Vascular endothelial growth factor levels in post-CABG pleural effusions are associated with pleural inflammation and permeability

Ioannis Kalomenidis\textsuperscript{a,\*}, Georgios T. Stathopoulos\textsuperscript{b}, Randal Barnette\textsuperscript{b}, Spyros Papiris\textsuperscript{c}, Timothy S. Blackwell\textsuperscript{b}, Charis Roussos\textsuperscript{a}, Richard W. Light\textsuperscript{b}

\textsuperscript{a}Department of Critical Care and Pulmonary Services, Athens Medical School, Evangelismos Hospital, 45-47 Ipsilandou Street, 10675 Athens, Greece
\textsuperscript{b}Department of Allergy Pulmonary and Critical Care Medicine, Vanderbilt University, Nashville, TN, USA
\textsuperscript{c}2nd Department of Pulmonary Medicine, Athens Medical School, Attico Hospital, Athens, Greece

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Summary

Background: Vascular endothelial growth factor (VEGF) participates in the pathogenesis of exudative pleural effusions (PEs). In the present study, we determined the pleural fluid (PF) and serum VEGF levels in patients with post-coronary artery by-pass grafting (post-CABG) PEs.

Methods: Thirty-eight patients with post-CABG (two with bilateral) PEs were studied. PEs were divided into "early" (occurring earlier than 30 days after surgery) and "late" ones. VEGF levels were measured using ELISA.

Results: (i) Serum and PF VEGF levels did not differ significantly when all the patients ($P = 0.053$) or those with late effusions ($P = 0.6$) were analyzed; serum VEGF levels were significantly elevated in comparison to PF VEGF levels in patients with early ($P = 0.007$) effusions. (ii) Serum VEGF levels were significantly higher in patients with early than in those with late effusions ($P = 0.033$), while PF VEGF levels were not significantly different between the two groups ($P = 0.77$). (iii) PF VEGF levels were higher than corresponding serum levels in 4/24 patients with early and in 10/16 patients with late post-CABG PEs ($P = 0.006$). (iv) In PEs VEGF levels significantly correlated with red blood cells ($P = 0.015$), nucleated cells ($P = 0.003$), protein levels ($P = 0.002$) and lactate dehydrogenase (LDH) levels ($P = 0.04$).

Conclusion: In post-CABG PEs, preferential local production of VEGF in the pleural cavity is most commonly observed a month or later after surgery. The fact that in PEs...
VEGF levels correlate with markers of pleural inflammation (inflammatory cells and LDH levels) and vascular hyperpermeability (protein levels) suggests that VEGF may be involved in the pathogenesis of post-CABG PEs.

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Introduction

Exudative pleural effusions (PEs) occur in 42–89% of patients subjected to coronary artery by-pass grafting (CABG). The majority of the post-CABG PEs are small, asymptomatic and resolve spontaneously. However, in some patients they are large enough to cause dyspnea and persist for months, hence requiring repeated therapeutic thoracenteses. Though very rare, pleural fibrosis and trapped lung may even develop. Sadikot and co-workers have previously observed that the pleural fluid (PF) features of PEs occurring within the first month after CABG differed substantially from those of PEs occurring later and they speculate that “early” effusions are secondary to surgical pleural trauma and bleeding in the pleural cavity while “late” effusions may be immune-mediated. However, the pathogenesis of post-CABG PEs remains largely unknown.

Accumulating evidence suggests that vascular endothelial growth factor (VEGF), a major angiogenic cytokine, promotes increased PF production and accordingly PF accumulation in inflammatory and malignant pleural diseases. This action of VEGF is mainly attributed to the fact that the growth factor is a potent enhancer of vascular permeability which represents the underlying pathology for fluid exudation in the pleural cavity. In this regard, previous studies have found higher PF VEGF levels in pleural exudates than in transudates and an association between PF VEGF and markers of pleural vascular hyperpermeability and pleural inflammation, such as PF protein level, PF/serum protein ratio, PF LDH levels, PF/serum LDH ratio, nucleated cell count and PF neutrophil count. It is thus reasonable to hypothesize that VEGF participates in the pathogenesis of post-CABG PEs.

In the present study, we determined PF and serum VEGF concentrations in patients with post-CABG PEs and we examined whether the presence of VEGF in the pleural cavity is associated with PF features indicating pleural inflammation and enhanced pleural vascular permeability. We hypothesized that PF VEGF levels would be significantly higher than corresponding serum levels in both early and late effusions and they would correlate significantly with PF total protein levels, PF lactate dehydrogenase (LDH) levels and PF nucleated cell counts.

Patients—methods

The study was approved by the Institutional Review Board of the Saint Thomas Hospital, Nashville, TN and every patient signed an informed consent. We studied 38 patients who had had CABG within the previous months and who underwent thoracentesis for a PE at the St. Thomas Hospital between December 2002 and September 2003. These patients were included in a previous study by our group. Thirty-eight patients, 36 with unilateral and 2 bilateral PEs were included in the study (40 effusions in total). Thoracentesis was performed 19 (range: 4–159) days after CABG. An effusion was defined as “early” when the time elapsed between surgery and thoracentesis was less than 30 days and “late” when this time period was at least 30 days. Among the 40 PEs, 24 were “early” and 16 were “late”. One patient with bilateral PEs had one “early” and one “late” effusions. PF and blood samples were obtained at the same time and centrifuged at 2000 rpm for 10 min. The supernatants and the serum samples were stored at −80 °C until the assays were performed. PF red and nucleated cells were counted by manual microscopy. The differential nucleated cell counts in PF were determined by manually counting 100 cells on a modified Wright–Giemsa-stained smear after cells had been concentrated by cytocentrifugation at 2000 rpm for 10 min. VEGF level was measured by ELISA using Quantikine human VEGF ELISA kit (R&D Systems Inc., Minneapolis, MN, USA). The minimum detectable level is 5 pg/mL.

Statistics: Values were reported as median (interquartile range, IQR) since they were not normally distributed. To assess differences between median values, the Mann–Whitney or the Wilcoxon signed rank tests were used, as appropriate. To assess differences between categorical parameters, the Exact Fisher’s test was used. To assess the correlation between two variables the Spearman’s test was used. For statistical analysis
and construction of figures the SPSS 11.0 statistical software (SPSS Inc., Chicago, IL) was used.

Results

PF VEGF levels [228 (103–612) pg/mL] did not differ significantly from the serum levels of the growth factor but tended to be lower [443 (285–808) pg/mL] (P = 0.053). Similarly, there was no significant difference between PF and serum VEGF when patients with late effusions were analyzed separately (P = 0.6, Table 1). On the contrary, in patients with early effusions serum VEGF levels were significantly higher than the PF VEGF levels (P = 0.007, Table 1). In this connection, serum VEGF levels were significantly higher in patients with early than in those with late effusions (P = 0.033), while PF VEGF levels did not differ significantly (P = 0.77) between the two groups (Table 1).

The PF VEGF levels tended to be relatively higher in the late effusions as compared to the serum levels than in the early effusions. PF VEGF levels were higher than the corresponding serum levels in only 4 of 24 patients with early but in 10 of 16 patients with late post-CABG PE (P = 0.006, Table 2), indicating that preferential production of the growth factor in the pleural cavity is significantly more common a month or later after surgery. There was a significant negative correlation between the number of days elapsed between surgery and thoracenteses and serum VEGF levels (r = -0.32, P = 0.045) or the difference between serum and PF VEGF levels (r = -0.42, P = 0.007). The number of days elapsed between surgery and thoracenteses did not correlate significantly with PF VEGF levels.

The levels of VEGF in the PF were significantly correlated with several PF measurements. A significant correlation between PF VEGF levels and the following PF features was observed: red blood cell counts (r = 0.384, P = 0.015), nucleated cell counts (r = 0.463, P = 0.003), number of neutrophils (r = 0.345, P = 0.032) and lymphocytes (r = 0.458, P = 0.003), total protein levels (r = 0.48, P = 0.002) and LDH levels (r = 0.34, P = 0.004) (Figs. 1–6). There was not a statistically significant correlation between PF and serum levels of VEGF in all patients (P = 0.9), in those with early (P = 0.47) or in those with late PE (P = 0.57).

Discussion

In the present study, we determined PF and serum concentrations of VEGF in patients with post-CABG PE. Our main findings were: (i) PF and serum VEGF levels did not differ significantly when patients were examined as a whole and in patients with late PE, while serum VEGF levels were significantly elevated compared to PF VEGF levels in those with early PE. (ii) Serum VEGF levels were significantly higher in patients with early than in those with late effusions, while PF VEGF levels did not differ significantly between the two groups. (iii) Preferential local production of VEGF in the pleural cavity, manifested by higher concentration of the

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<tr>
<th>Table 1</th>
<th>Serum and pleural fluid levels in early and late post-CABG pleural effusions.</th>
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<tbody>
<tr>
<td>Post-CABG PE</td>
<td>Serum VEGF (pg/mL)</td>
</tr>
<tr>
<td>Early</td>
<td>619 (311–855)</td>
</tr>
<tr>
<td>Late</td>
<td>320 (218–597)</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.033</td>
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</tbody>
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*Difference between serum and PF VEGF levels. **Difference between early and late effusions.

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<tr>
<th>Table 2</th>
<th>Post-CABG PE with higher or lower VEGF levels than the corresponding serum levels, occurring early and late after the operation (P = 0.006).</th>
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<tbody>
<tr>
<td>Post-CABG PE</td>
<td>Early</td>
</tr>
<tr>
<td>Serum VEGF &gt; PF VEGF</td>
<td>20</td>
</tr>
<tr>
<td>Serum VEGF &lt; PF VEGF</td>
<td>4</td>
</tr>
<tr>
<td>Serum VEGF = PF VEGF</td>
<td>24</td>
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cytokine in PF than in serum of a patient was significantly more common in patients with late than in those with early effusion. (iv) The time elapsed from surgery inversely correlated with serum VEGF levels and with the difference between serum and PF VEGF levels. (v) In PF, VEGF levels
correlated significantly with red blood cell, nucleated cell, neutrophils and lymphocyte counts, as well as total protein and LDH.

Enhanced permeability of the pleural vasculature typically accompanies pleural inflammation and both are essential for the pathogenesis of exudative PEs. VEGF, an angiogenic growth factor that induces vascular hyperpermeability and promotes transmigration of leukocytes into sites of inflammation, is thought to play an important role in the pathogenesis of pleural exudates. This notion is largely based on the findings of higher PF VEGF levels in exudative than in transudative PEs and an association between PF VEGF and PF features indicating pleural vascular hyperpermeability and inflammation. The PF and serum levels of VEGF in patients with post-CABG PEs have not been thoroughly examined before. There are two previous studies by our group that have reported measurements of PF VEGF in post CABG PEs. The first of these studies showed that PF VEGF levels in post-CABG PEs were significantly higher than those observed in transudative PEs due to heart failure but significantly lower than those observed in malignant PEs but these findings were not confirmed by the second study. In neither of these studies were the serum levels of VEGF measured. The present study shows that VEGF levels in post-CABG PEs correlate significantly with markers of pleural inflammation (PF nucleated cells, neutrophils and lymphocytes and PF LDH), as well as PF protein, a marker of pleural vascular permeability. These observations agree with previous studies which showed that PF VEGF levels correlate with PF protein and LDH levels and PF nucleated cell and neutrophil count in PEs of various etiologies and imply that VEGF participates in the pathogenesis of post-CABG PEs by promoting pleural inflammation and PF exudation. Nevertheless, it should be acknowledged that our data are merely suggestive and do not definitively prove a role for VEGF in the formation of post-CABG PEs. Interestingly, although VEGF is implicated in the pathogenesis of exudative PEs in general, direct evidence for such a role exists only for malignant effusions. In this regards, in vivo inhibition of VEGF activity suppressed PF accumulation in animal models of malignant PE. The lack of in vivo studies that would clarify whether VEGF is involved in the pathogenesis of exudative PEs of benign etiology can be attributed to the shortage of relevant animal models that closely mimic human disease.

The biological basis of the correlation between PF VEGF levels and RBC counts found in both the present study and in another study of ours on patients with PEs of different etiologies is unclear. An association between VEGF and the presence of blood in the pleural cavity has also been reported by Ishimoto et al. who found that the PF VEGF...
levels are significantly higher in hemorrhagic malignant PEs than in non-hemorrhagic ones. These observations agree with the findings of an in vivo study in which the administration of VEGF neutralizing antibodies in mice with malignant ascites not only suppressed peritoneal microvascular permeability and fluid accumulation but it also reduced the number of RBC in the peritoneal cavity. Since, neo-vessels formed in the presence of excess amounts of VEGF are leaky, we speculate that the association between VEGF and the presence of blood in the pleural cavity most likely reflects the presence of VEGF-associated angiogenesis.

In patients with post-CABG PEs, the presence of VEGF in the pleural cavity may be explained by either intrapleural production from mesothelial or inflammatory cells or diffusion from systemic circulation. We thus compared PF to serum levels of the cytokine in order to examine whether preferential local production of VEGF in the pleural space occurs in these patients. When patients were examined as a whole, we observed that serum VEGF tended to be higher compared to corresponding serum VEGF but the difference did not reach statistic significance. When patients with early or late PEs were examined separately, it became clear that VEGF was elevated in the blood of patients with early PE only. In keeping with this, preferential production of VEGF in the pleural space was significantly more common in patients with late effusions. These findings should be mainly attributed to the presence of elevated peripheral blood VEGF levels in the early post-operative period which may reflect increased VEGF production at sites of extra-pleural neo-vascularization (surgical wounds, re-perfused myocardium). The following are in favor of this assumption: (a) peripheral blood VEGF levels were significantly higher in patients with early than in those with late effusions; on the other hand, although the median level of VEGF in late effusions was higher than in the early ones, the difference was not significant. (b) Serum VEGF levels and the difference between the serum and the PF VEGF levels declined with time elapsed from surgery; (c) PF VEGF levels did not correlate with time from surgery. The above imply that in contrast to what is happening in extra-pleural sites, the rate of VEGF production in the pleural cavity does not change substantially with time and that the presence of higher serum than PF VEGF concentration in patients with early PEs does not preclude local production of the growth factor in the pleural cavity. This interpretation of our results is further supported by the fact that PF VEGF levels do not correlate with corresponding serum levels either in early or in late disease, indicating that the presence of the growth factor in the pleural cavity is not merely the result of diffusion from plasma. Previous studies have not definitively elucidated the issue whether preferential local production of VEGF in the pleural space, manifested by higher PF than serum levels of the cytokine, characterizes exudative PE of any etiology. Thus, although such a finding has been constantly reported in malignant and parapneumonic PEs or empyema, the relationship between PF and serum VEGF levels in tuberculous PEs is controversial: three studies showed that VEGF is elevated in PF while other authors reported no difference between PF and serum. Since VEGF inhibition may be useful in clinical practice to halt PF accumulation, resolving the issue of whether VEGF or any other hyperpermeability factor involved in the pathogenesis of pleural exudates is produced mainly in the pleural cavity may be taken into account to decide whether an inhibitor should be administered systemically or intrapleurally.

In conclusion, among patients with post-CABG PE, PF VEGF levels are higher than serum VEGF levels in the majority of those with PE occurring after the first month post-operatively suggesting that VEGF is produced in the pleural space. In contrast, PF VEGF levels are lower than serum levels in PEs that occur within the first month. This is most likely due to extra-pleural production of the growth factor during the first month post-surgery. Notwithstanding the source of VEGF, the fact that its PF levels correlate with markers of pleural inflammation and pleural vascular hyperpermeability indicates that the growth factor might be involved in the pathogenesis of post-CABG PEs. Further studies are required to examine whether VEGF blockade can be clinically beneficial in patients with persistent, symptomatic post-CABG PEs.

Acknowledgments

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References


