Usefulness of an IL-6 Semi-Quantification Kit in Patients with Perforative Peritonitis

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Objective: As gastrointestinal tract perforation frequently causes severe sepsis, it is associated with a poor prognosis. We investigated the usefulness of measuring blood interleukin-6 (IL-6) levels using a simple semi-quantitative analysis kit to predict the severity of perforative peritonitis.

Materials and Methods: Nineteen patients with generalized peritonitis due to gastrointestinal tract perforation who had undergone surgery in our department were eligible for this study. Preoperative blood IL-6 levels were measured using a semi-quantitative analysis kit and classified into two groups according to the color code: a dark-colored group (IL-6 ≥ 5,000 pg/ml) and a light-colored group (IL-6 < 5,000 pg/ml). The two groups were then compared in terms of preoperative inflammatory markers, severity scores (Mannheim Peritonitis Index (MPI), Acute Physiology and Chronic Health Evaluation (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score), postoperative factors, and outcome (survive/death).

Results: The median age of the patients was 70 years. The median interval between the onset of symptoms and surgery was 13 hours. Seventeen patients survived, and 2 patients died. Compared with the light-colored group, the preoperative white blood cell count was significantly lower; however, the MPI, postoperative SOFA score, and number of patients requiring vasopressors and hemoperfusion using polymyxin B-immobilized fiber column after surgery were significantly higher in the dark-colored group. Perforative peritonitis in the dark-colored group was more often associated with a fatal outcome (p = 0.04).

Conclusion: The results suggest that the semi-quantification of blood IL-6 levels is useful for predicting the outcome of patients with generalized peritonitis due to gastrointestinal tract perforation.

Key words: perforative peritonitis, interleukin-6 (IL-6), semi-quantitative analysis kit, systemic inflammatory response syndrome (SIRS), sepsis

Introduction

Since gastrointestinal tract perforation frequently causes disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) due to severe sepsis, its prognosis is poor and associated with a high mortality rate [1]. To date, the Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment (SOFA) score have been used to classify the severity of generalized peritonitis due to gastrointestinal tract perforation [2, 3]. However, their use is time-consuming because of the large number of examination items. Additionally, they mainly indicate organ damage and vital signs, not reflecting the pathological condition at the microscopic level. The inflammatory indicators include the white blood cell (WBC) count and C-reactive

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protein (CRP); however, they may not accurately reflect the severity of inflammation because the WBC count is low in patients with advanced sepsis and CRP levels may not be elevated early after the onset of sepsis.

Recently, an attempt was made to monitor the severity of inflammation in a more sensitive manner and in real-time by measuring the levels of cytokines, such as interleukin 6 (IL-6). However, it takes a long time to determine the results of such measurements; therefore, it is cumbersome for clinicians dealing with emergency cases to use cytokine measurements in clinical practice.

In this study, we investigated whether a simple semi-quantitative analysis kit that measures blood IL-6 levels (IL-6 STICKELISA, TORAY, Tokyo, Japan) is useful for predicting the severity of generalized peritonitis due to gastrointestinal tract perforation.

Materials and Methods

Between October 2013 and March 2016, a total of 19 patients with generalized peritonitis due to gastrointestinal tract perforation who had undergone surgery in our department were eligible for this study. This study was approved by the Institutional Review Board of Juntendo University Hospital (No.12-106) and registered under the University Hospital Medical Information Network (UMIN) 000009793 (https://www.umin.ac.jp/icdr/index.html). The IL-6 levels in blood samples were measured preoperatively using the IL-6 STICKELISA kit.

First, whole blood samples of 10-ml were collected and rapidly centrifuged at 4°C at 3,000 rpm for 10 min, and the plasma component was stored at -20°C. The plasma was thawed and 200-μl aliquots were used for the IL-6 assay. The semi-quantitative IL-6 STICKELISA kit can estimate the blood IL-6 levels by the intensity of the blue color that develops when the plasma is mixed with the reagent. In addition, it is possible to semi-quantify the IL-6 levels in the blood in about 45 min by comparing the blue color against the color code associated with concentration values (Figure-1). In this study, to test the accuracy of the semi-quantification of IL-6 levels using this kit, we used a Bio-Plex Suspension Array System to quantitatively measure the IL-6 levels in the blood samples taken from 19 patients before surgery, immediately after surgery, and on the postoperative days 1 and 7. These results were compared with the IL-6 levels determined by the semi-quantitative IL-6 STICKELISA kit. In this comparison, five surgeons blinded to the clinical information made decisions independently.

The blood IL-6 levels obtained using a semi-quantitative IL-6 STICKELISA kit were classified into dark- and light-colored groups. Additionally, the groups were compared in terms of their association with perioperative clinicopathological factors and postoperative results.

The examination items included preoperative factors (age, sex, cause of perforation, and site of perforation), preoperative inflammatory markers (WBC, CRP, and procalcitonin [PCT]), severity scores (Mannheim Peritonitis Index (MPI), APACHE II score, SOFA score), and postoperative factors (vasopressor, polymyxin B-immobilized fiber therapy (PMX), continuous hemodialfiltration (CHDF), presence or absence of mechanical ventilation, systemic inflammatory response syndrome...
(SIRS), DIC, presence or absence of postoperative complications, duration of ICU stay, duration of hospital stay, and death within 28 days after surgery).

Fisher’s exact probability test was employed to compare discrete variables. Continuous variables were compared using the Mann–Whitney U-test for individual comparisons and the Wilcoxon signed rank test for paired comparisons. The data were analyzed statistically using JMP 10 software (SAS Institute Inc., Cary, NC, USA). Differences were considered statistically significant at $p < 0.05$. Values are expressed as median (range).

Results

Table-1 shows the characteristics of the 19 patients. The median age was 70 years (range, 42–86 years). Nine and 10 patients were male and female, respectively. The primary diseases were diverticular perforation in 8 patients, cancer in 3 patients, and other diseases in 8 patients. A total of 15 patients had large bowel perforations, while 4 patients experienced small bowel perforations. The median time between the onset of symptoms and surgery was 13 hours (range, 4–120 hours). A total of 17 patients (89.5%) survived (survival group) and 2 patients (10.5%) died (death group).

Subsequently, we used a Bio-Plex Suspension Array System to quantitatively measure the preoperative IL-6 levels in the blood samples from the 19 patients and found that the IL-6 levels were significantly higher in the death group (median, 39,050 pg/ml) than in the survival group (median, 595 pg/ml; $p = 0.04$) (Figure-2).

Four concentration ranges of IL-6 were detected: $<1,000$ pg/ml, $\geq 1,000$ pg/ml or $<2,500$ pg/ml, $\geq 2,500$ pg/ml or $<5,000$ pg/ml, and $\geq 5,000$ pg/ml. Measurements with the IL-6 STICKELISA kit were in good agreement with the Bio-Plex Suspension Array System, with an agreement percentage of 90.7% (331/365 samples) (Table-2). In particular, the measurements in the range of $\geq 5,000$ pg/ml showed an accuracy of 100% when both tests were compared. When the blood IL-6 levels were classified into the dark-colored group (IL-6 $\geq 5,000$ pg/ml) and light-colored group (IL-6 $<5,000$ pg/ml), and compared in terms of perioperative results, a significant correlation was observed.

| Table-1 | Patients' characteristics |
|---------|--------------------------|
| Age\(^{a}\) (years) | 70 (42–86) |
| Sex | |
| Male | 9 (47.4) |
| Female | 10 (52.6) |
| Primary disease | |
| Diverticular disease | 8 (42.1) |
| Cancer | 3 (15.8) |
| Others | 8 (42.1) |
| NOMI\(^{b}\) | 1 (5.3) |
| Iatrogenic | 1 (5.3) |
| Anastomotic leakage | 1 (5.3) |
| Unknown | 5 (26.3) |
| Perforation site | |
| Small intestine | 4 (21.1) |
| Right-sided colon (cecum – transverse colon) | 5 (26.3) |
| Left-sided colon (descending colon – rectum) | 10 (52.6) |
| Preoperative duration of peritonitis\(^{a}\) (hours) | 13 (4–120) |
| Outcome within 28 days after surgery | |
| Survival | 17 (89.5) |
| Death | 2 (10.5) |

\(^{a}\) Median (min–max), \(^{b}\) Non-occlusive mesenteric ischemia
between the measurements at a cutoff value of 5,000 pg/ml and perioperative mortality (p=0.04) (Table-3). Therefore, in this study, a cutoff value of 5,000 pg/ml was used to define the dark-colored group (≥5,000 pg/ml) and light-colored group (<5,000 pg/ml).

We evaluated the association between clinicopathological factors and severity scores in the dark- and light-colored groups as determined by the IL-6 STICKELISA kit (Table-4). We found that the preoperative WBC count was significantly lower (p=0.02), the MPI and postoperative SOFA scores were significantly higher (p=0.04 and 0.03, respectively), and the number of patients requiring vasopressors and PMX after surgery was significantly higher (p=0.009 and 0.03, respectively) in the dark-colored group than in the light-colored group.

**Discussion**

Systemic inflammatory response syndrome (SIRS), caused by infection-induced sepsis, severe burns, or traumatic injuries, is a systemic inflammatory condition associated with a high mortality rate. Perforative peritonitis readily leads to sepsis, which causes SIRS, a systemic inflammatory condition associated with high mortality rates. Therefore, the biomarkers that can predict the severity of perforative peritonitis early after its onset and can be detected with a sensitive and simple method are important in determining the therapeutic approach. IL-6, a cytokine that controls the humoral immunity, in addition to IL-1 and TNF-α, is known as an inflammatory cytokine. In 1986, Hirano et al. cloned complementary DNA (cDNA), and subsequently clarified that IL-6 is closely involved in the pathogenesis of inflammation and immunologic diseases. On the other hand, IL-6 is considered to reflect the degree of hypercytokinemia, which is the underlying mechanism of SIRS.

However, IL-6 levels cannot be measured in all medical facilities; therefore, at present, it is necessary to request commercial clinical laboratories to measure the IL-6 levels in patients’ blood. An IL-6 assay kit that allows the quick and simple measurement of IL-6 levels in any institution is needed.

Thus, we evaluated the usefulness of the semi-quantitative IL-6 STICKELISA kit in predicting the severity of perforative peritonitis in patients who have undergone surgery.

Two patients (10.5%) died during the period of this study. This mortality rate was comparable to that previously reported. In the present study, inflammatory markers including the WBC count, CRP, and PCT were preoperatively measured. Preoperative PCT levels have been reported to

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**Table-2** Comparisons between reference values in IL-6 STICKELISA and perioperative mortality (Semi-Qualitative kit (IL-6 STICKELISA))

| Quantitative assay kit (Bio-plex) | <1,000 | ≥1,000 | ≥2,500 | ≥5,000 |
|----------------------------------|--------|--------|--------|--------|
| Survival group (n=17)            | 248    | 18     | 2      | 0      |
| Death group (n=2)                | 0      | 23     | 13     | 0      |
| p-value                          | 0.47   | 0.27   | 0.04   |

**Table-3** Comparisons between reference values in IL-6 STICKELISA and perioperative mortality

| Reference values in IL-6 STICKELISA (pg/ml) | Survival group (n=17) | Death group (n=2) | p-value |
|--------------------------------------------|-----------------------|-------------------|---------|
| <1,000                                     | 9                     | 0                 | 0.47    |
| ≥1,000                                     | 8                     | 2                 |         |
| <2,500                                     | 12                    | 0                 | 0.27    |
| ≥2,500                                     | 5                     | 2                 |         |
| <5,000                                     | 15                    | 0                 | 0.04    |
| ≥5,000                                     | 2                     | 2                 |         |
reflect the severity of sepsis, including secondary to perforative peritonitis). In this study, the 2 patients in the death group showed an increase in the preoperative IL-6 levels and PCT levels (survival group: 2.5 ng/dl vs. death group: 147.6 ng/dl, p=0.03, data not shown). The IL-6 level reaches a peak at about 6 hours after an insult, while PCT is induced by IL-6 and increases about 24–48 hours later. Therefore, IL-6 may be useful for the early detection of sepsis; however, its usefulness could not be estimated in this study because of the small number of patients in this study and awaits further studies.

Till date, several studies have evaluated the usefulness of MPI to stratify the prognosis of patients with sepsis due to intra-abdominal
infections\textsuperscript{15-17}.Billing \textit{et al.}\textsuperscript{18} reported that the mortality predicted by MPI was 2.3\% (MPI <21), 22.5\% (21 \leq MPI \leq 29), and 59.1\% (MPI >29), indicating the usefulness of a severity classification using MPI. In the present study, MPI was significantly higher in the dark-colored group, suggesting an association between mortality and MPI.

Additionally, the SOFA score was reported to be useful for assessing the severity of sepsis\textsuperscript{3}. In the present study, the SOFA score was significantly higher in the dark-colored group, suggesting that it is a useful severity score when using clinical factors.

Contrarily, the preoperative WBC count was significantly lower in the dark-colored group than in the light-colored group. As described in the diagnostic criteria for SIRS, the WBC count decreases to less than 4,000/μl in severe infections\textsuperscript{6}. Therefore, the above results suggest the possible presence of severe peritonitis in the dark-colored group. Damas \textit{et al.}\textsuperscript{19} reported that the tumor necrosis factor (TNF) level, which is a mediator of inflammatory response, was negatively correlated with the WBC count. This suggests a similar mechanism of action for IL-6.

In addition, regarding the postoperative factors, the number of patients requiring vasopressors and PMX was significantly higher in the dark-colored group, probably because it included patients with severe disease. Recently, Terayama \textit{et al.}\textsuperscript{20} conducted a meta-analysis of the efficacy of PMX for sepsis and reported the possibility of PMX being effective for high-mortality-risk patients. However, the indications for PMX remain controversial. Further studies are necessary to determine whether the evaluation of the severity of perforative peritonitis using the semi-quantitative IL-6 STICKELISA kit can further define the indications for PMX.

In the present study, the mortality rate was significantly higher in the dark-colored group. Moreover, the measurements using the semi-quantitative IL-6 STICKELISA kit and Bio-Plex Suspension Array Basic System were in close agreement. Therefore, the results suggest that the semi-quantitative IL-6 STICKELISA kit accurately reflects the IL-6 levels and is useful for predicting the outcome of patients with generalized peritonitis due to gastrointestinal tract perforations.

The present study has a few limitations. First, the number of patients studied was small. Because the number of patients with gastrointestinal perforation encountered at a single institution is limited, a multi-institutional study is needed to confirm the efficacy of the IL-6 kit used. Second, this study was performed retrospectively using a semi-quantitative kit with a cutoff of 5,000 μg/ml. Therefore, to confirm the usefulness of a cutoff of 5,000 μg/ml for the kit, a prospective study involving a large number of patients is needed. In addition to the one-point preoperative measurement, the subsequent follow-up may provide more important information.

Conclusions

This study found that the results of semi-quantification of blood IL-6 levels were in close agreement with those of quantification using the Bio-Plex Suspension Array Basic System and suggests the usefulness of the kit for predicting the outcome of patients with generalized peritonitis due to gastrointestinal tract perforation.

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Conflicting interest statement

The authors declare that there is no conflict of interest.

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