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SHORT COMMUNICATION

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Post-mortem markers of sepsis: an immunohistochemical study using VLA-4 (CD49d/CD29) and ICAM-1 (CD54) for the detection of sepsis-induced lung injury

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Abstract The up-regulation of different adhesion molecules such as VLA-4 (CD49d/CD29) and ICAM-1 (CD54) on the pulmonary endothelium and leukocytes, is a key event in sepsis-induced lung injury leading to inflammatory tissue alterations. The value of VLA-4 and ICAM-1 as micromorphological post-mortem markers for the detection of sepsis-induced lung injury, was evaluated in a semiquantitative immunohistochemical study. VLA-4 was strongly expressed on intravascular, interstitial and intra-alveolar leukocytes in sepsis-associated fatalities, whereas in non-septic fatalities an irregular weak immunoreactivity was observed on interstitial leukocytes and no positive immunohistochemical expression was detected on intravascular or intra-alveolar leukocytes. ICAM-1 was strongly expressed on endothelial cells of the pulmonary microvasculature and on pulmonary macrophages and lymphocytes in sepsis-associated fatalities. In contrast, an infrequent weak immunohistochemical reaction for ICAM-1 was found on pulmonary endothelium and on perivascular leukocytes in non-septic fatalities. Based on the results of the present preliminary study, VLA-4 and ICAM-1 can be considered as useful immunohistochemical post-mortem markers of sepsis.

Keywords Sepsis · Adhesion molecules · VLA-4 · ICAM-1 · Post-mortem · Diagnostics

Introduction

The antigen VLA-4 (very late activation antigen 4, CD49d/CD29) is a cell-surface molecule that is expressed on monocytes, eosinophils, basophils and lymphocytes [5, 16]. VLA-4 is involved in leukocyte adhesion to activated endothelial cells with subsequent migration from the vasculature into pulmonary tissue and the alveolar compartment during inflammatory processes and sepsis [10, 14, 16, 20]. ICAM-1 (intercellular adhesion molecule 1, or CD54) is a cell-surface protein that is expressed at very low levels on pulmonary endothelium, lymphocytes and macrophages [19]. The expression of ICAM-1 is up-regulated on stimulation by inflammatory mediators such as cytokines and bacterial lipopolysaccharides in septic processes [2, 11, 19]. ICAM-1 mediates inflammatory responses by adhesion of leukocytes to activated endothelium and subsequent leukocyte transmigration through the pulmonary endothelial layer [3, 15, 16, 20]. As a consequence of these intense cellular interactions resulting in pulmonary tissue injury, septic conditions often lead to adult respiratory distress syndrome, impaired lung function and death [4].

In a previous study we have shown that the immunohistochemical detection of an enhanced expression of E-selectin in lung tissue can be regarded as a post-mortem marker of sepsis [22]. The present preliminary study was undertaken to evaluate the potential value of VLA-4 and ICAM-1 as post-mortem immunohistochemical markers for the differentiation between death due to sepsis and death due to other causes.

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Material and methods

Tissue sampling

Lung specimens were obtained from four different lung lobes at autopsy from individuals (post-mortem interval between 1 and 4 days) with different causes of death. Pneumonic tissue alterations were not detected in any of the selected cases by gross examination of the lungs.

Study groups

1. Sepsis group (n = 8): autopsy cases with a well-documented previous history and clinical diagnosis of death due to sepsis, confirmed by post-mortem examination (3 males, 5 females, individ-

ual ages 36–83 years old, period of septic condition between 1 and 15 days)

2. Non-sepsis group I (n = 6): autopsy cases with death due to natural causes e.g. myocardial infarction (n = 2), intracerebral bleeding (n = 2), right cardiac failure (n = 2) (2 males, 4 females, individual ages 66–85 years old)

3. Non-sepsis group II (n = 16): autopsy cases with death due to unnatural causes e.g. trauma (n = 4), electrocution (n = 2), exsanguination (n = 2), drowning (n = 2), gunshot (n = 2), hanging (n = 1), polytrauma (n = 1), throttling (n = 1), carbon monoxide poisoning (n = 1) (13 males, 3 females, individual ages 21–82 years old)

Immunohistochemical staining

Details of the immunohistochemical staining procedure have been described elsewhere [22]. In brief, histological sections from the lung specimens as well as positive and negative control sections, were stained immunohistochemically for VLA-4 (CD49d/CD29, monoclonal, anti-mouse, 1:50, Immunotech, The Netherlands) and ICAM-1 (CD54, monoclonal, anti-mouse, 1:50, Dianova, Germany).

Immunohistochemical grading

The immunohistochemical staining pattern was assessed by using a combined modification of the semiquantitative scoring systems used by Ortmann and Brinkmann [18] and Dreßler and co-workers [6, 7] by evaluating the amount of positively stained endothelial cells of pulmonary vessels and intravascular, interstitial and intra-alveolar leukocytes, and 20 representative visual fields were analysed at 40 × magnification. The immunohistochemical expression of VLA-4 and ICAM-1 on leukocytes and endothelial cells, respectively, was graded separately in a four-category ordinal scale as negative (0), up to 30% of leukocytes and endothelial cells showing a positive staining reaction (1.0), 30–60% of the cells showing a positive immunoreactivity (2.0) and > 60% with a positive staining reaction (3.0). The average ratio of the immunohistochemical expression of VLA-4 and ICAM-1 was calculated separately for both antibodies and analysed statistically.

Statistical analysis

Statistical analysis of the data was performed using the Kruskal-Wallis test with $P < 0.01$ considered as significant.

Results

Expression of VLA-4

In all cases in the sepsis group, VLA-4 was strongly expressed on intravascular, interstitial and intra-alveolar pulmonary leukocytes (mean expression score 1.8) (Fig. 1). In the cases comprising the non-sepsis groups I and II, an irregularly weak positive immunoreactivity was observed on interstitial leukocytes (mean expression scores 0.1 and 0.7, respectively), whereas no immunopositivity could be detected on intravascular or intra-alveolar leukocytes. In comparison to the non-sepsis groups I and II, VLA-4 expression in the sepsis group differed significantly (P < 0.001). In the sepsis group, the intensity of the leukocyte immunoreactivity was homogeneous in all lobes of the lungs irrespective of the length of the post-mortem interval or the length of the sepsis period.

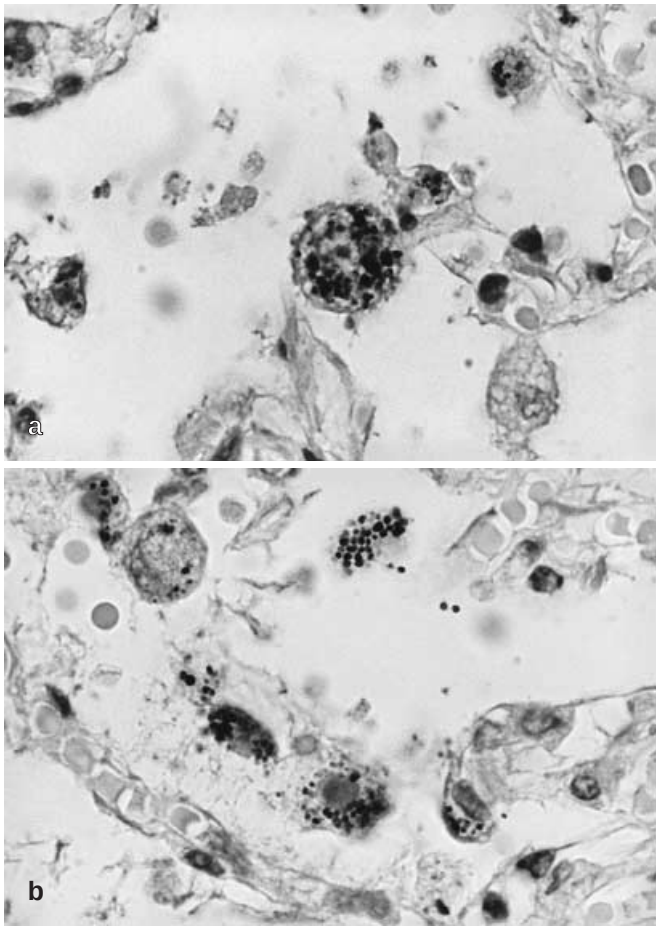

Fig. 1 a, b VLA-4 positive immunoreactivity in sepsis-induced lung injury. **a** Intra-alveolar and **b** intravascular leukocytes with strong positive staining reaction for VLA-4 (VLA-4 or CD49d/CD29, × 1000)

Expression of ICAM-1

A strong positive expression of ICAM-1 was detected on endothelial cells of pulmonary arteries, arterioles, precapillaries, alveolar capillaries, postcapillary venules and veins (mean expression score 2.2) in all cases in the sepsis group. Moreover, immunoreactivity for ICAM-1 was strongly positive on pulmonary macrophages and lymphocytes (mean expression score 2.2). Both, endothelial and leukocyte immunoreactivity for ICAM-1 showed a homogeneous staining pattern in the sepsis group, irrespective of the post-mortem period or the time period of the septic condition (Fig. 2). In the non-sepsis groups I and II, an infrequent weak immunopositivity for ICAM-1 was observed on pulmonary endothelial cells (mean expression score 0.1 in both study groups) and leukocytes (mean expression score 0.1 in both study groups), the lat-

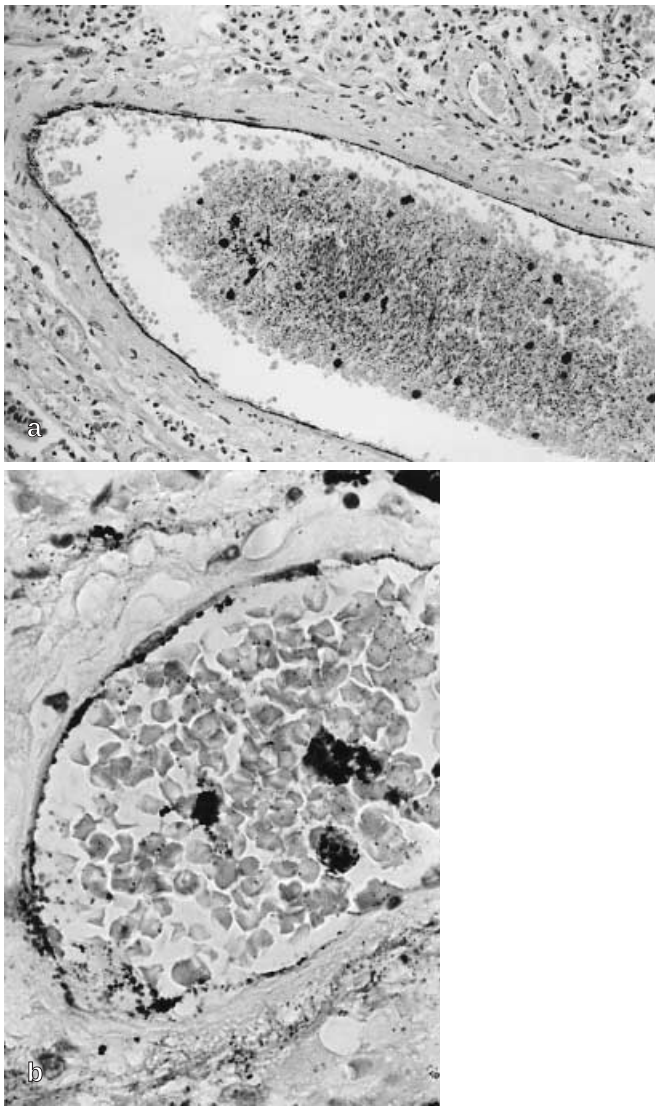

Fig. 2 a, b ICAM-1 positive immunoreactivity in sepsis-induced lung injury. **a** Strong endothelial ICAM-1 expression of the pulmonary microvasculature and intravascular leukocytes with positive staining reaction (ICAM-1 or CD 54, × 150). **b** High power view of strong ICAM-1 positive endothelial cells and intravascular leukocytes (ICAM-1 or CD 54, × 800)

ter mostly in a perivascular location. In comparison to the non-sepsis groups I and II, the immunohistochemical expression of ICAM-1 in the sepsis group differed significantly for endothelial cells ($P < 0.001$) and leukocytes ($P < 0.001$).

Table 1 shows the results of the semiquantitative morphological evaluation of the immunohistochemical expression of both markers in the different study groups.

Discussion

Our findings of an enhanced expression of VLA-4 and ICAM-1 on pulmonary leukocytes and endothelium in sepsis-induced lung injury and the observed distribution pattern are very similar to the results of other laboratory studies performed on in vitro cell lines and animal models identifying VLA-4 and ICAM-1 as playing a pivotal role in the pathogenesis of systemic inflammation [4, 13, 14, 16].

While recent emphasis has focused on the immunohistochemical detection of the inflammatory cellular response in human skin wounds and brain injury for a forensic wound age estimation [1, 6, 7, 8, 9, 12, 17], only very few studies have dealt with the expression pattern of immunohistochemical markers of inflammation in sepsis-induced lung injury in forensic autopsy material. Recently, two studies by Ortmann and Brinkmann [18] and our research group [22] demonstrated that the immunohistochemical detection of a different pulmonary expression pattern of cellular adhesion molecules, such as the selectins, can add relevant information for the forensic post-mortem elucidation of death due to sepsis.

Dreßler and co-workers investigated the usefulness of ICAM-1 for a forensic wound age estimation and found that it was not affected by decomposition or autolysis, even in skin wounds obtained at autopsy after a post-mortem interval of 7 days [6]. In our study the length of the post-mortem interval had no influence on the immunohistochemical staining intensity of VLA-4 and ICAM-1, therefore autolysis does not seem to have any considerable influence on these investigations in the early post-mortem period.

We conclude that VLA-4 and ICAM-1 can be considered useful immunohistochemical post-mortem markers of a previously undiagnosed sepsis and to confirm or rule out the presumed diagnosis of a sepsis-associated fatality. The use of these immunohistochemical methods will be particularly helpful when macroscopic and routine histological autopsy findings in cases of suspected fatal sepsis are not convincing [21, 23] and clinical data on the patient's previous history are not available.

Table 1 Results of the semiquantitative morphological evaluation. Immunohistochemical expression of VLA-4 on pulmonary leukocytes and ICAM-1 on endothelial cells and pulmonary leukocytes, respectively (*95% CI:* 95% confidence interval)

Study group	Cases ($n = 30$)	Expression of VLA-4 on pulmonary leukocytes	Expression of ICAM-1 on pulmonary leukocytes	Expression of ICAM-1 on pulmonary endothelium
Sepsis group	8	Mean 1.8, 95% *CI* 1.3–2.4	Mean 2.2, 95% *CI* 1.5–3	Mean 2.2, 95% *CI* 1.3–3
Non-sepsis group I	6	Mean 0.1, 95% *CI* 0–0.3	Mean 0.1, 95% *CI* 0–0.3	Mean 0.1, 95% *CI* 0–0.2
Non-sepsis group II	16	Mean 0.7, 95% *CI* 0.4–0.8	Mean 0.1, 95% *CI* 0–0.2	Mean 0.1, 95% *CI* 0–0.2

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