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ORIGINAL ARTICLE

Immunohistochemical expression of fibronectin in the lungs of fire victims proves intravital reaction in fatal burns

Michael Bohnert · Jürina Anderson · Markus A. Rothschild · Joachim Böhm

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Abstract Immunohistochemical studies about the presence of fibronectin in the lungs were performed in a group of 73 fire victims (63 cases of intravital and 10 cases of postmortem burn) as well as in an unselected control group of 55 individuals not exposed to fire before death. The cases of intravital burn showed a significantly stronger fibronectin expression than the control cases and the cases of postmortem burn. Fibronectin was mainly present in macrophages of the peribronchial lung parenchyma and, not associated with cells, in the matrix of peribronchial tissue. Our findings suggest that higher levels of fibronectin expression in the lung tissue of burn victims compared to fire-unrelated deaths may serve as an indicator of an early intravital inflammatory response to fire damage.

Keywords Fibronectin · Burns · Lungs · Inhalational trauma · Diagnosis of vitality

Introduction

The purpose of a medicolegal evaluation of fire-related deaths is not only to identify the victim and to determine the manner and cause of death, but also to investigate whether the individual had been still alive during exposure to the fire. Especially in cases with very short survival times, the classical vitality parameters like soot aspiration, swallowing of soot, and elevated COHb values may be discreet or even absent [5]. In this case, histomorphological and immunohistochemial changes of the bronchi and the adjacent pulmonary tissue may be of crucial importance. Whenever a living person is exposed to flames or heat, he will have to inhale hot fire fumes. The latter can damage the respiratory tract and lungs by two different ways. Firstly, by direct inhalation of hot and toxic fire fumes, and secondly by a vascular-mediated systemic inflammatory response (SIRS). It is assumed that toxic mediators, originating from thermally damaged tissue in the trachea and large bronchi may spread via bronchopulmonary shunts to induce the full-blown clinical picture of an inhalation trauma and thus contributing to the systemic reaction of the organism to the thermal injury [10, 13, 14].

Recent immunohistochemical studies have shown an increased expression of heat shock protein 70 (HSP 70) [11] and of the adhesion molecules von-Willebrand factor and P-selectin [15] in fire fatalities. Fibronectin is another glycoprotein, which is activated early during wound healing after traumatization [6]. It is capable of binding to numerous other macromolecules such as collagen, glucosaminoglycan, fibrinogen, and fibrin. Fibronectin is also important for cell adhesion, i.e., it makes cells adhere and bind to neighboring cells or extracellular structures [9]. Fibronectin is regarded as one of the earliest markers that become expressed in an inflammatory process. Betz et al. reported that skin wounds of victims after exposure to lethal traumatization showed clear fibronectin reactions already within survival times of only a few minutes and had markedly different immunohistochemical staining patterns than injuries sustained postmortem [1–3].

M. Bohnert (✉) · J. Anderson
Institute of Legal Medicine, Albert Ludwig University,
Albertstrasse 9,
79104 Freiburg, Germany
e-mail: michael.bohnert@uniklinik-freiburg.de

M. A. Rothschild
Institute of Legal Medicine, University of Cologne,
Melatengürtel 60-62,
50823 Cologne, Germany

J. Böhm
Institute of Pathology, University Hospital Freiburg,
Postfach 214,
79002 Freiburg, Germany

Fibronectin is involved in inflammatory processes in the deeper portions of the respiratory tract. This is due to a fibronectin receptor on the surface of alveolar macrophages, which play an important role in protecting the lung and repairing damages during inflammatory processes [16]. Moreover, fibronectin in the lungs may be present in vascular endothelia, smooth muscle cells of vascular walls, chondrocytes, and in fibroblasts [12].

The purpose of this paper was to investigate whether the extent of fibronectin expression in the lung tissue of fire-related fatalities is significantly higher than in a control group of deaths without a history of burn. In this case, fibronectin expression could serve as a marker to prove that a fire victim was still alive during exposure to thermal damage.

Material and methods

Our study group included 73 fire fatalities on which forensic autopsies were performed at the Institutes of Legal Medicine of the Universities of Freiburg and Berlin in the years 1996–2001. Among the fire victims there were 43 males (58.9%) and 30 females (41.1%) with an average age of 46.1 years (mean age, 48 years for males, and 44 years for females). The youngest victim was a 6-year-old girl; the oldest was a woman aged 92 years. The inclusion criteria were exposure to fire, no survival after recovery from fire and absence of late postmortem changes. In 64 of the cases (87.7%), the circumstances and autopsy findings proved that the victims had been still alive during exposure to fire (e.g., aspiration of soot, COHb concentration exceeding 10%). In 9 cases (12.3%), the exposure to fire occurred postmortem. The most common cause of death was peracute burn trauma followed by poisoning due to inhalation of toxic fire fumes. In those cases with postmortem exposure to fire, death resulted from mechanical injuries, which occurred mostly in traffic accidents. The majority of the fire victims were found in houses (56.4%), another 32.9% in vehicles, and 10.7% died outdoors.

For comparison, a control group of 55 unselected autopsy cases was investigated in the Institute of Legal Medicine of Freiburg University in the years 2000–2001. This group included fatalities with both, natural and non-natural causes of death. Exclusion criteria were any link with a fire, late postmortem changes, and chronic diseases or traumata with intensive medical care before death. The control group included 41 men (74.5%) and 14 women (25.5%) with an average age of 47.3 years. At the time of death, the mean age in this group was 43.9 years for men and 57.3 years for women. The youngest victim was a male infant aged 4 months; the oldest was a man aged 88 years. The most frequent cause of death in the control group was coronary heart disease (25.5% of the cases). Head injuries and polytraumata accounted for 14.6% each. All other causes of death made up less than 10%.

Immunohistochemical staining of fibronectin (Dako®, Rabbit Anti-Human, Fibronectin, Code No. A 0245, Lot No. 097, dilution 1:400, incubation for 3 h) was performed with the avidin–biotin method using the Vectastain Universal Elite ABC-Kits of Vector Laboratories (Burlingame, CA). In each case, 5 slides of peripheral lung tissue (one sample from every lobe) were examined. The slides were investigated under blinded conditions by the same investigator immediately after the staining was finished. The staining intensity was semiquantitatively graduated (negative, weak, moderate, and strong).

For statistical analysis, we used the Wilcoxon test for unpaired samples and the Kruskal–Wallis test. In both tests, an error probability of $p<0.05$ was considered as significant. With the help of logistic regression (Holm-corrected), further differences could be identified between the groups by means of backward and forward eliminations.

Results

Staining with anti-fibronectin (FN)

Burned victims

The percentage of cases with positive immunostaining is given in Table 1. In the group of victims that were still alive when exposed to fire, the expression of fibronectin was observed in fibroblasts, macrophages, alveolocytes, respiratory epithelium, glandular cells of the bronchi, interalveolar septa, vascular walls, and vascular lumina (Figs. 1, 2, 3, and 4). In fact, the same staining pattern was also observed in cases of postmortem burn, but it was much less intensive than in the victims with intravital exposure to fire. Positive staining in this group was present mainly in alveolar macrophages, alveolar epithelial cells and, not associated with cells, in the matrix of interalveolar septa (Table 2). In the group of victims burned postmortem, there was no expression of fibronectin in the endothelium of the blood vessels or in the vascular lumina.

Control group

The percentage of positive immunostaining within the group of control cases is given in Table 3. In this group of deaths, an expression of fibronectin was sometimes observed in alveolocytes, fibroblasts, alveolar macrophages, endothelium, and media of the great blood vessels (Fig. 4). In the respiratory epithelium and the capillary lumina, fibronectin staining was seen only rarely (Fig. 3).

Table 1 Frequency of positive staining with fibronectin antibody in intravital fire victims

Localization	Structures	Negative	Weak	Moderate	Strong
Peripheral bronchi	Respiratory epithelium	77.8	18.5	3.7	0
Peripheral bronchi	Free in the lumina	8.9	73.3	17.8	0
Peripheral bronchi	Macrophages	13.3	57.7	22.2	6.7
Peripheral bronchi	Fibroblasts	8.9	53.3	31.1	6.7
Pulmonary tissue	Alveolar macrophages	0	25	52.1	22.9
Pulmonary tissue	Desquamated cells	0	35.5	56.3	8.2
Pulmonary tissue	Free in the alveolar lumen	4.2	83.3	6.3	6.2
Pulmonary tissue	Alveocytes	2.1	47.9	43.8	6.2
Pulmonary tissue	Free in alveolar septa	8.3	58.3	31.3	2.1
Capillaries	Free	41.7	56.2	2.1	0
Pulmonary vessels	Leucocytes	70.8	27.1	2.1	0
Pulmonary vessels	Monocytes	54.2	41.7	4.2	0
Pulmonary vessels	Free	37.5	52.1	10.4	0
Pulmonary vessels	Endothelium	41.7	37.5	20.8	0
Pulmonary vessels	Tunica media	12.4	68.8	18.8	0

No fibronectin expression was observed in most of the monocytes and other leucocytes.

Statistical comparison between fire fatalities and control group cases

As the number of postmortem fire victims was very small, these cases were not included in the statistical analysis and subjected to descriptive evaluation only. The most important difference between the two groups was found to be the expression of not cell-bound fibronectin in the walls of peripheral bronchi and capillary lumina in the lung tissue with an χ^2 value of 10.75 and 8.942, and an error probability of $p=0.001$ and $p=0.002$, respectively. Another distinguishing feature between both groups proved to be the expression of fibronectin in macrophages of peripheral bronchi ($p=0.0164$). For all other features under investigation, no statistically significant differences were found. The results were checked with the help of Holm-corrected logistic regression by means of backward elimination, which confirmed the sequence of the Wilcoxon test.

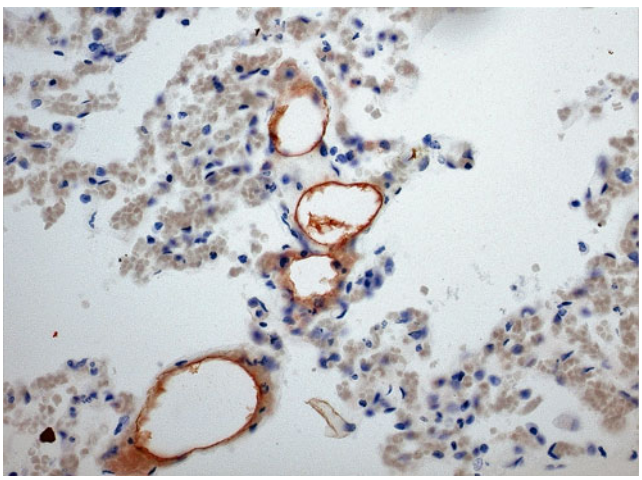

Fig. 1 Fire fatalities: positive staining of fibronectin in endothelial cells of pulmonary capillaries (magnification, ×400)

Discussion

In the present study, we were able to demonstrate that the expression of fibronectin as an early marker of inflammation in the respiratory tract and the lungs of fire fatalities is

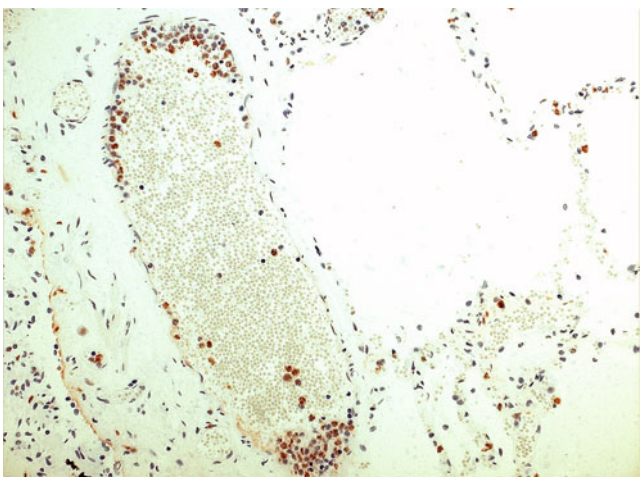

Fig. 2 Fire fatalities: intrapulmonal blood vessel. Note the margination of intraluminal blood leucocytes as an early sign of an inflammatory reaction. Many of the leucocytes show a fibronectin expression. There are some fibronectin-positive alveolar macrophages in the adjacent alveolar walls (magnification, ×200)

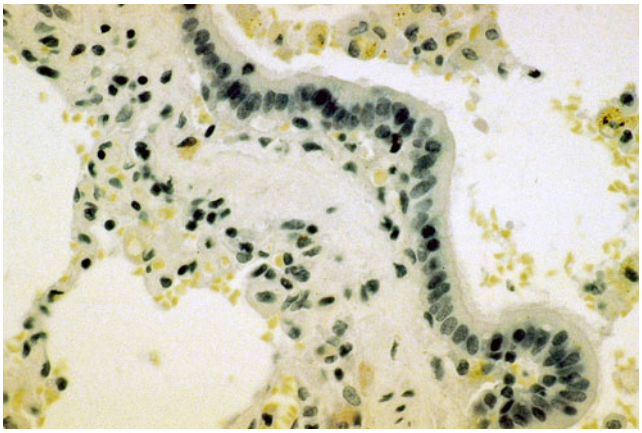

Fig. 3 Control group: peripheral bronchus with well-preserved respiratory epithelium. No positive staining of fibronectin

significantly different compared to other causes of death and to cases of postmortem burn. In all victims who were still alive during exposure to fire, the macrophages in the trachea, peripheral bronchi, and in the alveolar lumina showed a strong expression of fibronectin. On the other hand, there was only a weak expression of fibronectin in our control group of deaths unrelated to fire and in cases of postmortem burn.

In cases of intravital burn, the fibroblasts, which are often found in the vicinity of macrophages, also showed an enhanced expression of fibronectin. It may be due to a close interaction between macrophages and fibroblasts that the expression of fibronectin was found to be increased near the bronchi and in the adjacent pulmonary tissue. In the control group and in the cases of postmortem burn, however, fibroblasts mostly did not show any fibronectin expression.

As far as endothelial cells and smooth muscle cells of the vessels are concerned, the expression pattern of fibronectin was not different between the groups of burn victims on the one hand, and the fire-unrelated deaths on the other.

Another feature suitable for differentiating burn fatalities from fire-unrelated deaths was the expression of not cell-bound, "free" fibronectin, which was present especially in the vicinity of peripheral bronchi of intravital burn victims. On the contrary, in the control cases and in postmortem burn victims, the fibronectin staining was either negligible or absent. Deno et al. [7] attributed the increase of free fibronectin in the vicinity of the airways after heat exposure to the fact that plasma fibronectin in the presence of intense heat attaches to components of the surrounding traumatized tissue and thus can be more easily detected [7]. This is in analogy with the results of studies on wound age determination, which proved that skin wounds with survival times of only a few minutes already showed a clear fibronectin release that was distinguishable from unspecific postmortem artifacts [1, 3]. As it is the case in burn trauma, high temperatures and toxic fumes damage the lung tissue, which thereby can be regarded as wounds. This may explain our observation of an increased expression of non-cell-bound fibronectin in the tissue surrounding the peripheral bronchi and is in accordance with similar findings in the literature [1, 3]. Another explanation for this may be that the inflammatory reaction induced by the inhalation trauma attracts both alveolar macrophages and fibroblasts. As these two cell types are able to produce fibronectin in large quantities and to release it into the surrounding tissue, an increased presence of fibronectin in the thermally damaged parenchyma will result [4].

In our study, we also found different expression levels of free fibronectin in the lumina of capillary vessels in the lung. In contrast to intravital burn victims, who showed a weak but distinct fibronectin expression in their vessel lumina, the control group members and the cases of postmortem burn as a rule displayed no fibronectin expression at all. This did not only apply to the lung capillaries, but also to the vessels of the epiglottis and the trachea. In studies using rats that were treated with heat, Deno et al. [7] found that immediately after heat exposure, the plasma fibronectin concentration initially dropped but then rose to levels above the normal range within 8 h, while in the control group that was unexposed to heat, the plasma fibronectin concentration remained constant.

Interestingly, our studies showed that in the lumina of vessels, there was a gradient of fibronectin expression with distance from the bronchial airways. Thus, as a rule, fibronectin immunostaining was much stronger in large pulmonary vessels, which were located close to the bronchi, than in the capillary lumina remote from it. This phenomenon may be due to the fact that the lung is damaged by heat in two different ways. Firstly, by a systemic effect of toxins released in burn trauma and, secondly, by direct inhalation of fire fumes [8, 13]. Therefore, our observation of a stronger intensity of fibronectin staining in peribronchial vessels may be due to both, a local effect of hot fire

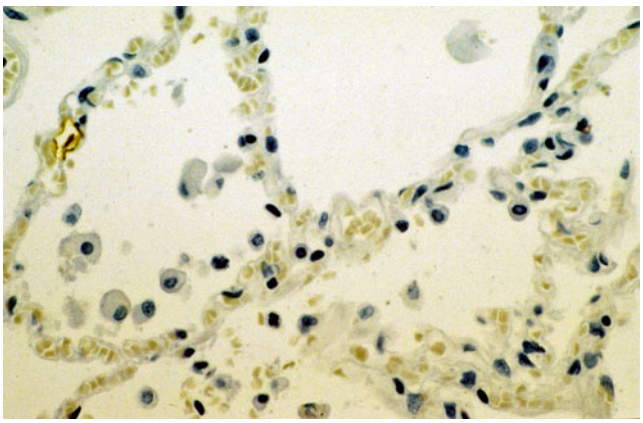

Fig. 4 Control group: No staining of alveolar macrophages

Table 2 Frequency of positive staining with fibronectin antibody in cases of postmortem burns

Localization	Structures	Negative	Weak	Moderate	Strong
Peripheral bronchi	Respiratory epithelium	100	0	0	0
Peripheral bronchi	Free in the lumina	25	75	0	0
Peripheral bronchi	Macrophages	12.5	62.5	25	0
Peripheral bronchi	Fibroblasts	0	50	25	25
Pulmonary tissue	Alveolar macrophages	0	50	37.5	12.5
Pulmonary tissue	Desquamated cells	0	12.5	62.5	25
Pulmonary tissue	Free in the alveolar lumen	12.5	62.5	12.5	12.5
Pulmonary tissue	Alveocytes	0	50	37.5	12.5
Pulmonary tissue	Free in alveolar septa	0	75	25	0
Capillaries	Free	75	25	0	0
Pulmonary vessels	Leucocytes	75	25	0	0
Pulmonary vessels	Monocytes	25	75	0	0
Pulmonary vessels	Free	0	75	25	0
Pulmonary vessels	Endothelium	50	12.5	25	12.5
Pulmonary vessels	Tunica media	0	75	25	0

fumes and to mediator-triggered inflammatory reactions on inhalative toxins. From there, these effects may systemically spread to the peripheral lung tissue via the blood circulation. This idea is in accord with the results of Loick et al. [10] who found in their studies on sheep that after exposure of the trachea and the right lung to fire fumes, both lungs were damaged, a finding that may be attributable to bronchopulmonary shunts [10].

Our studies of fibronectin in lung tissues of burn fatalities and of a control group of fire-unrelated deaths demonstrated an increased fibronectin expression in macrophages, fibroblasts, peribronchial tissue, and vascular lumina of the burn fatality group, which was most likely due to an inflammatory response caused by the inhalation trauma. Despite of the small number of cases of the postmortem victim group, we were able to demonstrate that their fibronectin expression pattern was similar to that of the control cases. Thus, our findings suggest that the vitality status of a victim during fire exposure may be reflected by its fibronectin expression level in the peribronchial tissue and lung parenchyma. Therefore, the immunohistochemical detection of fibronectin staining intensity in these tissues may help to distinguish between intravital burn victims (strong fibronectin expression) and fire-unrelated deaths (weak fibronectin expression). However, further studies on larger cohorts of victims are needed to verify this observation. Moreover, it has to be stated that the presence

Table 3 Frequency of positive staining with fibronectin antibody in control cases

Localization	Structures	Negative	Weak	Moderate	Strong
Peripheral bronchi	Respiratory epithelium	100	0	0	0
Peripheral bronchi	Free in the lumina	100	0	0	0
Peripheral bronchi	Macrophages	34.9	53.5	7	4.6
Peripheral bronchi	Fibroblasts	25.6	51.2	16.3	7
Pulmonary tissue	Alveolar macrophages	14.5	76.3	9.1	0
Pulmonary tissue	Desquamated cells	0	23.7	60	16.3
Pulmonary tissue	Free in the alveolar lumen	14.5	65.5	7.3	12.7
Pulmonary tissue	Alveocytes	0	45.4	47.3	7.3
Pulmonary tissue	Free in alveolar septa	9.1	63.6	25.5	1.8
Capillaries	Free	72.7	21.9	5.5	0
Pulmonary vessels	Leucocytes	85.5	14.5	0	0
Pulmonary vessels	Monocytes	70.9	25.4	3.6	0
Pulmonary vessels	Free	52.7	32.8	12.7	1.8
Pulmonary vessels	Endothelium	47.3	34.6	16.4	1.8
Pulmonary vessels	Tunica media	12.7	70.9	16.4	0

of fibronectin alone is not a reliable sign of vitality. According to our results, only the proof of an increased fibronectin expression in different cell types (macrophages, fibroblasts) and extracellular spaces (peribronchial tissue, capillary vessels) may be highly indicative of an intravital heat exposure.

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