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TITLE

Induction and Phenotyping of Acute Right Heart Failure in a Large Animal Model of Chronic Thromboembolic Pulmonary Hypertension

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KEYWORDS

Acute right heart failure; pulmonary hypertension; right ventricle; animal model; pressure-volume loops; echocardiography; myocardial ischemia

SHORT ABSTRACT

We present a protocol to induce and phenotype an acute right heart failure in a large animal model with chronic pulmonary hypertension. This model can be used to test therapeutic interventions, to develop right heart metrics, or to improve the understanding of acute right heart failure pathophysiology.

ABSTRACT

The development of acute right heart failure (ARHF) in the context of chronic pulmonary hypertension (PH) is associated with poor short-term outcomes. The morphological and functional phenotyping of the right ventricle is of particular importance in the context of hemodynamic compromise in patients with ARHF. Here, we describe a method to induce ARHF in a previously described large animal model of chronic PH, and to phenotype, dynamically, right ventricular function using the gold standard method (i.e., pressure-volume PV loops) and with a non-invasive clinically available method (i.e., echocardiography). Chronic PH is first induced in pigs by left pulmonary artery ligation and right lower lobe embolism with biological glue once a week for 5 weeks. After 16 weeks, ARHF is induced by successive volume loading using saline followed by iterative pulmonary embolism until the ratio of the systolic pulmonary

pressure over systemic pressure reaches 0.9 or until the systolic systemic pressure decreases below 90 mmHg. Hemodynamics are restored with dobutamine infusion (from 2.5 µg/kg/min to 7.5 µg/kg/min). PV-loops and echocardiography are performed during each condition. Each condition requires around 40 minutes for induction, hemodynamic stabilization and data acquisition. Out of 9 animals, 2 died immediately after pulmonary embolism and 7 completed the protocol, which illustrates the learning curve of the model. The model induced a 3-fold increase in mean pulmonary artery pressure. The PV-loop analysis showed that ventriculo-arterial coupling was preserved after volume loading, decreased after acute pulmonary embolism and was restored with dobutamine. Echocardiographic acquisitions allowed to quantify right ventricular parameters of morphology and function with good quality. We identified right ventricular ischemic lesions in the model. The model can be used to compare different treatments or to validate non-invasive parameters of right ventricular morphology and function in the context of ARHF.

INTRODUCTION

Acute right heart failure (ARHF) has been recently defined as a rapidly progressive syndrome with systemic congestion resulting from impaired right ventricular (RV) filling and/or reduced RV flow output¹. ARHF may occur in several conditions such as left-sided heart failure, acute pulmonary embolism, acute myocardial infarction or pulmonary hypertension (PH). In the case of PH, ARHF onset is associated with a 40% risk of short-term mortality or urgent lung transplantation²⁻⁴. Here, we describe how to create a large animal model of ARHF in the setting of chronic pulmonary hypertension and how to evaluate the right ventricle using echocardiography and pressure-volume loops.

Pathophysiological features of ARHF include RV pressure overload, volume overload, a decrease in RV output, an increase in central venous pressure and/or a decrease in systemic pressure. In chronic PH, there is an initial increase in RV contractility allowing to preserve cardiac output despite the increase in pulmonary vascular resistance. Therefore, in the context of ARHF on chronic PH, the right ventricle can generate nearly isosystemic pressures, particularly under inotropic support. Taken together, ARHF on chronic PH and hemodynamic restoration with inotropes lead to the development of acute RV ischemic lesions, as recently described in our large animal model⁵. The increase in inotropes creates an increased energetic demand that may further develop ischemic lesions, and finally lead to the development of end-organ dysfunction and poor clinical outcomes. However, there is no consensus about how to manage patients with ARHF on PH, mainly regarding fluid management, inotropes and the role of extra-corporeal circulatory support. Consequently, a large animal model of acute right heart failure may help to provide pre-clinical data on ARHF clinical management.

As a first step to quantify the response to therapy, simple and reproducible methods to phenotype the right ventricle are needed. To date, there is no consensus about how to better phenotype the RV morphology and function of patients with ARHF. The gold standard method to evaluate RV contractility (i.e., intrinsic capacity to contract) and ventriculo-arterial coupling (i.e., contractility normalized by ventricular afterload; an index of ventricular adaptation) is the analysis of pressure-volume (PV) loops. This method is twice invasive because it requires right heart catheterization and a transient reduction in RV preload using a balloon inserted in the inferior vena cava. In clinical practice, non-invasive and repeatable methods to evaluate the right ventricle are needed. Cardiac magnetic resonance (CMR) is considered as the gold

standard for non-invasive evaluation of the right ventricle. In patients with ARHF on chronic PH who are managed in intensive care unit (ICU), the use of CMR may be limited because of the patient's unstable hemodynamic condition; moreover, repeated CMR evaluations, several times a day, including at night, may be limited because of its cost and limited availability. Conversely, echocardiography allows non-invasive, reproducible and low-cost RV morphology and function evaluations in ICU patients.

Large animal models are ideal to perform preclinical studies focusing on the relationship between invasive hemodynamic parameters and non-invasive parameters. The large white pig anatomy is close to humans. Consequently, most of the echocardiographic parameters described in humans are quantifiable in pigs. Some minor variations exist between human and pig heart that must be taken into account for echocardiographic studies. Pigs present a constitutional dextrocardia and a slightly counterclockwise rotation of the heart axis. As a result, the apical 4-chamber view becomes an apical 5-chamber view and the acoustic window is situated below the xiphoid appendix. Additionally, parasternal long and short axis views acoustic windows are situated on the right side of the sternum.

Here, we describe a novel method to induce ARHF in a large animal model of chronic thromboembolic PH and to restore hemodynamic using dobutamine. We also report RV ischemic lesions present in the model within 2–3 hours after hemodynamic restoration with dobutamine. Moreover, we describe how to acquire RV PV-loops and echocardiographic RV parameters at each condition providing insights on the dynamic changes in RV morphology and function. As the large animal model of chronic thromboembolic PH and the PV-loop methods were previously described⁶, these sections will be briefly described. Also, we reported results of echocardiographic evaluations which are deemed potentially difficult in porcine models. We will explain the methods to achieve repeated echocardiographic in the model.

The model of ARHF on chronic PH reported in this study can be used to compare different therapeutic strategies. The methods of RV phenotyping can be used in other large animal models mimicking clinically relevant situations such as acute pulmonary embolism⁷, RV myocardial infarction⁸, acute respiratory distress syndrome⁹ or right heart failure associated with left ventricular failure¹⁰ or left ventricular mechanical circulatory support¹¹.

PROTOCOL

The study complied with the principles of laboratory animal care according to the National Society for Medical Research and was approved by the local ethic committee for animal experiments at Hospital Marie Lannelongue.

1. Chronic thromboembolic PH

1.1. Induce chronic thromboembolic PH as previously described^{6,12}.

1.2. Briefly, induce a model of chronic thrombo-embolic PH in around 20 kg large white pigs (*sus scrofa*). Perform a ligation of the left pulmonary artery ligation through a left thoracotomy at week 0 (closed pericardium); and perform weekly an embolization of the right lower lobe

pulmonary artery (0.2 mL to 0.4 mL per week) with a mixed solution composed with 1 mL of soft tissue glue including N-butyl-2-cyanoacrylate and 2 mL of lipidic contrast dye (lipiodol) for 5 weeks.

1.3. Perform a xyphoidectomy at week 0 at time of left pulmonary artery ligation to improve the echocardiography feasibility. To do this, perform a 4 cm longitudinal incision in front of the xiphoid process. Remove the xyphoid process using a diathermy knife. Close the subcutaneous plan and the skin with a running suture.

1.4. Perform an additional right lower lobe pulmonary embolism at week 10 by using the same protocol explained above (step 1.2).

1.5. Perform the ARHF induction (section 6) model 6 weeks after the last right lower lobe embolization (week 16) in order to avoid acute right heart lesions induced by acute pulmonary embolisms.

NOTE: Other large animal model of right heart failure can be used, or other pathological conditions can be induced in the chronic-thromboembolic PH model.

2. Animal positioning and catheter placements

2.1. Perform general anesthesia as previously described⁶.

2.1.1. Briefly, let the animal fast for 12 h. Then perform an intramuscular injection of ketamine hydrochloride (30 mg/kg) for premedication. Perform an intravenous bolus of fentanyl (0.005 mg/kg), Propofol (2 mg/kg) and cisatracurium (0.3 mg/kg) intravenously through an ear vein and intubate non-selectively the pig with a 7 French probe.

2.1.2. Maintain general anesthesia with inhaled 2% isoflurane, continuous infusion of fentanyl (0.004 mg/kg) and propofol (3 mg/kg).

2.2. After general anesthesia induction, position the pig on his back with its forelegs in a slightly spread position to allow parasternal echocardiographic acquisition (section 3).

2.3. Place the device electrodes on the arms and legs (echocardiograph, workstation for hemodynamic acquisitions) prior to the placement of the sterile fields.

2.4. Place an 8-French sheath into the jugular vein using the Seldinger method¹³.

2.4.1. Introduce an 18 G (1.3 mm x 48 mm) IV catheter into the jugular vein.

2.4.1.1. Perform a percutaneous puncture on the middle line at 2 cm above the manubrium with a 45° orientation.

2.4.1.2. After obtaining a venous reflux, insert a guidewire into the catheter (0.035 inch / 0.089 mm, 180 cm, angled).

2.4.1.3. Verify the correct placement of the guidewire into the superior vena cava with fluoroscopy and dispose the 8-French sheath on the guidewire into the superior vena cava.

NOTE: The guidewire is correctly placed when it goes through the inferior vena cava along the right border of the spine.

2.5. Perform a division of the right femoral vessels to introduce a fluid filled catheter into the right femoral artery for continuous systemic pressure monitoring and a balloon dilation catheter into the inferior vena cava through the femoral vein as follow.

2.5.1. Perform a 4 cm transverse incision at the groin.

2.5.2. Place a Beckman retractor and divide the anterior face of the femoral vein and of the femoral artery using a Debackey forceps and Metzenbaum scissors.

2.5.3. Place a 20 G catheter into the femoral artery under direct visual control and connect it to a disposable transducer with a fluid filled catheter to obtain continuous systemic blood pressure monitoring.

NOTE: The mean blood pressure should be continuously above 60 mmHg.

2.5.4. Use an 18 G catheter to insert a guidewire (0.035 inch / 0.089 mm, 180 cm, angled) into the femoral vein through the inferior vena cava under fluoroscopic control.

2.5.5. Insert a balloon dilation catheter on the guidewire through the inferior vena cava at the intrapericardial level under fluoroscopic control.

2.6. Perform the fluoroscopic control with a C-arm using an anteroposterior view. Place the visible markers of the balloon immediately above the diaphragm level under fluoroscopic control. Remove the guidewire when the balloon is placed.

2.7. Sew a purse with a 5.0 polypropylene monofilament suture around the venous dilation balloon catheter to avoid bleeding from the femoral vein.

3. Echocardiography

3.1. Perform the echocardiography right after the animal positioning and the catheter placement (section 2) in animals still under general anesthesia and mechanical ventilation.

3.2. Acquire each echocardiographic view in cine loop format for at least 3 cardiac cycles during end-expiratory apnea.

3.3. Acquire all views in 2-dimension and Tissue Doppler modes.

3.4. Acquire the apical 5-chamber view under the xiphoid process.

3.5. Acquire the parasternal short and long axis views on the right side of the sternum.

236
237 3.6. Acquire valvular flow using continuous and pulsed Doppler modes.
238

239 3.7. Acquire Tissue Doppler signals of the lateral tricuspid annulus and lateral and septal mitral
240 annulus.
241

242 NOTE: Use the latest guidelines for echocardiographic assessment in humans for
243 echocardiographic acquisitions and interpretations¹⁴.
244

245 4. Right heart catheterization 246

247 4.1. Perform the right heart catheterization after the cardiac echo (section 3) and prior the
248 pressure-volume loop acquisitions (section 5)
249

250 4.2. Link the Swan-Ganz catheter to the disposable transducer.
251

252 4.3. Introduce the Swan-Ganz catheter into the jugular 8-French sheath previously inserted
253 into the jugular vein (section 2.4) and acquire mean right atrial, right ventricular and
254 pulmonary artery pressures. Place the catheter under fluoroscopy if needed.
255

256 NOTE: Check that fluid filled catheters are well purged with saline and remove air bubbles to
257 avoid pressure signal damping.
258

259 4.4. After placing the Swan-Ganz catheter into the pulmonary artery, measure the cardiac
260 output with the thermodilution method as explained by the manufacturer's instructions;
261 measure simultaneously the heart rate for stroke volume calculation.
262

263 4.4.1. Ensure that the saline is at 4 °C to avoid overestimation of the cardiac output.
264

265 4.4.2. Connect the disposable transducer to the PV-loop work station for live acquisitions of
266 pressures derived from fluid filled catheters.
267

268 5. Pressure volume loop acquisition using the conductance method 269

270 NOTE: This section has been previously published¹⁵.
271

272 5.1. Introduce the conductance catheter into the right ventricle under fluoroscopic control.
273

274 5.1.1. Verify the quality signal using "in live" acquisition of pressure-volume loops.
275

276 5.2. Activate adequate electrodes to obtain optimal signal (i.e., counter-clockwise PV-loops
277 with physiological shape).
278

279 5.3. Follow-up the pressure and volume calibration steps of the workflow according to the
280 manufacturer's instruction (blood conductivity, parallel volume, stroke volume calibration =
281 alpha calibration).
282

NOTE: Stroke external with the Swan-Ganz catheter can be repeated for each conditions; whereas the other calibration steps can be performed only once.

5.4. Acquire PV-loop families in steady states and during acute preload reduction (i.e., acute occlusion of the inferior vena cava) during end-expiratory apnea.

5.5. Perform at least 3 acquisitions per condition (steady + IVC occlusion).

6. Induction of acute right heart failure by volume and pressure overload (Figure 1).

6.1. Induce volume overload using a 3-step saline infusion (around 2 h).

6.1.1. Start the first infusion of 15 mL/kg of saline with a free-flow infusion output.

6.1.2. Perform the measurements (right heart catheterism, PV-loops and echocardiographic) 5 min after hemodynamic stabilization after the end of each infusion.

6.1.3. Start the second volume infusion of 15 mL/kg immediately after the end of the measurements.

6.1.4. Start the third volume infusion of 30 mL/kg of saline immediately after the end of the measurements.

CAUTION: Volume loading can induce hemodynamic compromise or pulmonary edema depending on the animal model used. In this model, volume loading revealed an adaptive response characterized by increasing cardiac output, stable right atrial pressure and preserved ventriculo-arterial coupling.

NOTE: Volume loading can be stopped in case of poor respiratory or hemodynamic tolerance.

6.2. Induce pressure overload with iterative pulmonary embolism.

6.2.1. Insert a 5 French angiographic catheter through the jugular sheath into the right lower lobe pulmonary artery under fluoroscopic control.

6.2.2. Embolize the right lower lobe pulmonary artery with a bolus of 0.15 mL of a mixed solution composed with 1 mL of soft tissue glue including N-butyl-2-cyanoacrylate and 2 mL of lipidic contrast dye. Wash out the catheter with 10 mL of saline.

6.2.3. Evaluate the hemodynamic response 2 minutes after the embolization using the systemic pressure and pulmonary artery pressure.

6.2.4. Repeat embolisms of 0.15 mL every 2 minutes until obtaining hemodynamic compromise, (i.e., systolic systemic pressure <90 mmHg or systolic pulmonary pressure over systolic systemic pressure ratio >0.9).

CAUTION: Pulmonary embolism can induce severe hemodynamic compromise, sometimes irreversible, leading to immediate death. Prior starting the embolization step, be ready to start hemodynamic support (dobutamine protocol or epinephrine in case of circulatory arrest). Be ready to start PV-loops and echocardiographic monitoring. As this step may be associated with severe hemodynamic compromise, right heart catheterization using the Swan-Ganz catheter can be avoided so as to start dobutamine support sooner.

7. Induce restoration of the systemic hemodynamic with dobutamine

7.1. After reaching hemodynamic compromise and performing PV-loops and echocardiographic acquisitions, start dobutamine infusion at 2.5 µg/kg/min.

NOTE: Other drugs or treatments can be started at this time point.

7.2. Wait 10 to 15 min for hemodynamic stabilization.

7.3. Perform right heart catheterization, PV-loops and echocardiographic acquisitions.

7.4. Increase the dose of dobutamine infusion to 5 µg/kg/min.

7.5. Wait 15 min for hemodynamic stabilization and repeat acquisitions.

7.6. Repeat right heart catheterization, PV-loops and echocardiographic acquisitions.

7.6. Increase the dose of dobutamine infusion to 7.5 µg/kg/min.

NOTE: Other doses, drugs or treatments can be initiated.

8. Euthanasia and cardiac tissue harvesting

8.1. At the end of the protocol, perform a median sternotomy using an oscillating saw.

8.2. Open the pericardium and inject a lethal solution of potassium chloride (0.2 g/kg).

8.3. Harvest the heart; select samples of the right and left ventricular free-walls for pathological and molecular evaluations.

NOTE: The methods for the pathological evaluations of the right ventricle and for the statistics were previously reported⁵.

REPRESENTATIVE RESULTS:

Feasibility

We describe the results of 9 consecutive procedures of ARHF induction in a large animal CTEPH model previously reported⁵. The duration of the protocol was around 6 hours to complete, including anesthesia induction, installation, vascular access/catheter placements, induction of volume/pressure overload and hemodynamic restoration, data acquisitions and euthanasia.

Each hemodynamic condition requires around 40 minutes to achieve induction of the condition, hemodynamic stabilization and data acquisitions.

The protocol was achieved in 7 out of 9 animals, which represents the learning curve. Three additional protocols were successfully achieved after these described (not published). The cause of the 2 protocol failures was the induction of an irreversible hemodynamic failure after the pulmonary embolism phase.

PV loops were not acquired in 1 out of 7 animals at time of hemodynamic compromise because of the necessity to provide rapid systemic hemodynamic restoration with an epinephrine bolus after the right heart catheterism and the cardiac echo. In this case, dobutamine was started immediately after the restoration of the systemic hemodynamic with epinephrine.

Effects of volume and pressure overload on hemodynamics and RV function

Acute volume loading did not induce ARHF but rather highlighted the adaptive phenotype of the chronic PH model. With volume loading, the cardiac output increased without increase in right atrial pressure, while the ventriculo-arterial coupling remained stable (**Figure 2**).

Hemodynamic compromise criteria were achieved after 1 embolus in 1 animal, 2 emboli in 2 animals, 3 emboli in 5 animals and 4 emboli in 1 animal. Two animals died immediately after PE (1 animal with 1 embolus and 1 animal with 4 emboli). In another animal, severe hypotension required an epinephrine bolus and immediate starting of dobutamine prior to PV-loop and echocardiographic data acquisitions. The 2 deaths occurring immediately after acute pulmonary embolism were associated with acute thrombosis of the right heart cavities (as illustrated in **Figure 3**).

Hemodynamic compromise was associated with a significant decrease in cardiac output, stroke volume and ventriculo-arterial coupling (E_{es}/e_a), whereas RV contractility remained stable (**Figure 2**); there was a two-fold increase in right atrial pressure and mean pulmonary artery pressure.

Dobutamine effect on ARHF

Dobutamine restored a cardiac output, stroke volume, and ventriculo-arterial coupling within normal range (**Figure 2**).

Echocardiography

Echocardiography was feasible providing quantification of dynamic changes in RV size and function during the protocol (**Figure 4**). Echocardiographic parameters were not assessed in 1 animal with severe hemodynamic compromise after pulmonary embolism requiring an epinephrine bolus and immediate starting of dobutamine.

RV PV-loops

Pressure volume loop analysis allowed dynamic quantification of RV end-systolic elastance and ventriculo-arterial coupling (**Figure 2** and **Figure 5**).

Right ventricular ischemic lesions

After hematein, eosin, and saffron staining, we observed RV ischemic lesions in the subendocardial and in the subepicardial layers of the RV free-wall (**Figure 6**). The ischemic lesions were characterized by clusters of hypereosinophilic cardiomyocytes with picnotic nucleus.

FIGURE LEGENDS

Figure 1: Protocol summary. PH, pulmonary hypertension; VL1, volume loading with 15 mL/kg of saline; VL2, 15 mL/kg of saline; VL3, 30 mL/kg of saline; ARHF, acute right heart failure; PE, pulmonary embolism. *systemic systolic pressure <90 mmHg or systolic pulmonary/systemic pressures ratio >0.9. This figure has been modified from⁵.

Figure 2: Individual hemodynamic and pressure-volume loop dynamic changes. MPAP, mean pulmonary artery pressure; MAP, mean arterial pressure; RAP, right atrial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; Ees, right ventricular end-systolic elastance; Ea, arterial elastance. Plots are median and interquartile range. *P<0.05 compared to baseline; comparisons were performed using Wilcoxon matched-pairs signed rank tests with GraphPad Prism 6. This figure has been modified from⁵.

Figure 3: Example of cause of protocol failure: acute right heart thrombosis (arrow) after pulmonary embolism responsible for irreversible hemodynamic compromise, immediate death and protocol failure.

Figure 4: Representative echocardiographic windows and results. (A) Position for acquisition of the apical 5-chamber (A5C) view. (B) Position for acquisition of the parasternal short axis (PSSAX) view. (C) Dynamic echocardiographic evaluations of the A5C and the PSSAX views during the different steps of the protocol. VL, volume loading; PE, pulmonary embolism; Dobu 2.5, dobutamine 2.5 µg/kg/min; Dobu 7.5, dobutamine 7.5 µg/kg/min. *right ventricle; **left ventricle. This figure has been modified from⁵.

Figure 5: Representative dynamic RV multibeat pressure-volume loops. PH, pulmonary hypertension; PE, pulmonary embolism; Ees, end-systolic elastance (black line labelled *); Ea, arterial elastance (black line labelled **); Ees/Ea, ventriculo-arterial coupling. This figure has been modified from⁵.

Figure 6: Representative RV ischemic lesions in the subendocardium and in the subepicardium layers. (A) Subepicardial ischemic lesion; (B) Subendocardial ischemic lesions; (C) Magnification of a border of a subepicardial ischemic lesion with normal nuclei (1), intracytoplasmic vacuolization (2) and pyknotic nuclei (3). (D) individual numbers of subendocardic and subepicardic ischemic lesions in 2 cm length samples of RV free-wall from animals with acute right heart failure (ARHF) on chronic pulmonary hypertension (PH), animals with chronic PH and healthy controls; plots are medians. Comparisons were performed using Mann-Whitney test with GraphPad Prism 6. *P<0.05. This figure has been modified from⁵.

DISCUSSION:

We describe a method to model major pathophysiological features of ARHF on chronic PH in a large animal model including volume and pressure overload and hemodynamic restoration with dobutamine. We also reported how to acquire hemodynamic and imaging data to

phenotype the dynamic changes of the right ventricle at each condition created during the protocol. These methods can provide background data to build up future research protocols in the field of ARHF, particularly regarding fluid management and inotropic support.

Inducing hemodynamic compromise was a critical step in the model because of the risk of unexpected and immediate death of the animal. Consequently, we recommend inducing progressive pulmonary embolism with small embolus volumes. At time of pulmonary embolism, the investigators should be ready to immediately start data acquisitions and hemodynamic support. In our experience, we were able to realize the PV-loop acquisitions and the echocardiography prior to starting dobutamine in 6 out of 7 animals in whom the protocol was completed.

The critical step to phenotype the right ventricle is to obtain comprehensive hemodynamic, PV loop and echocardiographic data. Right heart catheterization allows one to estimate cardiac output and stroke volume changes for each condition. Changes in cardiac output and stroke volume can be further evaluated with echocardiography. This multimodal analysis of cardiac output and stroke volume changes better the external volume calibration of the PV-loops. Importantly, absolute values and rates of changes of PV-loop parameters can be more precisely quantified by including cardiac output and stroke volume changes with external methods performed for each situation.

We observed that volume loading did not induce hemodynamic compromise but rather revealed the adaptive phenotype of the PH model as we observed an increase in cardiac output, stroke volume and systemic pressure with preserved ventriculo-arterial coupling. Therefore, in our model, initial volume loading provided the conditions to observe a major drop in cardiac output and stroke volume after acute pulmonary embolism, hence increasing the sensitivity of the model. Future studies should determine the effect of volume loading or fluid depletion at the time of hemodynamic compromise.

Our protocol has several limitations. This protocol was not built to analyze the cause of the edema, but it may represent an interesting research area. Another limit of the protocol is the time-consumption and the skills required to perform all the steps. The volume loading phase can be shortened or removed from the protocol, but this may result in a lower decrease in the absolute value of the cardiac output and stroke volume after acute pulmonary embolisms. The skills required to perform the protocol require the collaboration of several investigators to place the catheter under fluoroscopy, perform the echocardiography, and analyze in real time the PV-loop quality. We acknowledge that we did not performed 3-dimensional evaluations of the RV volumes. We aim to develop 3-dimensional evaluations of RV volumes as it may provide more precision in the RV volume calibration for RV PV-loop evaluations. One of the first steps would be to evaluate the feasibility of the method. Furthermore, our protocol requires specific facilities such as an operating room and fluoroscopy for invasive RV evaluations.

To our knowledge, we have described the first animal model of ARHF with chronic PH. Previous studies reported dynamic changes of the right ventricle with dobutamine and levosimendan after acute pulmonary artery constriction⁷. In our group, we also quantified the RV reserve using dobutamine infusion in chronic PH without hemodynamic compromise¹⁵. Multibeat PV-

loops are considered the gold standard method to quantify the end-systolic elastance, which represents the ventricular contractility independently from loading conditions¹⁶. RV elastance (Ees=end systolic elastance) absolute values should be interpreted with caution as there are several methodological limits. The main limits are the definition of the end-systolic point and the precision of the volume calibration with external methods (thermodilution and echocardiography)¹⁷. The ratio of end-systolic elastance over arterial elastance (Ea=end-systolic pressure over stroke volume ratio), known as the ventriculo-arterial coupling (Ees/Ea) ratio, reduces the errors due to external volume calibration. The ventriculo-arterial coupling is of major interest in the field of pulmonary hypertension as it captures the adaptation of RV contractility to increased afterload. Methods measuring RV adaptation to afterload have gained major interest in recent years because it has better phenotyping of patients with PH¹⁸⁻²⁰.

Our methods provided values of ventriculo-arterial coupling (i.e., Ees/Ea) consistent with previously published values²¹ and with RV function estimation using echocardiography. In this protocol, we show that acute vena cava occlusion is safe when performed in the context of hemodynamic compromise. Moreover, RV echocardiographic evaluation in the large animal model was complementary from RV echocardiographic evaluation in small animals models as it allowed to quantify different RV function parameters compared with previously reported mice models with RV remodeling²².

The methods described in this study can be used for different research protocols aiming to address key questions in the field of ARHF. First, these methods can be used to perform research protocols aiming to compare different treatment strategies in the context of ARHF on chronic PH. Secondly, iterative and simultaneous PV-loop and echocardiographic evaluation can allow to validate echocardiographic indices in different situations of clinical interest.

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DISCLOSURE:

The authors have nothing to disclose.

REFERENCES:

- 1 Harjola, V. P. et al. Contemporary management of acute right ventricular failure: a statement from the Heart Failure Association and the Working Group on Pulmonary Circulation and Right Ventricular Function of the European Society of Cardiology. *European Journal of Heart Failure*. **18** (3), 226-241, doi:10.1002/ejhf.478 (2016).
- 2 Haddad, F. et al. Characteristics and outcome after hospitalization for acute right heart failure in patients with pulmonary arterial hypertension. *Circulation: Heart Failure*. **4** (6), 692-699, doi:10.1161/circheartfailure.110.949933 (2011).
- 3 Sztrymf, B. et al. Prognostic factors of acute heart failure in patients with pulmonary arterial hypertension. *European Respiratory Journal*. **35** (6), 1286-1293, doi:10.1183/09031936.00070209 (2010).

- 4 Huynh, T. N., Weigt, S. S., Sugar, C. A., Shapiro, S., Kleerup, E. C. Prognostic factors and outcomes of patients with pulmonary hypertension admitted to the intensive care unit. *Journal of Critical Care*. **27** (6), 739.e737-713, doi:10.1016/j.jcrc.2012.08.006 (2012).
- 5 Boulate, D. et al. Early Development of Right Ventricular Ischemic Lesions in a Novel Large Animal Model of Acute Right Heart Failure in Chronic Thromboembolic Pulmonary Hypertension. *Journal of Cardiac Failure*. **23** (12), 876-886, doi:10.1016/j.cardfail.2017.08.447 (2017).
- 6 Noly, P. E. et al. Chronic Thromboembolic Pulmonary Hypertension and Assessment of Right Ventricular Function in the Piglet. *Journal of Visualized Experiments*. (105), e53133, doi:10.3791/53133 (2015).
- 7 Kerbaul, F. et al. Effects of levosimendan versus dobutamine on pressure load-induced right ventricular failure. *Critical Care Medicine*. **34** (11), 2814-2819, doi:10.1097/01.ccm.0000242157.19347.50 (2006).
- 8 Ratliff, N., Peter, R., Ramo, B., Somers, W., Morris, J. A model for the production of right ventricular infarction. *The American journal of pathology*. **58** (3), 471 (1970).
- 9 Ballard-Croft, C., Wang, D., Sumpter, L. R., Zhou, X., Zwischenberger, J. B. Large-animal models of acute respiratory distress syndrome. *The Annals of Thoracic Surgery*. **93** (4), 1331-1339 (2012).
- 10 Dixon, J. A., Spinale, F. G. Large animal models of heart failure: a critical link in the translation of basic science to clinical practice. *Circulation: Heart Failure*. **2** (3), 262-271 (2009).
- 11 Letsou, G. V. et al. Improved left ventricular unloading and circulatory support with synchronized pulsatile left ventricular assistance compared with continuous-flow left ventricular assistance in an acute porcine left ventricular failure model. *The Journal of Thoracic and Cardiovascular Surgery*. **140** (5), 1181-1188, doi:10.1016/j.jtcvs.2010.03.043 (2010).
- 12 Mercier, O. et al. Piglet model of chronic pulmonary hypertension. *Pulmonary Circulation*. **3** (4), 908-915, doi:10.1086/674757 (2013).
- 13 Seldinger, S. I. Catheter replacement of the needle in percutaneous arteriography: a new technique. *Acta Radiologica*. (5), 368-376 (1953).
- 14 Lang, R. M. et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal - Cardiovascular Imaging*. **16** (3), 233-270, doi:10.1093/ehjci/jev014 (2015).
- 15 Guihaire, J. et al. Right ventricular reserve in a piglet model of chronic pulmonary hypertension. *European Respiratory Journal*. **45** (3), 709-717, doi:10.1183/09031936.00081314 (2015).
- 16 Burkhoff, D. Pressure-volume loops in clinical research: a contemporary view. *Journal of the American College of Cardiology*. **62** (13), 1173-1176, doi:10.1016/j.jacc.2013.05.049 (2013).
- 17 Sagawa, K. The end-systolic pressure-volume relation of the ventricle: definition, modifications and clinical use. *Circulation*. **63** (6), 1223-1227 (1981).
- 18 Amsellem, M. et al. Load Adaptability in Patients With Pulmonary Arterial Hypertension. *The American Journal of Cardiology*. **120** (5), 874-882, doi:10.1016/j.amjcard.2017.05.053 (2017).

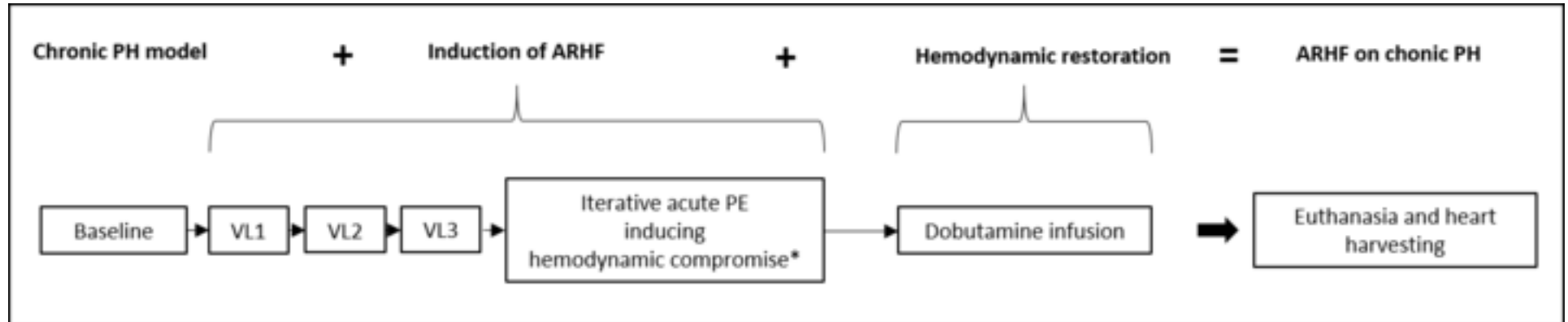
609 19 Dandel, M., Knosalla, C., Kemper, D., Stein, J., Hetzer, R. Assessment of right
610 ventricular adaptability to loading conditions can improve the timing of listing to
611 transplantation in patients with pulmonary arterial hypertension. *The Journal of*
612 *Heart and Lung Transplantation*. **34** (3), 319-328, doi:10.1016/j.healun.2014.11.012
613 (2015).

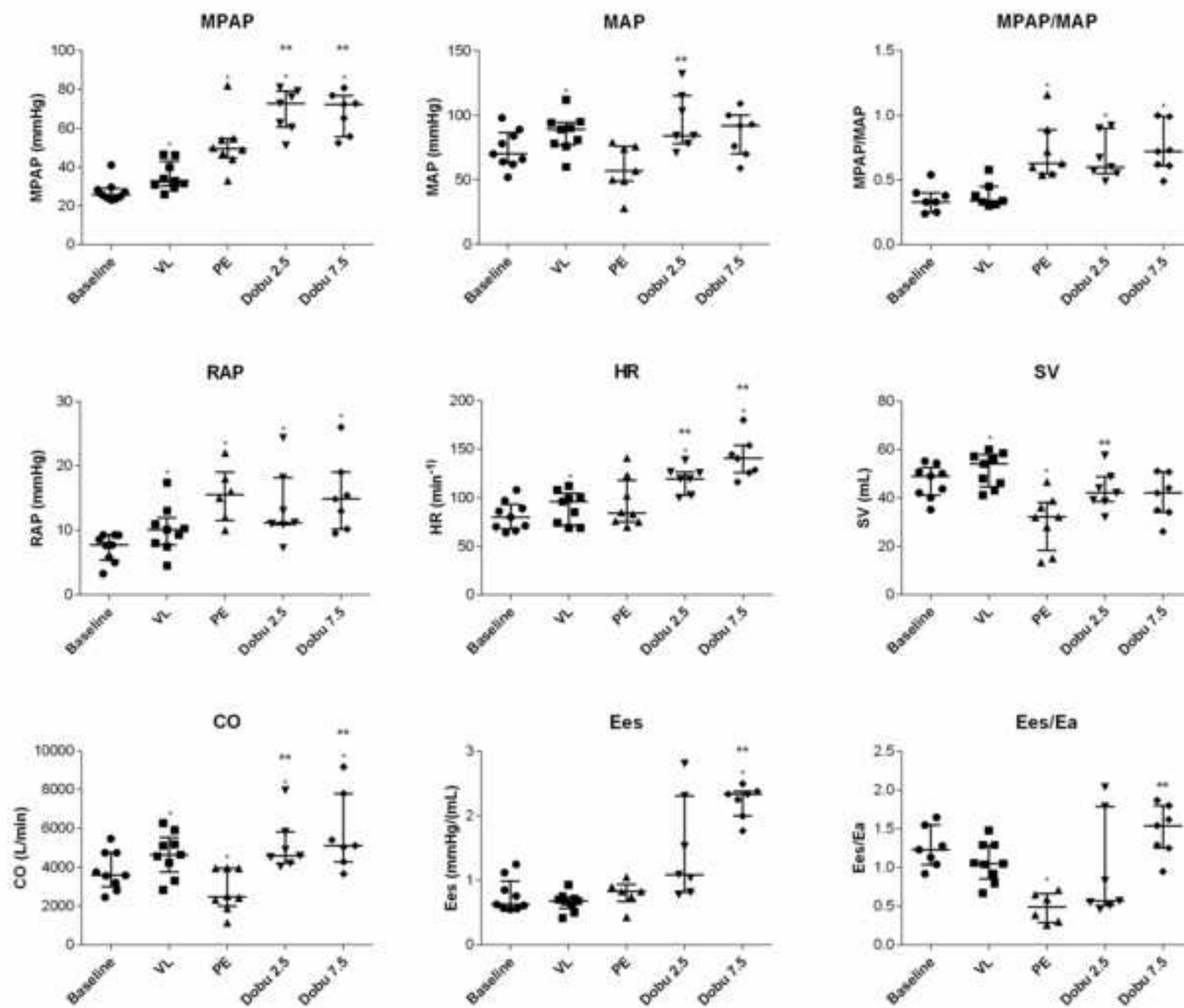
614 20 Vanderpool, R. R. et al. RV-pulmonary arterial coupling predicts outcome in patients
615 referred for pulmonary hypertension. *Heart*. **101** (1), 37-43, doi:10.1136/heartjnl-
616 2014-306142 (2015).

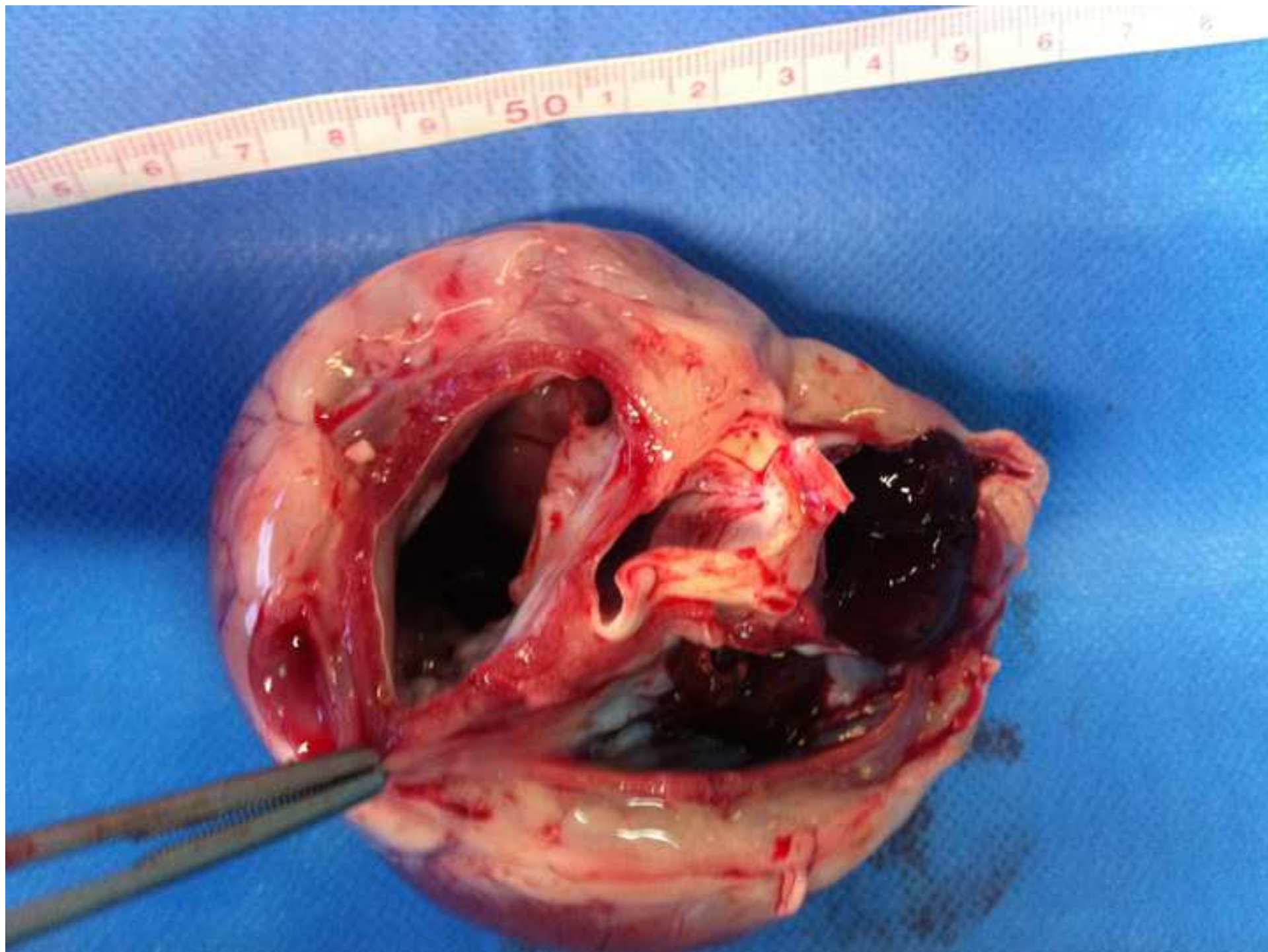
617 21 Boulate, D. et al. in *Pulmonary Hypertension* 241-253 (Springer, 2016).

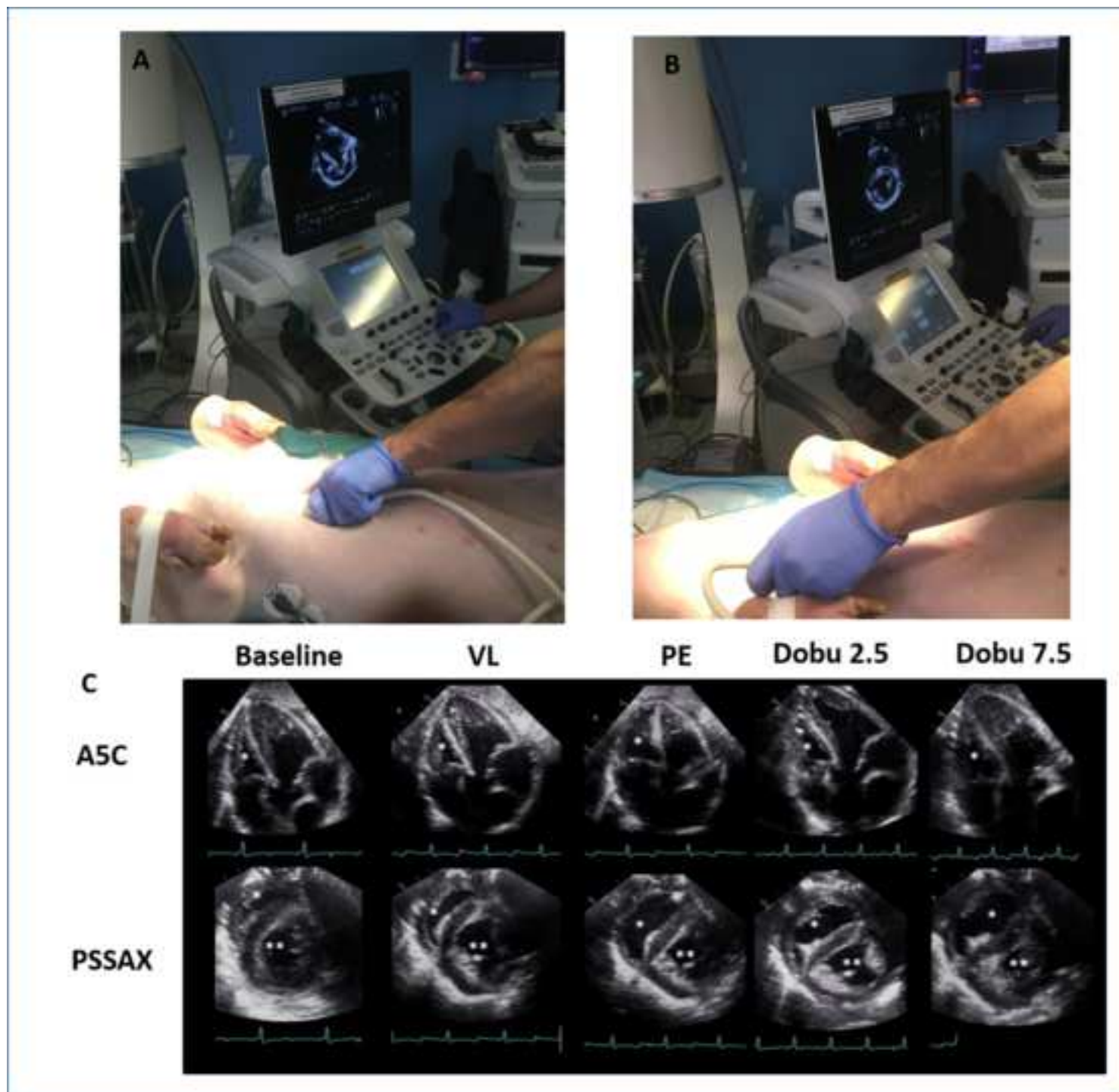
618 22 Cheng, H. W. et al. Assessment of right ventricular structure and function in mouse
619 model of pulmonary artery constriction by transthoracic echocardiography. *Journal*
620 *of Visualized Experiments*. (84), e51041, doi:10.3791/51041 (2014).

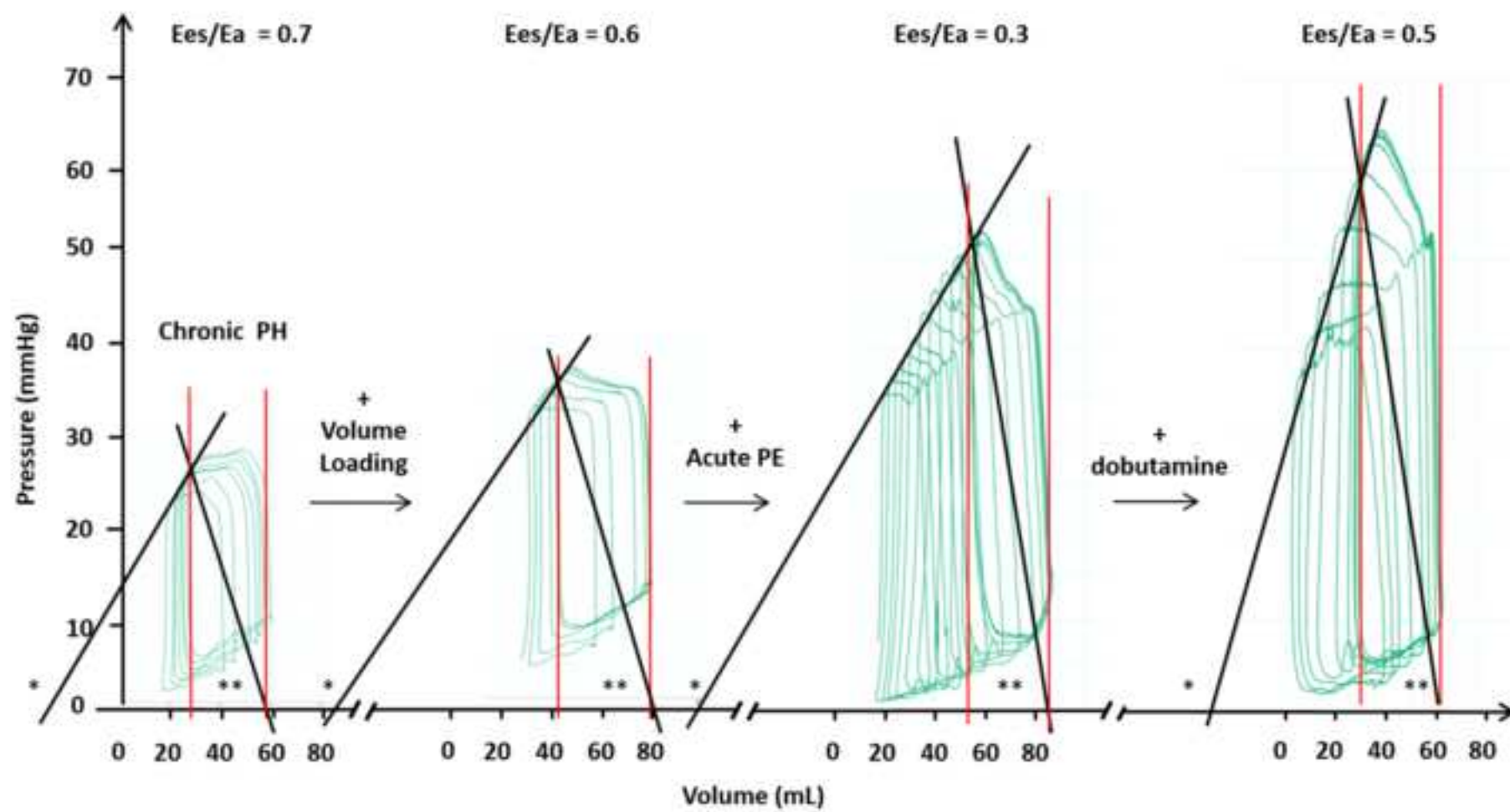
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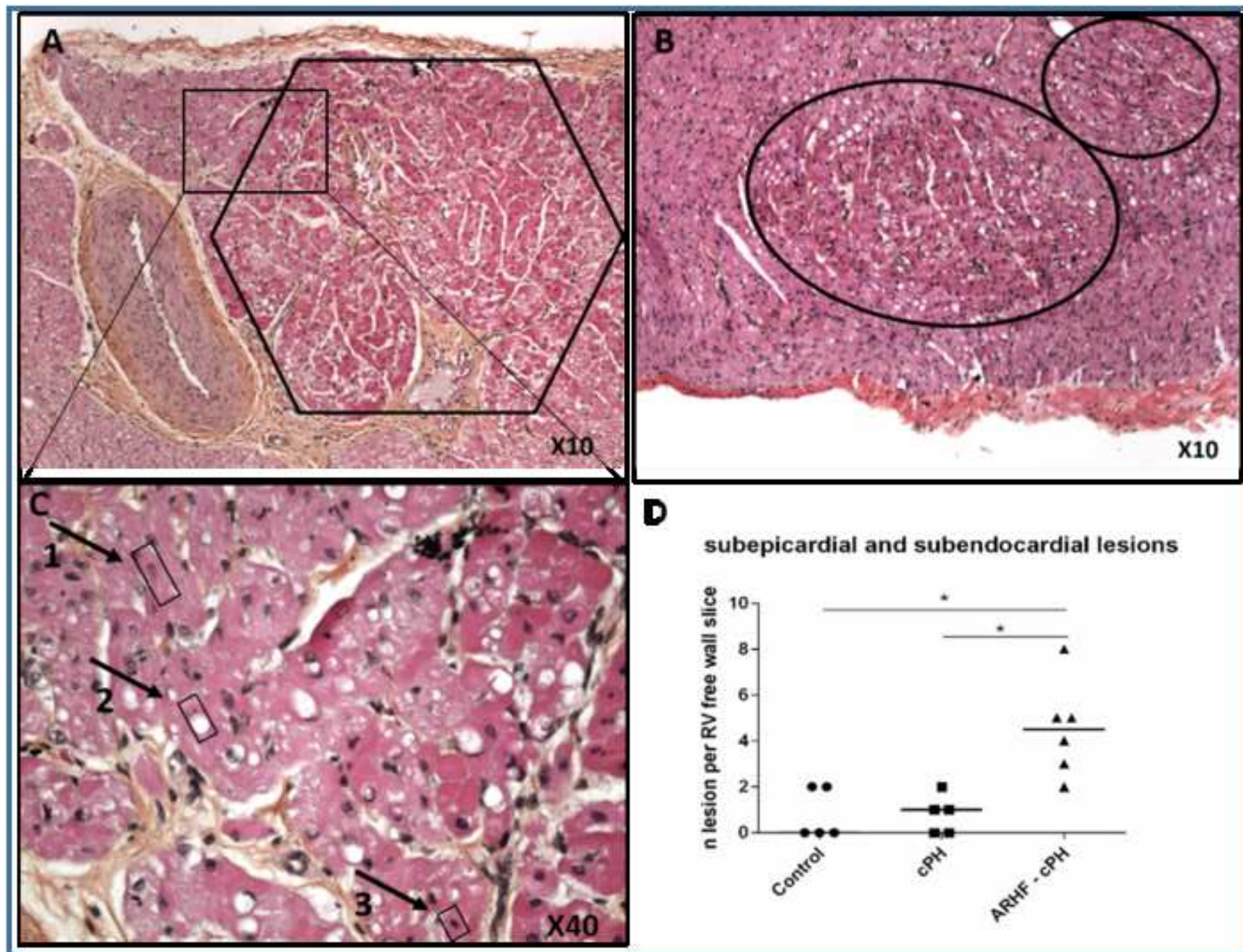












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