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Pneumonia after esophageal surgery for squamous cell carcinoma: impact of HLA-DR antigen expression on monocytes

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Abstract Although surgical techniques and perioperative management of esophageal surgery have improved remarkably, morbidity and mortality remain high in comparison with those associated with other procedures. Mortality after esophageal surgery is primarily determined by the development of pulmonary complications. This study included 41 patients who underwent esophageal resection and reconstruction for thoracic esophageal cancer. We evaluated changes in the levels of interleukin (IL)-6 and IL-10, the type 1/type 2 T-helper (Th1/Th2) cell balance, and the expression of HLA-DR antigen on the surface of monocytes after surgery.

The pneumonia group tended to have undergone surgeries of a longer duration and had higher serum IL-6 levels on postoperative day 1 than the no pneumonia group. However, serum IL-10 levels or the Th1/Th2 cell balance did not differ significantly between the 2 groups. HLA-DR antigen expression on monocytes was significantly lower in the pneumonia group than in the no pneumonia group on postoperative day 3. In conclusion, estimation of operative time, serum IL-6 levels, and HLA-DR expression on monocytes may provide a method for predicting postoperative pneumonia.

Key words: Pneumonia, esophageal cancer, surgery, HLA-DR antigen expression on monocytes

Introduction

Esophageal cancer is associated with an unfavorable prognosis. Surgery remains the standard therapy for esophageal cancer because it provides the best chance of a cure.¹ However, esophagus resection and reconstruction is one of the most invasive treatments for cancers of the digestive organs. Although the surgical techniques and perioperative management for esophageal surgery have improved remarkably in recent years, the morbidity and mortality rates remain high in comparison with those associated with other

procedures.²⁻⁵

Postoperative alterations in host immune functions after major surgical interventions have been extensively investigated and described.⁶⁻⁸ The body's response to surgical stress is evoked mainly by afferent nerve signals from the operative site and the release of cytokines from damaged tissue.⁹ Interleukin (IL)-6 and IL-10 are amongst the earliest cytokines observed. Recent studies have shown that a successful immune response is largely dependent on the activation of 2 subsets of committed T-helper cells, type 1 T-helper (Th1) and type 2 T-helper (Th2) cells. Th1 and

Th2 cells secrete different patterns of cytokines. In an effort to assess surgical stress by means of characterizing the immune response, we focused on changes in cell-mediated and antibody-mediated immunity as illustrated by the Th1/Th2 cell balance.¹⁰ In addition, downregulation of HLA-DR antigen expression on the surface of monocytes has been shown to correlate with a deficient immune response after surgery.⁶

Previous analyses have been conducted to determine the preoperative risk factors for complications¹¹; however, clinically practical methods for assessing operative risks are yet to be established. The present study aimed to prospectively evaluate immune response markers in perioperative peripheral blood to predict the postoperative occurrence of pneumonia. Our data indicates the relationship between the levels of IL-6 and IL-10, the Th1/Th2 cell balance, and HLA-DR expression on monocytes and the occurrence of pneumonia after esophagectomy.

Patients and Methods

Patients

This study included 41 participants between January 2000 and January 2010: 37 men and 4 women (median age: 67 years; age range: 51-78 years). These patients underwent esophageal resection and reconstruction for thoracic esophageal cancer at the Department of Surgery and Clinical Science, Yamaguchi University. All patients had a World Health Organization performance status of 0. Squamous cell carcinoma was diagnosed histologically in all patients. Patients were divided into 2 groups depending on the incidence of postoperative pneumonia: no pneumonia ($n = 32$) or pneumonia ($n = 9$). Pneumonia was defined as an abnormal shadow on a chest radiograph and the clinical manifestation of fever and purulent sputum.

Treatment procedure

Thoracic esophageal cancer was treated by subtotal esophagectomy with lateral right thoracotomy and cervical anastomosis. Reconstruction was accomplished using a gastric tube in all patients.

Peripheral blood analysis

Peripheral venous blood samples were obtained before surgery and on postoperative days 1, 3, and 14 (a total of 4 sampling time points). Blood samples were then assayed for IL-6 and IL-10 levels, the Th1/Th2 cell balance, and HLA-DR antigen expression on monocytes.

The concentration of serum IL-6 was measured using a chemiluminescent enzyme immunoassay using the Lumipulse forte system (Fujirebio Inc., Tokyo, Japan). The concentration of serum IL-10 was measured using an enzyme-linked immunosorbent assay (Bio Source Europe S.A., Nivelles, Belgium).

The Th1/Th2 cell balance was assayed by concomitantly staining cells with fluorochrome-conjugated monoclonal antibodies (mAb) directed against the cytokines interferon (IFN)- γ and IL-4. Cells were incubated with previously optimized concentrations of fluorescein isothiocyanate (FITC)-conjugated anti-IFN- γ mAb and phycoerythrin (PE)-conjugated anti-IL-4 mAb (BD Biosciences, Franklin Lakes, USA) at 4 °C for 30 minutes in the dark. Fluorescence was measured immediately after incubation on a FACSCanto II flow cytometer (BD Biosciences), allowing for the calculation of the percentage of cytokine-producing CD4+ T cells.

HLA-DR antigen expression among CD14+ monocytes was quantified by concomitantly staining monocytes with PE-conjugated anti-CD14 mAb and FITC-conjugated anti-HLA-DR mAb (BD Bioscience) at 4 °C for 20 minutes in the dark. Thereafter, cells were washed, pelleted, and suspended in staining buffer. Fluorescence was quantified using flow cytometry, and the percentage of HLA-DR-expressing CD14+ monocytes was calculated.

Statistical analysis

Statistical significance was determined using the unpaired Student's *t*-test, chi-squared test, or the Mann-Whitney *U*-test. All statistical analyses were performed using StatView-J 5.0 (Abacus Concepts Inc., Berkeley, USA). All two-sided *P* values < 0.05 were considered statistically significant.

Results

Patient characteristics according to the occurrence of postoperative pneumonia

Postoperative pneumonia occurred in 9 patients (21.9%, pneumonia group). The no pneumonia group included the remaining 32 patients (78.1%). The 2 groups did not differ with regard to mean age, sex, body mass index, hemoglobin level, albumin level, presence of diabetes mellitus, smoking status, or pulmonary function (Table 1). Perioperative factors in each group are presented in Table 2. The pneumonia group tended to have undergone surgeries of a longer duration than the no pneumonia group (439 ± 81 min versus 371

± 103 min; $P = 0.07$). However, the other perioperative factors did not differ between the 2 groups (Table 2).

Relationship between the development of postoperative pneumonia and cytokine levels

We measured and compared the serum IL-6 and IL-10 levels in 13 patients. Cytokine levels were compared between the pneumonia and no pneumonia groups. Patients in the pneumonia group tended to have higher levels of serum IL-6 than the no pneumonia group on postoperative day 1 (329.2 pg/mL versus 178.8 pg/mL; $p=0.158$). However, serum IL-10 levels did not differ between the 2 groups (Figure 1 and Figure 2).

Table 1: Patient characteristics according to the development of pneumonia

Group	No-pneumonia (n=32)	Pneumonia (n=9)	P value
Age (yr)	66.8 ± 6.6	68.2 ± 7.9	0.59
Sex (M/F)	28/4	9/0	0.26
BMI (kg/m ²)	20.6 ± 3.0	21.3 ± 2.0	0.51
Hemoglobin (g/dL)	13.2 ± 1.8	13.6 ± 2.1	0.63
Albumin (g/dL)	3.9 ± 0.44	3.8 ± 0.47	0.59
Diabetes mellitus (n)	2	1	0.62
Smoking (n)	25	8	0.47
VC (% predicted)	97.6 ± 20.2	102.8 ± 27.4	0.53
FEV1 (% predicted)	66.8 ± 6.6	68.2 ± 7.9	0.51

Table 2: Perioperative factors for esophageal cancer

Group	No-pneumonia (n=32)	Pneumonia (n=9)	P value
Intraoperative			
Operative time (min)	371 ± 103	439 ± 81	0.07
Bleeding (g)	580 ± 369	892 ± 711	0.80
Preoperative			
Oral care (n)	13	4	0.63
Administration of steroid (n)	17	4	0.59
Postoperative			
Enteral nutrition (n)	14	4	0.97

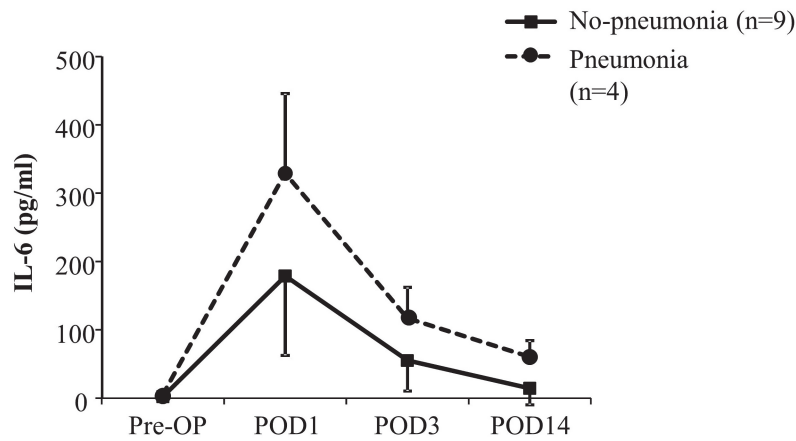


Figure 1: IL-6 levels after esophagectomy

Patients in the pneumonia group tended to have higher levels of serum IL-6 than the no pneumonia group on postoperative day 1. Error bars indicate \pm S.D..

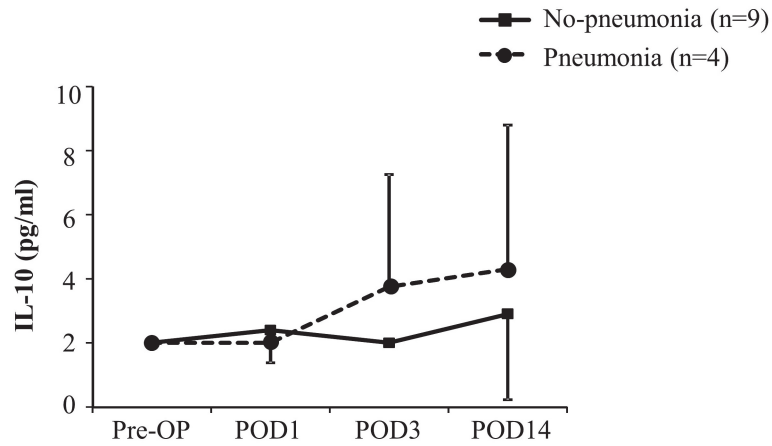


Figure 2: IL-10 levels after esophagectomy

Serum IL-10 levels did not differ between the 2 groups at all points in time. Error bars indicate \pm S.D.

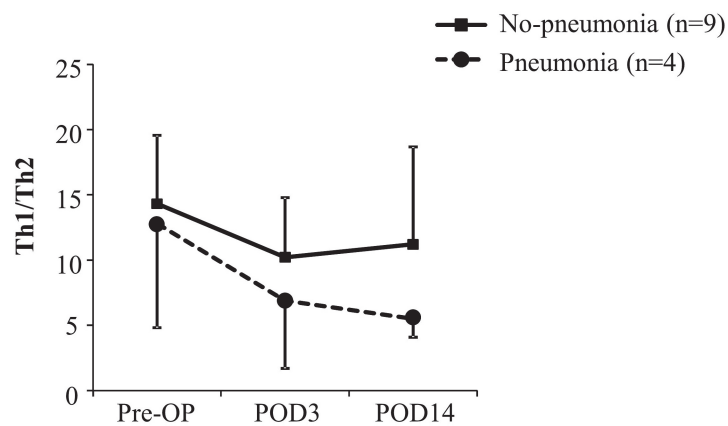


Figure 3: Th1/Th2 cell balance after esophagectomy

Th1/Th2 cell balance did not differ between the 2 groups at all points in time. Error bars indicate \pm S.D.

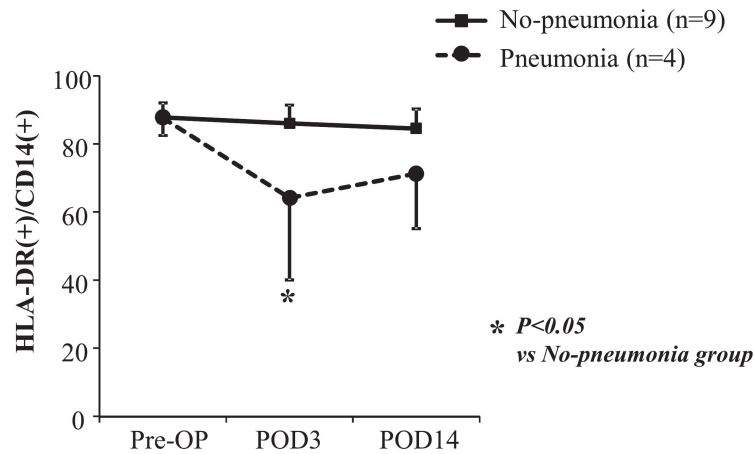


Figure 4: HLA-DR antigen expression on monocytes after esophagectomy

HLA-DR antigen expression on monocytes was significantly lower in the pneumonia group than in the no pneumonia group on postoperative day 3. Error bars indicate \pm S.D.

Relationship between immune response biomarkers and the incidence of postoperative pneumonia

Immunological parameters, including the Th1/Th2 cell balance and HLA-DR expression on monocytes was measured after surgery. The Th1/Th2 cell balance did not differ between the 2 groups (Figure 3). We investigated whether there was a relationship between HLA-DR antigen expression on monocytes and the incidence of postoperative pneumonia. HLA-DR antigen expression on monocytes was significantly lower in the pneumonia group than in the no pneumonia group on postoperative day 3 (64.1% versus 86.0%, $P < 0.5$) (Figure 4).

Discussion:

Mortality after esophageal surgery ranges from 2% to 25%² and is primarily determined by the development of pulmonary complications.^{3-5,16-18} Various risk analyses have also revealed that impaired heart, liver, and respiratory function in addition to the patient's age and general condition are independent predictors of morbidity and mortality.¹²⁻¹⁵ Therefore, the prevention of pneumonia is of utmost importance in decreasing the incidence of postoperative death. However, no perioperative biomarkers have been established to predict the incidence of postoperative

pneumonia. We evaluated the levels of IL-6 and IL-10, the Th1/Th2 cell balance, and the expression of HLA-DR on monocytes before and after surgery. For esophageal cancer patients, these biomarkers are considered representatives of the perioperative immune condition.

Surgical stress associated with esophagectomy is greater than that with any other general surgery as it often involves the abdomen, chest, and neck. Such an operative trauma activates peripheral blood neutrophils, monocytes, lymphocytes, and endothelial cells. This results in the release of pro-inflammatory and anti-inflammatory cytokines such as IL-6 and IL-10. We sought to measure and use serum IL-6 and IL-10 levels as a predictive biomarker for postoperative pneumonia following esophagectomy. In this study, we showed that serum IL-6 levels were higher in patients with pneumonia than those in patients without pneumonia on postoperative day 1. Therefore, early systemic release of IL-6 may affect the increased systemic inflammatory response 1 day after surgery. The duration of surgery also tended to be longer in the pneumonia group (Table 2). Therefore, elevated serum IL-6 levels and long operative times may be risk factors for postoperative pneumonia.

Patients with esophageal cancer have a diminished immune response characterized by a

reduced number of Th1 cells and depressed mitogenic response.¹⁹ Abnormalities in the Th1/Th2 cell balance are thought to be involved in the compromise of immune function after esophagectomy. A previous study showed that surgical stress induces a shift in the Th1/Th2 cell balance toward Th2 cells, suggesting that cell-mediated immunity is down-regulated and antibody-mediated immunity is upregulated after surgery¹¹. In addition, surgical stress is known to suppress Th1 cell function and enhance Th2 cell function, which also shifts the balance towards a Th2-dominant phenotype⁶. However, our results showed no significant differences in the Th1/Th2 cell balance.

Interestingly, HLA-DR antigen expression on monocytes was significantly lower in the pneumonia group than in the no pneumonia group on postoperative day 3. HLA-DR antigen expression on monocytes is thought to be required for major histocompatibility complex-restricted antigen presentation, a key step in the development of a specific immune response.^{20,21} It has been reported that the ability of monocytes to express HLA-DR correlates directly with the development of infection and death in severe trauma patients.^{22,23} In the pneumonia group, postoperative pneumonia occurred 5.3 ± 2.4 days after surgery and the duration of artificial ventilation was 12.6 ± 7.1 days (data not shown). In contrast, the duration of artificial ventilation was almost 1 day in the no pneumonia group. Therefore, low HLA-DR expression on monocytes in the early phase may be accompanied by immunosuppression, which exacerbates postoperative pneumonia and prolongs the duration of artificial ventilation. And we can analyze the HLA-DR expression using FACS analysis 3 days after surgery. So we can start to take measures before postoperative pneumonia occur.

In conclusion, the quantification of HLA-DR expression on monocytes in the early phase may be a valid biomarker to predict postoperative pneumonia in esophageal cancer patients after esophageal surgery. Thus, we pursue the minimally invasive surgery for esophageal cancer to reduce postoperative pneumonia.

Conflict of Interest

The authors declare no conflict of interest.

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