

Case Report

Immunohistochemical expression of MPO, CD163 and VEGF in inflammatory cells in acute respiratory distress syndrome: a case report

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Abstract: Acute respiratory distress syndrome (ARDS) is a serious medical condition occurring in patients with poly-trauma, pulmonary or non-pulmonary sepsis, pneumonia and many other circumstances. It causes inflammation of the lung parenchyma leading to impaired gas exchange with a systemic release of inflammatory mediators, causing consequential lung tissue injury, hypoxemia and frequently multiple organ failure. The aim of current study was to describe expression of inflammatory markers (myeloperoxidase, CD163 and vascular endothelial growth factor) by the cells in acute phase of ARDS. The lung samples of a 20-year-old man who had suffered a serious motorbike accident were obtained for histological examination. He died on the seventh day as a consequence of respiratory failure. Our results imply that expression of CD163 was restricted to activated alveolar macrophages and monocytes. Immunopositivity of MPO was observed in neutrophil granulocytes within lung alveoli and lung blood vessels. Myeloperoxidase positivity was observed in alveolar macrophages, too. Vascular endothelial growth factor was expressed in cytoplasm of neutrophil granulocytes, monocytes, small-sized alveolar macrophages and type II pneumocytes localized mostly inside lung alveoli. On the contrary, no positivity was observed in lung endothelial cells of blood vessels.

Keywords: ARDS, myeloperoxidase, VEGF, CD163, immunohistochemistry

Introduction

Acute respiratory distress syndrome (ARDS) is a severe condition occurring in intensive care units. It represents clinical syndrome of non-cardiogenic pulmonary oedema associated with bilateral pulmonary infiltrates, stiff lungs and refractory hypoxemia. ARDS is an inflammatory disease initiated by a wide variety of systemic and/or pulmonary insults that lead to disruption of the alveolar-capillary unit and to a breakdown in the barrier and gas exchange functions of the lung [1]. Berlin definition in 2012 corrected criteria for determination of ARDS consisting of acute onset (within one week of known clinical insult), CT or RTG findings, non-cardiogenic pulmonary oedema and oxygenation changes. A draft definition proposed 3 mutually exclusive categories of ARDS based on degree of hypoxemia: mild, moderate

and severe degree [2]. Histopathological features of the individual stages of ARDS are characteristic, but sometimes these stages cross each other. Most prominent and acute changes can be observed in exudative stage with formation of protein rich fluid in the interstitium and alveoli. In addition, hyaline membrane formation, necrosis of type I pneumocytes and destruction of alveolar basement membrane can be seen. Among several cells involved in this exudative stage play essential role lung neutrophils with their active substances. Their accumulation starts in pulmonary capillaries and during ARDS progress they migrate to the lung interstitium and alveolar lumen [3]. Many other cells such as macrophages, monocytes and vascular endothelium are involved in exudative stage of ARDS by secretion of inflammatory mediators [4, 5]. The aim of our work was to describe and identify inflammatory cells using

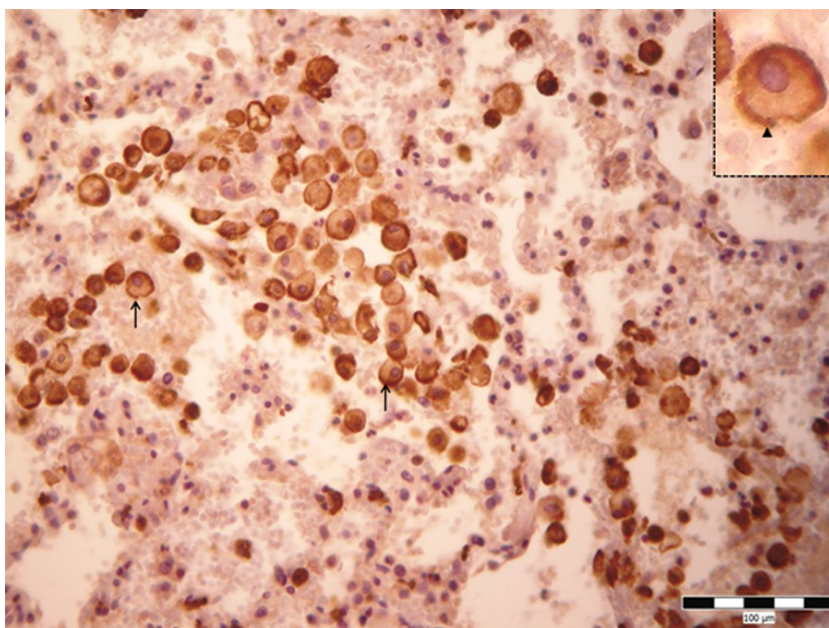


Figure 1. Cytoplasmic expression of CD163 in monocyte-macrophage cell population (arrows). Strong CD163-immunopositivity of cell membrane surface is seen (arrowhead, left corner-detail).

immunohistochemical detection of selected markers-myeloperoxidase (MPO), CD163 and vascular endothelial growth factor (VEGF).

Material and methods

Clinical features

A motorcycle rider (20-year-old male) was involved in a high-energy collision with a car, suffering traumatic amputation of the upper right arm in its middle third, compound fractures of the right thigh and right tibia with soft tissue devastation and problematic blood perfusion in the region. Patient was in hemorrhagic shock with 40% estimated blood loss (initial haemoglobin 59 g/l), 80/40 mmHg blood pressure, 120 beats per minute heart rate and mental confusion. Spiral head and torso CT scan revealed no other organ injury. After surgical soft tissue debridement and bone stabilization (external fixator, Kirschner-wire skeletal traction) the patient was transferred to the intensive care unit for further treatment. On the second day no signs of lung parenchyma infiltration were revealed by plain X-ray of the chest. There was an unsuccessful attempt to wean the patient from mechanical ventilation. On the third day after injury, diffuse crackles on chest auscultation and infiltration of the lung paren-

chyma at the right and left bases with right pleural effusion were revealed by X-ray. Positive end-expiratory pressure (PEEP) mode of mechanical ventilation was instigated. On the fourth day the patient became cyanotic with a drop in oxygen saturation to 72%. Emergency therapeutic fiberoptic bronchoscopy was carried out with suctioning of pink-tinged clear frothy fluid, and the right pleural space was drained with active suction. On the fifth day a CT scan of the lungs revealed signs of ARDS, atelectasis and minimal bilateral pleural effusions. Despite treat-

ment with higher levels of PEEP with an increased fraction of inspired oxygen in the gas and repeated therapeutic fiberoptic bronchoscopies, the patient died from his injuries on day seven.

Immunohistochemical procedure

Necroptic lung specimens were harvested and immediately fixated in 4% paraformaldehyde and embedded in Paraplast wax, sectioned in 4-5 μm tissue slices. Histological sections were deparaffinised and rehydrated. Endogenous peroxidase activity was blocked with 3% H₂O₂ with methanol. Pre-treatment was performed in a microwave oven at 600 W for 15 min in 0.01 M citrate buffer at pH 6.0. A primary anti-MPO rabbit polyclonal antibody (Thermo Scientific, MA, USA), primary anti-VEGF rabbit polyclonal antibody (Thermo Scientific, MA, USA) and anti-CD163 rabbit clonal antibody (DB-Biotech, Košice, Slovakia) were used. Primary antibodies were labelled using a two-stage indirect immunoperoxidase technique. Primary antibodies were applied at the appropriate titre. Biotinylated secondary anti-mouse IgG (H+L)/anti-rabbit IgG (H+L) (Thermo Scientific, MA, USA), was used in labelling with R.T.U. Vectastain ABC Reagent (Vector Laboratories) for detection of MPO-positive cells. Biotinylated second-

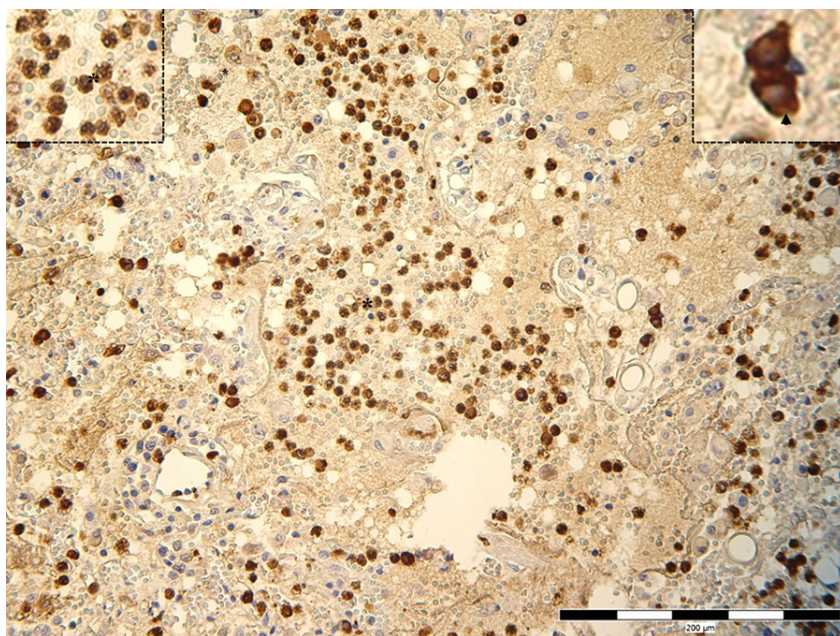


Figure 2. Cytoplasmic expression of MPO in neutrophil granulocytes within lung alveoli (asterisk). Strong MPO-immunoreactivity is seen in cytoplasm of small-sized macrophages within lung interstitium (arrowhead, left corner-detail).

ary goat anti-mouse IgG/goat anti-rabbit IgG (Millipore Bioscience Research Reagents, MA, USA) antibody was used in labelling with IHC Select® Immunoperoxidase Secondary Detection System (Millipore Bioscience Research Reagents) for detection of the CD163-positive cell population. Positive cells were visualized with diaminobenzidine, DAB (Sigma-Aldrich, MO, USA) and counterstained with Mayer's haematoxylin. Omitting the primary antibodies was considered as the negative control. The tissue sections were examined and photographed using an Olympus BX50 light microscope with an Olympus SP350 camera (Olympus, Japan) and were evaluated by two blinded and independent histologists.

Results

Alveolar macrophages demonstrated strong CD163-positivity of the cell membrane as well as distinct cytoplasmic positivity (**Figure 1**). In addition, much stronger CD163-immunopositivity was observed in small-sized cells morphologically similar to the monocytes or small macrophages, which were located especially inside interalveolar septa. Much higher CD163-positive cell concentration was found in alveolar space. Strong granular MPO expression was

evident within the cytoplasm of neutrophils which were present inside the lumen of dilated blood vessels as well as inside the fluid filling the alveoli (**Figure 2**). Minority of MPO-positive cells was detected in the lung interstitial tissue. Strong expression of MPO as a homogenous cytoplasmic positivity was observed in interstitial small-sized macrophages as well. Large active macrophages positive for CD163 were MPO-negative. The VEGF immunoreactivity was observed in the neutrophil granulocytes and small-

sized macrophages inside haemorrhagic lesions in lung parenchyma and inside dilated lung blood vessels (**Figure 3**). Distinct VEGF positivity was typical finding in cytoplasm of type II pneumocytes lining the alveolar surface and in the cytoplasm of detached cells within lung alveoli. Endothelial lining of pulmonary blood vessels showed no positivity for VEGF.

Discussion

A main feature of ARDS acute phase is influx of protein rich fluid into the lung alveoli, which is caused by increased permeability of alveolar-capillary membrane. This condition is a result of endothelial injury accompanied by damage of alveolar epithelium. In our study the high number of neutrophils showed a typical feature for acute exudative phase of ARDS. Normally, the neutrophils cause no damage, but following activation they release oxygen radicals and hydrolytic enzymes that damage the endothelium of the lung capillaries. The development of diffuse alveolar impair is connected with pulmonary oedema and hypoxemia with consequent formation of hyaline membranes and type I pneumocyte necrosis. Detailed histopathological description of that was published previously [6].

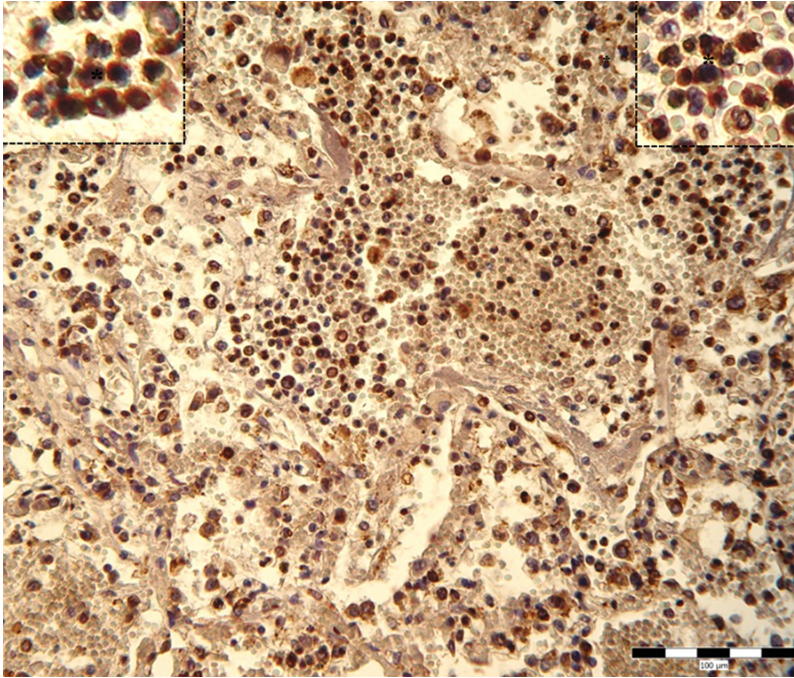


Figure 3. Cytoplasmic expression of VEGF in neutrophil granulocytes, small-sized macrophages and type II pneumocytes in lung lesions (asterisks).

Since neutrophils play critical and crucial role in ARDS development in acute phase, MPO expression as a part of neutrophil compartment is evident. Sugamata et al. studied MPO as a potent tissue damage factor and examined its contribution in influenza pneumonia by using mice genetically lacking in MPO. The absence of MPO reduced inflammatory damage with suppression of leakage of total bronchoalveolar lavage fluid (BALF) proteins associated with alteration of claudins in the lung. They showed that in mice genetically absent in MPO, inflammatory response was reduced as well as decreased leakage of total BALF proteins [7].

Grattendick et al. pointed out on fact that neutrophil-derived MPO and enzymatically inactive MPO stimulate lung alveolar macrophages, resulting in an increased inflammatory and cytotoxic state, and thereby contributing to the general lung inflammatory response [8]. Furthermore it was observed that some mediators like human recombinant complement factor 5a and human recombinant and granulocyte-macrophage colony-stimulating factor can inhibit rate of neutrophil apoptosis and that way increase life span of neutrophils in injured and inflamed tissue [9]. Our study showed defi-

nite granular MPO expression in the cytoplasm of neutrophils present inside the lumen of dilated blood vessels as well as inside the fluid filling the alveoli during acute phase of ARDS. During the resolution phase of an acute inflammatory response, infiltrated neutrophils undergo apoptosis and are subsequently cleared by resident macrophages [10]. In our study, MPO expression was found as a homogenous cytoplasmic positivity in interstitial small-sized macrophages as well. Rousseau et al. noted in a study on ARDS that the alveolar cells in the patients had a phenotype similar to blood monocytes [11]. Based on studies in the mouse, the same

group demonstrated that these cells are newly emigrated blood monocytes [12]. In the mouse blockade of monocyte immigration also blocked influx of neutrophils, indicating that the newly recruited monocytes are central to the inflammatory process [13]. MPO positivity found in the small macrophages in our case report needs further investigation and could be probably connected with their phagocytic activity. These cells located mostly in the interalveolar septa exhibit CD163-positivity as well. In human lung, CD163-positivity in both alveolar and interstitial mature macrophages was described [14]. The expression of CD163 can be regulated by a variety of factors which have been studied extensively in vitro. Consistent with the expression on mature macrophages in vivo, the in vitro differentiation of monocytes to macrophages strongly induces CD163 mRNA and protein expression. After in vitro treatment of monocytes with glucocorticoids the percentage of CD163 positive monocytes has been reported to rise from 10-30% to 90% [15]. When glucocorticoids were injected in vivo into human volunteers this also results in an increase of CD163 positive monocyte population of more than 80% within 6 h [16]. Recently was found, that interleukin-6 (IL-6) and tumor

necrosis factor- α up-regulated CD163 expression in mononuclear cells of normal subjects [17].

Besides MPO, VEGF is another inflammatory mediator which is expressed in neutrophils. Taichman et al. showed that human neutrophil granulocytes are source of VEGF in inflamed tissue. He mentioned that although the amount of secreted VEGF is relatively small, high accumulation of cells may be sufficient to contribute VEGF to the local cytokine pool [18]. In our study VEGF immunoreactivity was observed in the neutrophil granulocytes, small-sized macrophages and type II pneumocytes. VEGF is a potent regulator of vascular permeability, inflammatory response, and cell survival in the lung. Mura et al concluded that VEGF in type II pneumocytes helps protect alveolar epithelial cells from caspase-dependent apoptosis [19]. However, VEGF produced from type II cells may contribute to increased vascular permeability during acute lung injury. In the lung, alveolar epithelial cells and microvascular endothelial cells are highly sensitive to hypoxia and together orchestrate a rapid and sustained adaptive response. Signorelli et al. confirmed inducing effect of hypoxia on VEGF and IL6 secretion by alveolar epithelial cells and microvascular endothelial cells. During ARDS progression hypoxia plays important role and could participate to strong VEGF production by type II pneumocytes found in our observing. Increased level of concurrent pro-inflammatory IL-6 production could be explanation for up-regulation CD163 expression in monocyte/macrophage cells. The absence of type I pneumocytes indicates their higher sensitivity to acute alveolar injury. Type II pneumocytes were more resistant and they persist in alveolar lining in terminal phase of disease, although some of them were detached and were found in alveolar space [20].

In conclusion, the inflammatory cell population taking part in exudative phase of ARDS is represented by neutrophils, which exhibit MPO and VEGF immunopositivity. From the group of monocytes/macrophages the small-sized cells had the same character together with CD163 positivity. From alveolar epithelial lining, the type II pneumocytes were present and they reveal strong VEGF immunoreactivity. Majority

of immunoreactive cells were present inside alveolar air space spread in exudate.

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Disclosure of conflict of interest

None.

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