Study objectives: The purpose of this study was to analyze the relationship of the pleural fluid vascular endothelial growth factor (VEGF) level with the diagnostic category and with the pleural fluid characteristics in a group of 70 patients.

Design: The VEGF levels of consecutive patients undergoing therapeutic thoracentesis were determined with an enzyme-linked immunosorbent assay.

Setting: University-affiliated tertiary care center.

Results: The median level of pleural fluid VEGF in the patients with congestive heart failure (150 pg/mL) was significantly (p < 0.05) lower than the median level in the patients with coronary artery bypass grafting (357 pg/mL), which in turn was significantly lower (p < 0.05) than the median levels in the patients with malignancy (1,097 pg/mL). The overlap between groups, however, limits the diagnostic usefulness of pleural fluid VEGF levels. The VEGF level was most closely correlated with the lactate dehydrogenase level (r = 0.42, p < 0.001) and was also significantly correlated with the total pleural fluid protein level. The median VEGF levels in the pleural fluid of patients with breast cancer were significantly lower (p = 0.017) than in those with lung cancer. The VEGF level was very high (3,294 pg/mL) in the one patient with pulmonary embolism.

Conclusions: We conclude that the VEGF levels in pleural fluid differ significantly from one diagnostic category to another with the highest median levels occurring in patients with malignant pleural effusions. We speculate that VEGF may be responsible for the pleural fluid accumulation in at least some situations.

Key words: malignant pleural effusion; parapneumonic effusion; pleural effusion; vascular endothelial growth factor

Abbreviations: CAGB = coronary artery bypass grafting; LDH = lactate dehydrogenase; VEGF = vascular endothelial growth factor
dates as compared with transudates, and that malignant pleural effusions would have higher VEGF levels than would other exudative effusions. The basis for the first hypothesis is that VEGF is present in areas of inflammation and is thought to be at least partially responsible for inflammatory edema. The basis for the second hypothesis is the observation that many malignant tumors have high levels of VEGF and that higher levels of VEGF are associated with more tumor-associated edema.

**Materials and Methods**

Between August 1, 1997, and January 30, 1998, we prospectively studied 70 consecutive patients who underwent thoracentesis under ultrasound guidance in the Department of Radiology. The study was approved by the Institutional Review Board of the New York Presbyterian Hospital, and before the thoracentesis all patients signed an informed consent.

Standard definitions were used for identifying the cause of the pleural effusion. A pleural effusion was said to be caused by congestive heart failure if the patient had symptoms and signs of congestive heart failure that improved with appropriate therapy. A pleural effusion was said to be malignant if the patient had either a pleural fluid cytologic examination or a pleural biopsy specimen that was positive for malignancy, or if the patient had known metastatic malignancy with no other explanation for the pleural effusion. A pleural effusion was labeled post–coronary artery bypass grafting (CABG) when it occurred within the first few months after CABG and had no other obvious explanation (eg, congestive heart failure, chylothorax, or infection). A parapneumonic effusion was an effusion associated with bacterial pneumonia, lung abscess, or bronchiectasis.

At the time of the thoracentesis, pleural fluid was collected in a hematocrit tube for WBC count and differential, in a 5-mL citrated tube for VEGF measurement, and in a tube with no additives for glucose, protein, amylase, and lactate dehydrogenase (LDH) analysis. For VEGF measurement, the pleural fluid was immediately centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was separated from the cellular pellet and was frozen at −70°C until analysis. The total WBC count and the total RBC count were obtained manually by microscopy. The differential leukocyte count was immediately obtained by counting 100 cells on a Wright’s-stained smear after the cells had been concentrated by cytocentrifugation at 2,000 rpm for 10 min. The glucose, protein, and LDH measurements were obtained from fluid collected in a sterile tube with no additive using an automated analyzer (Vitros Model 950; Johnson & Johnson; Rochester, NY). VEGF concentrations were measured using an enzyme-linked immunosorbent assay (R&D Systems, Inc; Minneapolis, MN). The procedures were performed according to the manufacturer’s instructions with validation for pleural fluids including determination of recovery (99%). Measurements and standard curve fittings were performed with Bio-Rad Model 3550 microplate reader and Microplate Manager Software (Bio-Rad; Hercules, CA). Samples in which the levels were outside the linear part of the curve were diluted and reanalyzed. Interassay and in-assay coefficient of variance were < 15%.

**Statistical Analysis**

Because the pleural fluid VEGF, LDH, WBC, and individual cell counts were not normally distributed, Kruskal-Wallis one-way analysis of variance on ranks was used for comparing the VEGF levels among groups and Dunn’s method was used to perform multiple comparison procedures. Correlations were analyzed with the Spearman rank order correlation. Data were analyzed using SigmaStat v2.03 statistical software package (Jandel Scientific; San Rafael, CA).

**Results**

The diagnoses and the pleural fluid characteristics of the 70 patients studied are summarized in Table 1. The most common diagnosis, surprisingly, was pleural effusion after CABG. This hospital, however, performs > 2,500 CABG surgeries annually.

The distribution of the pleural fluid VEGF levels varied among groups (Fig 1). Patients with congestive heart failure had the lowest pleural fluid VEGF levels whereas the patients with malignancy had the highest levels, and the patients after CABG surgery had intermediate levels. The median level of VEGF in the patients with congestive heart failure (150 pg/mL) was significantly (p < 0.05) lower than the median level in the group with CABG (357 pg/mL), which in turn was significantly lower than the median level in the group with malignancy (1,097 pg/mL).

**Table 1—VEGF Levels and Characteristics of Pleural Fluid in Different Diagnostic Groups**

<table>
<thead>
<tr>
<th>Category</th>
<th>CAGB (n = 36)</th>
<th>Malignant (n = 18)</th>
<th>CHF (n = 11)</th>
<th>Pneum (n = 4)</th>
<th>PE (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF, pg/mL</td>
<td>357 (211–581)</td>
<td>1,097 (297–3,298)</td>
<td>150 (97–248)</td>
<td>1,376 (280–5,467)</td>
<td>3,294</td>
</tr>
<tr>
<td>Protein, g/dL</td>
<td>3.2 (2.0–3.6)</td>
<td>4.1 (3.7–4.8)</td>
<td>3.0 (1.9–4.0)</td>
<td>3.9 (3.4–4.25)</td>
<td>4.2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>110 (60–144)</td>
<td>105 (92–127)</td>
<td>104 (95–146)</td>
<td>101 (50–124)</td>
<td>149</td>
</tr>
<tr>
<td>LDH, IU/L</td>
<td>723 (503–1,555)</td>
<td>851 (402–1,257)</td>
<td>322 (267–435)</td>
<td>1,409 (728–5,152)</td>
<td>1,595</td>
</tr>
<tr>
<td>WBC, cells/mL</td>
<td>1,675 (575–7,350)</td>
<td>965 (530–2,225)</td>
<td>750 (330–1,150)</td>
<td>5,125 (3,087–5,187)</td>
<td>750</td>
</tr>
<tr>
<td>PMNs, cells/mL</td>
<td>255 (50–725)</td>
<td>38 (21–220)</td>
<td>60 (6–146)</td>
<td>2,250 (967–5,904)</td>
<td>105</td>
</tr>
<tr>
<td>Lymphs, cells/mL</td>
<td>350 (130–800)</td>
<td>280 (64–717)</td>
<td>212 (105–578)</td>
<td>432 (250–1,186)</td>
<td>503</td>
</tr>
<tr>
<td>Monos, cells/mL</td>
<td>498 (119–1,131)</td>
<td>280 (64–716)</td>
<td>212 (105–578)</td>
<td>1,094 (554–1,802)</td>
<td>120</td>
</tr>
</tbody>
</table>

*Data are presented as median (25th to 75th percentiles). CHF = congestive heart failure; Pneum = parapneumonic effusion; PE = pulmonary embolus; PMN = polymorphonuclear cell; Lymphs = lymphocytes; Monos = monocytes.
†p < 0.05 when VEGF levels of effusions caused by CAGB, malignancy, and CHF are compared with each other.
pg/mL). The pleural fluid VEGF levels in the patients with parapneumonic effusions were widely distributed (Fig 1). The pleural fluid from the one patient with a pulmonary embolus had a high (3,294 pg/mL) VEGF level.

We next analyzed the data for all 70 patients to determine whether there was any relationship between the pleural fluid VEGF levels and other characteristics of the pleural fluid. Because the VEGF levels were not normally distributed, we used the Spearman rank order correlation for this analysis. The closest correlation was between the VEGF levels and the pleural fluid LDH levels, but as seen in Figure 2, this was still not a close relationship ($r = 0.42, p < 0.001$). There were also a significant relationship between the pleural fluid VEGF level and the total protein level ($r = 0.28, p = 0.019$).

When the group of 18 patients with malignant pleural effusions was analyzed, the 7 patients with breast carcinoma had a significantly ($p = 0.017$) lower median pleural fluid VEGF level (279 pg/mL) than did the 7 patients with lung carcinoma (2,958 pg/mL). One exception to this pattern was a patient with breast carcinoma and a pleural fluid VEGF level of 5,930 pg/mL. When the medical records for this patient were reviewed, however, we found nothing striking that set her apart from the other patients with breast carcinoma. In this group of patients with malignant pleural effusions, the pleural fluid VEGF and LDH levels were significantly correlated ($r = 0.63, p = 0.005$). There was not a significant correlation between the VEGF levels and any other measurement in this subgroup of patients. The VEGF levels did not differ significantly according to whether the cytologic examination yielded positive or negative results.

When the group of 36 patients with pleural effusion after CABG surgery was analyzed, there was no significant relationship between the levels of VEGF in the pleural fluid and any other characteristics of the patients. Specifically, there was not a significant relationship between the pleural fluid VEGF levels and the pleural fluid LDH, WBC, differential WBC, or protein levels.

In this series there were only four patients who had parapneumonic effusions. In these patients, the pleural fluid VEGF levels were quite variable. Although the number of patients is very small, the level of VEGF in the pleural fluid was significantly correlated with the pleural fluid LDH level ($r = 0.97, p = 0.03$), the neutrophil count ($r = 0.99, p = 0.003$), and the glucose levels ($r = -0.99, p = 0.01$).

**Discussion**

The results of the present study demonstrate that VEGF is present in pleural fluid with marked variation in its levels. The median level of VEGF in patients with congestive heart failure was significantly ($p < 0.05$) lower (150 pg/mL) than the median level in patients after CABG (357 pg/mL), which in turn was significantly ($p < 0.05$) lower than the median level in the patients with malignancy (1,097 pg/mL). Overall, there was much overlap in the pleural fluid VEGF levels from category to category.
localized in the respiratory epithelium. Hypoxia has been well documented as a potent inducer of VEGF in many tissues and cells including pulmonary vascular smooth muscle cells. Conversely, it has also been shown that hyperoxic exposure leads to decreased VEGF in the lung. The role of oxygen tension in controlling the levels of VEGF in pleural fluid is not known. It is possible that the very high level of VEGF seen in the patient with pulmonary embolism might be related to an area of tissue ischemia.

In the present study, the levels of VEGF in the pleural fluid varied markedly from patient to patient. One might ask whether the VEGF that was present in the pleural fluid was related to the presence of cells in the pleural fluid. Indeed, Yeo and coworkers reported that the VEGF levels correlated strongly with the numbers of monocytes and macrophages present, but not with the total numbers of WBC, neutrophils, or lymphocytes. They postulated that the VEGF present in the pleural fluid was produced by the malignant cells, monocytes, or macrophages in the pleural fluid. In the present study, we could demonstrate no strong relationship between the pleural fluid VEGF levels and any other characteristic of the pleural fluid. Specifically, malignant effusions with cytologic examination yielding positive results did not have higher levels of VEGF than did malignant effusions with cytologic examination yielding negative results, and there was no strong correlation between the monocyte/macrophage count and the VEGF level in any category of pleural effusion. It appears, therefore, that the origin of the VEGF in the pleural fluid is probably not the cells in the pleural fluid.

Inasmuch as the pleural fluid VEGF levels are not closely correlated with any characteristic of the pleural fluid, it is likely that the VEGF in the pleural fluid is formed elsewhere. Pleural fluid in patients with congestive heart failure has its origin in the interstitial spaces of the lungs and is essentially an ultrafiltrate of serum caused by high pulmonary vascular pressures. The median level of VEGF in the pleural fluid of our patients with heart failure was 150 pg/mL, similar to the mean serum level for normal subjects that Donovan and coworkers reported (230 ± 127 pg/mL) using the same enzyme-linked immunoassay kit. It is likely that the VEGF in the pleural fluid of the patients with congestive heart failure originates in the blood.

The median levels of VEGF in the pleural fluid were much higher in the patients with malignancy. In the present paper, the pleural fluid VEGF levels in patients with lung cancer were significantly higher than the VEGF levels in patients with breast cancer. Yeo and coworkers also reported that the mean pleural fluid levels of VEGF in patients with lung cancer were higher than those in patients with breast cancer. The explanation for this difference is not clear. There is no evidence that breast carcinoma cells produce less VEGF than do lung carcinoma cells. Patients with both breast carcinoma and non–small cell lung carcinoma have elevated serum levels of VEGF.

The origin of pleural fluid in patients with malignancy is not definitely known. Although it has been stated that pleural fluid accumulation in malignancy is caused by lymphatic obstruction, this mechanism cannot be the entire explanation. If lymphatic obstruction were the only mechanism, the pleural fluid should be a transudate, but the fluid with malignancy is an exudate. Moreover, the rate of pleural fluid accumulation is often > 100 mL/d, and one would expect an accumulation of < 20 mL/d if the rate of pleural fluid formation was normal.

We believe that, at least in some cases, fluid from the tumor enters the pleural space. If the pleural surfaces are directly involved by the tumor, the fluid could enter the pleural space directly. If the tumor were in the lung, edema fluid could enter the interstitial spaces of the lung and then traverse the visceral pleura to enter the pleural space. It is also possible that VEGF in the effluent from the tumor that enters the pleural space could also increase the permeability of the capillaries in the pleura, leading to increased pleural fluid formation. The above
The fluid can be a transudate or an exudate, and the differential cell count is quite varied. The observation in the present study that the pleural fluid from the one patient with pulmonary embolism had a VEGF level of 3,294 pg/mL is noteworthy. The source of the VEGF in this situation is unknown, but it could possibly be the blood clot inasmuch as it is known that both the aggregation and activation of platelets result in the release of VEGF.

It is interesting that there was a significant relationship between the levels of VEGF in the pleural fluid and the LDH levels in the pleural fluid. Neutrophils also produce VEGF in response to different stimuli. Increased VEGF gene expression in pulmonary fibroblasts and pulmonary vascular smooth muscle cells is induced by proinflammatory mediators such as platelet-activating factor or platelet-derived growth factor. We hypothesize that the levels of VEGF and LDH in pleural fluid are roughly correlated because they are both crude markers of the inflammatory response.

What are the clinical implications of the present study? The present study demonstrates that there is much overlap between the VEGF levels in the various groups. Accordingly, VEGF levels are unlikely to be useful diagnostically. However, the elevated levels of VEGF in some of the pleural fluid samples are noteworthy and raise the possibility that the accumulation of pleural fluid, in at least some patients, may be related to VEGF. If this speculation is correct, then in the future, it may be possible to decrease the rate of pleural fluid formation by administering inhibitors of VEGF.

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