$CD4^+$ T-lymphocytes are activated by surgical stress following colorectal resection in cancer patients

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Abstract. The aim of the present study was to measure adenosine triphosphate (ATP) levels in $CD4^+$ T cells as a marker of T-cell activity following surgery for colorectal cancer using the ImmuKnow assay kit. A total of 16 consecutive patients who underwent surgical resection for colorectal cancer between August and December, 2012 were enrolled in this study, of whom 7 underwent laparoscopic resection and 9 underwent open abdominal surgery. The intracellular ATP levels in $CD4^+$ T-lymphocytes were measured using the ImmuKnow assay kit preoperatively and on the 1st, 4th and 8th postoperative days, as were the white blood cell (WBC) count, lymphocyte count and C-reactive protein (CRP) levels. The ATP level of the $CD4^+$ T-cells was significantly elevated on the 1st day following surgery compared to the preoperative level ($P<0.01$) and gradually returned to preoperative levels; the lymphocyte count was significantly decreased on the 1st postoperative day ($P<0.001$). In addition, the ImmuKnow assay demonstrated that only the ATP level, but not the WBC count, lymphocyte count or CRP level, exhibited a significant difference on the 1st ($P=0.080$) and 8th ($P=0.042$) postoperative days between the laparoscopic and open abdominal surgery groups. In conclusion, the ATP level of $CD4^+$ T-lymphocytes was increased in response to surgical stress, in tandem with a decrease in the lymphocyte count. Therefore, the ImmuKnow assay kit may be clinically applicable for monitoring the immune response following surgery, as it exhibits a higher sensitivity compared to other assays.

Introduction

The immune cell function assay (ImmuKnow; Cylex, Inc., Columbia, MD, USA), which enables the measurement of the $CD4^+$ T-cell activity, was approved for use by the US Food and Drug Administration in 2002 (1). To date, a number of studies (2-5) have investigated the correlation between the immune response and rejection in transplant recipients since Hooper *et al* (5) established immune response zones using the ImmuKnow assay in 2005. Those studies demonstrated that low adenosine triphosphate (ATP) levels in $CD4^+$ T cells are associated with increased risk of infection. In addition, it was suggested that monitoring using the ImmuKnow assay may reflect the dynamics of the immune status (4).

In contrast to transplantation, surgical stress induces postoperative immunosuppression, which has been evaluated by measuring interleukin (IL), cortisol and C-reactive protein (CRP) levels and/or the $CD4^+$ T-cell count (6,7). By contrast, Wu *et al* (8) reported the reference of CRP, IL-6, IL-8 and HLA-DR levels, but not the CD4/CD8 ratio between laparoscopic and conventional surgery for colon cancer and concluded that the extent of surgical trauma may affect those results. However, these parameters have not been proven sufficient to evaluate the postoperative immune status. Therefore, it is necessary to identify a novel parameter that may be used to accurately evaluate the immune status in postoperative patients.

To the best of our knowledge, there are currently no available studies evaluating the extent of surgical stress by assessing the ATP levels of $CD4^+$ T cells. The aim of this study was to measure ATP levels in $CD4^+$ T cells as a marker of T-cell activity in the acute phase following colorectal surgery using the ImmuKnow assay. We investigated the dynamics of postoperative ATP levels and compared these levels between laparoscopic and conventional open abdominal surgery as an endpoint in this study.

Patients and methods

Patients. A total of 16 consecutive patients with colorectal cancer who underwent surgery at the Department of Gastroenterological Surgery at Fukuoka University Hospital between August and December, 2012 were included in the present study. Patients who had received immunosuppressive drugs or steroids and underwent organ transplantation were excluded. Our sample included 13 men (81.2%) and 3 women (18.8%), with a mean age of 62.4 years (range, 41-84 years). The diagnosis was based on radiological examinations and histological confirmation for staging information. The clinicopathological characteristics of

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Table I. Clinical characteristics of the patients.

Characteristics	Patient no. (n=16)
Age, years	
Mean (range)	62.4 (41-84)
Gender	
Male	13
Female	3
Cancer location	
Colon (colectomy)	12
Rectum (anterior resection)	4
Surgery	
Open abdominal	9
Laparoscopic	7
pStage	
I	7
II	5
IIIa	0
IIIb	2
IV	2

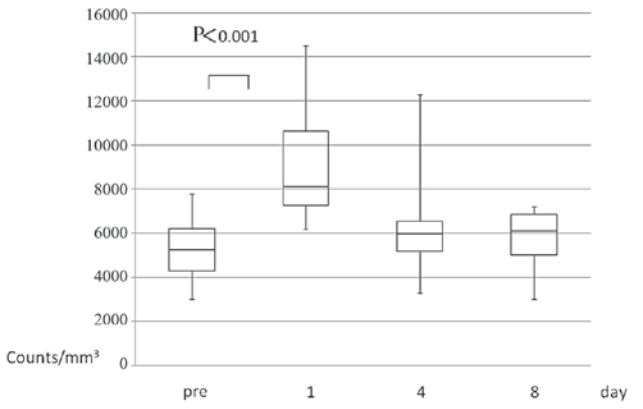

Figure 1. Change in white blood cell count (mean ± standard deviation) following surgery. On the 1st postoperative day, the level increased to a maximum, followed by a gradual decrease over time (P<0.001).

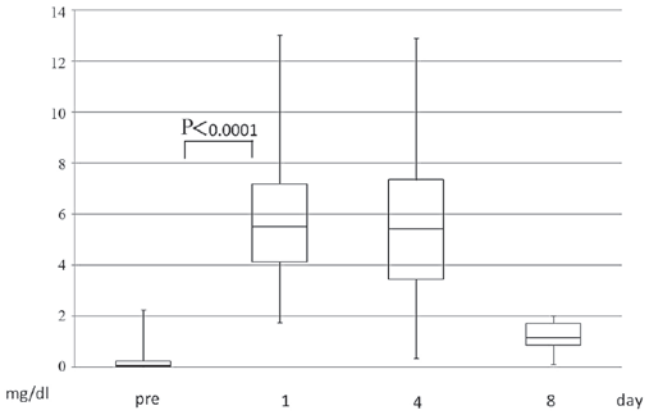

Figure 2. Change in C-reactive protein level (mean ± standard deviation) following surgery. On the 1st postoperative day, the level increased to a maximum, followed by a gradual decrease over time (P<0.0001).

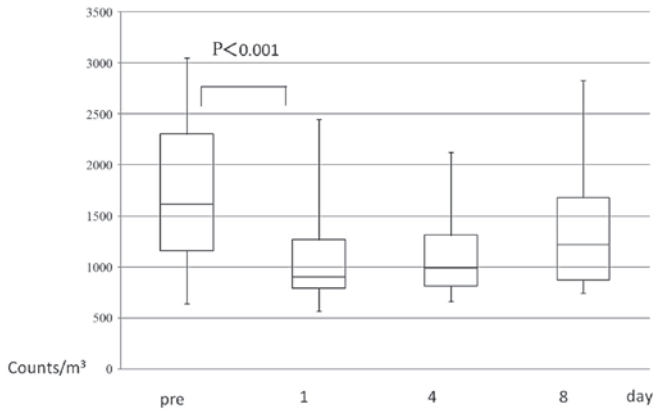

Figure 3. Change in lymphocyte count (mean ± standard deviation) following surgery. On the 1st postoperative day, the level decreased to a minimum, followed by a gradual increase over time (P<0.001).

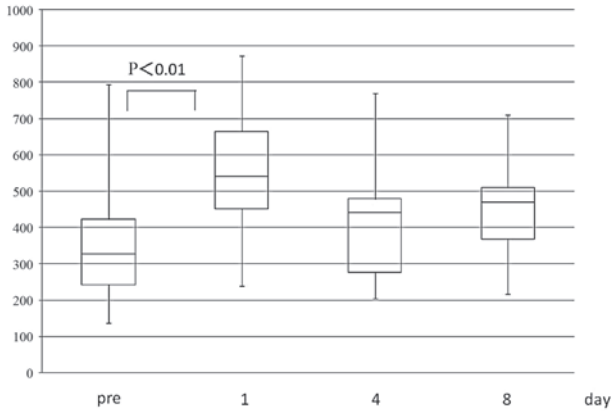

Figure 4. Change in ImmuKnow assay level (mean ± standard deviation) following surgery. On the 1st postoperative day, the level increased to a maximum, followed by a gradual decrease over time (P<0.01).

the patients are summarized in Table I. Pathological staging was performed based on the TNM classification of malignant tumors, 7th edition, published by the Union for International Cancer Control (9).

The present study was approved by the Medical Ethics Board of Fukuoka University, Faculty of Medicine (no. 12-6-15) and all the patients provided written informed consent prior to enrolment.

ImmuKnow assay. Whole-blood samples from the colon cancer patients were collected using sodium heparin anticoagulant tubes. The ImmuKnow assay was performed according to the manufacturer's protocol. In brief, the blood samples were diluted using sample diluents and incubated for 15-18 h with phytochemagglutinin-L-containing cell lysate at 37˚C, in a 5% CO_2 incubator. Following incubation, the CD4+ T cells were collected using magnetic particles coated with anti-human CD4 monoclonal antibodies. Following rinsing, the intracellular ATP released by cell lysis was measured using luciferin/luciferase and a luminometer (Tristar LB941; Berthold Technologies GmbH, Bad Wildbad, Germany). The ATP level was calculated in nanograms per milliliter. The ImmuKnow assay was performed in quadruplicates preoperatively and on postoperative days 1, 4 and 8.

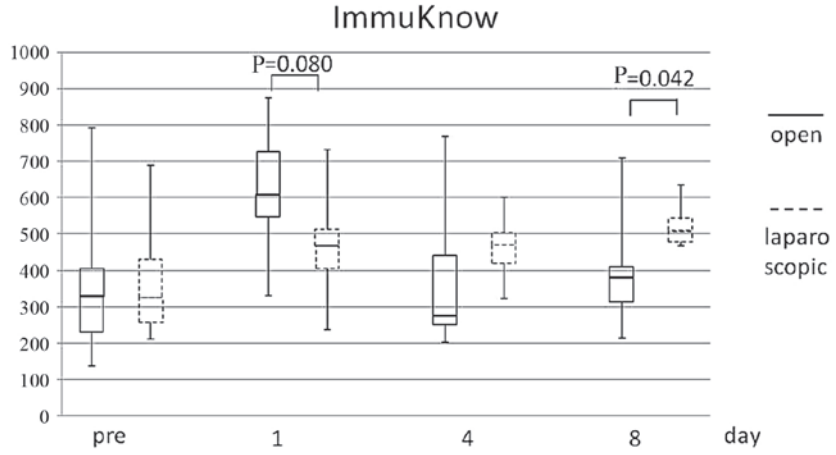

Figure 5. Comparison of ImmuKnow assay level (mean ± standard deviation) between open and laparoscopic surgery. On the 1st postoperative day, a smaller increase was observed in the laparoscopic surgery group (P=0.080). By contrast, the level in the open surgery group was lower compared to that in the laparoscopic group on the 8th postoperative day (P=0.042).

Statistical analysis. The correlations between the ImmuKnow assay data and the clinicopathological parameters were statistically analyzed. In addition, the blood laboratory data were examined on the same days as the ImmuKnow assay and these values were investigated for a correlation with the ImmuKnow data. The statistical analysis was performed by paired t-test using JMP Statistical Discovery software (SAS Institute Inc., Cary, NC, USA) and $P<0.05$ was considered to indicate a statistically significant difference.

Results

TNM staging. According to the TNM classification, 7th edition, 7 patients had stage I, 5 had stage II, 2 had stage IIIb and the remaining 2 had stage IV disease (Table I).

White blood cell (WBC) count. Open abdominal surgery and laparoscopic resection were performed in 9 and 7 patients, respectively (Table I). The WBC count was significantly increased on the 1st postoperative day [mean preoperative vs. 1st postoperative day level ± standard deviation (SD): 5,318.8±1,453.8 vs. 9,087.5±2,5878.6, respectively; $P<0.001$], followed by a gradual return to the baseline preoperative levels (Fig. 1).

CRP. The CRP level (mg/dl) was aberrantly high on the 1st postoperative day (mean preoperative vs. 1st postoperative day level ± SD: 0.27±0.55 vs. 5.91±2.95, respectively; $P<0.0001$) and the 4th postoperative day, but decreased to normal by the 8th day (Fig. 2).

Lymphocyte count. The lymphocyte count decreased significantly on the 1st postoperative day (mean preoperative vs. 1st postoperative day level ± SD: 1,740.7±758.7 vs. 1,095.1±515.8, respectively; $P<0.001$), but returned to normal on the 8th day (Fig. 3).

ImmuKnow value. The ImmuKnow value increased significantly on the postoperative 1st day (mean preoperative vs. 1st postoperative day level ± SD: 378.5±193.7 vs. 553.3±172.7, respectively;$P<0.01$), followed by a gradual return to the preoperative level by the 8th day (Fig. 4).

Comparison between surgical approaches. In a comparison between laparoscopic and open abdominal surgery, there were no significant differences in the WBC count, lymphocyte count or CRP value. However, the ImmuKnow assay demonstrated a tendency for the laparoscopic group to exhibit a lower response on the 1st postoperative day (open vs. laparoscopic surgery ± SD: 619.8±166.1 vs. 467.9±150.5, respectively; $P=0.080$) and a higher response on the 8th postoperative day (open vs. laparoscopic surgery ± SD: 394.0±141.0 vs. 522.1±60.6, respectively; $P=0.042$) (Fig. 5).

Discussion

Surgical stress induces postoperative immunosuppression. Ogawa *et al* (6) reported that the peripheral blood lymphocyte count and function were suppressed for ≥2 weeks postoperatively in 20 patients with stage I gastrointestinal cancer who underwent surgical resection. In particular, the CD4/CD8 T-cell ratio was decreased during the postoperative acute phase. Similarly, it was reported that the number of $CD4^+$ T cells was decreased on the 1st day post-cholecystectomy and returned to normal at 5-7 days postoperatively (10).

In contrast to those reports, in our study, the $CD4^+$ T-cell activity was significantly increased on the 1st postoperative day and was gradually reduced by day 4-7 postoperatively. This result may suggest that the $CD4^+$ T-cell activity was stimulated in order to compensate for the decrease in $CD4^+$ T cells due to the postoperative immunosuppression. Therefore, the ImmuKnow assay, which monitors the $CD4^+$ T-cell activity, may be clinically applicable for the monitoring of the postoperative immune status.

Furthermore, when the effects of laparoscopic and open abdominal surgery on the $CD4^+$ T-cell activity were compared, there was a tendency for the laparoscopic approach to exert a more limited effect compared to open surgery, although the difference did not reach statistical significance. Wu *et al* (8) reported that serum IL-6 and IL-8 levels were higher in the

early phase following surgery in the open surgery compared to the laparoscopic surgery group in patients with colorectal cancer. The results of that study were consistent with ours, which demonstrated that ImmuKnow value correlated with acute phase response following colorectal surgery, similar to proinflammatory cytokines. Brune *et al* (11) also evaluated the immune defense following laparoscopic and open cholecystectomy and reported a more prominent immune suppression in the open surgery group. In addition, Bessler *et al* (12) reported that the immune activity associated with T-cell function was maintained closer to normal following laparoscopic colorectal surgery compared to open surgery. Thus, ImmuKnow value, as well as cytokine levels, suggest that laparoscopic colorectal surgery was less traumatic compared to open surgery, from the view point of acute phase response.

In addition, the $CD4^+$ T-cell activity revealed a different type of reaction in patients subjected to surgical stress compared to those receiving immunosuppressive drugs. The ImmuKnow activity was found to be low in patients receiving immunosuppressive drugs following transplantation and its level may be used for risk monitoring of the patients to prevent transplant rejection and/or infection. Surgical stress is a well-known immunosuppressive factor following surgery, although the ImmuKnow activity was actually elevated on the 1st postoperative day, when the immunosuppression was considered to be at its peak. The ImmuKnow activity, therefore, may reflect the different mechanisms underlying the suppression of the immune system by surgical stress and pharmacotherapy (2-5). To elucidate the reasons for these differences, a subpopulation analysis of $CD4^+$ T cells may be undertaken in the future. A limitation of the present study was that ImmuKnow is a qualitative assay, but does not directly reflect the level of immunosuppression, as described in the manufacturer's manual.

In conclusion, the immune activity during the acute phase following colorectal surgery may be monitored using the ImmuKnow assay. In addition, the ImmuKnow value may demonstrate the significant difference of the acute phase response between laparoscopic and open colorectal surgery. Futher clinical applications using ImmuKnow assay are expected in the future.

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