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Vascular endothelial growth factor (VEGF) expression in the lung in toxic septic shock

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Abstract

Introduction: The need for reasoning with medical evidence the different types of shock, especially when there are medical and legal implications, has determined the search of biological markers of the shock. In the case of toxic septic shock, the most important markers to be used are: the cytokines, the tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6), procalcitonin, lactoferin and the vascular endothelial growth factor (VEGF). VEGF has an essential role in angiogenesis and vascular permeability. *Materials and Methods*: In our study group, we included 30 cases of different types of shock in which we studied the VEGF expression in the lungs. We added also 10 fragments of lung as control group. According to the etiology, the 30 cases of shock were: 15 with a toxic septic shock and 15 with a hemorrhagic shock. In all these cases we used the classical Hematoxylin and Eosin staining method and the immunohistochemical reactions for VEGF-A. Statistical analysis was performed using SPSS 13.0. *Results*: The VEGF expression was decreased in all the cases of toxic septic shock, in the endothelium and also in the alveolar epithelium, compared to a high level of expression in other cases of shock and in the control lung. *Conclusions*: These data allow us to appreciate that VEGF has a different expression in different types of shock and in the normal lung. We observed a statistically significant difference between VEGF expression in toxic septic shock and hemorrhagic shock (p=0.000001). There is a similarity of VEGF expression between hemorrhagic shock and the control lungs (p=0.00001). An obviously low VEGF expression in the toxic septic shock represents a useful biological marker in the forensic medical cases.

Keywords: toxic septic shock, VEGF-A, markers.

Introduction

In some cases, the clinical and morphological changes in the macroscopic and microscopic post-mortem forensic expertise cannot clearly differentiate between types of shock. There are studies showing the utility of biological markers in medical substantiation of the type of shock, especially when there are forensic medical implications on the legal aspects of cases [1, 2].

In toxic septic shock, the potential useful markers are: cytokines, particularly tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), procalcitonin, lactoferrin and VEGF [3, 4].

Normally, the pro-inflammatory mediators as cytokines (TNF-α and IL-6), are released following the assault of infections or other disorders in order to eliminate pathogens and promote healing. Vascular endothelial growth factor (VEGF) has an essential role in angiogenesis and vascular permeability [5].

VEGF has an uneven distribution in normal human tissues, being immunolocalized in both endothelial and also epithelial cells from different tissues. This suggests as possible mechanism of transport of plasmatic factors by increasing the permeability of both endothelial, as well as epithelial cells [6].

This research aims to study lung immunohisto-chemical VEGF expression in toxic septic and hemorrhagic shock, using some cases of forensic medicine, given the relatively limited information in the literature on this issue.

Materials and Methods

Our study was performed on 30 cases of shock to which we added a control group of 10 lung tissue fragments obtained from individuals who died of other causes than pulmonary diseases or shock.

Depending on the etiology, the cases were classified in two groups as follows:

- Group I: toxic septic shock – 15 cases;
- Group II: hemorrhagic shock – 15 cases.

The assessed parameters were:

- The number of cells with VEGF expression compared with the total number of cells counted;
- The intensity of immunohistochemical reaction;
- Localization of the marker at endothelial and epithelial level and in macrophages.

Lung fragments taken from these cases were processed by the classic histopathological technique of paraffin embedding, microtome sectioned in 5 μm thick slices and stained with the classical, standard Hematoxylin and Eosin method [7, 8].

The VEGF-A expression was subsequently revealed using the immunohistochemical technique [9]. Sections for immunohistochemistry (IHC) were displayed on special slides, Superfrost Ultra Plus, and incubated in the thermostat at 60^0C; they were dewaxed in toluene, dehydrated in alcohol and then we proceeded to expose the antigen in a steamer at 100^0C in EDTA–TRIS solution at pH 7.5 for 30 minutes.

Endogenous peroxidase was inhibited with 3% hydrogen peroxide for 3 minutes. Superblock was then used 30 minutes to block nonspecific sites.

The sections were then incubated with the primary antibody VEGF (A-20, code sc-152, lot C2106) rabbit polyclonal, Santa Cruz, 1:500 dilution, for 18 hours (overnight) at 4^0C. The working system was ENVISION DUAL (rabbit-mouse) for 30 minutes and the final reaction product was visualized with diaminobenzidine (DAB +) in brown color.

Nuclei were counterstained with Hematoxylin, followed by rehydration, and toluene passage. Finally, the sections were mounted in Canada balm. The external positive control was performed on normal pulmonary tissue and the internal positive control was the chondrocyte.

All cases were processed simultaneously under the same environmental conditions, using the same dilution of the marker, and a single container of staining reactant.

Examination of slides was performed with a Nikon Eclipse E600 microscope equipped with a DN100 digital camera with 1280×1024 pixels resolution. Quantification of VEGF expression was performed using the LuciaNet image analysis software.

Statistical analysis was performed with the SPSS 13.0 software.

For each case, 900 cells were randomly selected from several random non-overlapping fields using a ×400 magnification and evaluated, 300 of each main cellular type:

- endothelial cells;
- epithelial cells (bronchial and alveolar);
- macrophages.

Measurements were preceded by software calibration for the used magnification. The unit used was μm. The values obtained were recorded in tables of the LuciaNet software.

For each parameter, average values and standard deviations were estimated.

Results

Histopathologic examination showed different aspects regarding lung damage.

The toxic septic shock cases showed the appearance of cellular proliferative phase of diffuse alveolar damage (DAD), with pneumocyte hyperplasia, fibroblasts and inflammatory elements in the interstitial tissue and in the fibrinous exudate of alveoli.

It also showed the organization of fibrine exudate, with resultant intra-alveolar fibrosis. These facts indicate that in some cases, the clinical course exceeded seven days. In two of the 15 cases with toxic septic shock, we observed an exudative phase, with edema in the interstitial tissue and in alveoli, hyaline membranes and fibrin exudation in the alveoli and alveolar ducts, rare alveolar epithelial necrosis. In cases of hypovolemic shock, the aspect of lung parenchyma showed alveolar and interstitial areas of edema. The assessed parameters of cells expressing VEGF, the intensity and localization of the marker are presented in Table 1.

Table 1 –*VEGF parameters in studied groups*

Cases with shock and control group	Number of cells with VEGF expression / %	Marker localization			Intensity of the stain
		Endothelial cells	*Epithelial cells*	*Macrophages*	
Toxic septic shock	124/13.78%	59	46	19	Low
	116/12.89%	44	50	22	Low
	134/14.89%	61	42	21	Low
	152/16.89%	76	48	28	Moderate
	143/15.89%	64	43	36	Low
	179/19.89%	74	63	42	Low
	110/12.22%	35	44	31	Low
	0/0%	0	0	0	Null
	52/5.78%	24	16	12	Very low
	97/10.78%	41	35	21	Low
	73/8.11%	32	24	18	Low
	139/15.44	59	41	39	Low
	126/14.0%	47	42	37	Low
	163/18.11	71	54	38	Low
	104/11.56%	23	47	34	Low
Hemorrhagic shock	757/84.11%	267	251	239	Strong
	626/69.56%	222	206	198	Strong
	749/83.22%	305	276	168	Strong

Cases with shock and control group	Number of cells with VEGF expression / %	Marker localization			Intensity of the stain
		Endothelial cells	*Epithelial cells*	*Macrophages*	
	578/64.22%	211	208	159	Moderate
	665/73.89%	245	219	201	Strong
	579/64.33%	203	186	190	Strong
	445/49.44%	167	153	125	Moderate
	521/57.89%	213	173	135	Strong
	634/70.44%	179	247	208	Strong
	537/59.67%	204	135	198	Moderate
	786/87.33%	307	254	225	Strong
	694/77.11%	283	253	158	Strong
	632/70.22%	254	235	143	Strong
	587/65.22%	201	223	163	Strong
	621/69.00%	247	205	169	Strong
Control group	678/75.33%	267	231	180	Strong
	739/82.11%	324	286	129	Strong
	665/73.89%	261	256	148	Strong
	723/80.33%	301	305	117	Strong
	687/76.33%	321	267	99	Moderate
	653/72.56%	266	304	83	Strong
	732/81.33%	298	342	92	Strong
	654/72.67%	186	377	91	Strong
	696/77.33%	193	361	141	Strong
	578/64.22%	176	378	24	Moderate

The intensity of immunohistochemical stain in cases with toxic septic shock was significantly lower than both normal (χ^2=22.22; GL=2; p=0.00001), and also the cases with hemorrhagic shock (χ^2=27.0; G=2; p=0.000001) (Figure 1).

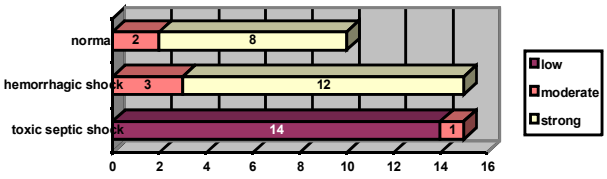

Figure 1 – ***The intensity of the staining reaction in the study groups.***

VEGF expression was different: in all cases of toxic septic shock, we obtained a low expression in both the endothelial and alveolar epithelium, particularly in type II pneumocytes (Figures 2 and 3).

Compared to control samples, the decrease of VEGF expression in toxic septic shock was measured as a percentage between 50 and 90%. Only one case showed complete absence of the marker.

In the other types of shock, VEGF expression was stronger in the endothelium and alveolar epithelium and also intense in the epithelium of bronchioles compared with VEGF expression in cases with septic shock (Figures 4–6).

On control sections, VEGF expression was strong in the epithelium of the alveoli, the bronchial epithelium, macrophages and in intra-alveolar macrophages (Figures 7 and 8).

Toxic septic shock cases with characteristic histopathologic expression of exudative phase showed stronger VEGF expression than those in proliferative phase (Figure 9).

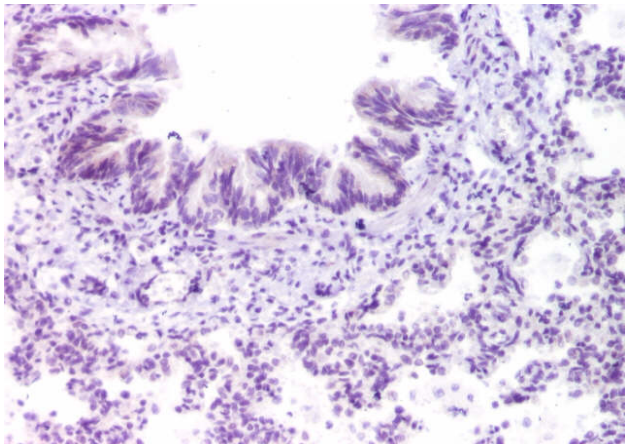

Figure 2 – ***VEGF expression in septic shock: endothelium and alveolar epithelium (much lower than in controls or in other cases with shock) (×100).***

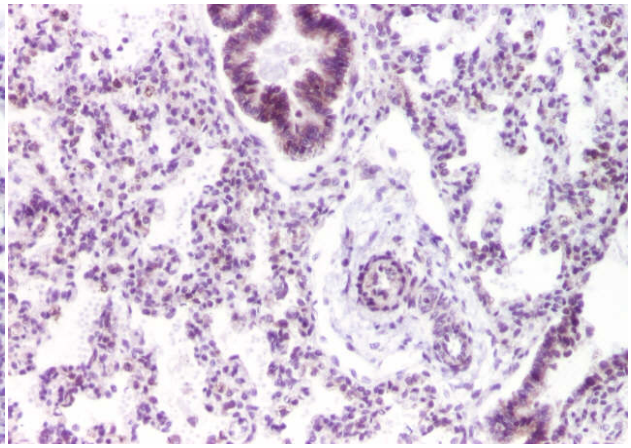

Figure 3 – ***VEGF expression in septic shock: endothelium and alveolar epithelium (much lower than in controls or in other cases with shock) (×100).***

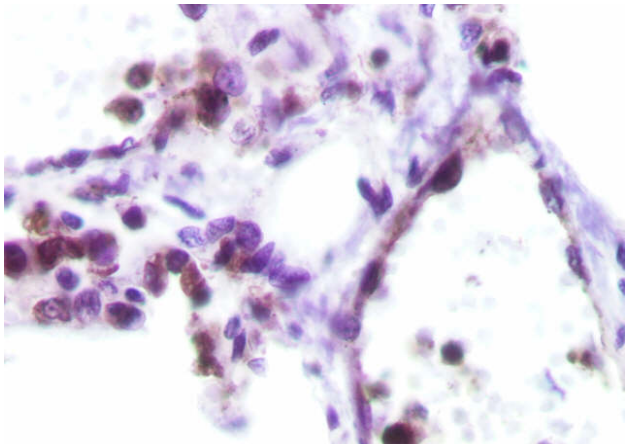

Figure 4 – ***Strong expression of VEGF in hemorrhagic shock: the alveolar epithelium, bronchioli, endothelium and cartilage (×200).***

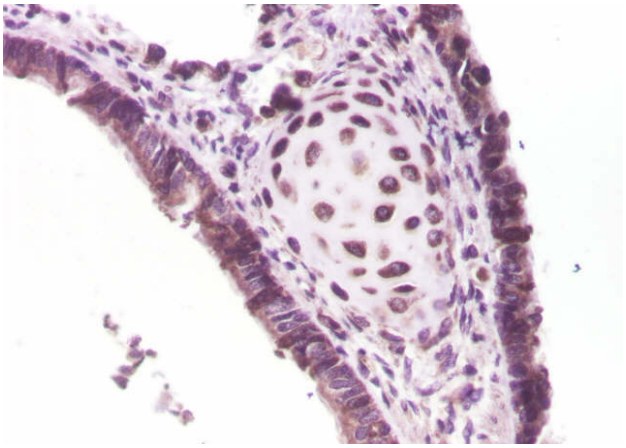

Figure 5 – ***Strong expression of VEGF in hemorrhagic shock: the alveolar epithelium, bronchioli, endothelium and cartilage (×200).***

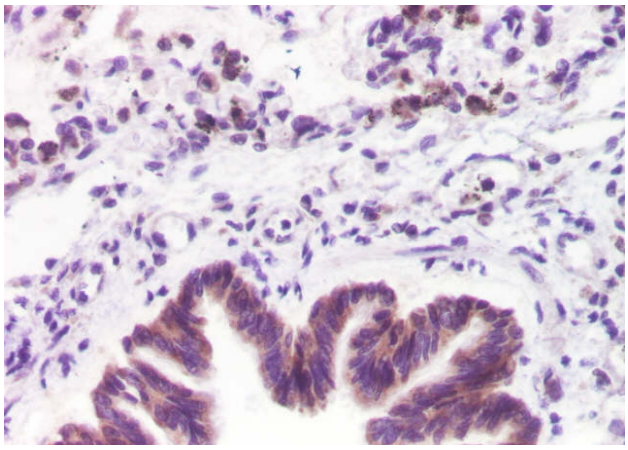

Figure 6 – ***Strong VEGF expression in alveolar macrophages in hemorrhagic shock (×200).***

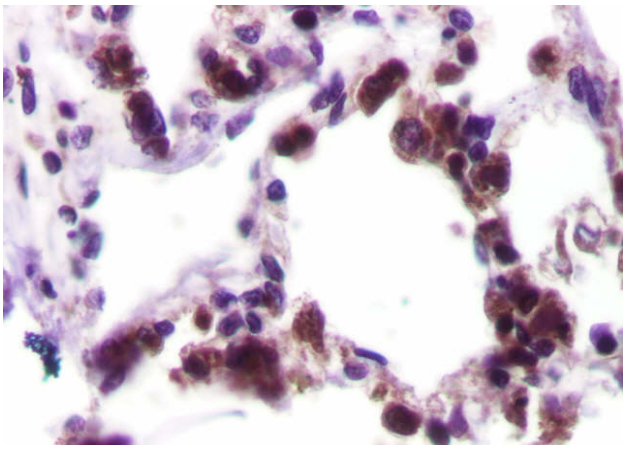

Figure 7 – ***Strong expression of VEGF in the alveolar epithelium (control) (×400).***

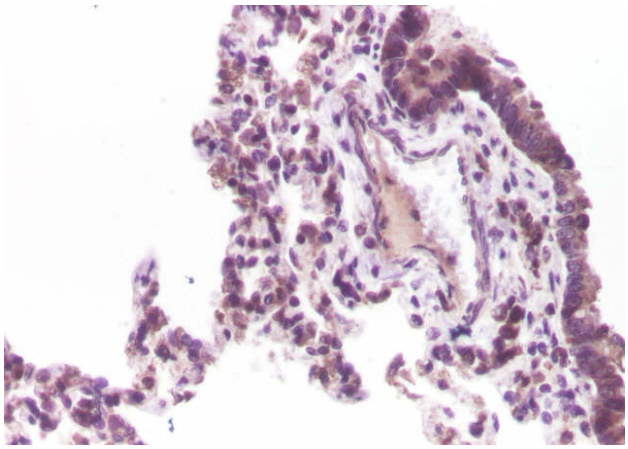

Figure 8 – ***Strong expression of VEGF in blood vessels, bronchioles and alveoli (control) (×200).***

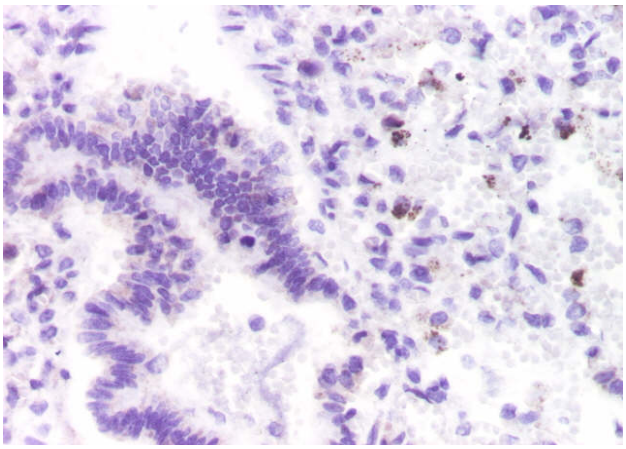

Figure 9 – ***Reduced VEGF expression in alveolar macrophages in septic shock (×200).***

Discussion

Vascular endothelial growth factor (VEGF) is a glycoprotein, which has six isoforms with 121, 145, 165, 183, 189 and 206 amino acids respectively, namely VEGF 121, VEGF 145, VEGF 165, VEGF 183, VEGF 189 and VEGF 206. These isoforms have different binding capacities for heparin, which affects their solubility. Many studies identified two types of tyrosine kinase VEGF receptors, flt-1 and flk-1/KDR. The two receptors can mediate different functions of VEGF [10, 11]. VEGF is a potent mitogen factor for vascular endothelium and stimulates vascular permeability.

These two properties are important in the cascade of biological events, which lead to angiogenesis and affects vascular permeability [12, 13]. Vascular endothelial growth factor (VEGF) has a mitogenic effect on endothelial cells *in vitro* and is a major angiogenic factor *in vivo*. VEGF is actually a family of growth

factors with multiple isoforms, including VEGF-A which has been shown to be angiogenic, and VEGF-C, which is the most powerful lymphangiogenic agent.

To study the lung VEGF expression in various types of shock it is necessary to know that usually there is a strong expression in normal lung, especially in the alveolar epithelium and bronchial glands, bronchial cells and alveolar activated macrophages [3, 14]. Also, there is normally an increased expression of VEGF in the cartilage, which increased the use of this marker as internal control [5, 15]. In our study, a decreased expression of VEGF in cases of toxic septic shock compared with its high expression in hypovolemic shock and in control group, allowed us to appreciate that VEGF expression is different in different types of shock, and compared to the normal lung.

Although the precise mechanism of this decrease of VEGF expression in toxic septic shock is not yet clear, many researchers believe that excessive release of pro- and anti-inflammatory mediators in septic shock could block VEGF expression [16].

It is clear now that VEGF expression may be an important determinant of sepsis morbidity and mortality, but also in other diseases such as hematologic malignancies [17–20].

Conclusions

Our results allow us to advance some conclusions, which at least statistically, argue that VEGF expression in the lung in septic shock is much reduced to its absence in the endothelium, bronchial and alveolar epithelium, and even in alveolar macrophages. Low expression of VEGF in cases of toxic septic shock compared with its high expression in hypovolemic shock, and in control group, allows us to appreciate that VEGF expression is different in different types of shock, and from normal lung (p=0.00001). There is enough evidence that the study of VEGF expression in toxic septic shock is a useful biological marker in forensic cases.

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