as follows. For the effector cells, lymphocytes centrifuged from 5 m*l* of blood sample (1,800 rpm for 20 min) were added to phosphate-buffered saline (PBS), and then centrifuged at 2,000 rpm for 5 min and the supernatants were aspirated off twice. The effector cells were resuspended at 10^6 cells/m*l*. For the target cells, K-562 tumor cells were labeled by ^{51}Cr (Perkin Elmer life and Analytical Sciences, USA). Ten microliters (0.01 m*l*) of the target cells at 10^6 cells/m*l* were added to 200 μl (0.2 m*l*) of the effector cells; then they were cultured at 37°C in 5%CO_2 for 3.5 h. Target cells are impaired by the effector cells, and ^{51}Cr is released from the impaired target cells. The released ^{51}Cr was measured by a gamma counter (Perkin Elmer life and Analytical Sciences, USA) and NK cell activity was calculated.

Lymphocyte subpopulations (CD4, CD8 and CD56) were analyzed by the flowcytometric immunofluorescence method[27] as follows. A 5 m*l* sample of heparinized blood was drawn, and the monoclonal antibodies used were T4 FITC, T8 RD1 and NKH1-RD1 (Beckman Coulter, USA) which are specific to CD4, CD8 and CD56, respectively. One hundred microliters (0.10 m*l*) of whole blood was incubated with one of the monoclonal antibodies at 4°C for 30 min in darkness. All antibodies were used at optimal dilutions with 0.2% of BSA-PBS (bovine serum albumin-phosphate buffered saline). After incubation, each sample was added to 5 m*l* of lysing solution and the samples were mixed gently and stood at room temperature for 10 min. The samples were centrifuged at 1,800 rpm for 5 min and the supernatants were aspirated; then, lysing solution was added again. After the samples had been re-centrifuged at 1,800 rpm for 5 min, their supernatants were removed again. The pelleted cells were resuspended in 5 m*l* of PBS, centrifuged (1,800 rpm for 5 min) and their supernatants were again removed. After re-centrifugation, the pelleted cells were resuspended in PBS. Lymphocytes were distinguished from other leukocytes (monocytes and granulocytes) on the basis of their forward narrow-angle and side angle light-scattering properties. CD4, CD8 and CD56 were also analyzed by a flowcytometer (FACS calibur, Becton Dickinson, USA). The numbers of each lymphocyte subpopulation were calculated by multiplying the lymphocyte count by the percentage of positive cells in each category, as determined by flowcytometry.

Statistical analysis

Mean ± standard deviation (mean ± SD) for continuous variables were computed by group and are shown in Tables 2–4. Statistical analyses were performed using SPSS version 6.1 for Windows.

For inter-group comparisons, homogeneity of variance was assessed by Levene's test. One-way analysis of variance (one-way ANOVA) was used to compare characteristics among the three groups (age and sleeping hours at home), workload of chronic overwork (excluding monthly night duties), and immune variables. The Bonferroni's multiple comparison was applied if the results of ANOVA were statistically significant.

Student's *t*-test was used to compare the frequency of monthly night duties between the traditional work group and the shift work group, since the day work group had no night duty.

Multiple logistic regression analyses were performed to provide odds ratios (adjusted relative risk estimates) indicating the magnitude of risk of lowering immune function. For the dependent variable, NK cell activity was classified into 2 groups, lower and upper group, less than 35% and 35% and over, respectively, because 35% was the mean of NK cell activity among the 74 physicians. Independent variables consisting of characteristics and workload were defined as follows. Characteristics: age, gender, position, regularity of meals, smoking, sleeping hours at home, and sampling timing. Workload: weekly

Table 2. Characteristics of 74 subjects

Characteristics			Shift work 27	Traditional work 39	Day work 8	Results of one-way ANOVA
Age		(yr)	34.4 ± 6.3	31.2 ± 5.6	41.3 ± 5.8	F=10.3, df=[2,71], p<0.01
				**	*	
Sleeping hours at home		(h)	6.4 ± 1.3	6.1 ± 0.9	6.9 ± 0.8	F=2.3, df=[2,71], p=0.11
Gender	Male	(N)	21	30	8	
	Female	(N)	6	9	0	
Position	Staff physicians	(N)	21	22	8	
	Residents	(N)	6	17	0	
Meals	Regular	(N)	8	11		
	Irregular	(N)	19	28	0	
Smoking	Non-smokers[a)]	(N)	24	29	8	
	Smokers	(N)	3	10	0	

[a)] Ex-smokers were included to non-smokers. *: p<0.05 by Bonferroni's multiple comparison. **: p<0.01 by Bonferroni's multiple comparison.

working hours, monthly night duties, and monthly days off. Categorizations of each variables are shown in Table 5 (as 35 yr old is used as a cut-off point in the health management of Japanese workers according to the Industrial Safety and Health Law, the categorical variable "Age" was classified into 2 categories, "<35 yr old" and "≥35 yr old"). Results were considered statistically significant at p<0.05.

Ethics

The study protocol was approved by the Ethical Review Committee of the School of Medicine, Kyorin University. Procedures were performed under the regulation of the Declaration of Helsinki.

Results

Seventy-four of the 89 volunteer physicians were analyzed in this study. Fifteen of the 89 physicians were excluded due to incompleteness of the questionnaires, recent long vacation or having worked before the blood examination (i.e. occasionally worked day time before their night shift). Twenty-seven of the 74 physicians were in the shift work group, 39 physicians in the traditional work group, and 8 physicians in the day work group (including 3 internists, 1 surgeon, 1 orthopedist, 1 urologist, 1 neurosurgeon, and 1 rehabilitation physician).

Table 2 shows the characteristics of subjects in each group (age, gender, position, regularity of meals, smoking, sleeping hours at home). According to Bonferroni's multiple comparison, the physicians of the day work group (41.3 ± 5.8 yr) were significantly older than EPs of the other two groups (34.4 ± 6.3 yr for the shift work group (p<0.05), 31.2 ± 5.6 yr for the traditional work group (p<0.01)).

Table 3 shows the workload of the 74 subjects during the month before this investigation. Among these three groups, significant differences were observed in weekly working hours (F=62.5, p<0.01), and monthly days off (F=52.2, p<0.01). Monthly night duties of the traditional work group (9.5 ± 3.2 times a month) were significantly more frequent than the shift work group (6.9 ± 2.6 times a month, p=0.01 by Student's t-test). According to Bonferroni's multiple comparison, weekly working hours of the traditional work group were significantly longer (77.2 ± 15.2 hours a week) than both the shift work group (43.6 ± 11.0 hours a week, p<0.01) and the day work group (42.4 ± 4.6 hours a week, p<0.01), and monthly days off of the traditional work group were significantly shorter (2.1 ± 1.8 days a month) than both the shift work group (6.5 ± 2.0 days a month, p<0.01) and the day work group (6.6 ± 1.7 days a month, p<0.01). Therefore, the results in Table 3 show that EPs of the traditional work group were worked most heavily.

The results of the blood analyses in Table 4 show no significant differences among the groups for WBC, CD4 (%), CD8 (number and %) and CD56 (number and %). Among these groups, significant differences were observed in lymphocytes (F=3.62, p=0.03), numbers of CD4 (F=4.13, p=0.02), and NK cell activities (F=11.2, p<0.01). Lymphocytes and the number of CD4 in the traditional work group were significantly lower than in the shift work group (both p<0.05 by Bonferroni, respectively). Lymphocytes and the number of CD4 in the traditional work group were lower than in the day work group (both not significant by Bonferroni). The NK cell activity of the shift work group was significantly lower than that of the traditional work group (p<0.01) and that of the day work group (p<0.01), according to

Table 3. Workload of 74 Japanese physicians for a month before the investigation

Workload		Shift work 27	Traditional work 39	Day work 8	Results of statistical testing
Weekly working hours	(h/wk)	43.6 ± 11	77.2 ± 15.2	42.4 ± 4.6	F=62.5, df=[2,71], p<0.01 (by one-way ANOVA)
		* (shift vs traditional)		* (traditional vs day)	
Monthly night duties	(times / month)	6.9 ± 2.6	9.5 ± 3.2	–	p<0.01[a)]
Monthly days off	(days/month)	6.5 ± 2.0	2.1 ± 1.8	6.6 ± 1.7	F=52.2, df=[2,71], p<0.01 (by one-way ANOVA)
		* (shift vs traditional)		* (traditional vs day)	

[a)]Student's t test was performed to compare the shift work group and the traditional work group.
*: p<0.01 by Bonferroni's multiple comparison

Table 4. Immune variables of 74 Japanese physicians

		Shift work 27	Traditional work 39	Day work 8	Results of one-way ANOVA
WBC	(number/μl)	5,967 ± 1,298.5	5,612.8 ± 1,696.8	4,725 ± 903.6	F=2.15, df=[2,71], p=0.13
Lymphocyte	(number /μl)	2,647 ± 732.2	2,195.1 ± 619.3	2,355 ± 711.5	F=3.62, df=[2,71], p=0.03
		* (shift vs traditional)			
Number of CD4	(number/μl)	1,138 ± 390.1	911 ± 250	967.3 ± 344.4	F=4.13, df=[2,71], p=0.02
		* (shift vs traditional)			
Percentage of CD4	(%)	42.8 ± 7.2	42.2 ± 7.4	41.1 ± 8.7	F=0.17, df=[2,71], p=0.84
Number of CD8	(number /μl)	923 ± 268.2	763.2 ± 345.8	851.8 ± 295.7	F=2.08, df=[2,71], p=0.13
Percentage of CD8	(%)	35 ± 5.1	34 ± 7.9	35.7 ± 4.6	F=0.32, df=[2,71], p=0.73
Number of CD56	(number /μl)	511 ± 261.8	437.4 ± 198.6	566 ± 250.8	F=1.49, df=[2,71], p=0.23
Percentage of CD56	(%)	19.2 ± 8	19.9 ± 6.6	24.7 ± 10.5	F=1.69, df=[2,71], p=0.19
NK cell activity (E/T ratio of 20:1)	(%)	25.6 ± 16	38.6 ± 13.2	49.8 ± 13.6	F=11.2, df=[2,71], p<0.01
		** (shift vs traditional); ** (shift vs day)			

*: p<0.05 by Bonferroni's multiple comparison.
**: p<0.01 by Bonferroni's multiple comparison.

Bonferroni's multiple comparison.

The results of multiple logistic regression analyses are shown in Table 5. The NK cell activity, the dependent variable of this analysis, was classified into 2 groups, lower (<35%) group and upper (>35%) group as described in "Statistical analyses." Both groups for the night sampling and the 0–3 days off per month were significantly associated with lower NK cell activity (OR=8.34, 95%CI: 1.95–35.6, p=0.004 for night sampling; OR=4.65, 95%CI: 1.27–17.0, p=0.02 for 0–3 days off per month).

Discussion

It is known that Japanese physicians tend to work heavily (long hours of working and frequent "tochoku" as emergency duties at night) and suffer high stress as a result[1, 2]. Despite their serious occupational health problems, few studies have investigated them until now. As few English publications have been published on the occupational health of Japanese physicians, this is probably the first article to show the actual conditions of work of Japanese EPs, and to study their occupational health problems.

Figure 1 illustrates representative samples of Japanese physicians' work schedules during a week. Traditional work EPs work long hours at a stretch (32 h), such as day-*tochoku*-day duty (e.g., physician D's working style

Table 5. Results of multiple logistic regression analyses for under 35% in NK cell activity

Independent Variables	Categorization	N[a]	OR	95% CI	*p* value
Age	<35 yr old	45 (25)	1		
	≥ 35 yr old	29 (12)	0.6	0.27–1.33	0.210
Gender	male	59 (29)	1		
	female	15 (8)	0.71	0.28–1.80	0.470
Position	staff physicians	51 (24)	1		
	residents	23 (13)	1.02	0.47–2.19	0.970
Meals	regular	27 (14)	1		
	irregular	47 (23)	0.53	0.25–1.13	0.100
Smoking	non smoker	61 (31)	1		
	smoker	13 (6)	1.12	0.48–2.59	0.790
Sleeping hours at home	≥6 h	58 (26)	1		
	< 6 h	16 (11)	1.84	0.88–3.83	0.100
Sampling timing	in the morning	47 (18)	1		
	at night	27 (19)	8.34	1.95–35.6	0.004
Weekly working hours	< 65 h	44 (24)	1		
	≥ 65 h	30 (13)	1.95	0.66–5.73	0.220
Monthly night duties	0–3 times	10 (3)	1		
	4–7 times	24 (16)	1.34	0.38–4.74	0.650
	≥8 times	40 (18)	0.29	0.07–1.19	0.090
Monthly days off	≥4 days	41 (20)	1		
	0–3 days	33 (17)	4.65	1.27–17.0	0.020

NK cell activity was determined at an E/T ratio of 20:1. [a]: Number of physicians who had a NK cell activity value under 35% in parentheses.

from Tuesday to Wednesday in Fig. 1). Some EPs often carry on this pattern 2 or 3 times a week (e.g., work styles of physicians E and F in Fig. 1).

With regard to occupational safety and health in Japan, Wada reviewed the literature concerning overworked workers' health[28] in 2002, and Liu *et al*. reported that overtime work might be related to the risk of acute myocardial infarction[29]. The Expert Committee of the Ministry of Health, Labour and Welfare (MHWL) compiled a report on the relationship between overwork and workers' health[30, 31], and found that more than 100 h of overtime a month introduces a high risk both of brain/heart vascular disease and mental disorder. In 2005, health guidance for overworked workers was enacted in a revised Japan's Industrial Safety and Health Law (article 66–8)[32]. Based on this law, since April in 2006, it has become mandatory upon employers for workers who work more than 100 h of overtime a month to have a doctor's interview. One hundred hours of overtime a month is equivalent to about 65 working hours a week. Since Japanese traditional EPs are likely to work more than 65 h a week if they take the day-*tochoku*-day duty twice a week (e.g., work style of physician E in Fig. 1), this would constitute a serious occupational health risk.

Generally, shift work is characterized by much stress[10, 11] and overload[15]. Shift work sometimes causes EPs' retirement from emergency medicine outside Japan[8, 11]. However, the results of this study suggest that shift work conditions are actually better than the traditional work conditions (Table 3 and Fig. 1) in Japan. In fact, shift work could improve Japanese EPs' total weekly work schedule (Table 3) and avoid the heavy workload of the day-*tochoku*-day duty (Fig. 1).

This study evaluated physicians' workload of chronic overwork in terms of working hours, numbers of night duties and total days off. Physicians' workload cannot be simply estimated from the number of patients they deal with, since it is difficult to adequately evaluate the workload due to differences among patients with various diseases, clinical job contents and job burdens (i.e., differences among consulting outpatients and inpatients, operations, and performing examinations). Parameters such as the number of patients with different diseases, differences in the quality of clinical job contents, and differences in clinical job burdens should be taken into account in future studies.

Several studies reported that a reduction in T cell number was associated with depression[18], job stress[19–22], and burnout[23]. In a study about physicians, Bargellini *et al*. reported that a high degree of personal accomplishments in terms of Maslach Burnout Inventory (MBI) was significantly associated with an increase in the number of T cells (CD3), T helper cells (CD4), and T suppressor cells (CD8) in Italian physicians[23]. Another study in Israel reported that there was no significant difference of immune variables (CD4, CD8, and NK cell activity) between anesthetists and healthy controls who were not physicians[33]. Few researchers have reported relationships between T cell number and workload or work conditions. In this study, no significant difference of T cell number was observed among the physicians' groups except for the number of T helper cells (CD4). No apparent relationship was observed between T cell subpopulations and workload of EPs.

It is well known that NK cells defend against tumor cell growth and viral infections in human cellular immune systems, and that NK cell functions are variously influenced by workload[15–17], mental health[20–22, 24, 25], and life style[34–50]. As for life style, it was reported that reduction of NK cell functions was associated with cigarette smoking[34–38], shortened daily sleep[17], disturbed sleep[39–41], circadian rhythm (high activity in the morning and low activity at night)[42–45], and excessive physical exercise[46, 47]. Conversely, healthy life styles (i.e., good sleep, adequate physical exercise, and so on) were associated with increases of NK cell functions[48–50].

Several occupational health studies[15–18, 20–25] of workers have reported that reduction of NK cell functions (including CD56) was associated with night shift work[15], long work[16, 17], depression[18], job stress[20, 25], and burnout[24]. Morikawa *et al*. recently reported that increased workload quantified by the Nursing Job Stressor Scale (NJSS) was significantly related to decreased NK cell activity among healthy Japanese nurses[25].

The present study indicates that NK cell activity was significantly lower at night (at the beginning of the shift work group) than in the morning (at the beginning of both the traditional work group and the day work group as shown in Table 4). Results in Table 5 show that NK cell activity was significantly lower at night (note: "at night" includes "in the evening" because no difference of NK cell activity was observed, though not shown here, between those taken at night and in the evening) as compared to the morning, which might be explained by circadian rhythm[42–45]. Further studies of the relationships between immune function and workload will be required to clarify the role of circadian rhythm.

Levi *et al*. reported that numbers of NK cells from January to June were significantly less than those in August to November[51], but it is unknown how seasonal variations influence NK cell activities.

It appears that lack of rest, such as 0–3 days off per month, would cause job fatigue and impair NK cell activity and ultimately EPs' physical health. Thus, impaired EPs' physical health could lower the quality of emergency medical services. For the sake of Japanese EPs' physical health and the quality of Japanese emergency medical services, it is desirable for EPs to take 4 or more days off per month.

Despite fact that a significant association existed between monthly days off and NK cell activity and that an opposite relation existed between fewer days off and longer working hours, it was difficult to explain why weekly working hours did not significantly affect NK cell activity (Table 5). It is also difficult to explain why irregular meals had a positive effect on NK cell activity and why monthly night duties of more than 8 times positively affected NK cell activity (Table 5), since irregular meals and frequent night duties would seem to be bad life styles.

There were several limitations to this study. The first was the relatively small number of subjects: a larger number of subjects is required for better multiple logistic regression analyses. Even though we were able to perform statistical analyses, we should discuss and interpret the results of these analyses with care.

The second limitation was the number of physicians in the day work group was small, just 8 physicians, because it was difficult to find Japanese physicians who were under 50 yr of age and worked in hospitals without "tochoku" or night duty in Japan. Thus, these 8 physicians were indeed precious samples for an occupational health study.

The third limitation existed in the measurement of NK cell activity. The measurement of NK cell activity was likely to be affected by the conditions of the culture phase. Ideally, all blood samples should have been measured at the same time. We were careful to collect all the data of immune functions in the same manner in this research, but as the blood samples were not measured at the same time, we need to be careful about interpreting the results. Moreover, in spite of using multiple effector:target (E/T) ratios (5:1, 10:1, 20:1 and 40:1) in measurement of NK cell activity, we could not get the values of NK cell activity at E/T ratios of 5:1, 10:1 and 40:1 because the NK cell activity was measured by SRL Inc. at an E/T ratio of 20:1 only. However, since other studies have reported NK cell activity also using an E/T ratio of 20:1[15, 16, 25], our data of NK cell activity are useful for comparative studies.

Concerning the influence of gender on NK cell activity, it has been reported that NK cell activity of males is significantly higher than that of females with a regular menstrual cycle[52]. Similarly, the results in Table 5 show that NK cell activities of females were lower than those of males, but no significant differences were observed.

As for the influence of the menstrual cycle on NK cell activity, it has been reported that the menstrual cycle had no significant effect on NK cell activity[52], and that the menstrual cycle had some effects on NK cell activity[53]. The menstrual cycle might have influenced NK cell activity in female physicians in the present study, and the fact that we did not investigate the menstrual cycle in this study could be considered as another limitation of the study design.

No differences existed in the emergency medical services among the listed facilities when medical facilities No.11 & 12 were excluded from Table 1. Each facility was selected as a representative emergency center of a region of Japan. However, differing characteristics and numbers of admitted patients among the facilities (e.g., primary-tertiary emergency medicine (No.1–4), tertiary emergency medicine (No.5–10) in Table 1) could have affected the results of this study. In the present study, we could not collect data on admitted patients consulting physicians during the month before this investigation. Thus, we could not search for relationships between the data of admitted patients as a workload and NK cell activity. Further studies are required taking into account both the characteristics and the numbers of admitted patients.

Despite the limitations described above, some conclusions may be drawn. First, EPs of the traditional work group worked very heavily (i.e., 32 continuous hours working of the day-*tochoku*-day duty, and had fewer days off per month), while the workload of EPs of the shift work group was less than that of the traditional one. Second, significant reductions in NK cell activity were associated with fewer days off. These results suggest that the low NK cell activity might be putting Japanese EPs' health and the quality of emergency medical services at risk. Future studies, with samples of a larger size and with consideration of circadian rhythm of NK cell function are required.

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