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Increased Serum Vascular Endothelial Growth Factor Following Major Surgical Injury

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Abstract

Objectives: Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis. We evaluated the changes in serum levels of VEGF following major surgical trauma and postoperative inflammatory complications.

Materials and Methods: The serum concentration of VEGF was measured with enzyme-linked immunosorbent assay in 41 patients with esophageal cancer who underwent right transthoracic esophagectomy with extensive lymphadenectomy and in 13 patients with gallstones who underwent less-invasive laparoscopic cholecystectomy for comparison. Serum and plasma samples were obtained before the operation and on postoperative days (PODs) 1, 7, 14, 21, and 28. The changes in serum VEGF levels were compared among groups categorized by age, sex, blood loss volume during operation, amount of transfusion, pathological stage of the tumor, and postoperative inflammatory complications. The correlation between serum VEGF levels and inflammatory factors, such as peripheral blood cell count, interleukin-6 (IL-6), C-reactive protein (CRP), and severity of postoperative inflammatory complications, was also investigated. Furthermore, because platelets are a potential source of serum VEGF, platelet-poor plasma (PPP) was prepared from plasma samples, and the VEGF concentration in PPP was measured to compare with those in sera.

Results: Serum VEGF levels increased significantly postoperatively. After reaching maximal levels on POD 14, VEGF levels gradually decreased until POD 28. The increase in the tranthoracic esophagectomy group was approximately twice that in laparoscopic cholecystectomy group on POD 14. Serum VEGF levels were not correlated with sex, age, blood loss, amount of transfusion, or tumor stage. However, serum VEGF levels were significantly higher in patients with postoperative inflammatory lung complications than in patients without such complications, and the maximal level of serum VEGF correlated with the severity of postoperative lung complications. However, there were no significant correlations between the increase in the level of serum VEGF and that of serum IL-6 or CRP. The increase of platelet counts in the peripheral blood correlated with that of the serum VEGF level, and VEGF levels in PPP were significantly lower than those in sera.

Conclusions: Serum VEGF levels increased in the angiogenesis phase of wound healing following major surgical injury. Platelets are a potential source of increased serum VEGF levels, whereas inflammatory lung complications might also be related to increased serum VEGF levels.

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Key words: vascular endothelial growth factor, major surgery, platelet, wound healing, angiogenesis

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Introduction

Cytokines and other growth factors are involved in the maintenance of homeostasis during wound healing following surgical trauma[1,2]. Angiogenesis is an important mechanism in various physiological and pathological events, such as major trauma and tumor growth. Vascular endothelial growth factor (VEGF) is a relevant factor in these processes[3,4]. However, the serum level of VEGF has not been thoroughly investigated.

The surgical treatment of esophageal cancer induces marked inflammatory responses because the surgical methods include thoracotomy and laparotomy for esophageal resection and for constructing a esophageal substitute with the stomach. Furthermore, extensive lymph-node dissection is commonly performed. Consequently, this type of major surgery often causes severe systemic inflammatory responses and serious complications postoperatively[5,6]. In the present study, we investigated serum levels of VEGF in the angiogenesis phase following major surgical injury and examined the correlation with clinical variables and inflammatory markers. Because platelets are a potential source of serum VEGF[7,8], we measured VEGF levels in platelet-poor plasma (PPP) prepared from plasma samples. Furthermore, the correlation between serum levels of VEGF and the severity of postoperative inflammatory complication was also investigated.

Materials and Methods

Patients

As a major-surgery group, 41 patients (35 men and 6 women) with a mean age of 60 years who underwent surgery for esophageal cancer were enrolled in this study. The preoperative diagnosis of esophageal cancer showed that 11 patients had Stage I disease, 11 had Stage II, 9 had Stage III, and 10 had Stage IV. The surgical methods used were right transthoracic esophagectomy followed by laparotomy for construction of an esophageal substitute with the stomach. In each patient, systematic lymphadenectomy in the neck, mediastinum, and abdomen was performed. Bolus intravenous injections of urinary trypsin inhibitor were given at the induction of anesthesia (2,000 U/kg body weight) and on postoperative days (PODs) 1 through 5 (6,000 U/kg body weight). However, no glucocorticoids or any other agents that could affect the results of this study were given. For comparison, the results were compared with those of a group of patients who underwent less-invasive laparoscopic cholecystectomy as a minor-surgery group. This group consisted of 13 patients (4 men and 9 women) with a mean age of 50 years. None of the patients in this study had acute inflammation, diabetes mellitus, any vascular disorders, or other angiogenesis-related diseases.

Postoperative inflammatory complications were found in 11 patients with esophageal cancer (**Table 1**). All these patients had pneumonia, and 4 patients also had atelectasis and pleural effusion. Pyothorax caused by anastomotic leakage was diagnosed in 2 patients. Informed consent was obtained from each patient. No patients died of postoperative complications.

Blood Samples

Serum and plasma samples were obtained before the operation and on PODs 1, 7, 14, 21, and 28. Serum samples were immediately separated with centrifugation (1,250 *g* for 15 min) and then stored at −70℃ until assay. The PPP was prepared according to the method of Wynendaele, et al.[9]. Briefly, the citrated blood was centrifuged at 180 *g* for 10 minutes at 20℃ to generate platelet-rich plasma. After removal of platelet-rich plasma, the samples were again centrifuged at 3,000 g for 10 minutes at 20℃ to obtain PPP and were also stored −70℃ until assay.

Assay

The concentration of VEGF A-165 in the serum and PPP was measured with an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the method described elsewhere[10]. Briefly, the system used a solid-phase monoclonal antibody and an enzyme-linked polyclonal antibody raised against recombinant human VEGF A-165. In each analysis, 100 μL of serum or PPP was used. All analyses and calibrations were performed in duplicate. The

Table 1 Patients with lung complication following esophagectomy

Age	Gender	Lung Complication	Lung Injury Score
58	M	pneumonia, atelectasis, pleural effusion	2.7
51	F	pneumonia, atelectasis, pleural effusion	4.0
69	M	pneumonia, atelectasis, pleural effusion	1.3
52	M	pneumonia, pyothrax, pneumothorax	3.0
57	M	pneumonia	3.7
61	M	pneumonia, pyothorax	3.3
63	M	pneumonia, pneumothorax	2.0
61	M	pneumonia	3.0
60	M	pneumonia	1.7
44	M	pneumonia, pneumothorax	1.7
53	M	pneumonia, atelectasis, pleural effusion	1.0

M: male; F: female

calibrations on each microtiter plate included recombinant human VEGF A-165 standards. The optical densities were determined by absorption at 450 nm. Levels of interleukin (IL)-6 in sera were measured with an ELISA kit according to the manufacturer's instructions (Fujirebio, Tokyo, Japan)[11]. Blood analysis and measurement of the serum C-reactive protein (CRP) level were performed in the central laboratory of our hospital.

Postoperative Complications

Pneumonia was the most frequent postoperative complication following this major surgery[6] and was defined by a mixture of abnormal chest X-ray findings with lung infiltrates, elevated body temperature, elevated peripheral blood leukocyte counts, identification of bacteria in the airway, and abnormal arterial blood gas analysis. The severity of lung injury was assessed according to the Murray's Lung Injury Score[12]. Atelectasis and pleural effusion were diagnosed with plain chest X-ray films and computed tomography. Pyothorax was confirmed with the finding of purulent chest drainage and identification of bacteria.

Evaluation

The postoperative changes in serum levels of VEGF were followed until POD 28 in 41 patients with esophageal cancer. The serum levels of VEGF were compared with those in 13 patients undergoing minor surgery for gallstones. The relationship between serum levels of VEGF and the clinical variables, such as sex, age, blood loss during operation, amount of transfusion, pathological stage of cancer, and postoperative inflammatory complications, was evaluated. As inflammatory variables, peripheral blood cell counts, and levels of IL-6 and CRP in sera were measured to investigate the relationship with serum levels of VEGF. Furthermore, the correlation between serum levels of VEGF and the severity of postoperative inflammatory complications was also analyzed. The changes in VEGF levels in PPP were compared with those in sera, because some studies have shown that VEGF in serum is derived mainly from platelets[7,8].

Statistical Analysis

All the results are expressed as means ± standard deviation of the error (SEM). The Wilcoxon signed-rank test was used to compare VEGF levels before and after surgery. Repeated measure analysis of variance (ANOVA) was used to compare changes in VEGF levels. The correlation between two variables was examined with Spearman's rank correlation test. A result was considered statistically significant when the p value was less than 0.05.

Results

The serum VEGF level increased significantly from 337 ± 41 pg/m*l* before major surgery to a maximal level of 1,109 ± 109 pg/m*l* on POD 14 ($p < 0.0001$), then gradually decreased until POD 28. There was a slight increase in the serum VEGF level on POD 14 after minor surgery, but the maximal increase in the major surgery group was approximately twice that in the minor surgery group. There was significant difference in the levels

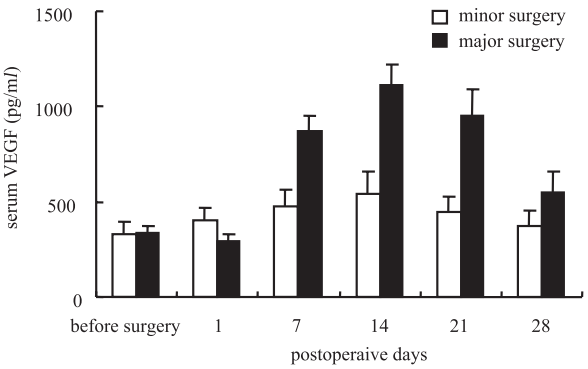

Fig. 1 Serum VEGF had a significant increase following major surgery compared with minor surgery (p=0.0067, repeated measure ANOVA).

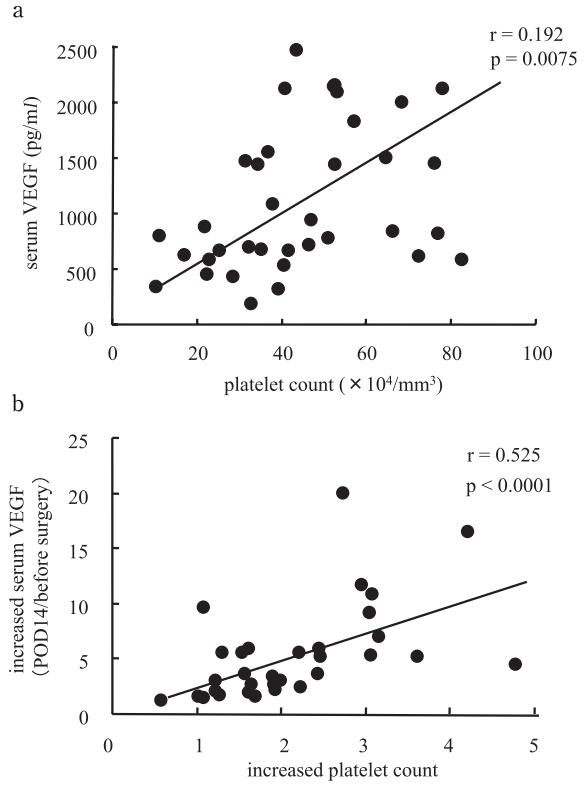

Fig. 2 **2a**: There was a significant correlation between the postoperative maximal levels of platelet count and those of serum VEGF (p=0.0075, r=0.192, Spearman's rank correlation test).
2b: The increased ratio of platelet count in the postoperative level compared with the preoperative level was also correlated with that of serum VEGF (p<0.0001, r=0.525, Spearman's rank correlation test).

of serum VEGF between the major and minor surgery groups **(Fig. 1)**. However, there was no significant relationship between the serum level of VEGF and sex, age, blood loss volume during operation, amount of transfusion, or pathological stage of the tumor (data not shown).

Serum levels of IL-6 and CRP reached maximal levels on POD 1 and POD 2, respectively. Therefore, serum levels of IL-6 on POD 1 and of CRP on POD 2 were each analyzed for correlation with the maximal serum levels of VEGF on POD 14, but there was no correlation among these factors. However, there was a significant correlation between the postoperative maximal levels of platelet counts in peripheral blood and maximal serum VEGF levels on POD14 **(Fig. 2a)**, and the increase in platelet count from the preoperative level was strongly correlated with the increased serum VEGF level **(Fig. 2b)**.

After major surgery, the VEGF level in PPP was significantly higher than the preoperative value (15 ± 4 pg/m*l*) on PODs 1, 7, and 14. The VEGF level reached a maximum of 56 ± 8 pg/m*l* on POD 1 and then gradually decreased. However, the levels of VEGF in PPP were significantly lower than those in sera **(Fig. 3)**.

Eleven patients had postoperative inflammatory complications. All these patients had lung complications as summarized in **Table 1**. Serum VEGF levels in these patients were significantly higher than those in patients without lung complications **(Fig. 4)**. Furthermore, the maximal serum levels of VEGF were significantly correlated with the lung injury score **(Fig. 5)**. In patients with lung complications, however, the changes in platelet counts in the peripheral blood were not significant correlated with changes in serum levels of VEGF (p=0.0795, r=0.587).

Discussion

In this study, the increase in the serum VEGF level was most prominent on POD 14. Wound repair is most active in the first few weeks after surgery[2]. During this period, many new capillaries are produced in the granulation tissue indicating the angiogenesis phase of the postoperative wound healing process[2]. VEGF mediates this angiogenesis[13]. Because the increased serum levels of VEGF were

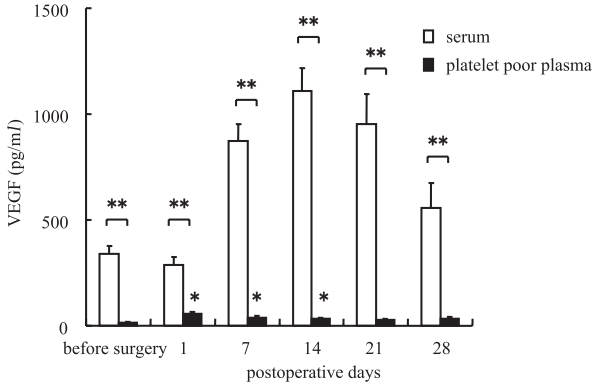

Fig. 3 *VEGF in PPP significantly increased on POD 1, 7 and 14 compared with the postoperative value ($p<0.05$, Wilcoxon signed-rank test). However, **the levels of VEGF in PPP were significantly low compared with those in sera ($p<0.0001$, repeated measure ANOVA).

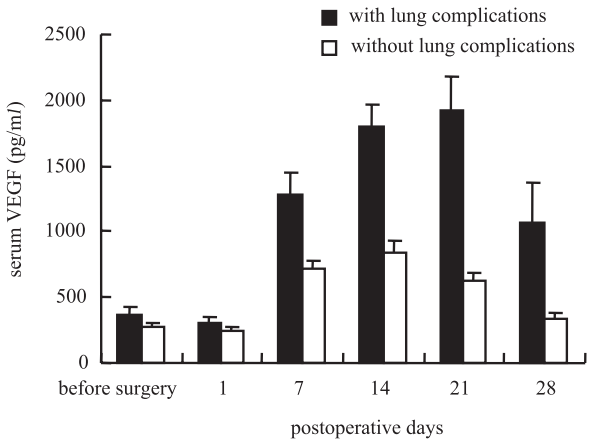

Fig. 4 Patients with lung complications and without lung complications were compared for the change of serum VEGF levels. Serum VEGF significantly increased in patients with lung complications compared with that in patients without lung complications ($p<0.0001$, repeated measure ANOVA).

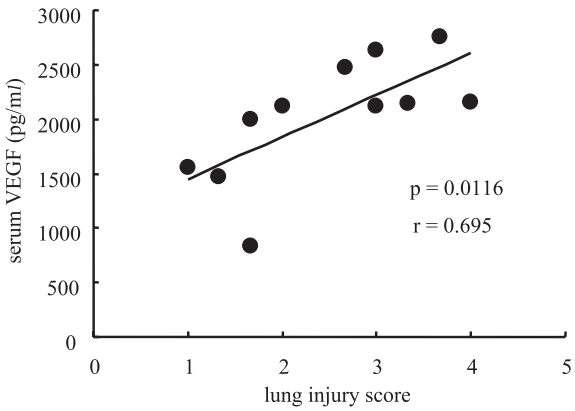

Fig. 5 In patients with lung complications, serum maximal levels of VEGF were significantly correlated to the lung injury score. ($p=0.0116$, $r=0.695$, Spearman's rank correlation test).

more prominent after major surgery, it is thought that major surgery requires a greater response of angiogenesis for tissue repair. There are many angiogenesis factors, such as VEGF, nitric oxide, and fibroblast growth factor[14–16]. There are also some antiangiogenesis factors, such as angiostatin, endostatin, and interferon[17–19]. In our preliminary study, only VEGF was elevated in the patients' sera postoperatively, whereas serum levels of basic fibroblast growth factor and endostatin showed no significant change. Therefore, VEGF was selected as a potential marker of the postoperative signal for angiogenesis in this study. Age and sex have been reported to correlate with serum VEGF level[20]. However, neither age nor sex showed any effects on serum levels of VEGF in the present study. It has been reported that bleeding or transfusion affects serum VEGF levels[21–24], but no clear relationship was found in the present study. It has also been reported that serum VEGF levels increase in advanced stages of various tumors[25,26] and in patients with liver metastasis[27]. In this study, there was no significant correlation between the postoperative of serum levels VEGF and tumor stage, although the preoperative levels of serum VEGF were varied in each patient.

VEGF is an angiogenesis factor that selectively acts on vascular endothelial cells[28]. The mRNA of the three isoforms of VEGF (121, 165, and 189 amino acids) is expressed in megakaryocytes[29]. Furthermore, some studies have shown that VEGF in the serum is derived mainly from platelets[7,8]. Our present study also found that the increased platelet count in the peripheral blood matched the increase in the serum level of VEGF. To examine whether platelets are a potential source of VEGF in serum, PPP was prepared. The concentration of VEGF in PPP was much lower than that in sera. All these findings suggest that platelets may be a potential source of the increased serum VEGF levels following surgical injuries. Our findings also indicate the

important role of platelets in surgical wound healing. However, the mechanism of platelet activation to produce VEGF under these conditions is poorly understood. It has been reported that IL-6 is a differentiation factor for megakaryocytes in vitro in the presence of IL-3[30], but our present study did not find a correlation between postoperative serum levels of IL-6 and platelet counts. Furthermore, no relationship between IL-6 and the serum level of VEGF was found. It has been reported that VEGF production is increased by many factors, including transforming growth factor-β (TGF-β), IL-1, platelet-derived growth factor (PDGF), tumor necrosis factor-α, IL-13, IL-8, lipopolysaccharide, and hypoxia[31–34]. Furthermore, the stimulatory effects of TGF-β and IL-1 on VEGF secretion are additive, and hypoxic culture conditions doubles the rate of VEGF secretion stimulated by the cytokines TGF-β and IL-1[34]. The VEGF secretion stimulated by various cytokines and the additive VEGF secretion by hypoxic condition or compound of cytokines, such as TGF-β and IL-1, may be responsible for the lack of correlation with IL-6 or CRP in the present study. The mechanism of platelet activation, including other growth factors such as PDGF, needs to be investigated in the future.

Another important aspect of increased serum levels of VEGF may be an association with postoperative inflammatory complications. Patients with postoperative inflammatory lung complications showed a significant increase in the serum VEGF level. However, in this group, there was no significant increase in platelet count (data not shown), and the correlation between serum VEGF and platelet count was not statistically significant. These findings suggest the existence of another major source of serum VEGF. It has been reported that serum levels of VEGF in children with pneumonia were significantly higher than those in control subjects and that in the systemic circulation inflammatory cells cause the elevation of serum levels of VEGF[35]. During the acute phase of pneumonia, inflammatory cells, including peripheral blood mononuclear cell, mainly produce VEGF in sera. However, our present study shows that serum VEGF levels were elevated for several weeks after resolution of pneumonia and that the severity of lung injury correlated with the maximal serum levels of VEGF. Furthermore, several reports have suggested bronchoalveolar lining cells, alveolar macrophages, and alveolar neutrophils release VEGF during the resolution of lung injury[31–33]. Therefore, the increase in serum VEGF levels may be associated with the damage to and the repair of lung tissues in the postoperative inflammatory lung complications.

In conclusion, serum VEGF levels increased in the angiogenesis phase of wound healing following major surgical injury, and this increase continued for several weeks until reaching a maximal level on POD 14. Platelets may be the main source of serum VEGF, and, furthermore, increased serum VEGF is also associated with lung inflammatory conditions.

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