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The Impact of Hemorrhagic Shock on Lung Ultrasound Induced Pulmonary Capillary Hemorrhage

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Running Title: Impact of Shock on LUS Induced PCH

Abstract

Objectives: Lung ultrasound (LUS) exposure can induce pulmonary capillary hemorrhage (PCH), depending on biological and physical exposure parameters. This study was designed to investigate the variation in the LUS induction of PCH due to hemorrhagic shock, which itself can engender pulmonary injury.

Methods: Male rats were anesthetized with isoflurane in air. Shock was induced by withdrawal of 40% of blood volume, and assessed by the blood pressure detected by a femoral artery catheter and by blood glucose tests. B mode ultrasound was delivered at 7.3 MHz with a low output (-20 B) for aiming, and with the maximal output (0 dB) for exposure. PCH was quantified by assessment of comet tail artifacts in the LUS images and by measurement of PCH areas on the surface of fresh lung samples.

Results: Tests without shock or catheterization surgery gave results for PCH similar to those of previous studies using different methods. Exposure before hemorrhagic shock gave 24.8 ± 9.2 mm^2 PCH area on the ultrasound scan plane, while exposure after shock gave zero PCH ($p< 0.001$, $n=7$).

Conclusions: The presence of hemorrhagic shock significantly reduces the occurrence of LUS induced PCH.

Key Words: Lung ultrasound; B lines; Bioeffects of ultrasound; Ultrasound dosimetry; Diagnostic ultrasound safety

Introduction

Lung ultrasound (LUS) has found valuable application to situations when simple and rapid examination is needed for patient assessment in pediatrics, emergency, intensive care, and point-of-care. Clinical LUS examinations are valuable in the diagnosis of pneumonia, pulmonary edema, pulmonary embolism, atelectasis, diffuse parenchymal disease, respiratory distress syndrome, and lung cancer [1-3]. Diagnostic ultrasound does not penetrate well into normal lung but the lung surface image can show signs of injury, edema and interstitial lung disease. An important sign of lung disease is the detection of multiple reflection artifacts known as comet tail artifacts (CTA, also known as B lines), which extend inward from the bright reflection of the pleura [2]. For example, LUS assessments of CTA and other features are valuable in emergency medicine for trauma [4,5]. The specific RUSH (Rapid Ultrasound in Shock) examination is commonly applied to the critically ill for assessing pneumothorax, pulmonary edema and hemorrhage, and hydrothorax [6]. In risk benefit considerations, ultrasound can avoid ionizing radiation and present an exceptional safety profile for the patient. However, LUS presents the sonographer with a potential for local lung injury in some patients [7,8]. The usage of LUS is widespread particularly in point of care settings for monitoring patient status. Therefore, the specific usage is uncertain in relation to biological and physical dosimetric parameters associated with potential injury by exposure to LUS.

Pulmonary injury from diagnostic ultrasound takes the form of capillary hemorrhage into the alveoli [7,9]. In LUS imaging of rat, the ultrasound image displayed CTA associated with

the occurrence and progression of pulmonary capillary hemorrhage (PCH) with time [10]. Animal research has shown that PCH depends on the pulse amplitude, indicated by the on-screen Mechanical Index, and follows a threshold dose response dependence. Additional physical parameters are also important, including the ultrasound frequency [11], imaging duration [12], and ultrasound mode [13]. An important feature of PCH is that biological factors also are very important, including sedation [14], ventilation [15], age and lung position [16], and animal species [17]. The large possible range of patient physical ultrasound exposure and of biological factors greatly complicates the assessment of possible clinical risk. The CTA displayed when diagnostic imaging induces PCH would not useful for real-time safety guidance because the same sign is used for the diagnosis of patient conditions [8]. During an exam, not only could LUS conceivably display ongoing CTA due to PCH induction, and not be recognized as iatrogenic lung injury which should be mitigated, but also confuse the interpretation CTA as diagnostic information.

Several factors common for emergency and clinical patient treatment have been investigated using our rat model of lung ultrasound. Fluid infusions commonly used for patient stabilization and resuscitation were tested as a perturbation of the blood side of the blood air barrier. PCH was virtually eliminated in rats by rapid venous infusion of 35 ml/kg saline [18]. Intermittent positive pressure ventilation in anesthetized intubated rats, which perturbed the air side of the blood-air barrier, was also tested with exposure to diagnostic imaging [15]. When rather small end expiratory pressures of ±4 cm H_2O were applied, the PCH was virtually

eliminated by positive pressure, but strongly enhanced by negative pressure. The fraction of inspired oxygen (FiO_2) also was investigated for its influence on LUS-PCH induction using Telazol anesthesia with and without xylazine [19]. Adding xylazine tends to enhance susceptibility to PCH. A reduced PCH effect with 10 %FiO_2 was enhanced with use of xylazine, while 60 %FiO_2 enhanced the PCH effect especially for anesthesia with Telazol only.

These results show that interventions (fluid infusion ventilation and hyperoxia) associated with emergency patient management had important but varied influences on PCH induction by LUS. Other medical interventions or patient conditions might also yield important variations in LUS induced PCH. Shock due to hemorrhage or trauma itself can engender pulmonary injury [20,21]. This tendency for shock related lung injury led us to an hypothesis that animals in shock could have a greater susceptibility to ultrasound induced PCH, which might worsen the impact of shock injury by adding LUS induced PCH. Animal models are extensively utilized for clinically relevant studies of shock [22]. Isoflurane is the common anesthesia for the rat model of hemorrhagic shock [20,21,23], and has been shown to be useful in ultrasound PCH research [29]. This study was designed to investigate the variation of the diagnostic ultrasound induction of PCH due to the existence of hemorrhagic shock in isoflurane anesthetized rats.

Methods

Animal preparation

All *in vivo* animal procedures were conducted with the approval and guidance of the

Institutional Animal Care and Use Committee, University of Michigan, Ann Arbor, MI. Male Sprague Dawley rats (Charles River, Wilmington, MA, USA) were used for this research and maintained in the animal housing rooms of the Unit of Laboratory Animal Medicine. The rats weighed an average of 428 ± 36 gm at the time of testing. Isoflurane in medical air was used to induce anesthesia (5%) and maintain the suitable level of anesthesia (0.5-1.5 %). The right thorax of each rat was shaved and depilated for ultrasound transmission.

The rats were mounted on a plastic holder fitted with a nose cone that was connected to the anesthesia machine (Surgivet Isotec 4, Smiths Medical ASD Inc., St. Paul, MN, USA). The rats were placed in left lateral recumbency, and instrumented with a heart rate (HR) and SpO_2 sensor on a front paw (SurgiVet V3395 TPR, Smiths Medical Inc. St Paul, MN USA). For exposure to ultrasound, the rat and mounting holder were placed horizontally on warming pad to maintain body temperature, monitored by a rectal probe. Coupling of the ultrasound to the chest wall was accomplished with a shallow dish filled with warm water. An ultrasound transmission window in the bottom of the dish was coupled to the chest wall with ultrasound gel. This water stand-off allowed consistent aiming and placement of the focal zone of the diagnostic ultrasound probe at the lung surface for imaging-exposure.

A femoral vein catheter was set for blood withdrawal. Hemorrhagic shock was produced by slowly withdrawing blood over 15 min totaling an estimated 40% of total blood volume. The total volume needed in ml was calculated using a standard formula for rats of 0.06 times body weight (BW) in gm plus 0.77 ml [30]. For example, 40 % of blood volume was estimated to be

10.5 ml for a 425 gm rat.

A non-invasive blood pressure measurement system (Coda monitor; Kent Scientific Corp, Torrington, CT USA) was used on the tail of some rats to noninvasively measure blood pressure. The system involved placement of an occlusive cuff and a sensing cuff on the rat tail. The blood pressure signals were sent to a personal computer via a USB port, recorded, and analyzed with the aid of the Coda Software. The system automatically made a series of blood pressure determinations, which stabilized after several readings. The 10^{th} reading was chosen as the optimal determination and used for this study for readings at initial, intermediate and final time points. This system was initially considered for use as the primary measurement method during shock, to avoid the trauma and procedure delay of femoral artery catheterization. However, the results were not reliable for blood pressure values much less than normal and could not be used for the low blood pressures produced by the hemorrhage procedure.

Blood pressure measurements for the tests of hemorrhagic shock were made using a Millar blood pressure catheter (SPR-320 size 2F, Millar Inc. Houston TX USA). The catheter was inserted into a femoral artery and the sensor advanced to the abdominal aorta. This surgical procedure was impactful and often involved a lengthy period of time, lasting up to 60 min, in the handling of the rats for the experiment. The blood pressure signal was amplified with a bridge amplifier and calibrated using a calibration kit (FE221 and MLA1052, ADInstuments, Inc., Colorado Springs, Co, USA). The signals were digitized (Powerlab 4/30, ADInstruments Inc., Colorado Springs, CO, USA), recorded, and analyzed with the aid of software (Chart Pro 5, v.

5.5.5, ADInstruments Inc., Colorado Springs, CO, USA). Systolic blood pressure (SBP) and diastolic (DBP) blood pressures were recorded. A shock index (SI) was calculated as HR/SBP [23,24]. This index was indicative of the shock severity, with increases to greater than 200 % suggestive of a lethal hemorrhage [23].

In addition, blood glucose was measured as a general indicator of trauma and blood loss [25,26] that has proven useful in shock research in rats [27,28]. A blood glucose meter (Contour 7151H, Bayer Health Care) was used for measurements using the standard human Contour Test Strips with a drop of blood. The blood glucose tends to rise after hemorrhagic shock and the ratio of blood glucose before and after shock was also used as a shock indicator.

Ultrasound

The diagnostic ultrasound machine used for imaging and exposure was a clinical GE Vivid 7 Dimension (GE Vingmed Ultrasound AS, Horten, Norway) with the 10L linear array probe. The probe was held by a rigid gantry in fixed position above the rat, and aimed between ribs using a gimbal mount. B mode was used with 3 cm image depth and 1.3 cm single focus which was placed at the lung surface. Parameters of the ultrasound pulses were measured at the positon of the maximum (1.3 cm depth on the probe axis) peak rarefactional pressure amplitude (PRPA) using a calibrated hydrophone with a 0.2 mm sensitive spot (model HGL-0200, Onda Corp., Sunnyvale, CA) and are listed in Table 1. To approximate in situ values at the lung surface, the digitized pulse pressure waveform, measured in water, was derated by an attenuation factor of 1.2 dB/cm-MHz [13], which was – 6.0 dB for the mean chest wall thickness of 0.68 ±

0.07 cm. The measured ultrasound frequency was 7.3 MHz, with 60.2 frames per second, a 96 μs pulse repetition period and 270 ns pulse duration. The -6dB width of the beam was 1.2 mm. A -20 dB power setting (pressure amplitudes reduced by a factor of ~10) was used for aiming and recording images before and after ultrasound images. For exposure, the power was quickly raised to the maximum setting (0 dB) and timed for 5 min, after which the power was quickly lowered again to -20 dB.

The probe was carefully aligned between two ribs to give a bright image of the lung surface, seen as a curved line across the image (Fig. 1). The image indicated approximately perpendicular incidence by presentation of a multiple reflection artifact (A line) at about 2.5 cm in the 3 cm deep image. During and after ultrasound exposure, the images provide information about the PCH effect by displaying comet-tail artifacts (CTA) projecting inward perpendicular to the lung surface image. This artifact gives a qualitative real-time indication of PCH induced by the ultrasound exposure. The width of the bright-line lung surface in the image was measured, together with the width of the line that was involved with the CTA. The percentage width of CTA across the bright-line image was calculated as one measure of the PCH effect.

At the end of the tests, each rat was euthanized under anesthesia by exsanguination of the inferior vena cava, as described previously [15]. The trachea was tied off, and the lungs and heart removed intact. Photomicrographs of the effected right cranial and medial lobes were used to measure the approximate area of the PCH regions on the lung surface (Spot v. 5.1, Diagnostic Instruments, Inc., Sterling Heights, MI USA), which was used as the quantification of the overall

PCH impact.

Experimental Plan and Statistics

For this study, a total of 35 male rats were randomly assigned to 5 groups of 7 rats each. The key test, to determine the impact of hemorrhagic shock on LUS induced PCH, was to perform ultrasound exposure either before (BSK) or after (ASK) hemorrhagic shock. Initial planning had included groups to build data for an exposure-response PCH threshold determination for the BSK and ASK tests, but the results were so definitive (see Results) that only the 0 dB exposure was used. The methods used in this study differed substantially from our previous studies [11, 29]. Previous work had used female rats, vertical positioning of the rats for exposure in a water bath, and did not require lengthy surgery for arterial catheter placement. Three groups of rats were used for gauging the possible changes in PCH results due to the different methods used for this study. Sham (Sham), and ultrasound with 30 min (UD30) and 60 min (UD60) delays in the procedure, were performed with male rats and horizontal position, but without surgery or shock, for comparison to previous measurements. Statistical analysis was performed using SigmaPlot for Windows V. 14.0 (Systat Software Inc., San Jose CA, USA). The Student's t-test was used for comparisons between groups as the two-tailed test (i. e. including the possibility of a reduction in the PCH effect as well as the hypothesized increase) with statistical significance assumed at $p<0.05$.

Results

The results for the physiological parameters are listed in Table 2. The HR, SpO_2 and blood pressure were somewhat suppressed in all rats, presumably owing to the anesthesia protocol. For BSK and ASK groups, the ultrasound exposure time corresponded to the intermediate time point. The shock produced by the blood withdrawal was evidenced by the statistically significant reduction in blood pressure for BSK (end relative to the intermediate time point) and for ASK (intermediate relative to the start time point). In addition, the blood glucose after the blood withdrawal was increased for ASK ($p<0.05$), but not significantly so for BSK ($p=0.09$).

Before and after LUS images of one BSK and one ASK exposed rat are shown in Figure 1. The normal images from aiming (-20 dB) before the power was raised are the top images. With the LUS looking downward, the water standoff is seen as a black layer above the skin surface, that appears as a curved white line. The skin layer has a granular appearance and, horizontally, the sternum is shown to the left. The intercostal space, which also has some indications of nearby ribs, extends downward to the pleurae and lung gas surface, which is displayed as a bright white curved line at about 1.3 cm depth. All echoes shown below this bright line present image artifacts, because the lung surface has mirror-like acoustical reflectivity. The strong reflection from the lung generates a second lung surface image artifact (an A line) at about 2.6 cm depth due to reflection from the surface of the probe and secondary reflection from the lung surface received by the probe and displayed as the deeper image. The A line indicates a clear image path and good alignment of the probe for exposure. If PCH starts, a perturbation of

the bright line surface image begins to be discernable, and gradually grows during the exposure to form the CTA that appear to grow downward from the lung air surface. The final BSK image shows an extensive CTA distribution penetrating downward to the bottom of the image in some of the width of the bright line lung image. The final ASK image shows absolutely no indication of CTA. The photographs of the freshly removed lung samples (bottom) show the PCH effect as the irregular blood-red pattern. For BSK the PCH extends along a line set by the ultrasound scan plane and tracks the CTA distribution. For ASK, there was no discernable indication of PCH on the lungs, again reported by the CTA observation by its absence.

The shock indicators and measured results for the PCH areas are presented in Table 3. The shock indicators of SI and BG ratio were useful, and confirmed that shock had resulted from the hemorrhage. SI strongly indicated shock for the BSK group, with the BG ratio only giving a weak indication. For the ASK group, the SI increase was not statistically significant but the BG ratio increase was a statistically significant indication of shock. As noted in methods, the groups designated as Sham, UD30 and UD60 where included to assess the possible differences in the PCH effect from previous work, which might help to interpret the BSK versus ASK results. In particular, a possible strong reduction in the PCH was postulated to occur due the relatively long delay after the start of anesthesia before the ultrasound exposure. However, the UD30 and UD60 exposures produced the expected PCH effect ($p<0.001$ relative to Sham), and there was no statistically significant difference between the UD30, UD60 and BSK results. The ASK result was the same as the Sham result and was highly significantly different from the BSK result

($p<0.001$). The PCH results are graphically displayed in Figure 2.

Discussion and Conclusion

These results clearly show that our initial hypothesis, that hemorrhagic shock might enhance susceptibility to the PCH bioeffect of LUS, was incorrect. The withdrawal of blood leading to shock completely eliminated the PCH effect obtained by the maximal exposure for our exposure system without shock. Apparently, the low blood pressure minimizes the tendency for capillary hemorrhage, certainly a plausible result. No further groups, e. g. a dose response or graded variation of blood withdrawal were performed due to the variability of the observed shock level in our tests and the perfectly clear result at 0 dB of a strong PCH effect for ultrasound before shock (BSK) and zero for ultrasound after shock (ASK).

The PCH results here seemed somewhat less than the effect for female rats with ketamine plus xylazine anesthesia using the Vivid 7 machine with 10L probe [13]. However, the isoflurane anesthesia method was previously shown to yield less PCH effect [29], similar to the result found here. In addition, the use of male rats, which have a thicker chest wall of 6.8 mm compared to 5 mm for the female rats would be expected to give somewhat lower results, due to the increased attenuation (-6 dB compared to -4.4 dB for the previous study with females). The horizontal position with standoff for these methods was somewhat more difficult to use in terms of aiming than the vertical mounting for the water-bath setup used previously, but again appears to give similar results. Overall, this study gave results comparable to those of earlier work, and those

other methods would likely produce the same positive BSK and negative ASK result shown here for hemorrhagic shock.

The conclusion from this study is that shock does not enhance susceptibility to LUS induced PCH and in fact hemorrhagic shock appeared to eliminate the effect obtained without shock. Examinations such as the RUSH protocol should not involve consideration of increased patient risk to PCH. This finding, together with the observation that saline infusion also seems to mitigate the PCH bioeffect [18] indicates that intervention on the blood side of the blood air barrier induces physiological changes less conducive to PCH induction. The interventions on the air side of the blood air barrier by ventilation or hypoxia, in contrast, readily gave increased as well as decreased changes in observed PCH. Possibly, blood-side perturbation giving higher blood pressure, such as hypertension, may yield enhanced PCH susceptibility. A study using spontaneously hypertensive rats is in progress to test this hypothesis.

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References:

1. Sartori S, Tombesi P. Emerging roles for transthoracic ultrasonography in pleuropulmonary pathology. World J Radiol. 2010; 2:83-90.
2. Ahmad S, Eisen LA. Lung ultrasound: the basics. In: Lumb P and Karakitsos D eds. Critical care ultrasound. Philadelphia: Elsevier, 2015. Ch 19, pp 106-110.
3. Dietrich CF, Mathis G, Cui XW, Ignee A, Hocke M, Hirche TO. Ultrasound of the pleurae and lungs. Ultrasound Med Biol. 2015;41:351-65.
4. Dietrich CF, Mathis G, Blaivas M, Volpicelli G, Seibel A, Wastl D, Atkinson NS, Cui XW, Fan M, Yi D. Lung B-line artefacts and their use. J Thorac Dis. 2016;8:1356-1365.
5. Volpicelli G, Mattia T, Lamorte A. Lung Ultrasound in Trauma. In: Lumb P and Karakitsos D eds. Critical care ultrasound. Philadelphia: Elsevier, 2015. Ch 21, pp 115-118.
6. Perera P, Mailhot T, Riley D, Mandavia D. The RUSH exam: Rapid Ultrasound in SHock in

the evaluation of the critically lll. Emerg Med Clin North Am. 2010;28:29-56

7. Church CC, Carstensen EL, Nyborg WL, Carson PL, Frizzell LA, Bailey MR. The risk of exposure to diagnostic ultrasound in postnatal subjects: nonthermal mechanisms. J Ultrasound Med. 2008;27:565-92.
8. Miller DL, Abo A, Abramowicz JS, Bigelow TA, Dalecki D, Dickman E, Donlon J, Harris G, Nomura J; American Institute of Ultrasound in Medicine Bioeffects Committee. Diagnostic Ultrasound Safety Review for Point-of-Care Ultrasound Practitioners. J Ultrasound Med. 2020;39:1069-1084.
9. Child SZ, Hart man CL, Schery LA, Carstensen EL. Lung damage from exposure to pulsed ultrasound. Ultrasound Med Biol. 1990;16:817-25.
10. Miller DL. Induction of pulmonary hemorrhage in rats during diagnostic ultrasound. Ultrasound Med Biol. 2012;38:1476-1482.
11. Miller DL, Dou C, Raghavendran K. The dependence of thresholds for pulmonary capillary hemorrhage on diagnostic ultrasound frequency. Ultrasound Med Biol 2015;41:1640-1650.
12. Miller DL, Dong Z, Dou C, Raghavendran K. Influence of scan duration on pulmonary capillary hemorrhage induced by diagnostic ultrasound. Ultrasound Med Biol. 2016;42:1942-1950.
13. Miller DL, Dong Z, Dou C, Raghavendran K. Pulmonary Capillary Hemorrhage Induced by Different Imaging Modes of Diagnostic Ultrasound. Ultrasound Med Biol. 2018;44:1012-1021.

14. Miller DL, Dou C, Dong Z, Raghavendran K. The influence of dexmedetomidine on ultrasound-induced pulmonary capillary hemorrhage in rats. Ultrasound Med Biol. 2016;42:964-970.
15. Miller DL, Dong Z, Dou C, Raghavendran K. Pulmonary Capillary Hemorrhage Induced by Diagnostic Ultrasound in Ventilated Rats. Ultrasound Med Biol. 2018;44:1810-1817.
16. O'Brien WD Jr, Simpson DG, Ho MH, Miller RJ, Frizzell LA, Zachary JF. Superthreshold behavior and threshold estimation of ultrasound-induced lung hemorrhage in pigs: role of age dependency. IEEE Trans Ultrason Ferroelectr Freq Control. 2003;50:153-169.
17. O'Brien WD Jr, Yang Y, Simpson DG, Frizzell LA, Miller RJ, Blue JP Jr, Zachary JF. Threshold estimation of ultrasound-induced lung hemorrhage in adult rabbits and comparison of thresholds in mice, rats, rabbits and pigs. Ultrasound Med Biol. 2006;32:1793-804.
18. Miller DL, Dong Z, Dou C, Raghavendran K. Does Intravenous Infusion Influence Diagnostic Ultrasound-Induced Pulmonary Capillary Hemorrhage? J Ultrasound Med. 2018;37:2021-2028.
19. Miller DL, Dou C, Raghavendran K and Dong Z. Variation of Diagnostic Ultrasound Induced Pulmonary Capillary Hemorrhage with Fraction of Inspired Oxygen Ultrasound Med Biol. 2020; 46:1978-1985.
20. Fukudome EY, Li Y, Kochanek AR, Lu J, Smith EJ, Liu B, Kim K, Velmahos GC, deMoya MA, Alam HB. Pharmacologic resuscitation decreases circulating cytokine-induced neutrophil chemoattractant-1 levels and attenuates hemorrhage-induced acute lung injury.

Surgery. 2012;152:254-61.

21. Kochanek AR, Fukudome EY, Li Y, Smith EJ, Liu B, Velmahos GC, deMoya M, King D, Alam HB. Histone deacetylase inhibitor treatment attenuates MAP kinase pathway activation and pulmonary inflammation following hemorrhagic shock in a rodent model. J Surg Res. 2012;176:185-94.
22. Fülöp A, Turóczi Z, Garbaisz D, Harsányi L, Szijártó A. Experimental models of hemorrhagic shock: a review. Eur Surg Res. 2013;50:57-70.
23. Kim KA, Choi JY, Yoo TK, Kim SK, Chung K, Kim DW. Mortality prediction of rats in acute hemorrhagic shock using machine learning techniques. Med Biol Eng Comput. 2013;51:1059-67.
24. Olaussen A, Blackburn T, Mitra B, Fitzgerald M. Review article: shock index for prediction of critical bleeding post-trauma: a systematic review. Emerg Med Australas. 2014;26:223-8.
25. Laird AM, Miller PR, Kilgo PD, Meredith JW, Chang MC. Relationship of early hyperglycemia to mortality in trauma patients. J Trauma. 2004;56:1058-62.
26. Kreutziger J, Wenzel V, Kurz A, Constantinescu MA. Admission blood glucose is an independent predictive factor for hospital mortality in polytraumatised patients. Intensive Care Med. 2009;35:1234-9.
27. Subeq YM, Peng TC, Hsu BG, Lin NT, Chao YF, Hu TM, Lee RP. Effects of different fluid resuscitation speeds on blood glucose and interleukin-1 beta in hemorrhagic shock. J Trauma. 2009;66:683-92.

28. Mueller F, Teloh-Benger JK, Hussmann B, Lendemans S, Waack IN. Malate Protects the Kidneys from Hemorrhagic Shock-Induced Injury in an Experimental Rat Model. J Surg Res. 2020;245:225-233.

29. Miller DL, Dou C, Raghavendran K. Anesthetic techniques influence the induction of pulmonary capillary hemorrhage during diagnostic ultrasound in rats. J Ultras Med. 2015;34:289-297.

30. Lee HB, Blaufox MD. Blood volume in the rat. J Nucl Med. 1985;26:72-6.

Table 1. The ultrasound exposure parameters obtained with the two exposure power settings for the observed ultrasound frequency of 7.3 MHz and derated (-6 dB) for attenuation *in situ*.

Setting	MI_{OS}	PRPA	PMPA	MI_{IS}	I_{SPPA}
dB	MPa $MHz^{-½}$	MPa	MPa	MPa $MHz^{-½}$	W cm^{-2}
0	1.2	2.6	4.2	0.95	307
-20	0.12	0.31	0.33	0.11	2.8

PRPA, peak rarefactional pressure amplitude; PMPA, pulse mean pressure amplitude; I_{SPPA}, spatial peak pulse average intensity; MI_{OS}, the Mechanical Index presented on-screen; MI_{IS}, Mechanical Index calculated in situ value.

Table 2. Physiological parameters (n=7, mean and standard deviation), of heart rate (HR) and percent oxygen saturation (%SpO_2), systolic and diastolic blood pressure, and blood glucose at the initial, intermediate (exposure before shock for BSK and after shock for ASK) and final time points. The hemorrhagic shock was seen as a significant decrease in systolic and diastolic blood pressure (BSK and ASK) and an increase in blood glucose (ASK).

Group	Time point	HR bpm	SpO_2 %	Systolic mmHg	Diastolic mmHg	Glucose mg/dL
Sham	start	326 ± 39	87 ± 4	86 ± 6	57 ± 9	196 ± 35
Sham	end	355 ± 49	90 ± 5	82 ± 9	61 ± 8	187 ± 27
UD30	start	342 ± 21	91 ± 3	75 ± 16	59 ± 9	228 ± 40
UD30	end	359 ± 22	90 ± 5	75 ± 12	58 ± 6	225 ± 62
UD60	start	357 ± 27	87 ± 4	96± 26	73 ± 19	194 ± 73
UD60	end	358 ± 39	87 ± 8	88 ± 16	65 ± 12	215 ± 87
BSK	start	323 ± 22	89 ± 7	105 ± 20	70 ± 12	191 ± 37
BSK	inter	307 ± 28	84 ± 7	103 ± 17	68 ± 15	204 ± 41
BSK	end	292 ± 51	79 ± 9	42 ± 6^{H}	24 ± 6^{H}	250 ± 59
ASK	start	320 ± 24	88 ± 7	81 ± 15	56 ± 9	207 ± 37
ASK	inter	295 ± 127	82 ± 8	53 ± 12*	33 ± 7^{H}	293 ± 105*
ASK	end	293 ± 71	87 ± 8	69 ± 15	37 ± 12	291 ± 98

Statistical significant difference: * $p<0.05$, ^{H} $p<0.001$

Table 3. Results for the shock parameters for LUS exposures and the measurements of the resulting CTA and PCH (n=7, mean and standard error). Statistical significance was shown for exposure versus sham or ASK,[H] and for hemorrhage SI for BSK and blood glucose for ASK.*

Group	SI start	SI inter	SI end	BG ratio inter/start	BG ratio end/inter	CTA %	PCH mm^2
Sham	4.3 ± 0.2	n/a	4.3 ± 0.2	n/a	0.98 ± 0.07	0	0
UD30	4.7 ± 0.3	n/a	4.8 ± 0.4	n/a	1.00 ± 0.08	55.6 ± 6.5**H**	15.7 ± 2.7**H**
UD60	3.9 ± 0.3	n/a	4.2 ± 0.2	n/a	1.13 ± 0.11	47.8 ± 6.5**H**	13.9 ± 3.0**H**
BSK	3.2 ± 0.2	3.0 ± 0.1	7.0 ± 0.3*	1.09 ± 0.09	1.24 ± 0.12	47.2 ± 7.5**H**	24.8 ± 9.2**H**
ASK	4.0 ± 0.3	5.6 ± 0.7	4.3 ± 0.4	1.41 ± 0.15*	1.00 ± 0.04	0	0

SI= shock index, BG=blood glucose, CTA=comet tail artifacts, PCH=pulmonary capillary hemorrhage.

Figure Captions

Figure 1. Examples of the lung ultrasound images before (top) and after (bottom) exposure and the PCH seen on the lungs for BSK (left) and ASK (right). The CTA showing in the images faithfully indicated the distribution of PCH occurrence along the line corresponding to the ultrasound scan plane on the right medial lobes.

Figure 2. Results for PCH areas (mean and standard error) measured on the fresh lung samples for the maximal 0 dB exposure in groups of 7 rats. The PCH results for UD30, UD60 and BSK were highly significantly different from the Sham and ASK results, which were both zero.

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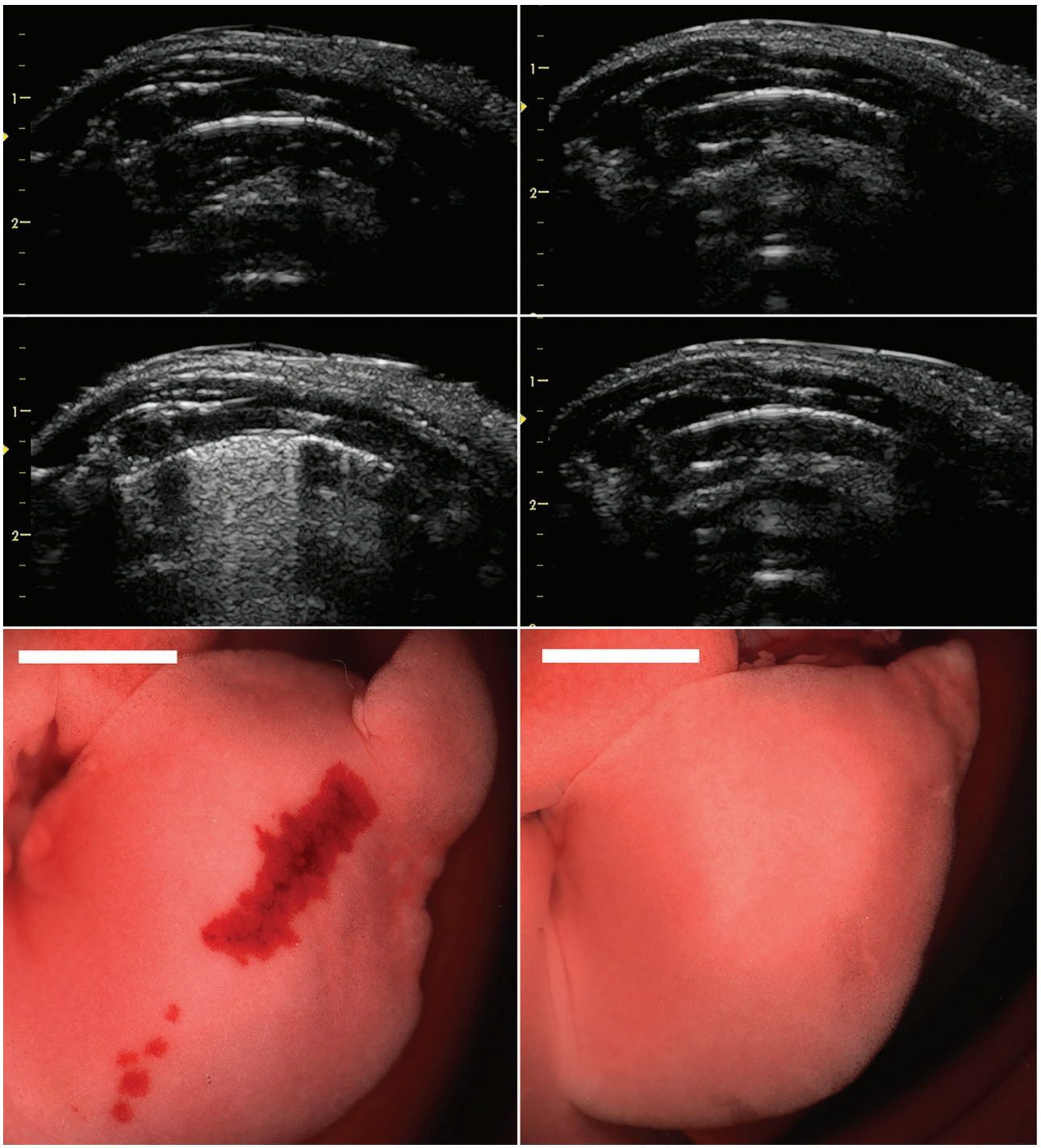

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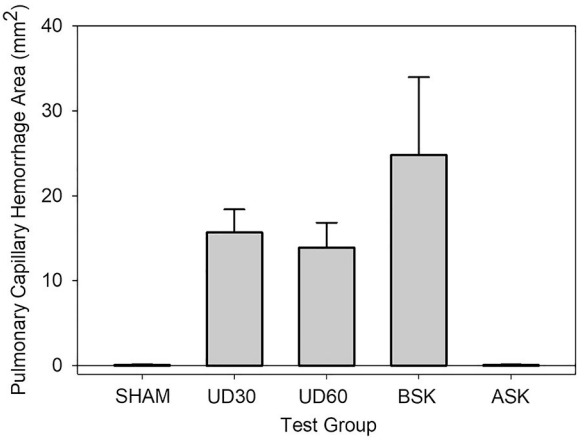

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