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Significance of vascular endothelium growth factor testing in exhaled breath condensate of patients with acute respiratory distress syndrome

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Abstract.
OBJECTIVE: We aimed to observe and investigate the clinical significance of vascular endothelium growth factor (VEGF) levels in exhaled breath condensate (EBC) from patients with acute respiratory distress syndrome (ARDS).
METHODS: An improved EcoScreen condenser was used to collect EBC from 31 ARDS patients on mechanical ventilation and from 22 healthy subjects. Serum and EBC VEGF levels were analyzed with ELISA. VEGF levels in the EBC of patients with different grades of lung injuries were analyzed. The correlation between VEGF levels and clinical indicators was analyzed.
RESULTS: Serum and EBC VEGF levels were linearly and positively correlated with a correlation coefficient of 0.694 ($P < 0.01$). The VEGF level in the EBC of ARDS patients was significantly lower than that in the control group ($P < 0.01$). The VEGF level in the EBC of the mild ARDS group was higher than that in the moderate-severe ARDS group ($P < 0.01$). The VEGF level in the EBC of the survival group was higher than that in the mortality group. The VEGF level in the EBC of ARDS patients was positively correlated with PaO_2/FiO_2 and PaO_2 and was negatively correlated with lung injury score (LIS) and A-aDO_2/PaO_2.
CONCLUSION: The changes in VEGF levels in the EBC of ARDS patients can Respiratory Medicine, reflect the severity of lung injury. Therefore, VEGF level in EBC can be used as an auxiliary index for judging the severity and prognosis of ARDS patients.

Keywords: Vascular endothelium growth factor, exhaled breath condensate, acute respiratory distress syndrome, mechanical ventilation, ELISA, PaO_2/FiO_2, lung injury score

1. Introduction

Acute respiratory distress syndrome (ARDS) is a diffused non-cardiogenic pulmonary edema that presents with impaired alveolar epithelial cells, increased pulmonary microvascular permeability and increased pulmonary interstitial and alveolar exudate proteins [1]. ARDS clinically manifests as acute

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respiratory failure that is characterised by reduced pulmonary compliance and refractory hypoxia. The mortality rate of ARDS in clinical practice is very high [2].

Systemic inflammatory response syndrome is a common route of infection, trauma and other factors that lead to ARDS. Inflammatory response is an important mechanism of ARDS pathogenesis. Neutrophils, alveolar macrophages and endothelial cells, which are inflammatory factors, mediate the occurrence and development of ARDS [3]. The accumulation and activation of neutrophils in the lung release injurious substances, such as proteolytic enzymes, superoxide and cytokines. Alveolar macrophages promote neutrophil infiltration and aggregation via the release of tumor necrosis factors (TNFs), interleukins (ILs) and leukotrienes. Pulmonary capillary endothelial cells release oxygen-free radicals and induce inflammatory cell injury in the vascular endothelium. These two processes can cause pulmonary edema. Alveolar epithelial cells and pulmonary vascular endothelial cells synthesize and release a variety of factors that mediate inflammation. These factors include oxygen free radicals, nitric oxide, 8-iso-prostaglandin, endothelin-1 and vascular endothelial growth factor (VEGF) [4].

VEGF is a multifunctional cytokine that promotes vascular permeability and induces angiogenesis. There are six subtypes of VEGF: A, B, C, D, E and placental growth factor (PLGF) [5]. Under normal physiological conditions, VEGF is moderately expressed in alveolar epithelial cells, bronchial epithelial cells and bronchial glandular cells.

Inflammatory reactions can be monitored as a simple, noninvasive, reliable and reproducible laboratory objective index for ARDS [6]. Therefore, this study aimed to determine VEGF levels in the exhaled breath condensate (EBC) of ARDS patients, to explore the relationship between VEGF levels and ARDS severity and to evaluate the clinical value of VEGF detection in EBC.

2. Objects and methods

2.1. Research object

We selected 31 patients with ARDS who underwent mechanical ventilation at the Intensive Care Unit (ICU) of the Second Affiliated Hospital of Nantong University from September 2015 to June 2018. None of the patients had a primary pulmonary disease prior to mechanical ventilation. The ARDS group comprised of 18 males and 13 females with ages of 21–84 years, with an average of 60.1 ± 15.7 years. Underlying diseases included multiple injuries, acute perforation of the digestive tract, acute intestinal obstruction, acute upper gastrointestinal bleeding, pelvic fractures, femoral fractures, postoperative ovarian cancer and placenta previa. The average time of stay in the ICU was nine days. Mechanical ventilation time was seven days. Mechanical ventilation followed by protective lung ventilation strategy. All patients received broad-spectrum antibiotics and comprehensive support therapy. Patients met the 2012 ARDS Berlin diagnostic criteria [7]. The patients were classified into the mild ARDS (200 mmHg $<$ $PaO_2/FiO_2 \leqslant 300$ mmHg) and moderate-severe ARDS groups ($PaO_2/FiO_2 \leqslant 200$ mmHg) in accordance with the oxygenation index [8]. The mortality rate among the patients was 32.3% given that 10 out of the 31 ARDS patients died. These 10 patients died of shock, gastrointestinal bleeding and arrhythmia, amongst others. All 31 ARDS patients were excluded from acute respiratory failure caused by chronic pulmonary diseases, such as active pulmonary tuberculosis, lung cancer, bronchial asthma, bronchiectasis, interstitial lung disease and acute exacerbation of chronic obstructive pulmonary disease. The general clinical situation of patients is presented in Table 1. During the same period, 22 healthy persons were selected for the normal control group. This group comprised of 13 males and nine females with ages of 23–81 years with an average of 62.4 ± 13.8 years. No statistical differences in age and gender were found between the ARDS and control groups.

Table 1
General clinical situation of ARDS patients

Name of underlying diseases	Cases	Operation cases	Mild ARDS mortality (%)	Moderate-severe ARDS mortality (%)
Multiple injuries	15	8	33.3 (2/6)	44.4 (4/9)
Acute perforation of digestive tract	4	4	0.0 (0/2)	50.0 (1/2)
Acute upper gastrointestinal bleeding	3	1	100.0 (1/1)	50.0 (1/2)
Acute intestinal obstruction	3	2	0.0 (0/2)	0.0 (0/1)
Pelvic fractures	2	0	0.0 (0/1)	0.0 (0/1)
Femoral fractures	2	1	0.0 (0/1)	0.0 (0/1)
Postoperative ovarian cancer	1	1	0.0 (0/1)	0.0 (0/0)
Placenta previa	1	0	0.0 (0/0)	100.0 (1/1)
Total	31	17	21.4 (3/14)	41.2 (7/17)

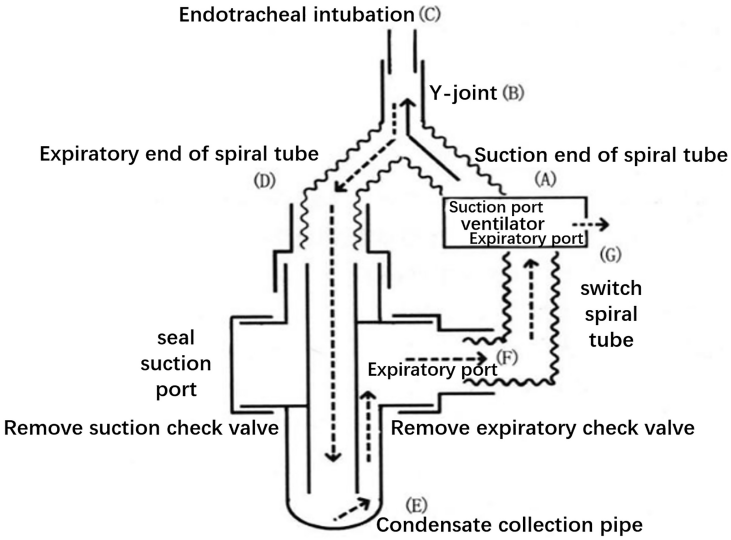

Fig. 1. Schematic diagram of the connection between the modified condenser and the ventilator.

2.2. Methods

2.2.1. Sample collection

EBC specimens were collected within 24 h after definitive ARDS diagnosis. Prior to collection, the ventilator pipe was replaced with a dry threaded pipe and disconnected from the humidifier. The modified EcoScreen condenser was connected in series to the end of the ventilator pipe (Fig. 1). The condenser collected 2 ml of EBC for 20 min. EBC was collected from the control group by mouth breathing. A total of 5 ml of venous blood was simultaneously collected per subject. To extract serum, blood samples were centrifuged at 4000 rpm for 5 min. The samples were preserved in a −70°C freezer [9]. The environment of the ICU ward was maintained at 20°C–25°C and 45%–50% humidity [10].

2.2.2. Arterial blood gas analysis

Prior to EBC collection, arterial blood (radial artery or femoral artery or dorsal artery) was collected with a Roche OMNIC blood gas analyser.

2.2.3. Chest X-ray examination

Prior to EBC collection, patients were examined with a bedside X-ray.

Table 2
Lung injury score (LIS)

Score	0	1	2	3	4
X-ray score	0	$0 \sim 1/4$	$1/4 \sim 2/4$	$2/4 \sim 3/4$	$3/4 \sim 1$
PaO_2/FiO_2 (mmHg)	$\geqslant 300$	$225 \sim 299$	$175 \sim 224$	$100 \sim 174$	$\leqslant 99$
PEEP (cmH_2O)	$\leqslant 5$	$6 \sim 8$	$9 \sim 11$	$12 \sim 14$	$\geqslant 15$
Static compliance (ml/cmH_2O)	$\geqslant 80$	$60 \sim 79$	$40 \sim 59$	$20 \sim 39$	$\leqslant 19$

Table 3
Comparison of EBC and serum VEGF levels between ARDS and the healthy group ($\bar{x} \pm$ s)

	N	EBC	Serum
		VEGF (ng/L)	VEGF (ng/L)
ARDS group	31	49.22 ± 7.70	1248.12 ± 315.24
Healthy group	22	56.62 ± 7.06	1373.11 ± 375.84
t		3.566	1.312
P		< 0.01	0.195

2.2.4. Detection method

VEGF levels in EBC and serum were measured by enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's protocols [11]. The kits were purchased from Bender Med Systems GmbH (Austria).

2.2.5. Observation index

The lung injury score (LIS) included PaO_2/FiO_2, positive end expiratory pressure (PEEP), X-ray score and static compliance of respiratory system = VT/(P_{plat}-PEEP). LIS was divided by the sum of the number of projects. The LIS is shown in Table 2. We also used A-aDO_2/PaO_2 as the oxygenation index.

2.3. Statistical analysis

Statistical analysis was performed with SPSS13.0 statistical software. Quantitative data were subjected to the normal distribution test and χ^2 test. Normally distributed quantitative data were presented as mean $\pm$ standard deviation ($\bar{x} \pm$ s). Two samples were compared using the t-test. The relevance of VEGF in EBC and in serum was determined using correlation analysis. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Comparison of EBC and serum VEGF levels between ARDS group and healthy group

The VEGF level in the EBC of the ARDS group (49.22 ± 7.70 ng/L) was significantly lower ($P < 0.01$) than that in the healthy group (56.62 ± 7.06 ng/L, Table 3).

3.2. Correlation between EBC and serum VEGF levels

Serum and EBC VEGF levels were positively correlated with a correlation coefficient of 0.694 ($P < 0.01$, Fig. 2).

Table 4
Comparison of EBC and serum VEGF levels in mild ARDS group and moderate-severe ARDS group ($\bar{x} \pm$ s)

	N	EBC	Serum
		VEGF (ng/L)	VEGF (ng/L)
Mild ARDS	14	55.44 ± 3.50	1150.62 ± 348.56
Moderate-severe ARDS	17	44.09 ± 6.28	1328.41 ± 269.17
t		6.031	1.603
P		< 0.01	0.120

Table 5
Comparison of EBC and serum VEGF levels in survival and mortality groups ($\bar{x} \pm$ s)

	N	EBC	Serum
		VEGF (ng/L)	VEGF (ng/L)
Survival group	21	52.25 ± 5.88	1282.84 ± 341.10
Mortality group	10	42.84 ± 7.35	1175.21 ± 252.94
t		3.843	0.885
P		< 0.01	0.383

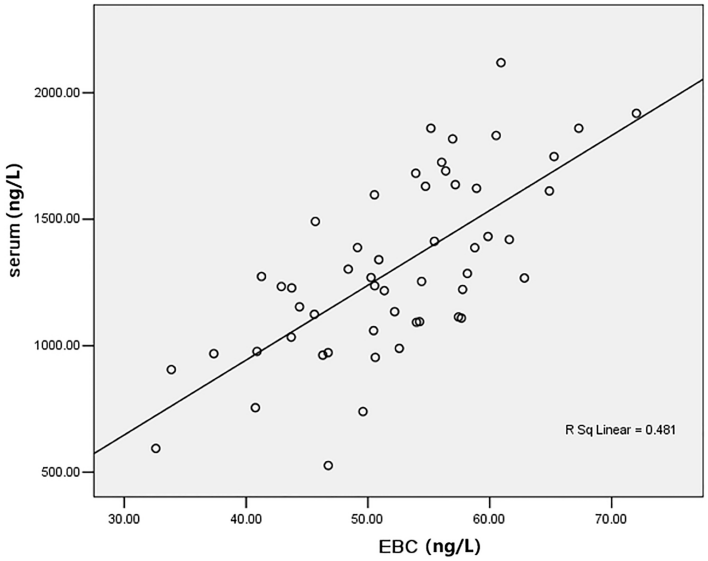

Fig. 2. Correlation scatter diagram of EBC and serum level of VEGF (ng/L).

3.3. Comparison of EBC and serum VEGF levels in mild ARDS group and moderate-severe ARDS group

The VEGF level in the EBC of the mild ARDS group (55.44 ± 3.50 ng/L) was significantly higher ($P < 0.01$) than that in the moderate-severe ARDS group (44.09 ± 6.28 ng/L, Table 4).

3.4. Comparison of EBC and serum VEGF levels in survival and mortality groups

The VEGF level in the EBC of the survival group (52.25 ± 5.88 ng/L) was significantly higher ($P <$ 0.01) than that in the mortality group (42.84 ± 7.35 ng/L, Table 5).

Table 6
Correlation analysis between VEGF and clinical indexes in patients with ARDS

Clinical index	EBC VEGF		Serum VEGF	
	r	P	r	P
LIS	−0.406	< 0.05	−0.074	> 0.05
Static compliance	0.132	> 0.05	0.069	> 0.05
PaO_2/FiO_2	0.579	< 0.01	0.256	> 0.05
A-aDO_2/PaO_2	−0.490	< 0.05	0.078	> 0.05
PaO_2	0.601	< 0.01	0.271	> 0.05

3.5. Correlation analysis between VEGF and clinical indexes in patients with ARDS

Table 6 shows that the VEGF level in EBC was positively correlated with PaO_2/FiO_2 and PaO_2 and was negatively correlated with LIS score and A-aDO_2/PaO_2. However, there was no significant correlation with lung compliance. In addition, VEGF level in serum and the above indexes were not significantly correlated.

4. Discussion

ARDS is caused by excessive and uncontrolled inflammatory reactions that are triggered by neutrophil activation and oxygen-free radical injuries. Inflammation changes lung microvascular permeability and the morphology and function of pulmonary microvascular endothelial cells [12]. Moreover, inflammation causes the vascular leakage of albumin to tissue, which results in hypoxemia. In this process, VEGF increases endothelial cell vesicles to act as vascular permeability regulator. VEGF is 50,000 times more potent than histamine [13]. The effects of VEGF cannot be blocked by antihistamines and platelet activating factor inhibitors. VEGF has a protective effect on ARDS and an important role in the survival of epithelial cells that are cultured *in vitro*. Alveolar epithelial cells proliferate in the presence of exogenous VEGF. Moreover, VEGF can repair damaged alveolar capillary membrane barriers, improve pulmonary edema and promote ARDS recovery [14].

The diagnosis and staging of ARDS mainly depends on comprehensive judgment that uses patient history, chest X-rays and arterial blood gas results. Clinical workers have focused on identifying the severity of ARDS by detecting the level of inflammatory factors and changes in the patient's body. The detection of EBC components has provided a new way to further study the mechanism of oxidative stress response in critically ill patients.

EBC, a mixture of mucus and volatile substances in respiratory tract liquid, can reflect various physiological and pathological changes in the body [15]. The collection and detection of EBC are advantageous in detecting early pathological changes in the lower respiratory tract and lung parenchyma. Given that EBC is directly derived from the airway, the physiological and biochemical function of the lungs can be detected without damaging bronchial mucosa [16]. In addition, EBC can produce reliable and reproducible test results and can be collected using simple methods that are suitable for patients of any age and any illness [17]. EBC collection is especially advantageous for diagnosing patients on mechanical ventilation. The repeated collection of EBC can dynamically detect airway inflammation and oxidative stress in critically ill patients [18,19].

This research revealed that VEGF levels in the EBC of ARDS patients on mechanical ventilation were lower than those in the control group. And our results demonstrated that VEGF levels in the EBC of

the mild ARDS group were significantly higher than those in the moderate-severe ARDS group and significantly higher in the survival than that in the mortality group. These results were consistent with decreased VEGF levels in the lung tissue and bronchoalveolar lavage fluid (BALF) of ARDS patients. VEGF levels decreased because: 1) The pulmonary vascular permeability of ARDS patients increased and more liquid accumulated in the alveolar dilution [20]. 2) Pulmonary edema directly damaged alveolar epithelial cells. The expression of IL-6, IL-8, TNF and other cytokines can indirectly damage alveolar epithelial cells. 3) Proteolytic enzymes that were released from neutrophils and other inflammatory cells accelerated the degradation of VEGF [21]. 4) Damaged alveolar capillary barriers released VEGF from the lungs to the blood. 5) VEGF was released as a self-protective mechanism. Given that the level of VEGF in EBC reflects the severity of lung injury and contributes to disease prognosis, EBC is a good objective index for evaluating the condition of ARDS [22].

We found that VEGF levels in EBC were significantly correlated with the index of oxygenation, positively correlated with PaO_2/FiO_2 and PaO_2 and negatively correlated with A-aDO_2/PaO_2 and LIS score. VEGF levels in EBC may be associated with damaged alveolar epithelial integrity, disordered surfactant synthesis, imbalanced ventilation/perfusion and decreased dispersion function. Therefore, decreased VEGF in EBC is a good index that reflects the function of pulmonary ventilation and oxygenation [23].

Although the differences in EBC VEGF levels among groups were statistically significant, there was no significant difference in serum VEGF levels. These results implied that changes in VEGF levels in EBC reflect the severity of lung injury, as previously reported by Pickkers [24].

In conclusion, the EBC assay can be used to analyze the changes of VEGF content in the epithelial lining of the respiratory tract to directly understand the degree of inflammation in the lung [25]. This diagnostic method is suitable for ARDS patients on mechanical ventilation. Hence, EBC levels of VEGF can be used as an index for judging the severity of illness and evaluating therapeutic effect.

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Conflict of interest

None to report.

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