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Morphological Signs of Intravital Contraction (Retraction) of Pulmonary Thrombotic Emboli

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Abstract The goal of the study was to establish whether contraction (retraction) of thrombi and/or thrombotic emboli occurs in vivo using morphological signs of blood clot compression, such as characteristic deformation of erythrocytes and redistribution of fibrin toward the surface of a thrombus/embolus. Three postmortem human pulmonary thrombotic emboli were examined by scanning electron microscopy and light microscopy after staining with hematoxylin and eosin as well as with Mallory's stain. In two pulmonary emboli, which were extracted at 7 and 15 h after death of the patients, compressed polyhedral erythrocytes (polyhedrocytes) were revealed that were formed due to mechanical deformation under the action of contractile forces generated by activated platelets. In addition, the uneven distribution of fibrin within the emboli was found with accumulation of fibrin at the periphery of the emboli, which is another structural characteristic of a contracted blood clot. In one of the three emboli analyzed, which was extracted 38 h after the patient's death (the "oldest" embolus), the morphological signs of contraction were absent, which was likely related to the partial postmortem autolysis of the embolus or intravital impairment of contraction. The ex vivo thrombotic emboli have morphological signs of clot contraction, suggesting intravital compression of the primary thrombi and/or thrombotic emboli. The in vivo contraction of thrombi and emboli may be an important pathogenic mechanism for modulation of blood flow past otherwise obstructive clots at the sites of thrombotic occlusion of a vessel. The presence of compressed erythrocytes inside and the predominant location of fibrin around the periphery of a thrombus or embolus can potentially serve as additional pathomorphological criteria for the intravital contraction of thrombi and thrombotic emboli.

Keywords Thrombosis · Thromboembolism · Clot contraction · Clot retraction

1 Introduction

Venous thromboembolism, which includes deep vein thrombosis, either isolated or associated with pulmonary embolism, is the third most common cardiovascular disorder and a major cause of morbidity and mortality worldwide [1]. Venous thromboembolism causes from 60,000 to 300,000 deaths each year among approximately 900,000 new cases in the USA [2]. Thrombotic emboli are the most frequent type of embolism that develop due to detachment of an entire thrombus or separation of its part. The size of thrombotic emboli can vary up to several centimeters. Venous thromboembolism complicates cancer, infections, cardiovascular diseases, and many other pathological states [3, 4]. Despite tremendous clinical importance, the mechanisms of thrombotic embolism remain largely unclear and the results of prophylaxis and treatment remain unsatisfactory.

In the vast majority of cases, the primary thrombotic occlusion occurs in the veins of the systemic circuit or in the right heart chambers, where fragments of the thrombus are carried by the blood flow into the pulmonary artery, leading to pulmonary embolism [4, 5]. Pulmonary embolism causes right ventricular failure and/or pulmonary infarction (when small and medium branches of the pulmonary artery are blocked) or sudden death (when the embolus is located in the trunk and in the large branches of the pulmonary artery). In the latter case, the mechanism of death includes a cardiopulmonary reflex accompanied by the coronary spasm. Thus, venous thromboembolism is a potentially lethal complication of thrombosis [4]; therefore, its prevention needs thromboprophylaxis or thrombectomy performed in a timely manner [6].

Blood clots normally undergo contraction or mechanical compression occurring under the action of the contractile

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forces produced by activated platelets adherent to fibrin [7]. Contraction can shrink blood clots, i.e., reduce the degree of occlusion of the blood vessels, and enhance the blood flow past the otherwise obstructive thrombotic masses [8–10]. In addition, contraction makes a clot more compact and less permeable due to dramatic changes in its structure and mechanical properties [11, 12]. One of the possible causes of thrombotic embolism is reduced contraction, resulting in formation of a less compact and mechanically unstable thrombus the may contribute to the mechanical breakage and embolization of a loose primary clot [9, 10, 13]. Surprisingly, while clot contraction has been investigated for decades, it remains one of the least studied reactions of blood coagulation, hemostasis, and thrombosis. Moreover, it has not been demonstrated so far that contraction of venous blood clots and/or thrombi happens in vivo in thrombotic patients. It is even less clear whether venous emboli can undergo contraction.

It was shown recently that contraction of blood clots in vitro has two structural consequences: (i) redistribution of platelets and fibrin and their accumulation on the surface areas of a clot and (ii) compression of erythrocytes with transformation of their shape from a biconcave to polyhedral (hence, the cells named polyhedrocytes) [14]. Based on these observations, formation of compressed polyhedrocytes inside and redistribution of fibrin to the surface of a clot can be considered as the objective morphological criteria of clot contraction [8, 15]. Moreover, these two morphological alterations could be used as a proof of the intravital compression of ex vivo clots and thrombi extracted during interventional thrombectomy or autopsy.

In this study, we investigated the composition of human pulmonary emboli obtained postmortem and confirmed the presence of morphological signs of the intravital contraction.

2 Materials and Methods

2.1 Scanning Electron Microscopy

Thrombotic emboli from three deceased patients were removed from the pulmonary arteries during autopsy, rinsed in phosphate-buffered saline, and fixed in 2% glutaraldehyde in phosphate-buffered saline. The fixed clots were washed in 50 mM sodium cacodylate, containing 150 mMNaCl (pH 7.4), then dehydrated in ascending concentrations of ethanol (30–100 *v*/*v*%), and dried using hexamethyldisilazane. The samples were sputter coated with gold-palladium. The emboli were cut open and the inner parts of the specimens were studied in a scanning electron microscope FEI Quanta 250 FEG (FEI, Hillsboro, OR, USA). For each sample, 10–15 images were taken randomly.

2.2 Light Microscopy

The emboli were fixed in 10% neutral buffered formalin, washed in water, cut, consequently dehydrated in ascending concentrations of ethanol and xylene, and embedded in paraffin. Four-micrometer-thick sections were stained with hematoxylin and eosin and with Mallory's stain which includes fuchsine acid, phosphomolybdic acid, aniline blue, orange G, and oxalic acid.

3 Results

3.1 Case 1

A 56-year-old male, autopsy was performed 7 h postmortem. No history of antemortem use of any medications affecting blood clotting.

3.1.1 Autopsy Summary

Principal Diagnosis A malignant neoplasm of the anterior mediastinum with tissue destruction. The tumor was 20 × 18 × 15 cm in size, had a mass of 2000 g, surrounded the pericardium and compressed the right ventricle of the heart.

Complications of the Underlying Disease Thrombosis of the right ventricle of the heart and pulmonary embolism with occlusion of small branches. Hypercoagulable state (the fibrinogen level was 8.54 g/L, the ethanol gelation test was positive). Bilateral hydrothorax (300 mL), hydropericardium (80 mL), parenchymatous dystrophy of the myocardium, liver, and kidneys.

Associated Diseases Atherosclerosis of the abdominal aorta, constrictive coronary atherosclerosis, focal cardiosclerosis. Hypertension with cardiac hypertrophy.

3.1.2 Morphology of the Embolus

Macroscopically, the thrombotic embolus was 9–9.5-cm-long with a diameter varying from 0.5 to 1.5 cm. The texture was moderately dense. The surface was smooth, grayish-reddish with an alternating brown color. On the interior observed with cut opening the tissue had a relatively homogeneous structure, a maroon color with light gray inclusions.

Histologically, there was clear segregation into "red" and "white" zones packed into layers with well-discernible red and white blood cells, respectively (Fig. 1a, b). At a magnification of ×400, the Mallory-stained sections contained clearly visible tightly packed polyhedrocytes. In both the "red" and "white" areas, aggregates of polymorphonuclear leukocytes were found that were accumulated predominantly near the borders between fibrin and erythrocytes. Fibrin formed condensed clusters as well as fibrous structures, mostly at the periphery

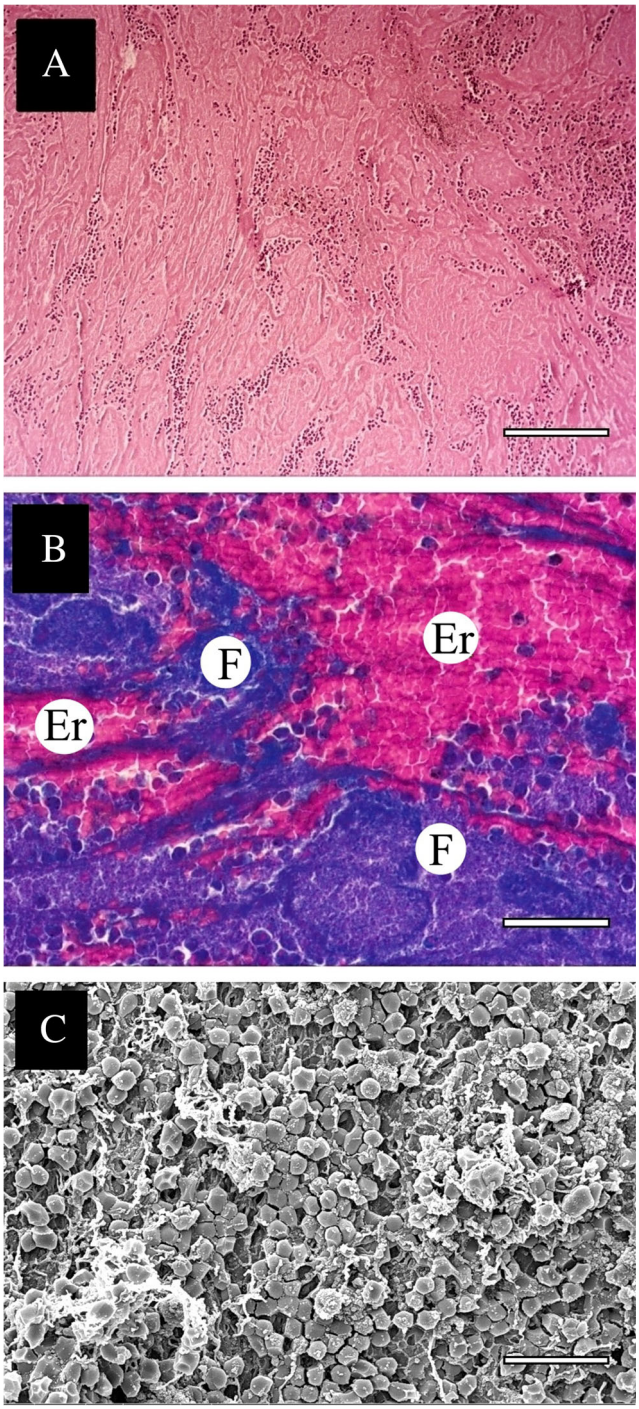

Fig. 1 Morphological characterization of a pulmonary thrombotic embolus from Patient 1. **a** Layered structure of the thrombotic mass with well-discernible blood cells. Hematoxylin and eosin, ×100; magnification bar = 0.2 mm. **b** Erythrocytes (Er) are densely packed and have a polyhedral shape, fibrin (F) forms amorphous deposits and fibrous structures. Mallory's stain, ×400; magnification bar = 0.05 mm. **c** Scanning electron microscopy shows fully compressed polyhedral and partially compressed flattened erythrocytes covered sparsely with fibrin fibers. Magnification bar = 20 μm

of the clot. Deep inside the clot we observed a sharp border between compacted but intact and damaged erythrocytes. Scanning electron microscopy of the core of the embolus (Fig. 1b) revealed many tessellated polyhedrocytes. Slightly misshapen erythrocytes that were only partially flattened and not so densely packed were located closer to the periphery. Sparse fibrin fibers covered the deformed red blood cells.

3.2 Case 2

A 66-year-old male, autopsy was performed 15 h after death. No history of antemortem use of any medications affecting blood clotting.

3.2.1 Autopsy Summary

Principal Diagnosis Cancer of the lower lobe of the left lung with destruction of the tumor and pleural dissemination on the left, metastases in the adrenal glands, paratracheal and paragastriclymph nodes (poorly differentiated spindle cell carcinoma of the lung). Left minithoracotomy, pleura biopsy, and drainage of the pleural cavity were performed.

Complications of the Underlying Disease Shift of the mediastinum to the right. Mural thrombosis of the right ventricle of the heart. Venous thromboembolism of the main trunk of the pulmonary artery. General venous stasis of parenchymal organs. Pulmonary edema. Right-sided hydrothorax (400 mL). Hepatic failure (AST 559 U/L, ALT 110 U/L, reactive hepatitis, centrilobular focal liver necrosis). Anemia (RBC count 3.57×10^{12}/L, hemoglobin 81 g/L, hematocrit 26.3%).

Associated Diseases Atherosclerosis of the aorta, iliac, and coronary arteries (without stenosis). Diffuse cardiosclerosis. Hypertension (eccentric cardiac hypertrophy with a mass of 430 g, thickness of the left ventricle was 1.8 cm, right ventricle 0.3 cm). A simple cyst in the right lobe of the liver.

3.2.2 Morphology of the Embolus

Macroscopically, the embolus was slightly flattened, 6.5–7 cm in length and 0.5–1.2 cm in diameter. The texture was moderately dense and elastic. In the interior on cutting, the tissue looked heterogeneous, with a light gray color closer to the surface and a maroon color closer to the interior of the embolus. There was a tendency to an alternation of dark and light areas.

Histologically, streaky structure was observed because of alternations of red and white portions of the embolus (Fig. 2a). In the more superficial white zones (Fig. 2b), fibrin formed a network, and a large number of diffusely located polymorphonuclear leukocytes were visualized. In the red zones that comprised more central areas, various forms of erythrocytes, including polyhedral erythrocytes, were found in combination with platelet aggregates and fibrin. At the same time, some areas (stained yellowish by Mallory's stain) contained a fraction of lysed erythrocytes.

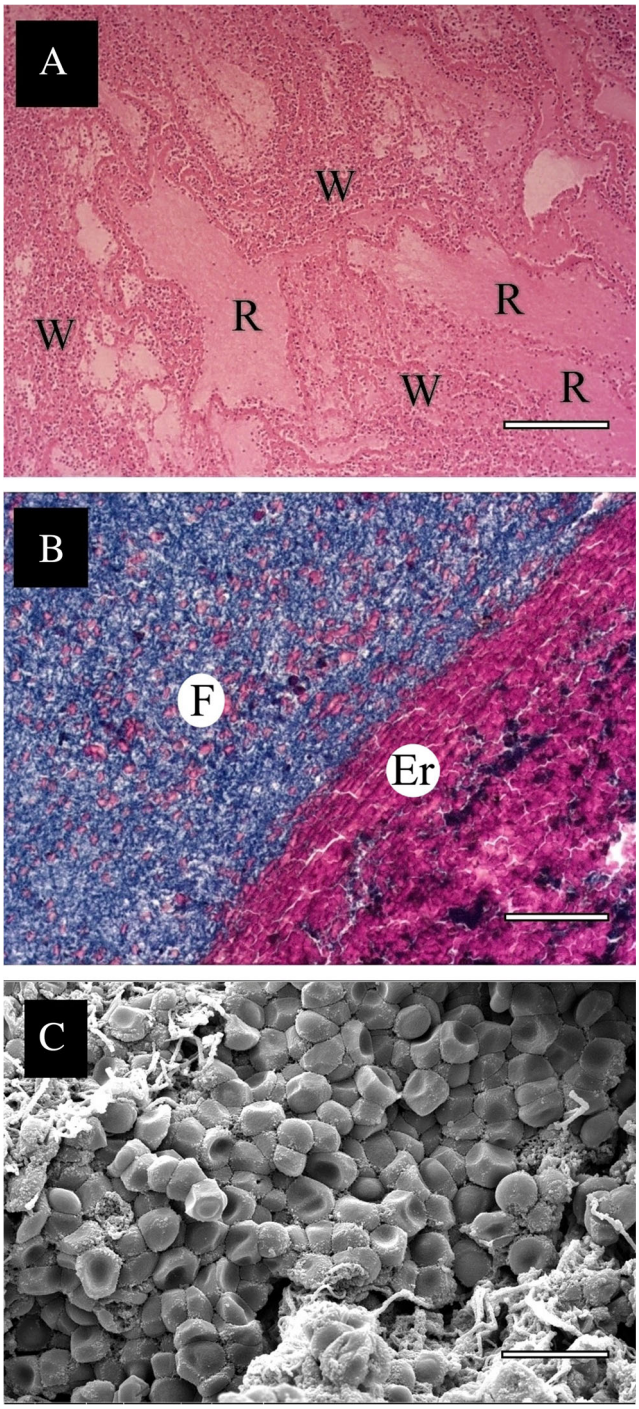

Fig. 2 Morphological characterization of a pulmonary thrombotic embolus from patient 2. **a** Layered structure of the thrombotic mass with a clear division between red (R) and white (W) zones. Hematoxylin and eosin, ×100; magnification bar = 0.2 mm. **b** Fibrin network (F) located on the periphery, erythrocytes (Er) of a various shape visualized in more centrally located areas. Mallory's stain, ×400; magnification bar = 0.05 mm. **c** Scanning electron microscopy shows a majority of erythrocytes are flattened and have a polyhedral form, fibrin predominates in the peripheral areas of the embolus. Magnification bar = 10 μm

Scanning electron microscopy (Fig. 2c) most areas, especially those that were closer to the core of the embolus, revealed erythrocytes that were either flattened or tightly packed in polyhedral form, with minor to moderate amount of fibrin on the cell surface but not between polyhedrocytes. Only a few erythrocytes were intact and had a biconcave shape. Fibrin strands prevailed at the periphery and on the surface of the embolus.

3.3 Case 3

A 70-year-old female, autopsy was performed 38 h after death. The patient had a history of antemortem use of anticoagulants and antiplatelet drugs, namely, unfractionated heparin, enoxaparin, fraxiparine, and aspirin.

3.3.1 Autopsy Summary

Principal Diagnosis Cancer of the uterine body with pelvic invasion. Hysterectomy, bilateral salpingo-oophorectomy, resection of greater omentum, and drainage of the abdominal cavity were performed.

Complications of the Underlying Disease Pelvic deep vein thrombosis and pulmonary embolism of the main trunk of the pulmonary artery. General venous stasis of parenchymal organs. Pulmonary edema. Anemia (RBC count 2.96×10^{12}/L, hemoglobin level was 81 g/L, hematocrit 24%). Thrombocytopenia (platelet count 63×10^{9}/L). Hypercoagulable state (fibrinogen level was 6.7 g/L, the ethanol gelation test was positive).

3.3.2 Morphology of the Embolus

Macroscopically, the thrombotic embolus had a length of 9 cm and a diameter of 1.5–1.6 cm with a dense texture. On the interior with cutting, the tissue was somewhat heterogeneous; it had a lighter surface and darkened gradually towards the core. Overall, the structure was relatively homogeneous.

Histologically, the clot had a very polymorphic structure with quite a vague border between red and white areas. In many areas, we found a mixture of leukocytes, fibrin, aggregated platelets, and erythrocytes (Fig. 3a). Layers of fibrin (Fig. 3b) were found throughout the entire embolus; between the fibrin fibers, intact erythrocytes and neutrophils were found, with a few mononuclear leukocytes. The fraction of erythrocytes only slightly increased from the surface towards the core of the embolus. A large fraction of lysed erythrocytes was seen closer to the core of the clot.

Scanning electron microscopy (Fig. 3b) revealed a large number of slightly altered tuberous, somewhat swollen or almost intact biconcave erythrocytes and leukocytes, richly covered with fibrin depositions in the form of amorphous mass or long filaments. The components of the embolus were relatively loosely packed.

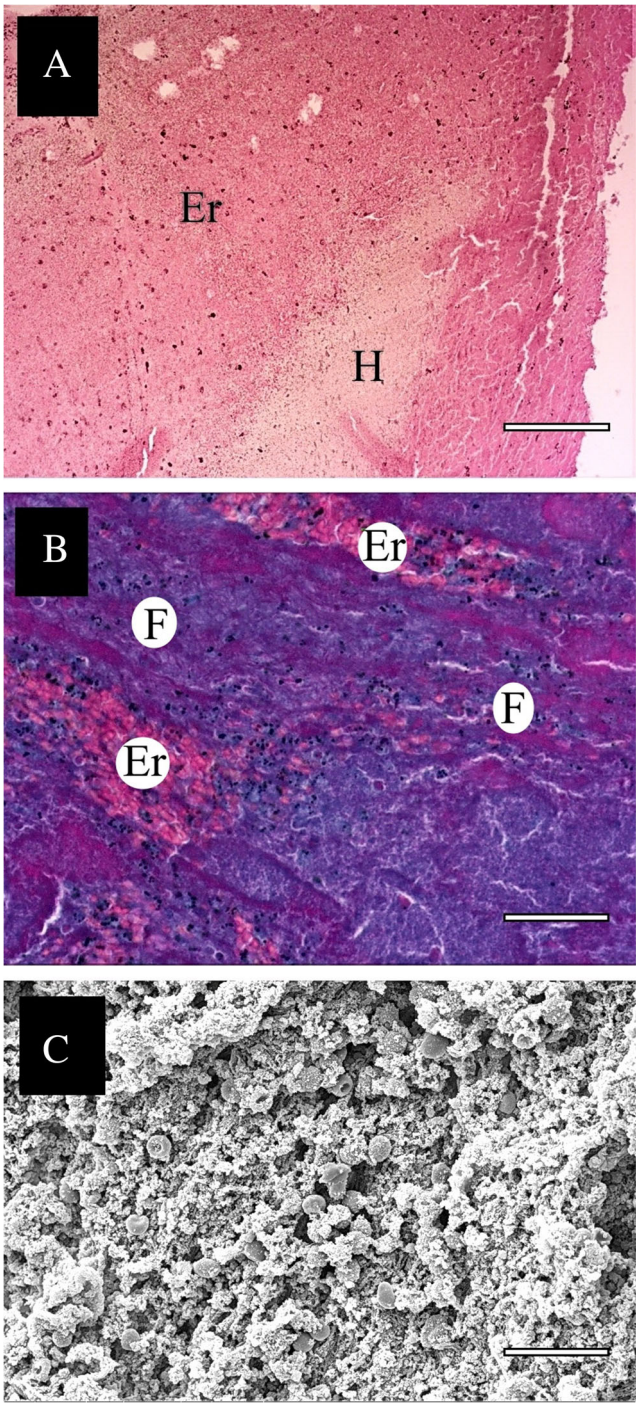

Fig. 3 Morphological characterization of a pulmonary thrombotic embolus from Patient 3. **a** The lack of layered structure (Er) and polymorphism with a large number of destroyed erythrocytes (H). Hematoxylin and eosin, ×100; magnification bar = 0.2 mm. **b** Fibrin is distributed evenly in the layers (F), erythrocytes are intact (Er). Mallory's stain, ×400; magnification bar = 0.05 mm. **c** Scanning electron microscopy shows blood cells loosely packed, most erythrocytes are intact, non-deformed, fibrin richly covers blood cells. Magnification bar = 20 μm

4 Discussion

The results of the study showed that pulmonary emboli in patients 1 and 2 contained erythrocytes with a polyhedral shape (polyhedrocytes) that were formed as a result of mechanical compression under the action of the contractile forces generated by activated platelets [14]. In addition, uneven distribution of fibrin within the thrombotic emboli was revealed, namely, the accumulation of fibrin near the periphery of the emboli, as in venous mouse clots [15]. These two structural features, i.e., the presence of polyhedrocytes and redistribution of fibrin towards the periphery, are the morphological criteria of platelet-driven contraction (compression) of a blood clot [14]. These observations strongly suggest that the emboli underwent pathophysiological contraction in vivo. It is noteworthy that both emboli with the morphological evidence for the intravital contraction were extracted and fixed 7 and 15 h after death, when postmortem changes of thrombotic emboli are not pronounced [16].

In one of the three pulmonary emboli studied (patient 3), we did not see the morphological signs of contraction (there were no polyhedrocytes and no fibrin redistribution to the periphery). Fibrin was located almost evenly throughout the embolus.

Because the embolus of patient 3 was extracted and fixed 38 h after death, it is possible that the morphologic characteristics were associated with postmortem autolysis and the related cell damage [16]. At the same time, the lack of the signs of compression may reflect the absence or a substantial reduction of the intravital contraction of the embolus. This impairment of the intravital clot contraction could be due to the anticoagulants and antithrombotic drugs taken by the patient, as well as to platelet dysfunction and low platelet count. Indeed, patient 3 had profound thrombocytopenia, which has been shown to be associated with reduced platelet contractility and low extent of blood clot contraction [17].

Based on the brightness of T1-weighted MR images of ex vivo postmortem pulmonary thromboemboli, Tratar et al. [18] concluded that most of the lethal pulmonary emboli were likely derived from retracted/contracted thrombi. Our results confirm this presumption but provide much better structural evidence for contraction of primary thrombi and/or secondary pulmonary thrombotic emboli.

5 Conclusions

Ex vivo thrombotic emboli of the pulmonary artery have morphological signs of contraction that include the presence of compressed erythrocytes of a polyhedral shape and accumulation of fibrin on the periphery. These results suggest the intravital contraction of primary thrombi and/or thrombotic emboli, which may be a pathogenic mechanism of blood flow

modulation at the sites of thrombotic occlusion. The absence of the morphological signs of contraction of emboli may either reflect impairment of the in vivo clot contraction or result from postmortem changes, such as autolysis.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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