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Original Article

A model of massive pulmonary embolism, development and characterization

The pre-clinical steps forward and details of the progress

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Abstract

BACKGROUND: Massive pulmonary embolism (MPE) is in most cases an unpredictable, life-threatening lung injury. In order to test this shock and its natural sequence, MPE animal model was established. Based on previous models, discussed within the article framework, this model was designed to closely narrate clinical pulmonary embolism.

METHODS: MPE was induced by a single injection of minced radioactive blood thrombi into an internal jugular vein. Thrombi were prepared from the autologous blood of each animal. Using rabbit model allowed sampling and recording additional data necessary for better analysis. Clotting additives were used for rapid clot stabilization. Clot was stabilized at room temperature for one hour and separated into micro-emboli of comparable size prior to the intravenous injection. A radioactive tracer, I-125 labeled rabbit fibrinogen, was added into thrombi to measure dynamic lung thrombi-turnover.

RESULTS: Thrombolysis dynamic efficacy was characterized by presence of high statistical significant difference ($P < 0.001$) found between released radioactive I-125 fibrin degradation products (FDPs) at 10 minutes and all others FDP time points until 60 minutes. Pulmonary thrombolysis was characterized by measuring residual radioactivity of the lungs at 10 and 60 minutes and was found statistically significant ($P < 0.05$) during the period of 50 minutes. For the purpose of model validation, systemic blood pressure, measured in carotid artery, significantly increased from the base-line point 47 mmHg to 80 mmHg at the first 10 minutes. Enormous mechanical thrombus injury of lung vasculature was depicted by MSB staining.

CONCLUSIONS: This MPE model contains a set of important and original patho-physiological data mimicking the fundamental characteristics of this shock situation in humans, which enhances the understanding of MPE, and leads to better characterization of this critical clinical condition.

KEYWORDS: Massive pulmonary embolism, animal model, thrombolysis dynamic efficacy.

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Massive pulmonary embolism (MPE) is a vessel obstruction triggered by escaped thromboemboli leading to a hemodynamic shock with vast impact on lung circulation. Patients die because of escaped emboli, most likely from the deep vein system of the leg, traveling upstream and obstructing different lung vessel regions, mainly the pulmonary artery. MPE patients usually die within 2 hours of the onset of symptoms, with right-sided heart failure- the primary cause of death. [1] MPE shock mechanism has been known for many years, but the model that would extrapolates this event from bench to bedside still lack its quality and full reproducibility. Currently, there is no laboratory animal

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design unifying the pre-clinical test of animal pulmonary embolism with clinical PE. Such model would describe, for example, how to reliably prepare thrombo-emboli of a certain size and by using clotting additives with detection tracers to expand our knowledge about this life-threatening condition. Knowing that MPE involves hemodynamic shock triggered by escaped thromboemboli, testing non-clotted blood, as described previously, [2-6] might not reveal the actual process and its complexity. For example, the constant lysis and clot accretion with platelets and other plasma member interactions (i.e., thrombin, fibrin, protein C) is considered critical by many when proposing a clinically relevant PE model. Consequently, a successful model that would allow emergency medical personnel to assess MPE and its derived complexity must combine knowledge from at least four interrelated fields: hemostasis and coagulation, pulmonary critical care, medical imaging, and surgery. The MPE model has to further not only address the time-to-treat window, but also advance and construct a multidisciplinary and clinically relevant MPE replica.

A model has to fulfill certain criteria in order to be practical, repeatable, and useful. Current scientific literature describes an enormous number of MPE models, most studying MPE while characterizing single underlying principles (i.e., pulmonary clot hemodynamic, [7] radiological visualization, [8,9] surgical access into the pulmonary artery, [10] without accounting for uniformity, standardization, reproducibility, or the overall patho-physiology of autologous MPE description. Certain disadvantages can be observed when animal models trying to extrapolate MPE to humans. For that purpose often tested mouse, rabbit and rat, rather than animals prevalently most affected with MPE, such as ferrets, cats, [11] rabbits and dogs can be seen as contra-productive. By examining MPE in large animals such as pigs or sheep, authors often study pulmonary mechanics. Visualizing pulmonary vessels in the parenchyma by detection of injected radioactive labeled emboli [12] allows development of improved surgical techniques that could be used in humans to safely evacuate life-threatening thrombi from the main pulmonary artery. It is the size of the pulmonary artery and, overall, the size of the lung parenchyma, [8,13] that prevent radiologists and surgeons to utilize the most prevalent animal models to train students in emergency surgical MPE techniques. Middle-sized animals (i.e., dogs, minipigs, hamsters and rabbits) are usually selected for more precise and detailed research. These models are easily blood sampled and visualized for detection of the thrombo-embolism, and as well for their limited requirements for an extensive food and water supply, and cost associated drug injections (i.e., thrombolytic therapy). [14] These models have the potential to become primary research models in the future.

A common characteristic to every MPE model is the method of initiation for the embolism. One approach is through the single short clot injection. Another, not so common, is to inject the clot repeatedly (over 5-10 minutes). [7] The preferred method generally varies with the methodology of each study. The induction sites also differ from the most proximal (a direct injection into the pulmonary artery in the pig model [13]), to the most distal (into the tail vein of a rat [15]). When thrombi are aimed directly towards the heart and large lung vessels, the patho-physiological alterations such as acute pulmonary hypertension complicated by a decrease of cardiac output can be intercepted almost instantly. [16] Moreover, passing thrombi can be visualized almost immediately if they are labeled. The overall processes occurring after MPE is characterized rather incompletely given the importance of MPE course of action. Authors usually concentrate on describing the percentage increase in pulmonary arterial blood pressure after embolization, such as the cases of visualization. [7,13] The death rate is rarely published, [15] and a standard histological assessment of the lungs post-embolism is seldom included. [4] Since clot structure differences are significant factors of MPE severity, a number of recent studies have provided details of a clot type employed, describing clotting addi-

tives and methods of clot preparation. Some groups have also tried to employ plasma clots harvested from humans and injected into animals, [15,17] while others have used autologous animal whole blood clot injections, [7,16] or a combination of human clots and autologous clots from animals. [18] Based on previously MPE animal model design, this model is based on the autologous blood thrombi injection centrally into a jugular vein (close to the final organ impact injection) while using the medium sized animal model. The uniqueness of this strategy was based on in vitro production of net clot radioactive (NCR) thrombi from the autologous animal blood, prepared one hour prior to the induction. Moreover, the baseline measurement of central arterial pressure, complete blood count (CBC) before and after embolism, radioactive fibrin degradation products measured in whole blood along with presenting lungs histological changes and measurement of radioactive thrombi presence in 60 minutes, assisted in better characterization of this life-threatening condition.

Methods

125-Iodination of Rabbit Fibrinogen

Iodobeads (Pierce, Rockford, IL, USA), were place the in eppendorf tube and 1 ml of 0.5 M phosphate buffer was added and removed immediately. 100 µL of 0.2 N phosphate buffer (pH 7.5) was added along with 9.1 µL (1 mCi) of I-125. Then, it was incubated for 5 minutes and added 200 µL of rabbit fibrinogen (Sigma, Oakville, Ontario, Canada), r-Fg and again incubated for 5 minutes. After incubation, solution was gently shaken every 30 seconds. The reaction stopped by removing solution from iodobead and onto the G-25 Sephadex (Pharmacia) PD-10 column. After fractionation, the tests of precipitation and clottability were run and fractions were aliquot into 50-µL fractions for future use.

Autologous thrombi preparation

The whole blood (0.5 ml) drawn from the left auricular marginal vein from each rabbit was slowly emptied into a 5 ml Sarstedt centrifuge tube with 10 µL of radioactive I-125 labeled rabbit fibrinogen and mixed on a vortex shake. Blood was then transferred into 5 ml Sarstedt centrifuge tube with Thrombosil (Hemoliance, Raritan, NJ, USA) (5 µL), the rabbit brain phospholipid reagent containing a silica activator of Factor XII, where it was slowly mixed for 10 seconds. The final concentration of Thrombosil in clot was 1 IU/µL. Blood was allowed to clot in room temperature for the period of one hour. Shortly after, five times passage through 20 GA needle was carried out in order to mince the stabilized clot. Before thrombi injection, it was necessary to determine the amount of radioactivity. By subtractions of counts per minute (CPM) of equipment used for clot preparation and stabilization, i.e., 20 GA needle, syringe, 1 ml syringe-tip, the 14 GA intravenous catheter and empty 5 ml mixing tube, where clot was allowed to stabilize, from the total CPM of the clot prior to the mincing NCR was determined. Later, CPM from each lung lobe added together and divided by net clot radioactive CPM illustrated clot lysis in lungs.

Animals and Experimental Study Design

The Animal Research Ethics Committee of McMaster University, Hamilton, Ontario, Canada along with the Veterinary Faculty, Veterinary and Pharmaceutical University Brno, Czech Republic approved all procedures performed on animals. All experiments were performed according to the Guidelines to the Care and Use of Experimental Animals.

Animals

Twenty-two New Zealand White (NZW) rabbits males, specific pathogen free, (average weight 3.5+/- 0.4 kg) over 90 day old, purchased from Charles River Laboratories (St. Constant, Quebec, Canada) were included into the study protocol.

Experimental protocol

Rabbits were pre-anesthetized by Ketamine (MTC Pharmaceuticals Cambridge, Ontario, Canada) 50 mg/kg IM, Xylazine (Bayer Inc, Animal Health Toronto, Ontario, Canada) 2 mg/kg IM. Ears and chest cavity were shaved and surgically prepared. The Left Central

Auricular Artery and the Left Auricular Marginal Vein were cannulated by 22 GA catheters (Angiocath). After an induction of anesthesia, rabbits were intubated via tracheotomy by 3.5 Portex blue line uncuffed endotracheal tube and ventilated by combination of 2.5 % of Isoflurane and 3L/min of oxygen by means of Penlon AM1000 (Abingdon, Oxon England). The respiratory rate was maintained at 30-40/min. The right carotid artery was then catheterized with Polyethylene tubing (PE 190) (Becton and Dickinson, Franklin Lakes NJ, USA) for blood sampling and recording blood pressure by PC Scout transport monitor 90309. The pressure in carotid artery was maintained on baseline anesthesia pressure (40-50 mmHg) to compare the level of the arterial blood pressure before and post embolism (figure 3). It was also recorded before the injection of the minced clot and during each experiment. After the thrombi injection, oxygen was increased to 5 L/min and isoflurane concentration was decreased to one volume percent. Once stable, (10- 15 minutes), animals were maintained on one and half volume percent of isoflurane and on 3 L / min of oxygen. The left jugular vein was cannulated, with a 14 GA catheter (Insyte), for minced clot delivery (for detail descriptions please see table 1, timeline of the experiment).

Table 1. Experiment outline.

Preparation	**Baseline blood sampling and Emboli Injection.**	**Experiment endpoint**
Animal Pre-medication Weight Surgery Prep. ***Ear Vascular Cannulation*** ***Left Central Auricular Artery*** **Left Auricular Marginal Vein** Intubations tracheotomy ***Vascular Cannulation*** **Right Carotid Artery** **Left Jugular Vein**	Left Central Auricular Artery (cells + hematocrit) 0,30,60 minutes. **0.5 ml of blood for thrombi preparation from Left Auricular Marginal Vein* **Right Carotid Artery** (thrombosis-thrombolysis) 0,10,20,30,40,50,60 minutes. and measurement of systemic blood pressure Right Carotid Artery (plasma) 0,10,20,30,40,50,60 minutes. Emboli Injection Autologous emboli IV delivery into Left Jugular Vein	1000UI of Heparin Last blood samples Last measurement of blood pressure **Right Carotid Artery** Euthanasia Pentobarbital Sodium **Left Auricular Marginal Vein** Inspection of chest for residual clotted blood Lungs and heart sample-established N.C.R and counted Thrombolysis dynamic efficacy and Lung pulmonary thrombolysis

One milliliter of the whole blood was taken from the left central auricular artery before an induction of coagulation, and at 30 and 60 minutes of experiment into Vacutainer K2 EDTA 7.2 mg was purchased from (Becton and Dickinson, Franklin Lakes NJ, USA) for Complete Blood Count (CBC) measurements. Samples were analyzed one hour after each experiment by Beckman Gen S Hematology analyzer, to quantify white blood cells, (WBC), red blood cell (RBC), platelets (PLT) and hematocrit parameters. One ml of the whole blood at 0, 10, 20, 30 40, 50, 60 minutes was obtained from the right carotid artery into 5 ml plastic tubes for measuring CPM of I-125 on Clinigamma 1272 LKB, (Wallac-Gamma counter) immediately after the end of experiment, to assess FDPs, released from injected clot. Two ml of the whole blood was collected from the right carotid artery at 0, 10, 20, 30, 40, 50, 60 minutes into 3 ml syringes pre-filled with 0.2 ml of 3.8% sodium citrate and immediately transferred into the eppendorf tubes and stored on ice for plasma collection.

After experiment, citrated blood was spanned on Centrifuge (Brinkmann instruments, Westburg NY) for 5 minutes with speed of 10,000 RPM for separation of plasma and citrated plasma. Then, it was stored in -70°C freezers for future analysis. Experiments were terminated at 60 minutes. Each rabbit received 1000 IU of Heparin (Hepalean, Organon Teknika, Toronto, Canada) as an intravenous bolus through left auricular marginal vein. One minute later, animals were euthanised with euthanyl (pentobarbital sodium, 360 mg/rabbit) via left auricular marginal vein. Thoracic cavity was opened and inspected for residual clot presence. Heart along with lungs was removed (for detail descriptions please see table 1, Experiment Outline). Each lobe of the lungs with heart was inspected macroscopically, sectioned into corresponding pieces and inserted into plastic tubes for radioactivity measurement. Thereafter, lobes were sectioned and deposited into OmniSette tissue cassettes. Cassettes were placed into formalin solution and later cut and stained with Martius Scarlet Blue (MSB) and Hematoxylin and Eosin (H & E), then analyzed under different magnifications. Damaged along with the healthy sites were mounted on a single slide, to help determine cell and fibrin presence. However, the vital conditions of each animal were well monitored. About 4% of animals died within the period of five to ten minutes within the minced clot delivery.

Thrombolytic- dynamic efficacy measurement (TDE)

Thrombolytic dynamic efficacy (TDE) measurement concentrates on the amount of radioactive FDPs released into the blood. Samples were drawn at pre-set time points as were released from the NCR injected into left jugular vein and trapped predominantly in the lungs. Radioactive FDPs released in blood were calculated and corrected as follows: radioactive counts released in each time point (FDP t) were multiplied by the weight of rabbit and further multiplied by an amount of his whole blood (61 ml/kg of total body weight) and were divided by the total net clot radioactivity injected, times hundred percent as depicted by the equation (a).

$$\text{(a)}\quad TDE = \frac{\text{FDP t.} \times \text{t. b. w.} \times 61}{\text{NCR}} \times 100\%$$

Measurement of pulmonary thrombolysis (PT-L)

After each experiment, lungs were measured for the presence of Residual I-125 counts per minute of captured thrombi. Clot lysis was described as CPM of radioactive I-125 thrombi, which were found not to be lung entrapped. For the purpose of presenting lung thrombolysis (lysis), all lobes were added together and divided by CPM of corrected NCR (as described in section thrombi preparation), times hundred percent. For the purpose of presenting thrombolysis (lysis), all lobes were added together and divided by CPM of NCR injected, times hundred percent as depicted by equation (b).

$$\text{(b)}\quad PT\text{-}L = \frac{\Sigma\text{CPM of each lobe}}{\text{CPM of NCR}} \times 100\%$$

Statistical analysis and blinding

Statistical analysis was performed using analysis of variance (ANOVA) for the time points of thrombosis and thrombolysis measurements, fibrin degradation products (FDPs) from radioactive blood, plasma, complete blood count (CBC) and blood pressure measurements. The data measured as baseline in case of level of FDPs, WBC and BP before the experiment, served as control (time-point 0 minute). Furthermore, for systemic blood pressure detection, the recording was established 5 minutes prior to each experiment, which later served as control. All data were expressed as mean+/- SEM. The P value in two tailed t-tests less than 0.05 was considered statistically significant. The P value < 0.001 was considered as highly significant. Blinding was assured for the assessment of the outcomes, while data collection was not blinded.

Results

Thrombolytic dynamic efficacy (TDE)

Amount of FDPs released into the whole blood from the NCR increased in 10 minutes to 17.09

% and then, in 20 minutes slightly declined to 15.23 % (figure 1). In 60 minutes, the radioactive fibrin degradation products were released with frequency of 14.39% of the total NCR. Using the ANOVA, high statistical significant difference ($P < 0.001$) was found between FDPs at 10 minutes and all others FDPs until 60 minutes, and significant difference ($P < 0.05$) between those of 20, 30, 40, 50 minutes and 60 minutes. Overall, FDP products showed the highest dynamic lysis at the first minute, which provided evidence that lung vasculature make up an extremely viable pro-thrombolytic environment.

Pulmonary thrombolysis (PT-L)

PT-L measured by the absence of radioactivity at 10 minutes showed 27.6 % lysis of injected NCR. After 60 minutes, lung lysis was found to be 35.7 % of NCR (figure 2). Lysis during the period of 50 minutes was found statistically significant ($P < 0.05$).

Systemic blood pressure

Systemic blood pressure (BP) (figure 3) significantly increased from the baseline point of 47 mmHg to 80 mmHg at the first 10 minutes. By ANOVA, significant difference was found between BP at 0 and 10 minutes, 10 and 20 minutes, 10 and 30 minutes and 10 and 40 minutes. After 10 minutes, BP abruptly dropped to 60 mmHg and further almost to the baseline levels, where it remained until the end of 60 minutes. After 10 minutes on MPE, BP significantly dropped to about 58 mm of Hg ($P < 0.05$). At 20, 30, 40, 50, and 60 minutes, compared to baseline there were no significant differences in BP

Platelets (PLT) levels

There was observed statistically significant change within 60 minutes in PLT levels that dropped from the baseline level of 296 to 253 x 10^9 /L (figure 4).

Histological assessment

The samples of injured lung parenchyma from the apical and distal lung lobes were compared (slides at figure 5). Slides were taken from the distal lung lobes. Figure 5, describes healthy and mechanically damaged lungs by fresh thrombi inside the lung vasculature after 60 minutes of MPE. Passing thrombi caused enormous abrasive mechanical damage to the lung vascular endothelium. Movement of diaphragm and pleurae, which pushed thrombi deeper into the pulmonary vasculature, caused more damage to smaller vessels and capillaries. Histological evidence indicated that distal lung lobes were damaged more than apical ones. The primary damage was caused by the mechanical clot trauma, which followed a massive perivascular interstitial edema and emphysema of damaged regions.

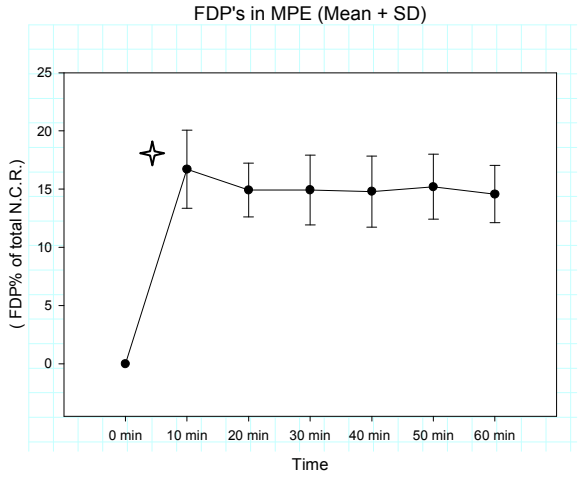


Figure 1. Thrombolytic Dynamic Efficacy (TDE) was measured based on the formula in the text. Fibrin Degradation Products released into the circulation after the MPE in a rabbit model. The stars (*) indicate high significant difference ($P < 0.001$) between the 10 minute point and all other measured time points.

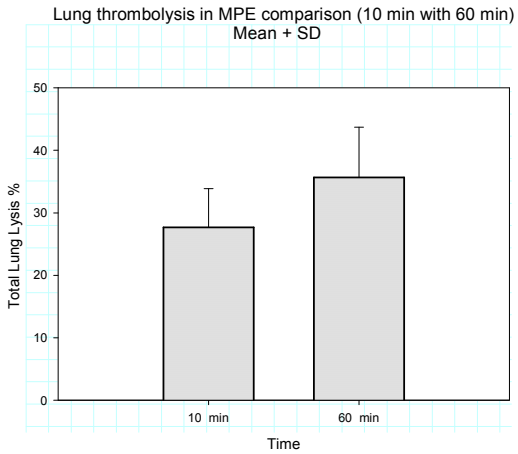


Figure 2. The precise radioactive labeled thrombi counts (NCR) just before the MPE induction makes this model unique, because of the ability to accurately portray pulmonary thrombolysis (PL-T) in time. As it is depicted in this figure, PL-T increased in 60 minutes ($P < 0.05$). Later, by using TDE and PL-T together, the clot lung turnover can be estimated.

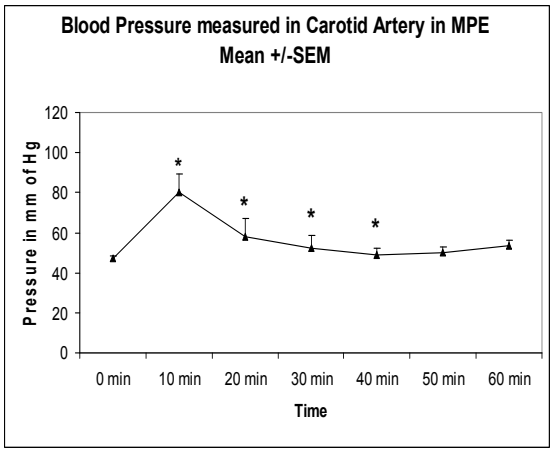


Figure 3. Systemic blood pressure measured in carotid artery showed significant increase after the first ten minutes of MPE, whereas later significantly dropped and difference can be observed between 10 and 20, 10 and 30, and 10 and 40 minutes. Stars (*) indicate the significant difference ($P < 0.05$).

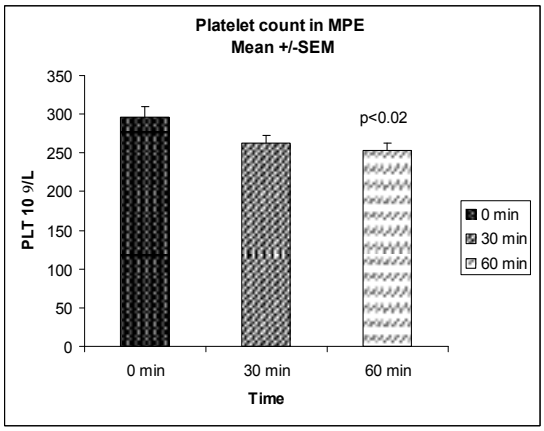
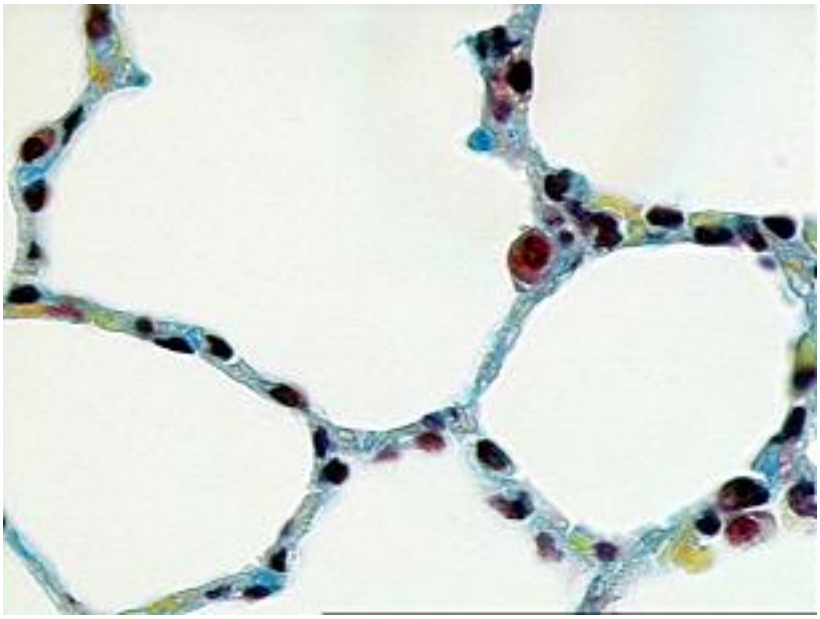


Figure 4. As reported in patients with deep vein thrombosis (DVT) and those scintigraphically PE positive, patients usually demonstrate fall in platelets count. This graph describes difference of platelets between baseline and 60 minutes of MPE ($P < 0.02$).



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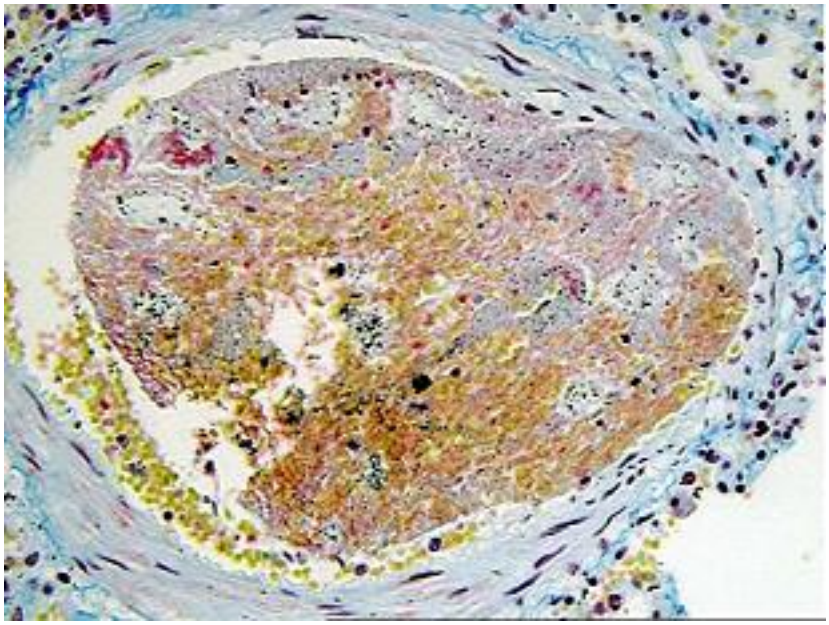

(2)

1. Structure of intact lung parenchyma (MSB staining).
2. The clot abrasive action in middle size pulmonary vessel (MSB staining).

Figure 5. A. Pulmonary alveoli of healthy lungs (MSB stain x1000)
Healthy Lung P1- Pneumocyte I type, squamous alveolar cell and P2-II type of Pneumocyte- typical pulmonary surfactant secretory cell are depicted by this image. P2 might be found where the alveolar walls unite and form angles. The capital letter C, Capillary lumen of E- Endothelial cell with Erythtrocyte presence, might be seen in the left corner of the slide. Nuclei of endothelial cells in the alveolo-capillary membrane are extremely thin and elongated; more than P1 type alveolar Pneumocyte, with which they are frequently confused.

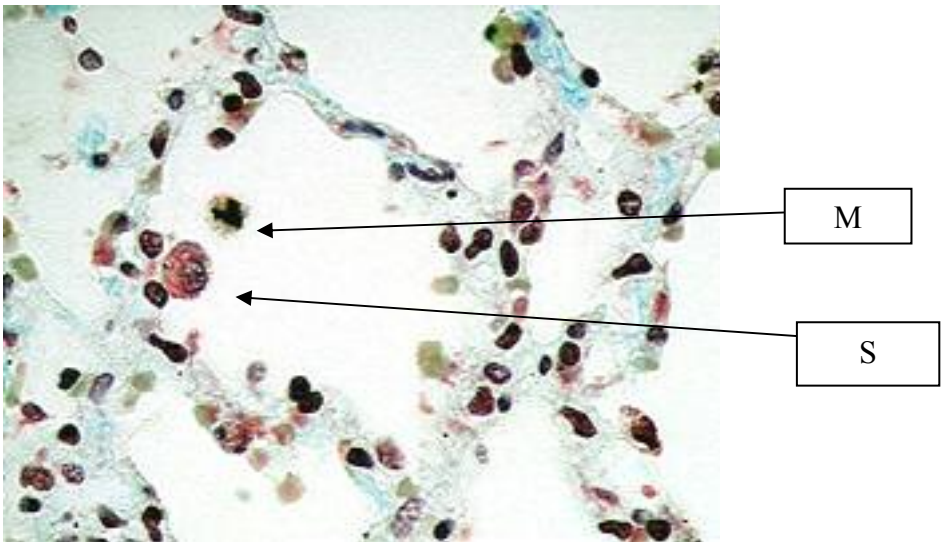

Figure 5. B. Pulmonary alveoli after the MPE (MSB stain x 1000)
A massive swelling of interalveolar septum caused by the perivascular edema of interstitium. The interstitium around P2 II, pulmonary surfactant secretory cell (arrow S) is massively swollen, the cell have edematically enlarged cytoplasm and is detached from the alveolar wall. Above the P2 is free floating pulmonary macrophage (arrow M) with visible V shaped nucleus.

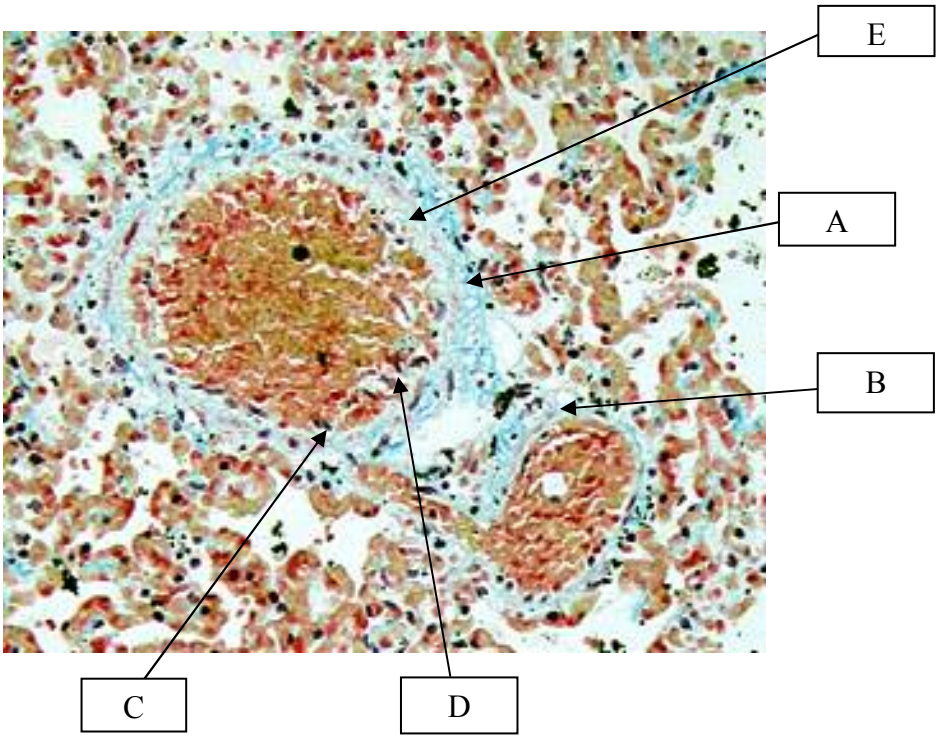

Figure 5. C. Cross-section of two medium sized pulmonary venulae after MPE (MSB stain x 400)
Two medium pulmonary venules were captured in damaged lung parenchyma. Tunica Adventitia, the outermost tunica of venulae (letter A), the capital letter B, tunica media, and by letter C Tunica intima. At 60 minutes after embolisation, Endothelial cells shed off from Tunica intima were found free floating and some already incorporated in the clot, surrounded by Erythrocytes and plasma, inside of the lumen of venula (letter D). The peeling processes of endothelial cells from it's lining and filling the gap with perivascularly escaping plasma could be seen around letter E. Growing amount of plasma escaping into the subintimal space of venula caused pressure built up, which further increased cell dislodgement in the area.

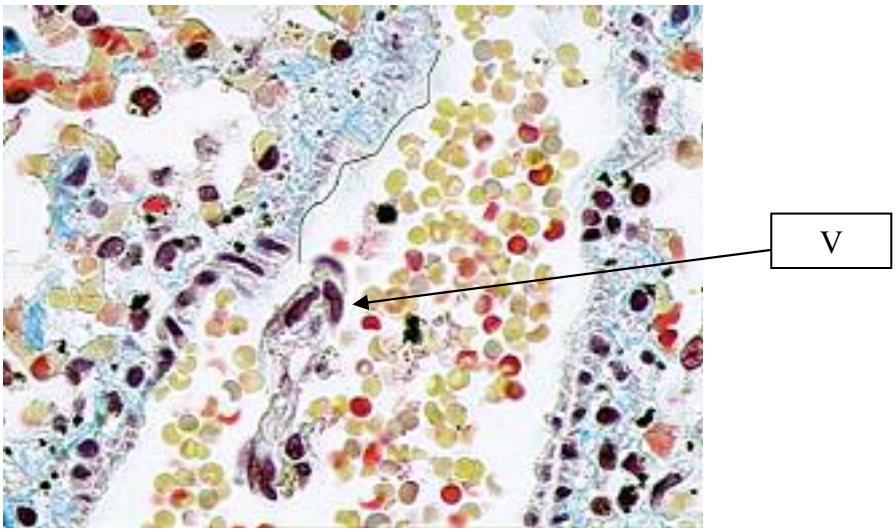

Figure 5. D. Longitudinal section through the medium size pulmonary venula (MSB stain x 400)

The line drawn at the left side of the picture, inside of the venous lumen, shows detachment of the portion of Endothelium that is liberated by an enormous mechanical force of the embolus that have recently passed through this area. The Endothelial cells with visible nuclei might be seen free floating in-between Erythrocytes (V).

Discussion

Literature search with the key words such as "model of acute pulmonary embolism", or "animal model of massive PE", provides an extensive and general synopsis of models characterizing this kind of hemodynamic instability. Although animals cannot be used to study complex human behavior, they do have similar basic functions. We may concur that human disorders that have animal models are better understood than disorders that do not. An ideal animal model should be similar to the disorder it models in terms of etiology, biochemistry, symptomatology, and treatment. As authors in pulmonary embolism studies tested the condition on pulmonary models, they were inclined to use a variety of animals to mostly test the same hypothesis with only limited field interrelation such as hemostasis coagulation, with pulmonary critical care, medical imaging with hemostasis, and coagulation. Moreover, there was certain delineation when animal PE models were used for imaging and surgical studies. Pigs, sheep, and dogs, rather than rodents, mostly for the convenience of a visualization and surgical access were presented. Subsequently and often mistakenly assumption of similarity of pig's branching of the major pulmonary arteries to that in ruminants leads to non-ability of further generalization. Consequently, sheep PE visualization and detection might give an observer the set of data, which might completely miss out the clinical topic. Thus, for the field of pulmonary imaging, testing hemodynamic instability in pig rather than ruminant model might be more practical. [8,9] An ideal model of MPE has to include thrombosis approach to introduce MPE, which will mimic the fundamental characteristics of this shock situation in humans (face validity). It should further conform its theoretical rationale by the animal model characterization i.e., thrombi generation, repeatable size of injury, clot detection, blood pressure parameters (construct validity), to better predict MPE, in clinical settings (predictive validity).

Using large animals like pig or sheep for PE study has many advantages such as the ability to study the pulmonary mechanics and visualization of pulmonary vessels in parenchyma, for its simple detection; i.e., injected radioactive labeled emboli [8,19,20], or contrast material to

visualize the involved area. [5,8,21] They have lately been used for the development of better surgical techniques that could be used in humans to safely evacuate the life-threatening thrombus (i) from the main pulmonary artery. It is the size of pulmonary artery and overall the size of lung parenchyma, allowing radiologist and surgeon to better train emergency surgical techniques and visualizations. [5,8,21] Once middle-to small sized animals like dogs, rabbits, hamsters, rats and mice are proposed to pre-clinical study of PE, they are usually selected for more precise and detailed research, [2,18-20,22-26] with only limited interrelations. Such models are used mostly due to the fact that they could be easily blood sampled for detection of induced pulmonary hypertension, [24] the thrombo-embolism can be monitored by sampling levels of t-PA, NO and AT-III [22] D-dimer [19] and plasminogen, tPA, fibrinogen, α 2-antiplasmin, [18,20,27] without requirements for an extensive food and water supply, and lastly because of the cost associated with drug injections (i.e., thrombolytic therapy). [20,22,23,25] They hence have the potential to become primary research models in the future. It is though regrettable that we opt for pre-clinical models that are supposed to extrapolate thromboembolic delivery from animals to human using mouse, rabbit, rat and dog models; animals that hardly ever experience this condition, rather than utilize the most affected PE animal like cat. [11] It is still not settled what makes the adequate thromboembolus to follow the clinical hypothesis. As is widely known, the composition of thromboembolic material varies, depending on particular clot-vessel endothelial interactions and flow conditions. Aged, platelet-rich, and well-organized thrombi formed under the flow conditions have been shown to be more resistant to thrombolysis than fresh, fibrin or red blood cell-rich clots formed under static conditions. [28,29] In addition, the clot structure may substantially differ, based on circumstances of the embolic source. As many clinicians are familiar with or on many occasions may have observed, thrombi engrafted in a proximal atherosclerotic lesion, is different than clots formation and passage through the cardiac cavities.

Since the difference of clot structure is a significant factor of PE severity, number of studies recently provided details of the type of used clot with clotting additives and methods of clot preparation. In that respect, plasma clot harvested from humans injected into animals was employed, [15,17] while others still using autologous animal whole clot injections [7,16], or variety of human clot mixed with autologous from animals. [18] When we examine the fate of thrombus or multiple thrombi produced by, or started with a silicone plugs, sephadex microspheres, colored methacrylate beads, microbeads, or an exposure of jugular vein to sodium morrhuate and other non-blood derived methods to simulate PE [2-5], clinicians may not be able to draw any conclusion. Moreover, since only mechanical properties of artificial embolus are tested, all other parameters are neglected (clot-vessel interactions, endogenous pulmonary thrombolytic ability, protein C and TM relations and so on), making such PE model incomplete and gathered values non-uniform, with very low specificity, repeatability and validity.

Characteristic of every PE model is how embolism is initiated. One approach of induction is a single short clot injection. Another is to inject clot repeatedly (over 5-10 minutes) [7] and lastly, not so common is by continuous introduction of venous thrombi into the left jugular vein. [26] It is generally up to the investigator to rationalize study methodology and further provide a clear description and rationale for the type and clot injection approach. The induction site varies as well from the most proximal, which is a direct injection into the pulmonary artery in the pig model [13], to the tail vein of a rat. [15] It is apparent that when thrombi aim directly to heart and the big lung vessels, the patho-physiological alterations such as acute pulmonary hypertension complicated by a decrease in cardiac output could be intercepted almost instantly. [16] Moreover, if labeled thrombi are injected, the passing ones could be visualized almost immediately. The

thrombi blood route can be controlled by measurement of mean pulmonary artery or aortic pressure. Thrombi can be at the same time traced by variety of techniques based on an employed tracer or implemented contrast. Later on, during the histo-pathological examination they can be visualized, while at the same time the level of thrombolysis can be measured. The uniqueness of this strategy is based on in vitro produced net clot radioactive thrombi from the autologous animal's blood, prepared not more than one hour prior to an induction and then, induced by close-to-heart injection. By precise control of injected tracer (radioactivity), it is possible to produce very solid and repeatable data, which could be in the future, used as a foundation for surgical or thrombolytic treatment. Visualized and gathered thrombi might be later on characterized by their size, volume, age (fibrin cross-linking). New accumulated non-radioactive platelets, cells and other blood plasma elements can be further analyzed to better understand their roles in pulmonary thrombus lysis and constant accretion.

The overall processes that take place after MPE, are characterized rather incompletely given the course of action, and mostly with no interrelations, where authors usually concentrate on description of percentage of an increase of pulmonary artery blood pressure after the embolization, in cases of visualization, [3,7,13,24,30] very rarely survival rate is published [2,15] and seldom standard histological assessment of lungs after the embolism is included. [4] The optimalization of PE model might be through an autologous blood derived thrombi injection, measured before an injection for the presence of radioactive or other tracer, [23] and injected centrally or into venus jugularis. The injection of autologous blood clot rather then human or mixed creates the possibility of an early antigenic response. Moreover, if thrombi are prepared inside the venous circulation, additives or tracer may escape (in case of thrombin-clot enrichment into ligated or balloon obstructed luminal segment) and create microembolization or tracer leakage before the initiation of an experiment. This medium size rabbit MPE model was developed, with simple, repeatable and easy to perform surgery. Excellent cannulation sites allowed to conveniently taking blood samples and monitor the systemic blood pressure (figure 3). Easy to repeat, and close to heart thrombus delivery, further enabled to monitor and address pathophysiological changes instantly after the embolization. Model enabled to test not only the pathophysiology of a massive hemodynamic shock, also the process of lung vascular thrombolysis of the original delivered thrombi. Description of dynamic lung fibrinolysis (figure 1) and lung thrombolysis at 10 and 60 minutes (figure 2) show that this experimental model is easy to repeat and gives the unique opportunity to test different types of thrombolytics in the near future. Although the lung homogenates in vitro possess the most intense thrombolytic activity compared to other organs, in vivo, emboli dissolution showed certain limitations, such as the pulmonary vessel recruitment, that hindered more clot thrombolysis. Due to the relative small lung parenchyma, all lobes were measured for the residual radioactive thromboemboli presence and histopathological changes after the end of each experiment, which could have not been easily executed in larger types of MPE models.

In this model, description of type of injection, clot maturation, used additives, and time to clot maturation is provided. Furthermore, its NCR thrombi characterization by age, size and volume might more accurately describe a series of patho-physiologic events, including MPE systemic blood pressure changes, death rate reports, and histological assessment during MPE. Radioactive labeled clot with the precise counts (NCR) present in thrombi just before the MPE induction into the right jugular vein makes this model unique, due to its ability to more accurately depict clot radioactive degradation products, TDE and PL-T and thus, more precisely can estimate the clot lung turnover. The precise control of injected NCR thrombi helps create a set of data, both repeatable and very statistically coherent, which

could be used in future MPE drug treatment studies. Adhering to the premise presented at the beginning, this preclinical model could offer a complex of steps and path-physiological events that could be later repeated for further conclusion; i.e., fibrinolytic MPE mechanism explanation, MPE pathway description and decision to employ treatment either surgically or medically. Forgetting the importance of clotted blood (blood-derived clot structure), the MPE causing factor, and selecting silicone plugs, or sephadex microspheres might not fully describe the thrombotic event and questions the validity of the studies.

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