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ORIGINAL ARTICLE

Immunohistochemical identification of prevalent right ventricular ischemia causing right heart failure in cases of pulmonary fat embolism

Tony Fracasso · Bernd Karger · Heidi Pfeiffer · Cristina Sauerland · Andreas Schmeling

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Abstract Pulmonary fat embolism is a life-threatening event that may result to potentially determining right ventricular failure. Even if the pathophysiology of this phenomenon has been widely investigated, no immunohistochemical demonstration of right ventricular failure following pulmonary fat embolism has been reported till now. We performed an immunohistochemical investigation with the markers fibronectin and C5b-9 in 21 cases of polytrauma with bone fractures (study group—nine females and 12 males; mean age 64.6 years) compared to a control group of 21 forensic cases with various causes of death (nine females and 12 males; mean age 68.6 years). In each case at least one tissue slide from both cardiac ventricles (free wall of the right ventricle, anterior and/or posterior wall of the left ventricle) was available. The reactions were semi-quantitatively classified, and the two groups were compared. In the study group, the occurrence of ischemic changes at the right ventricle was significantly higher than in controls. The determining aspect, however, seems to be the prevalent ischemic lesion at the right ventricle compared to the left one. This may indicate the primary involvement of the right ventricle, thus, demonstrating a right ventricular failure.

Keywords Ischemic damage · Pulmonary fat embolism · Right ventricular failure · Acute cor pulmonale · Immunohistochemistry

This work is dedicated to Prof. Dr. Drs. h. c. Stefan Pollak on the occasion of his 60th birthday.

T. Fracasso · B. Karger · H. Pfeiffer · A. Schmeling
Institute of Legal Medicine, University Hospital Münster, Münster, Germany

C. Sauerland
Institute of Medical Informatics and Biomathematics, University Hospital Münster, Münster, Germany

T. Fracasso (✉)
Institut für Rechtsmedizin, Universitätsklinikum Münster, Roentgenstr. 23, 48149 Münster, Germany
e-mail: Tony.Fracasso@ukmuenster.de

Introduction

Fat embolism (FE) is a known and common complication of blunt force injuries, which occurs in at least 90% of major traumas, especially if fractures of long bones are present [1, 2]. In the majority of the cases FE occurs without clinical manifestations [3].

In case of symptomatic FE, clinicians distinguish two different forms: the fulminant FE syndrome and FE syndrome [4–6]. The clinical course is strongly dependent on the quantity and rapidity of fat droplets occupying the pulmonary vessels. In the first case, the sudden massive obstruction of the pulmonary circle determines a rapid and often lethal increase in the impendence to right ventricular ejection [7–9]. At echocardiography, pulmonary and right heart dilatations are detected [8]. Death usually occurs in the first 12 h because of acute right ventricular failure [4, 6, 10]. At autopsy, the right side of the heart is dilated with thinning of the wall of the ventricle; the lungs are moist and anaemic [4]. The FE syndrome is, instead, a more complex clinical entity, which usual occurs after a free interval of

Table 1 Study group

ID	Sex (female/male)	Age	Cause of death	Main pathological findings	FE	RV Fib	RV C5b-9	LV fib	LV C5b-9	Δfib	ΔC5b-9
1	f	86	Fat embolism	Multiple fractures, fresh bleedings	3	3	1	1	0	2	1
2	f	12	Polytrauma	Multiple fractures	3	2	0	1	0	1	0
3	f	86	Fat embolism	Multiple fractures, arteriosclerosis	3	2	1	0	0	2	1
4	f	84	Fat embolism	Pelvis fracture, arteriosclerosis	3	0	0	1	0	−1	0
5	m	72	Fat embolism	Leg fracture, chronic hepatic congestion	3	3	0	0	0	3	0
6	m	58	Polytrauma	Multiple fractures, arteriosclerosis	3	3	0	0	0	3	0
7	f	48	Polytrauma	Multiple fractures	2	2	0	1	0	1	0
8	m	44	Polytrauma	Multiple fractures, fatty liver	2	1	0	2	0	-1	0
9	m	23	Polytrauma	Multiple fractures	2	3	2	3	1	0	1
10	m	75	Cardiac death	Multiple fractures, fresh bleedings	2	3	0	1	0	2	0
11	m	79	Polytrauma	Multiple fractures, arteriosclerosis	2	2	0	0	0	2	0
12	f	66	Fat embolism, acute haemorrhage	Multiple fractures, erosive esophagitis	2	1	0	0	0	1	0
13	m	54	Polytrauma	Multiple fractures, portal fibrosis	2	1	0	0	0	1	0
14	m	75	Polytrauma	Multiple fractures, chronic hepatic congestion	2	0	0	0	0	0	0
15	m	74	Shock	Multiple fractures, shock lungs	2	2	0	2	0	0	0
16	f	90	Fat embolism	Multiple fractures, arteriosclerosis	2	2	2	0	0	2	2
17	f	84	Fat embolism	Multiple fractures, cardiac hypertrophy	2	1	0	1	2	0	-2
18	m	18	Polytrauma	Multiple fractures, blood aspiration	1	1	0	1	0	0	0
19	f	81	Fat embolism	Multiple fractures, chronic hepatic congestion	1	1	0	2	0	−1	0
20	m	91	Shock	Multiple fractures, arteriosclerosis	1	1	0	0	0	1	0
21	m	56	Myocarditis	Humerus fracture, hepatic cirrhosis	1	0	0	1	0	−1	0

24–72 h [4–6, 10] and involves the respiratory tract and the nervous system with the classical triad of cerebral symptoms, respiratory distress, and petechiae of the skin and mucosae [11, 12]. In lethal cases, the FE is responsible for multiple organ failure. At external examination, petechial bleedings are usually observed at the anterior axillary fold, the root of the neck, the mucosa of the mouth, and the conjunctiva [3]. At autopsy, cerebral purpura is a characteristic [13]; the lungs are edematous; and the visceral pleura has a speckled appearance with bloody foamy fluid in the bronchi [4, 14].

If the clinical and pathophysiological aspects of the FE have been widely described and debated, its pathology has still not been completely elucidated. Many authors have intensively investigated the morphology and extension of the FE in different organs with special interest in the lungs, brain, heart, and kidney [i.e., 15, 16].

From a medico-legal point of view, the most important investigated matter is the effective lethality of FE [17–21], and the question is still debated [22]. The most accepted cause of death in cases of pulmonary FE is an acute cor pulmonale with subsequent right ventricular failure [23, 24]. In cases of massive fat embolism, the fat droplets occupy the pulmonary circle rapidly, which determines increasing pulmonary pressure directly by obstruction [7, 25] and indirectly due to reactive vasoconstriction following hypoxemia [26, 27]. A transitory increase of the pulmonary pressure is well tolerated by the heart that reacts with a double inotropic compensative mechanism: from one side an acute dilatation of the right ventricle occurs (Anrep's effect) [28, 29], while from the other side, a better perfusion of the myocytes is determined by increased coronary flow [7]. However, if the pulmonary hypertension persists, the compensative mechanisms will fail contributing to ischemia [30, 31]. In fact, at normal intra-ventricular pressures, the right coronary artery flow is continuous (occurs equally during systole and diastole), which is different from the left ventricle where the coronary perfusion only occurs during diastole [32]. When the right ventricular pressure is severely increased, the right coronary artery flow mainly occurs also in diastole: the combination of increased demand and decreasing blood flow determines ischemic damage [31], primarily occurring at the sub-endocardial regions [32, 33]. Ischemic changes at the right ventricle in cases of fat embolism were demonstrated in animal experiments [34–37]. Moreover, Friedberg [38] described leukocyte infiltration and late necrotic changes at the right ventricle in four of eight cases of post-traumatic FE in the man.

Table 2 Control group

ID	Sex (female/male)	Age	Cause of death	Main pathological findings	FE	RV Fib	RV C5b-9	LV fib	LV C5b-9	Δfib	ΔC5b-9
1	m	78	Sudden cardiac death	Severe coronarosclerosis	0	0	0	0	0	0	0
2	f	55	Hyperglycemic coma	Moderate arteriosclerosis	0	2	0	2	0	0	0
3	m	53	Asphyxia, inhalation injury	Minor burns, airway obstruction	0	1	0	1	0	0	0
4	m	68	Bleeding	Carotid bleeding, arteriosclerosis	0	2	0	2	0	0	0
5	f	81	Bleeding	Suicidal incised wrist wounds, chronic hepatic congestion	0	2	0	2	0	0	0
6	f	56	Bleeding	Hepatic cirrhosis, ruptured esophageal varices	0	2	0	2	0	0	0
7	f	82	Ileus	Intestinal obstruction, arteriosclerosis	0	2	0	2	0	-1	0
8	m	75	Myocardial infarction	Cardiac hypertrophy, fresh infarction	0	2	2	2	1	0	1
9	f	88	Rec. myocardial infarction	Old ischemic myocardial scars, fresh infarction	0	2	0	2	0	-1	-1
10	m	60	Rec. myocardial infarction	Old ischemic myocardial scars, fresh infarction	0	2	0	2	0	0	0
11	m	41	Rec. myocardial infarction	Old ischemic myocardial scars, fresh infarction	0	3	0	3	2	0	-2
12	f	68	Hemolysis and shock after hemotransfusion	Breast cancer, signs of shock	0	1	0	2	2	-1	-2
13	m	61	Esophageal carcinoma	Metastatic carcinoma	0	2	0	2	0	0	0
14	m	62	Rec. myocardial infarction	Old ischemic myocardial scars, fresh infarction	0	2	0	3	3	-1	-3
15	m	48	Terminal hepatic cirrhosis	Hepatic cirrhosis	0	1	0	2	1	-1	-1
16	f	80	Myocardial infarction	Coronarosclerosis, fresh infarction	0	2	1	3	1	-1	0
17	m	86	Myocardial infarction	Coronarosclerosis, fresh infarction	0	1	2	2	2	-1	0
18	m	87	Myocardial infarction	Coronarosclerosis, fresh infarction	0	2	2	2	2	0	0
19	f	87	Heart infarction, hypotermia	Coronarosclerosis, fresh infarction	0	2	0	0	2	2	-2
20	f	59	Sudden cardiac death	Coronarosclerosis	0	0	0	0	0	0	0
21	m	66	Rec. myocardial infarction	Old ischemic myocardial scars, fresh infarction	0	2	0	2	0	0	0

The scope of the present investigation was to evaluate whether the occurrence of right ventricular failure is morphologically detectable at immunohistochemistry in cases of FE compared to controls.

Materials and methods

We compared 21 cases of polytrauma with bone fractures (study group—nine females and 12 males; mean age 64.6 years) to a control group of 21 forensic cases with various causes of death (nine females and 12 males; mean age 68.6 years). All the cases were collected at the Institute of Legal Medicine of the University Hospital in Münster, Germany. In every case, information about circumstances of death as well as autopsy record was available. An overview of the investigated cases is given in Tables 1 and 2. Histological investigation of the heart, lung, liver, brain, kidney, pancreas, and spleen with haematoxylin and eosin was performed according to standard medico-legal investigation [39]. In each case, immunohistochemical reactions with the antibodies fibronectin (polyclonal rabbit anti-human, DAKO Deutschland GmbH, Hamburg, Germany) and C5b-9 (monoclonal mouse anti-human, DAKO Deutschland GmbH, Hamburg, Germany) of at least one tissue slide from both cardiac ventricles (free wall of the right ventricle, RV; anterior and/or posterior wall of the left ventricle, LV) were prepared as described elsewhere [40, 41]; in every staining procedure, negative controls were used to exclude artefacts. A blind investigation of the slides was performed by two different observers with final consensual evaluation. The positive reactions were semi-quantitatively classified as follows (Figs. 1 and 2):

Grade 0. Negative;
Grade 1. Single cells necrosis;
Grade 2. Group cells necrosis; and
Grade 3. Diffuse necrosis.

For the evaluation of the pulmonary FE at least two frozen sections of the lungs stained with Sudan III were available for

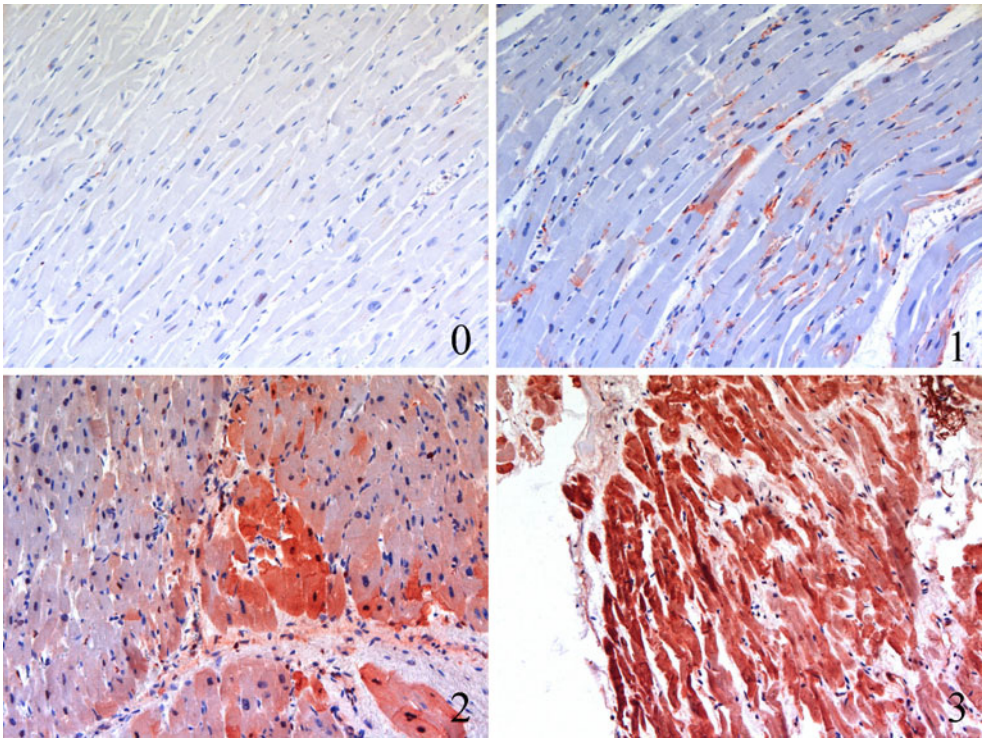

Fig. 1 Different grades of necrosis with the antibody fibronectin (×200). *0* no necrosis, *1* single cells necrosis, *2* group cell necrosisand, and three different: diffuse necrosis

each case in both groups. The grade of FE was consensually determined by two pathologists on a scale from 0 to 3, according to the classification proposed by Falzi et al. [20, 21].

The grade of necrosis in each ventricle for each antibody was compared to the corresponding value of the control group (grade of necrosis RV fibronectin study group versus grade of necrosis RV fibronectin control; grade of necrosis RV C5b-9 study group versus grade of necrosis RV C5b-9 control; grade of necrosis LV fibronectin study group versus grade of necrosis LV fibronectin control; and grade

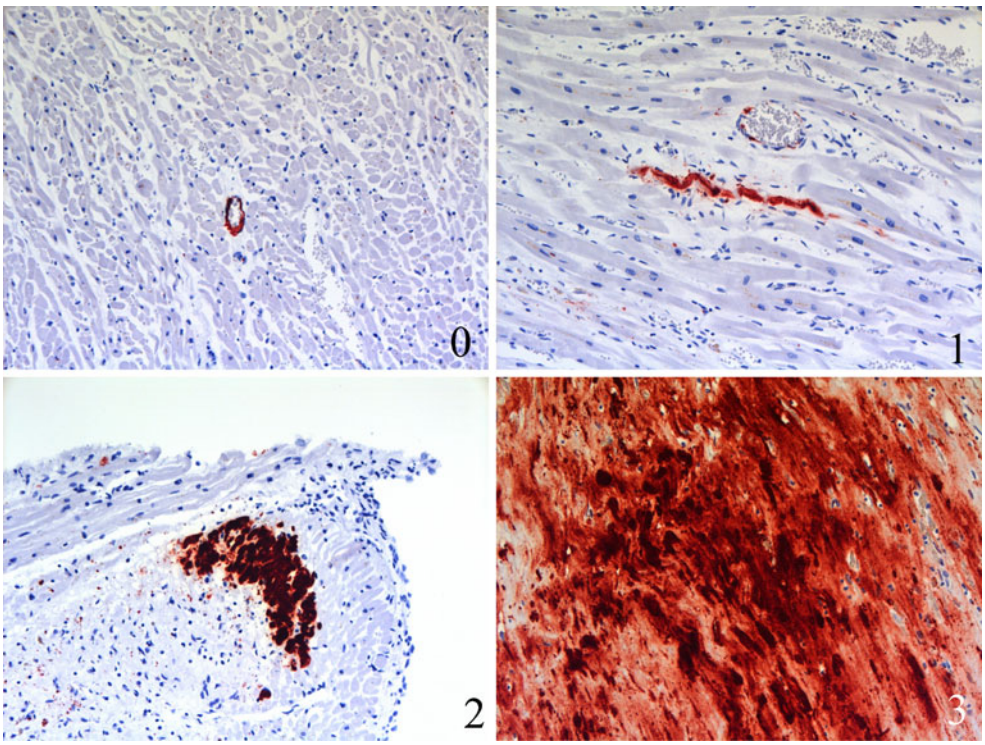

Fig. 2 Different grades of necrosis with the antibody Cb-9 (×200). *0* no necrosis, *1* single cells necrosis, two group cell necrosis and three: diffuse necrosis

of necrosis LV C5b-9 study group versus grade of necrosis RV C5b-9 control).

The difference of the grade of the necrosis between right and left ventricle was calculated in each case with both antibodies (Δ=grade necrosis RV—grade necrosis LV). A comparison of these differences between study and control group was performed.

Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Fisher's exact test was used for all comparisons between the study and the control group. The result was considered significant in case of the two-sided $p<0.05$.

Results

The comparison of the grade of necrosis in the right ventricles showed a prevalent ischemic damage in the FE group with the antibody fibronectin ($p=0.0014$). No statistically significant difference between the two groups with the antibody C5b-9 could be detected ($p=0.1301$).

The comparison of the grade of necrosis in the left ventricles showed a prevalent involvement of this ventricle in the control group with both antibodies. These results are statistically significant (fibronectin, $p<0.0001$; C5b-9, $p=0.0063$).

The comparison of the difference of the grade of the necrosis between right and left ventricle (Δfibronectin and ΔC5b-9) in the two groups showed a statistically significant difference between study and control group with both antibodies (fibronectin, $p<0.0001$; C5b-9, $p<0.0095$).

Discussion

In the investigated groups, the expression of the immunohistochemical markers is very different. In the cases of FE, the ischemic damage is prevalently localised at the right ventricle as detected with the antibody fibronectin, while the expression of the terminal complement complex C5b-9 is similar in both groups. This is not surprising. Fibronectin is a well-known marker of very early ischemic damage [41]; C5b-9 is, instead, known to react later [41, 42]. The possibility of detecting necrosis only with a very early marker probably indicates a very rapid evolution from the occurrence of the ischemic damage to death. Inflammatory changes at the sub-endocardial region were reported in cases of pulmonary embolism [43, 44] and may represent a late reaction to ischemia in cases of non-fatal embolism.

The discriminating element for the diagnosis of the occurred right ventricular failure is the evaluation of the difference between ischemic damage at the right and the left ventricle. This parameter allows the identification of the localisation of the prevalent ischemic damage. The simple detection of ischemic lesions at the right ventricle is, in fact, not indicative of primary affection of this ventricle since the main cause of right ventricular failure is the failure of the left ventricle [45]. In the control group, we could identify ischemic damage at the right ventricle in 90% of the cases with fibronectin and in 19%, with C5b-9. These results indicate an important impairment of the right ventricle, which is the consequence of the primary impairment of the left ventricle, as the prevalent localisation of the ischemic damage at this ventricle in this group demonstrates.

In the study group, the more evident is the primary involvement of the right ventricle, the higher the degree of the FE. A positive value of Δfibronectin was observed in five of six cases with FE grade 3, in six of eleven with grade 2, and in one of four with grade 1. The low number of investigated cases does not allow us to draw any definitive conclusion, but a tendency is evident. We think that a routine application of this method in cases of FE would facilitate the interpretation of the effective role played by FE in the determination of death. The FE should be considered as the primary cause of death only in cases with demonstrated prevalent ischemic damage at the right ventricle. In the other cases, the identified FE probably plays a concomitant role with other factors such as multiple fractures or major bleeding.

Similarly to other recent publications [46–49], this manuscript confirms the importance of the histological and immunohistochemical investigations in the routinely medico-legal practise.

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