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VEGF levels in the alveolar compartment do not distinguish between ARDS and hydrostatic pulmonary oedema

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ABSTRACT: Although overexpression of vascular endothelial growth factor (VEGF) 165 in the lung causes pulmonary oedema, its role in human acute lung injury (ALI) is unclear. VEGF levels are reported to be lower in bronchoalveolar lavage from ALI patients compared with normals, but these studies did not include a comparably ill control group with noninflammatory pulmonary oedema.

The current authors hypothesised that VEGF levels in pulmonary oedema fluid would be lower in ALI patients compared with control patients with severe hydrostatic pulmonary oedema. VEGF was measured in pulmonary oedema fluid and plasma from 56 patients with ALI and 46 controls with severe hydrostatic pulmonary oedema.

Pulmonary oedema fluid levels of VEGF did not differ between patients with hydrostatic oedema (median 799 pg·mL^{-1}, interquartile range (IQR) 226–2,281) and ALI (median 507, IQR 0.8–1,031). Plasma levels were also the same (median 20.5 pg·mL^{-1}, IQR 0–152 *versus* 4.8, IQR 0–99.8). There was no association between plasma or oedema fluid VEGF levels and outcomes including mortality.

Vascular endothelial growth factor levels in pulmonary oedema fluid were depressed both in acute lung injury and hydrostatic pulmonary oedema. The decrease in air space concentrations of vascular endothelial growth factor in acute lung injury may not be a function of the degree of lung injury, but rather may result from alveolar flooding.

KEYWORDS: Acute pulmonary oedema, acute respiratory distress syndrome

Vascular endothelial growth factor (VEGF) is an endothelial-specific mitogen that can induce endothelial permeability. There are four VEGF products of alternative splicing, VEGF 121, VEGF165, VEGF189 and VEGF206 [1]. VEGF121 and VEGF165 are secreted and have both mitogenic and permeability-inducing properties. VEGF165 is the predominant form in humans. Acute overexpression of VEGF165 in the lung causes pulmonary oedema and increased lung vascular permeability [2]. However, the role of VEGF in the pathogenesis of human acute lung injury (ALI) is unclear. Furthermore, the factors that modulate the release of VEGF in the lung are not well defined.

The normal lung is rich in VEGF [3]. The primary source of VEGF is alveolar epithelial cells [4] and levels of VEGF in the epithelial lining fluid are 500-fold higher than plasma levels [5]. In experimental studies, exposure to hyperoxia decreased VEGF expression in the rat lung [6]. Conversely, exposure to hypoxia stimulates VEGF expression in alveolar epithelial cells in both *in vitro* and *in vivo* models [7]. These findings suggest that VEGF levels might be elevated in the acutely injured alveolus as a response to hypoxia.

However, clinical studies have not supported this hypothesis. Several groups have measured VEGF in the plasma and bronchoalveolar lavage fluid (BAL) from patients with ALI and acute respiratory distress syndrome (ARDS). Levels of VEGF in the plasma were higher in ARDS patients compared with normal controls [8], but levels of VEGF in BAL were lower in acute ARDS compared with normal controls, mechanically ventilated controls without pulmonary oedema or at-risk patients. Furthermore, as the acute lung injury resolved, levels of VEGF in BAL rose [9, 10]. One possible explanation for this finding is that alveolar epithelial injury is the primary modulator of alveolar levels of VEGF in the acutely injured lung. However, a comparably ill

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control patient population with pulmonary oedema from a noninflammatory cause was not included in any of these studies.

Based on the prior BAL studies, the current authors hypothesised that undiluted pulmonary oedema fluid levels of VEGF would be lower in patients with ALI/ARDS than in a control population of patients with severe hydrostatic pulmonary oedema. The patients with hydrostatic pulmonary oedema have been previously described [7]. The aim of the current study was to determine whether alterations in VEGF levels present in the alveolar compartment were specific for ALI/ARDS.

METHODS

Patient selection

Patients with acute pulmonary oedema from a hydrostatic mechanism or ALI/ARDS were identified from patients admitted to the intensive care units of Moffitt Hospital, University of California, San Francisco or San Francisco General Hospital, CA, USA. Inclusion criteria included acute respiratory failure requiring mechanical ventilation and ability to aspirate pulmonary oedema fluid within 30 min of intubation. Patients were classified as having hydrostatic pulmonary oedema or ALI/ARDS based on the definitions outlined below, in addition to measurement of the oedema fluid-to-plasma protein ratio. Patients were excluded if the cause of pulmonary oedema was unclear or features of both hydrostatic oedema and ALI/ARDS were present. There were 46 patients with hydrostatic pulmonary oedema and 56 patients with ALI/ARDS in this study. The protocol was approved by the Human Research Committee of the University of California, San Francisco, CA, USA, with a waiver of informed consent.

Definition of hydrostatic pulmonary oedema

Hydrostatic pulmonary oedema was defined as central venous pressure $\geqslant$14 mmHg or pulmonary artery occlusion pressure $\geqslant$18 mmHg if measured, ejection fraction $\leqslant$45% if measured, 3rd heart sound or jugular venous distension, no risk factor for ALI/ARDS and a clinical history consistent with cardiac dysfunction or volume overload. The initial oedema fluid to plasma protein ratio was $\leqslant$0.65.

Definition of acute lung injury

The American European Consensus Conference definition of acute lung injury was used [11]. This included an arterial oxygen tension (P_{a,O_2})/inspiratory oxygen fraction (F_{i,O_2}) ratio $<$300, acute onset of bilateral infiltrates on chest radiograph, no clinical evidence of left atrial hypertension and a pulmonary artery wedge pressure $<$18 mmHg, if measured. In addition, patients were required to have a clinical risk factor for ALI/ARDS. The initial oedema fluid to plasma protein ratio was $>$0.65.

Collection of pulmonary oedema fluid

Undiluted pulmonary oedema fluid was suctioned through a 14 Fr suction catheter passed through the endotracheal tube and wedged in a distal airway. A simultaneous EDTA plasma sample was obtained. All samples were centrifuged at $3000 \times g$ for 10 min and supernatants were stored at -70℃.

Clinical data

Relevant clinical data were obtained from the medical record including attributable cause of pulmonary oedema, demographic data, physiological variables including haemodynamic, respiratory and ventilatory parameters, indices of multi-organ system function and medications. The Simplified Acute Physiology Score (SAPS II) [12], a measure of severity of illness, and the Lung Injury Score [13] were calculated.

Measurements

All measurements were made in duplicate in thawed plasma and oedema fluid samples. VEGF165/121 levels were measured by sandwich ELISA using commercially available kits (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Clinical characteristics that were continuous variables were normally distributed and were compared between acute lung injury and hydrostatic oedema using an unpaired t-test. Clinical characteristics that were categorical variables were compared between patient groups using Chi-squared analysis. VEGF levels were not normally distributed and, thus, nonparametric statistics were used for analysis. For comparison of multiple groups, the nonparametric Kruskal Wallis test was used, followed by Dunn's test for multiple comparisons to determine significant differences between individual groups [14]. Comparison between two groups was made using the nonparametric Mann-Whitney U-test. For bivariate correlations, Spearman rank correlation coefficients were reported. For all analyses, a p-value $<$0.05 was considered to be significant.

RESULTS

Patient characteristics

There were 56 patients with ALI/ARDS and 46 patients with hydrostatic pulmonary oedema in the study. Patient characteristics are summarised in table 1. The most common cause of ALI/ARDS was nonpulmonary sepsis (14/56, 25%), followed by pneumonia with sepsis (10/56, 18%), aspiration of gastric contents (8/56, 14%), and pneumonia without sepsis (5/56, 9%). Other causes included transfusion-related ALI, pancreatitis,

TABLE 1 Clinical characteristics of 46 patients with severe hydrostatic pulmonary oedema and 56 patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS)

Characteristic	Hydrostatic	ALI/ARDS
Male	59	66
Caucasian	65	60
Age	55±17	43±17*
SAPS II	42±15	51±18*
Hospital mortality	26	61*
Ventilator-free days	19±11	7±10*
$Pa,O_2/Fi,O_2$ ratio	124±74	81±42*
Lung injury score	2.6±0.7	3.0±0.7*
Tidal volume mL·kg^{-1}	11.2±2.2	11.2±2.4
Oedema fluid:plasma protein	0.49±0.16	0.91±0.32*

Data presented as mean±SD or %. SAPS II: Simplified Acute Physiology Score; Pa,O_2: arterial oxygen tension; Fi,O_2: inspiratory oxygen fraction. *: $p<0.05$ *versus* hydrostatic.

trauma, drug reaction and reperfusion pulmonary oedema. The most common cause of hydrostatic pulmonary oedema was acute myocardial infarction/ischemia (16/46, 35%), followed by volume overload/diastolic dysfunction (11/46, 24%), chronic congestive heart failure (11%), and neurogenic pulmonary oedema (4/46, 9%). Other causes included valve dysfunction and post-obstructive pulmonary oedema.

VEGF levels in ALI/ARDS compared with hydrostatic oedema

Pulmonary oedema fluid levels were not significantly different between hydrostatic oedema (median 799 pg·mL^{-1}, interquartile range (IQR) 226–2281) compared with patients with ALI/ARDS (median 507 pg·mL^{-1}, IQR 0.8–1031, p=NS; fig. 1). Median levels in the undiluted pulmonary oedema fluid of both patient groups were substantially lower than reported levels in the epithelial lining fluid of the normal lung (~11,000 pg·mL^{-1}) [5]. Plasma levels were also not significantly different between patients with hydrostatic oedema (median 20.5 pg·mL^{-1}, IQR 0–152) and patients with ALI/ARDS (median 4.8 pg·mL^{-1}, IQR 0–99.8, p=NS; fig. 1).

In all patients, VEGF levels were significantly higher in the pulmonary oedema fluid than in simultaneous plasma samples ($p<0.01$; fig. 1). The gradient between oedema fluid and plasma VEGF levels tended to be higher in patients with hydrostatic oedema (median gradient 705 pg·mL^{-1}, IQR 140–2,153) than acute lung injury (median gradient 385 pg·mL^{-1}, IQR 0–1,010), although this difference did not quite reach statistical significance (p=0.07).

VEGF levels and clinical outcomes

In the group of patients with ALI/ARDS, VEGF levels in the pulmonary oedema fluid were not significantly different between survivors and nonsurvivors (p=0.39; fig. 2). Plasma VEGF levels were also not different in survivors *versus* nonsurvivors (median 10 pg·mL^{-1}, IQR 0–109 *versus* 1.1 pg·mL^{-1} IQR 0–105, p=0.84). There were also no significant differences between survivors and nonsurvivors in the hydrostatic oedema group. In plasma, the median VEGF level was 38 pg·mL^{-1} (IQR 0–152) in survivors *versus* 11 pg·mL^{-1} (IQR 0–133) in nonsurvivors (p=0.14). In oedema fluid, the median VEGF level was 590 pg·mL^{-1} (IQR 172–2,268) in survivors *versus* 1658 pg·mL^{-1} (IQR 612–3,157) in nonsurvivors (p=0.71). VEGF levels also did not differ significantly in patients with a longer duration of mechanical ventilation (<14 days of unassisted ventilation) in either patient group, nor was there any correlation between VEGF levels and the degree of hypoxemia as measured by the $P_{a,O_2}/F_{i,O_2}$ ratio (r=0.24) or the degree of physiological respiratory impairment as measured by the lung injury score (r=-0.31). Finally, in the ALI/ARDS group there was no association between VEGF levels (plasma or oedema fluid) and the presence or absence of pneumonia, sepsis or shock.

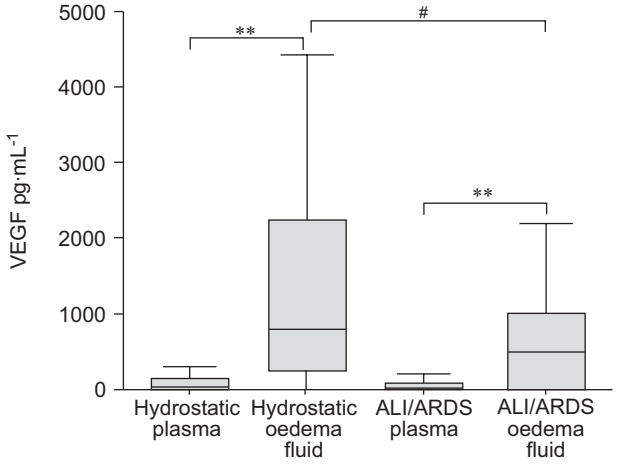

FIGURE 1. Soluble vascular endothelial growth factor (VEGF) levels in pulmonary oedema fluid and plasma from 56 patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) compared with 46 control patients with hydrostatic pulmonary oedema. n=41 (hydrostatic plasma), 43 (hydrostatic oedema fluid), 55 (ALI/ARDS plasma), 50 (ALI/ARDS oedema fluid). **: $p<0.01$; #: p= nonsignificant.

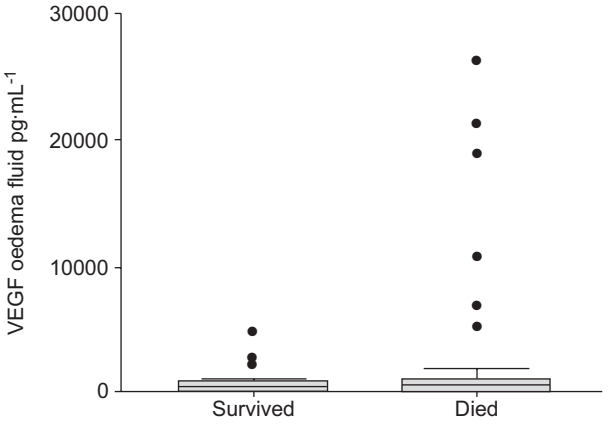

FIGURE 2. Vascular endothelial growth factor (VEGF) levels in the pulmonary oedema fluid in acute lung injury/acute respiratory distress syndrome patients. ●: outliers. n=20 (survived), 30 (died).

DISCUSSION

VEGF levels have been measured in a variety of acute and chronic lung diseases. BAL levels are lower than normal controls in smokers, patients with pulmonary fibrosis and sarcoidosis [15], and patients with high altitude pulmonary oedema [16]. In addition, very low levels have been reported in BAL fluid from patients with ALI/ARDS [9, 10], while plasma levels in ALI/ARDS were higher than normal controls [8]. Based on these reports the current authors hypothesised that levels of VEGF in undiluted pulmonary oedema would be lower in patients with ALI/ARDS compared with a severely ill group of mechanically ventilated control patients with hydrostatic pulmonary oedema. Contrary to the current authors' hypothesis, levels of VEGF in the alveolar compartment were not significantly different in patients with pulmonary oedema due to ALI/ARDS compared with hydrostatic causes. Plasma levels also did not differ by cause of pulmonary oedema. Thus, a reduction in alveolar levels of VEGF is not specific for ALI/ARDS and occurs in acute pulmonary oedema regardless of the aetiology.

The mechanisms that determine the levels of alveolar VEGF in the setting of acute pulmonary oedema are unclear. Since alveolar epithelial cells are an important source of VEGF in the

lung, possible modulators of VEGF levels include alveolar epithelial injury [17] and hypoxic upregulation of alveolar epithelial VEGF synthesis [7]. The overall drop in VEGF levels and lack of association between VEGF levels and the degree of hypoxia suggests that hypoxic upregulation of VEGF production is probably not the predominant determinant. The lack of difference between levels of VEGF in ALI/ARDS, characterised by alveolar epithelial injury [18], and hydrostatic oedema, typically not associated with alveolar epithelial injury, argues against a major contribution of alveolar epithelial injury to the low levels of VEGF measured in the oedema fluid. Median levels in the undiluted pulmonary oedema from both ALI/ARDS and hydrostatic pulmonary oedema were less than one tenth of the calculated levels of ~11,000 $pg \cdot mL^{-1}$ in normal epithelial lining fluid [5]. These low levels suggest that dilution as a result of alveolar flooding, rather than modulation of alveolar epithelial production of VEGF, may be the primary mechanism for the low VEGF levels observed in both the hydrostatic and ALI/ARDS oedema fluid.

The current findings can be contrasted with studies of other mediators of acute lung injury; for many mediators, there are substantial differences between alveolar concentrations in ALI/ARDS compared with hydrostatic pulmonary oedema. For example, levels of interleukin (IL)-8 in the oedema fluid of patients with ALI/ARDS are much higher than in hydrostatic oedema, suggesting a role for IL-8 in the neutrophil-dependent lung injury that may occur in some patients with lung injury [19]. Levels of plasminogen activator inhibitor-1 in the distal airspaces of patients with ALI/ARDS are also much higher than in hydrostatic oedema controls [20]. In addition, elevated levels of plasminogen activator inhibitor-1 were strongly associated with mortality.

What are the consequences of reduced levels of VEGF in the alveolar lining fluid in patients with acute pulmonary oedema? In both *in vitro* [21, 22] and *in vivo* studies [2], VEGF can increase endothelial permeability leading to a protein-rich oedema fluid. Alveolar-capillary barrier permeability as measured by the oedema fluid to plasma protein ratio (table 1) was markedly different between patients with hydrostatic oedema and those with ALI/ARDS, despite similarly low levels of VEGF in the pulmonary oedema fluid and plasma. It has been suggested that increased epithelial permeability in the setting of ALI may allow VEGF to have access to the lung microvascular endothelium, leading to increased endothelial permeability [23]. In support of this hypothesis, the oedema fluid to plasma gradient of VEGF tended to be higher in patients with hydrostatic oedema than ALI/ARDS ($p=0.07$), suggesting that differences in alveolar capillary barrier permeability might affect leakage of VEGF from the epithelial lining fluid permitting access to endothelial receptors from which they are normally restricted by compartmentalisation. However, plasma levels of VEGF were not different between the two groups. The findings from the current study do not resolve the issue of whether accessibility of VEGF to the endothelium under conditions of epithelial injury may be a mechanism of increased endothelial permeability in ALI/ARDS, exacerbating oedema formation.

Are there other potential consequences of the decline in VEGF levels in the alveolar compartment in acute pulmonary oedema? VEGF may play an important role in modulation of alveolar repair. TANG *et al*. [24] reported that lung-targeted VEGF inactivation in mice led to alveolar septal wall destruction, loss of lung elastic recoil and increased alveolar septal and bronchial epithelial cell apoptosis. Thus, a fall in VEGF levels might favour apoptosis of alveolar cells, specifically endothelial and epithelial cells, rather than proliferation and repair, leading to altered permeability of the alveolar capillary barrier. In cell culture, impaired proliferation of acid injured alveolar epithelial (A549) cells can be restored by exposure to VEGF. Thus, the rise in alveolar VEGF levels that has been reported during the resolution of ALI/ARDS [9, 10] may help to promote alveolar epithelial and endothelial proliferation and repair.

There are some limitations to the current study. First, oedema fluid and plasma were studied at only one time point in each patient, within the first few hours of intubation and, thus, the levels of VEGF later in the course of ALI/ARDS were not investigated. The time point for study was governed predominantly by the fact that pulmonary oedema fluid can usually only be collected in the first few hours after endotracheal intubation. As it is not diluted, oedema fluid has many advantages over BAL; no adjustment for dilution is necessary and simultaneous levels in the plasma and alveolar compartments can be compared. A second limitation is that the source of VEGF cannot be determined from the current studies. In order to better understand the complex role that VEGF plays in ALI and other forms of pulmonary oedema, more in depth experimental studies are needed of the cellular modulation of VEGF expression. A third limitation that is common to all of the published studies of VEGF in patients is that the precise biological activity of VEGF cannot be determined based on protein measurements alone. This is a common limitation of clinical studies of putative mediators of lung injury, although some studies have shown that the elevated levels of VEGF in the plasma of patients with ALI are associated with an increase in vascular permeability using *in vitro* assays [8].

In conclusion, these results emphasise the importance of having an appropriate control population of mechanically ventilated patients with hydrostatic pulmonary oedema when studying patients with acute lung injury. Vascular endothelial growth factor levels in pulmonary oedema fluid are depressed in patients with acute lung injury/acute respiratory distress syndrome and patients with hydrostatic pulmonary oedema. The mechanism for the decrease in air space concentrations of vascular endothelial growth factor in acute lung injury/acute respiratory distress syndrome may not be a function of the degree of lung injury but rather may result from alveolar flooding.

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