Number of Patients Examined May Affect Natural Killer Cell Activity in Japanese Emergency Physicians: A Preliminary Study

Hiroteru OKAMOTO¹,², Koji TERUYA¹,², Akinori NAKATA³, Yoshihiro YAMAGUCHI⁴, Takeaki MATSUDA⁴ and Tooru TSUNODA²

The purpose of this study was to investigate whether the number of patients examined could be a useful indicator of workload influencing natural killer cell activity (NKCA) among Japanese emergency physicians (EPs). The subjects were 34 healthy EPs from six critical care emergency centers in Japan. They completed a questionnaire regarding their workload and provided blood samples at the beginning and end of work shift for the measurement of NKCA in 2005. The subjects stayed at the hospital from the morning to the following evening for approximately 32 continuous hours. Relative to residents, staff members examined a significantly greater number of seriously ill inpatients (p<0.01). NKCA at the end of shift was significantly lower than the beginning of shift among staff members (p<0.01), while no significant change was observed among residents. Multiple linear regression analysis (stepwise method), using change in NKCA as a dependent variable, showed that the number of seriously ill inpatients examined was the only workload factor significantly associated with change in NKCA (standard partial regression coefficient = -0.59, p<0.01). Additionally, the confounding factor of sex was significantly associated with change in NKCA (standard partial regression coefficient = -0.33, p=0.04). The results suggested that physicians’ NKCA may be affected by the number of seriously ill patients they examined, although circadian rhythm and sex may confound this relationship; thus, number of seriously ill patients may serve as an indicator of workload in Japanese EPs.

Key words: Physicians, Workload, Natural Killer cell activity (NKCA), Number of Patients Examined

Introduction

Emergency physicians (EPs) are likely to experience a substantial level of occupational stress because they engage in highly demanding tasks on a daily basis. In addition to these daily tasks, shift work, night shift, and weekend work are common among EPs¹,², and could add extra burden to their professional lives³. Among several other workload-associated variables, hours worked is used as...
a health-related factor and an indicator of workload in job stress research\(^4\)\(^\text{-}^9\). However, in a similar manner to hours worked, the number of patients examined may serve as an index of quantitative workload among EPs because of the nature of the job. Indeed, several studies showed that the number of patients examined reflects a physician’s workload\(^9\)\(^\text{-}^\text{10}\), suggesting that a rise in number of patients examined by physicians could reduce job satisfaction or heighten job stress.

Natural killer (NK) cells are known to defend against tumor cell growth and virus-infected cells. Level of NK cell function reportedly serves as a useful index of overall health status\(^1\)\(^\text{-}^2\). Numerous studies have demonstrated relationships between level of NK cell function and health-related risk factors in workers. These studies revealed that risk factors for reduced NK cell function, such as NK cell activity (NKCA), include work itself\(^1\)\(^3\); increased workload\(^1\)\(^4\); night shift in Japanese nurses\(^1\)\(^5\)\(^\text{-}^1\)\(^6\); long working hours among truck drivers\(^1\)\(^3\), male engineers\(^1\)\(^7\), and white-collar employees\(^1\)\(^8\); sleep deprivation\(^1\)\(^7\); a few days off per month in Japanese EPs\(^1\)\(^9\); job stress among nurses and university workers\(^1\)\(^4\)\(^,\)\(^\text{-}^\text{20}\), feelings of job-related fatigue in Japanese nurses\(^1\)\(^6\); and poor mental health conditions such as anxiety, burnout, and depression\(^2\)\(^1\)\(^\text{-}^\text{24}\). We considered a reduction in NKCA an important indicator of both job stress reaction and lower immunity in health workers.

In addition, reduced NK cell function has been associated with poor lifestyle factors, including cigarette smoking\(^2\)\(^5\)\(^\text{-}^\text{28}\), reduced daily sleep\(^1\)\(^7\), sleep disturbances\(^2\)\(^4\)\(^,\)\(^\text{-}^\text{30}\), and disrupted circadian rhythm (high activity in the morning and low activity at night)\(^2\)\(^9\)\(^,\)\(^\text{-}^\text{33}\). Conversely, a healthy lifestyle (e.g., adequate sleep and regular physical exercise) has been associated with increased NK cell function\(^1\)\(^1\)\(^,\)\(^\text{-}^\text{34}\). In many such studies, researchers have utilized NKCA as a health index.

The purpose of the present study was to investigate whether the number of patients examined could serve as an indicator of physicians’ workload consequently influencing NKCA. As shown in Figure 1, we hypothesized that number of patients examined could affect NKCA among EPs.

![Figure 1](image_url)
Subjects and Methods

Subjects
The study subjects were 34 healthy, non-shift-working EPs (age: 24–49 years) from six tertiary critical care emergency centers in Japan. All participating EPs were informed of the study procedures and provided written informed consent. Eighteen of the 34 EPs were staff members, and the remaining 16 were residents. This investigation was conducted on weekdays during January and June, 2005.

In 2005, the majority of Japanese EPs who worked in the major tertiary emergency medicine centers were non-shift workers. When they were assigned to night shift, they worked for 32 hours in one cycle, which consisted of a continuous day–night–day shift. In addition, their tasks were to examine seriously ill outpatients in the emergency room, perform urgent operations on traumatic patients, and provide care for seriously ill inpatients in the intensive care unit or trauma care unit.

The study was conducted with the approval of the ethical review committee of the School of Medicine, Kyorin University (No. 127). Procedures were performed in accordance with the Declaration of Helsinki.

Questionnaire
We developed a self-administered questionnaire to assess the work conditions and workload of EPs, since no questionnaire for this purpose had existed. In this study, the workload of EPs were assessed via four items regarding 1) working hours, 2) resting hours, 3) number of seriously ill outpatients examined, and 4) number of seriously ill inpatients examined. Seriously ill outpatients were patients in critical condition who had been transported by ambulance, including those experiencing cardiopulmonary arrest on arrival. Inpatients were those who had been admitted to the ICU/TCU. Relative to residents, staff members examined a greater number of inpatients because they supervised residents and thus administered the examinations performed by residents and other staff members. The questionnaire assessed demographic and lifestyle characteristics (age, sex, smoking, and regular hours slept at home), and subjects were asked to complete the questionnaire during their shift. However, we failed to measure job stress of subjects.

NKCA
We collected blood samples from all subjects in the morning (at the beginning of shift) and in the following evening (at the end of shift) and compared NKCA between these two points. An independent laboratory measured NKCA (SRL Inc., Tokyo, Japan). The laboratory technicians used the chromium–51 (51Cr) release method with an effector: target (E/T) ratio of 20:1. Although multiple E/T ratios (5:1, 10:1, 20:1, and 40:1) can be used to measure NKCA, we chose an E/T cell ratio of 20:1 because a large study of a Japanese population (N = 3625) showed that differences in individuals’ cytotoxic activity were most distinguishable when this ratio was used. To prepare samples of effector cells, the laboratory technicians added lymphocytes centrifuged from a 5-ml blood sample (1,800 rpm for 20 min) to phosphate-buffered saline (PBS), which was then centrifuged at 2,000 rpm for 5 min; the supernatants were aspirated twice. They resuspended the effector cells in PBS at 10⁶ cells/ml. To prepare the target cells, they labeled K-562 tumor cells with 51Cr (51Cr: Perkin Elmer Life and Analytical Sciences, Waltham, USA). They added 10 µl (0.01 ml) of the target cells at 10⁶ cells/ml to 200 µl (0.2 ml) of the effector cells, and cultured the cell mixture at 37°C in 5% CO₂ for 3.5 h. Target cells are impaired by the effector cells, and 51Cr is
released from the impaired target cells. They measured the released $^{51}$Cr using a gamma counter (Perkin Elmer Life and Analytical Sciences, Waltham, USA) and used this information to calculate NKCA.

**Statistical analysis**

We performed all statistical analyses using SPSS for Windows version 20.0. The mean and standard deviation (SD) for the continuous variables are shown in Tables 1 and 2.

We used a Student’s t test to compare the characteristics (age, regular hours slept at home, working hours, and resting hours) of the staff group with those of the resident group (Table 1). We used the Chi–squared test to analyze sex and smoking data and the Mann–Whitney U test to analyze number of seriously ill patients examined (Table 1), as they are continuous variables but did not conform to a normal distribution in the Shapiro–Wilk test ($p<0.01$). We performed parametric analysis of NKCA (Table 2), as it was a continuous variable and conformed to a normal distribution in the Shapiro–Wilk test ($p>0.05$).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics and workload of 34 Japanese emergency physicians in the investigation</th>
<th>Staff members</th>
<th>Residents</th>
<th>Results of statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics:</td>
<td></td>
<td>N=18</td>
<td>N=16</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>32.8 (4.4)</td>
<td>26.9 (2.0)</td>
<td>$p&lt;0.01$ *a)</td>
</tr>
<tr>
<td>Regular hours slept at home (hours)</td>
<td></td>
<td>6.2 (0.9)</td>
<td>5.8 (0.7)</td>
<td>$p=0.26$ *a)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td></td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td></td>
<td>2</td>
<td>6</td>
<td>$p=0.11$ *b)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-smokers</td>
<td></td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>smokers</td>
<td></td>
<td>6</td>
<td>7</td>
<td>$p=0.73$ *b)</td>
</tr>
<tr>
<td>Workload:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hours at hospital (hours)</td>
<td></td>
<td>33.1 (0.9)</td>
<td>33.9 (1.4)</td>
<td>$p=0.06$ *a)</td>
</tr>
<tr>
<td>Working hours (hours)</td>
<td></td>
<td>26.5 (3.4)</td>
<td>27.8 (3.4)</td>
<td>$p=0.24$ *a)</td>
</tr>
<tr>
<td>Resting hours (including a nap) (hours)</td>
<td></td>
<td>6.7 (3.4)</td>
<td>6.1 (3.3)</td>
<td>$p=0.61$ *a)</td>
</tr>
<tr>
<td>Number of seriously ill outpatients examined *e)</td>
<td></td>
<td>3.5 *e)</td>
<td>2.0 *e)</td>
<td>$p=0.33$ *d)</td>
</tr>
<tr>
<td>Number of seriously ill inpatients examined *f)</td>
<td></td>
<td>5.0 *e)</td>
<td>2.0 *e)</td>
<td>$p&lt;0.01$ *d)</td>
</tr>
</tbody>
</table>

Values are means (SD). [minimum–maximum]

a) The Students’ t test was used to compare values for staff members and residents.
b) Chi–square test
c) Ex-smokers were included in the non-smoker group.
d) Mann–Whitney U test was used to compare values for staff members and residents.
e) Outpatients consisted of seriously ill patients transported by ambulance cars, including those with cardio pulmonary arrest on arrival (CPAOA).
f) Inpatients consisted of seriously ill patients in Intensive Care Unit/Trauma Care Unit.
g) Values are median.
We performed multiple regression analysis to obtain partial and standard partial regression coefficients (Table 3; stepwise method). Changes in NKCA, that is, NKCA at the beginning of shift subtracted from that at the end of shift, were set as the dependent variable. The independent variables were workload and confounding factors. Workload consisted of three factors: working hours, number of seriously ill outpatients examined, and number of seriously ill inpatients examined. We did not treat resting hours as the independent variable, because resting hours and working hours were significantly negative correlated ($r^{-0.93}$, $p<0.01$). Additionally, age, sex, smoking, and job class were confounding factors in this analysis. The threshold for statistical significance was set at $p<0.05$.

**Results**

Table 1 shows the characteristics (age, sex, smoking, and regular hours slept at home) and workloads (working hours, resting hours, and number of seriously ill outpatients /inpatients examined) of the subjects in each group. The EPs in the staff group (age: 32.8 [4.4] years) were significantly older than those in the resident group (age: 26.9 [2.0] years, $p<0.01$). No significant differences in sex, smoking, or number of regular hours slept at home were found between the two groups.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>NKCA at the beginning of shift</th>
<th>NKCA at the end of shift</th>
<th>Results of paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergency physicians ($N=34$) (%)</td>
<td>37.4 (13.7)</td>
<td>31.9 (14.1)</td>
<td>$p=0.02$&lt;sup&gt;b)&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>[39.0]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staff members ($N=18$) (%)</td>
<td>38.5 (14.1)</td>
<td>27.3 (11.0)</td>
<td>$p&lt;0.01$&lt;sup&gt;b)&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>[40.0]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residents ($N=16$) (%)</td>
<td>36.1 (13.6)</td>
<td>37.0 (15.8)</td>
<td>$p=0.74$&lt;sup&gt;b)&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>[35.5]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means, (SD), and [median].

- a) Value of NK cell activity was indicated at E/T ratio of 20:1.
- b) Paired t-test was used to compare NKCA between the start and at the end of work period.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>NKCA at the beginning of shift</th>
<th>NKCA at the end of shift</th>
<th>Results of paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>adjusted $R^2=0.34$, $F_{(2, 31)}=7.90$, $p&lt;0.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>$\beta$</td>
<td>t</td>
</tr>
<tr>
<td>Numeric constant</td>
<td>15.47</td>
<td>2.18</td>
<td>0.04</td>
</tr>
<tr>
<td>confounding factors:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sex</td>
<td>-9.99</td>
<td>-0.33</td>
<td>-2.13</td>
</tr>
<tr>
<td>Workload:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of serious inpatients examined</td>
<td>-2.11</td>
<td>-0.59</td>
<td>-3.84</td>
</tr>
</tbody>
</table>

The three confounding factors (age, smoking, and job class) and the two independent factors (working hours and number of serious outpatients examined) were excluded in this analysis.

B: partial regression coefficients

$\beta$: standard partial regression coefficients
Relative to the staff members, the residents examined significantly fewer seriously ill inpatients ($p < 0.01$). No significant differences between the two groups were found in terms of the other workload variables (working hours, resting hours, and number of seriously ill outpatients examined).

Table 2 and Figure 2 show NKCA for the 34 subjects. Among the 18 staff members, NKCA differed significantly between the beginning (38.5 [14.1] %) and the end (27.3 [11.0] %) of shift ($p < 0.01$). However, among the 16 residents, NKCA showed no significant change between the beginning and the end of shift. Moreover, in the 34 EPs, NKCA at the end of shift (31.9 [14.1] %) was significantly lower than that at the beginning of shift (37.4 [13.7] %, $p < 0.05$).

Table 3 shows the results of the multiple regression analysis. Among the seven independent variables consisting of the four confounding factors and the three workload factors, the results of the multiple regression analysis (stepwise method) show that number of seriously ill inpatients examined was the only workload factor that was significantly associated with change in NKCA (standard partial regression coefficient $\beta = -0.59$, $p < 0.01$, Table 3); additionally, sex was the confounding factor significantly associated with change in NKCA ($\beta = -0.33$, $p = 0.04$, Table 3).

Discussion

The results of the present study suggest three major points. First, NKCA among residents of Japanese EPs may not have decreased because residents were required to examine a few seriously ill inpatients (Table 2). Second, changes in NKCA in Japanese EPs may be influenced by the number of seriously ill inpatients they examined although the effect of circadian rhythm may explain this rela-
tionship to some extent (Tables 2 & 3, and Figure 2). Finally, the workload factor of examining seriously ill inpatients had a greater influence on the change in NKCA than the confounding factor of sex did (Table 3).

While no significant change in NKCA was found in residents, all 34 EPs and 18 staff members showed a significant reduction in NKCA between the beginning and the end of shift (Table 2). This change in all 34 EPs and 18 staff members might be due to the influence of circadian rhythm on NKCA\(^{20,31-33}\); it is known that NKCA levels are high in the morning and low in the evening. However, despite the influence of circadian rhythm, residents’ NKCA levels were higher in the evening relative to those in the morning. In addition, at the end of shift, residents’ NKCA were higher relative to those of staff members (Table 2 and Figure 2). The changes of NKCA by shift may not have been influenced solely by circadian rhythm, but by other factors, as well. Excluding number of seriously ill inpatients examined, no significant differences were observed between staff members and residents in any of the workload variables (Table 1). Therefore, the findings delineated in Table 2 and Figure 2 suggest that the difference of change in NKCA might have been due to the difference between the number of seriously ill inpatients examined by staff members and residents. Further, we suggest that the reduction of NKCA was suppressed in the residents because their workloads were smaller than those of staff members with regard to the number of seriously ill inpatients examined. Conversely, we consider that the reduction in staff members’ NKCA, induced by a heavy workload (examination of a large number of seriously ill inpatients), in staff members might have been masked by circadian rhythm fluctuations (Figure 2). In addition, the results of multiple regression analysis showed that the number of seriously ill patients examined was the only workload factor that was significantly associated with lower NKCA among Japanese EPs (Table 3).

Results from 2005 showed that Japanese EPs stayed at the hospital continuously for approximately 32 hours; this time consisted of approximately 26-27 hours on duty and 6-7 hours off duty (resting time; Table 1). Their resting time was too short considering a high number of hours worked. In addition, their resting time was not continuous; it consisted of disturbed and reduced sleep. Because of the relationship between NKCA and workload variables (long work hours, shortened rest, and disturbed naps), we expected that the Japanese EPs in this study would show reduced NKCA, and the changes in their NKCA would be associated with workload factors\(^{13,17,18,24,29,30}\). However, the results show that the measured workload variables were not associated with changes in NKCA (Table 3).

This study is subject to four major limitations. The first is the relatively small sample size. A larger sample is required for conclusive results. The second limitation is the influence of circadian rhythm on NKCA\(^{20,31-33}\). Ideally, blood samples for NKCA measurement should be collected at three or more additional time points (the evening of the first day of duty, the midpoint of the night of duty, and the morning of the second day of duty, or after a nap or rest). The third limitation is that we did not use standardized job stress questionnaires such as the NIOSH (National Institutes of Safety and Health) Job Stress Questionnaire\(^{38}\) or the Brief Job Stress Questionnaire\(^{39}\) to assess subjects’ job stress. As the results of this study may have been influenced by stress, the impact of stress must be controlled for in future research. Finally, we used the seven independent variables including the four confounding factors for NKCA in the analysis (stepwise method) even though this study sample was
small in size. Previous studies suggested that reduced NK cell function was associated with cigarette smoking\(^{25-28}\), and NKCA declines with age\(^{40,41}\) and may be higher in men relative to women\(^{40,42}\). In addition, Nakata et al.\(^{43}\) reported that, in white-collar employees, the association between job satisfaction and NKCA might be stronger in women than in men. This result also suggests that NKCA might be influenced by the confounding factor of sex. Considering the above-mentioned limitations and the confounding factor of sex, this result suggests that the workload factor of number of seriously ill inpatients examined may be associated with a lower NKCA in Japanese EPs.

The work schedules of Japanese EPs have improved since 2005 under the guidance of the Labor Standards Inspection Office; currently, many Japanese EPs do not work for 24 consecutive hours because the number of tertiary critical care emergency centers with shift work has increased. However, this recent development is not relevant to the present findings.

A prior study of physicians (senior house officers) showed a weak association between lower job satisfaction and the number of patients examined per year (standard regression coefficient = -0.13, \(p<0.05\))\(^ {9}\). Patients with mental health problems are an additional source of job stress for physicians\(^ {10}\).

Based on our experience, we feel that there is a difference in workload intensity between examining inpatients and outpatients. However, evidence of such a difference had not been previously identified. The present results demonstrate that number of seriously ill inpatients examined is the only workload associated with changes in NKCA; thus, among Japanese EPs, the workload associated with examining seriously ill inpatients may be more demanding than that associated with examining seriously ill outpatients.

**Conclusion**

First, the present results suggest that residents may not exhibit a reduction in NKCA, because they examine fewer seriously ill inpatients relative to the number of those examined by other Japanese EPs. Second, changes in NKCA in Japanese EPs may be influenced not only by circadian rhythms but also by the number of seriously ill inpatients examined. Finally, these results support our hypothesis and suggest that the number of seriously ill inpatients examined, which we considered a potential indicator of Japanese EPs workload, may be related to changes in NKCA. The results also suggest that the workload intensity associated with examining seriously ill inpatients had a greater impact on NKCA than the confounding factor of sex did. Future studies that measure NKCA in samples with identical culture phases and control for the effects of circadian rhythms are necessary. With regard to future studies on workload and health management among physicians, not only the number of hours worked but also the number of patients examined should be considered as indicators of workload.

**Acknowledgments**

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We thank all of the emergency physicians at Kyorin University Hospital, Nihon University Itabashi Hospital, Nippon Medical School Hospital, Osaka General Medical Center, Saitama Medical Center, and Teikyo University Hospital who participated in this study despite their busy schedules.

The authors declare no competing interests.

**References**


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Number of Patients Examined May Affect Natural Killer Cell Activity in Japanese Emergency Physicians: A Preliminary Study

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目的: 本研究は、ナチュラルキラー細胞活性（NKCA）を通して、診察した患者数が医師の労働負荷を反映する有用な指標になるかどうかについて検討する目的で行った。その際、医師の労働負荷との関係から、診察患者数が救急医師のNKCAに影響を与えるかもしれない仮説を立てた。

方法: 日本の主要な救命救急センターに勤務していた救急医師34名（スタッフ医師が18名、研修医師が16名）を対象に、2005年夏季に調査を行った。被験医師の勤務中に、彼らの労働負荷（勤務時間、休息時間、診察患者数）について質問票調査を行い、NKCA測定のために勤務開始時（朝）と終了時（翌夕）に採血を行った。解析には統計ソフトSPSS version20.0を用いた。本研究は、杏林大学医学部倫理委員会の審査を受け、承認された（承認番号127）。

結果: 連続約32時間も病院内に拘束されて勤務していた当時の救急医師において、労働負荷については、スタッフ医師に比べ研修医師が診察した重症病棟患者数が有意に少なかった（p<0.01）。スタッフ医師では勤務後のNKCAが勤務開始時に比べ有意に低値であったが（p<0.01）、研修医師のNKCAでは勤務前後の有意差はなかった。NKCAの変動（勤務終了時のNKCA-勤務開始時のNKCA）を従属変数とした重回帰分析の結果、NKCAの変動に有意な関係を示した独立変数は医師が診察した重症病棟患者数だけであった（標準化偏回帰係数=-0.49, p<0.01）。

考察: 今回の所見に対してNKCAの日内変動の影響を十分に考慮できていない可能性はあるが、医師が診察した重症病棟患者数がNKCAの増減に影響を与えるかもしれないことを示唆した。従って、医師が診察した重症病棟患者数は、日本の救急医師の労働負荷の一指標として用いられるかもしれない。