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Project Page Link: https://review.jove.com/files_upload.php?src=21417148

Title: Fixed Volume-Intercept Approach for Serial Contractility Assessment with Closed-Chest Pressure-Volume Loops in a Porcine Model

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 15

Number of Shots: 46

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: The video files can be found in the following path: 1. Video footage -> PRIVATE-> M4ROOT -> CLIP.

If there is a double digit number in the last slate number for a shot, fx. 2.11.12, it means that there is an extra take of 2.11.1.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Oskar Hørsdal:** This protocol describes a practical method to assess left ventricular contractility in fragile hemodynamic states.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Oskar Hørsdal:** Traditional ESPVR measurement requires repeated IVC occlusions causing instability; this protocol enables serial Ees estimation without repeated preload reduction.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Oskar Hørsdal:** This protocol enables serial, load-independent measurement of left ventricular contractility in models with fragile hemodynamic states without repeated destabilizing IVC occlusions.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Roni Nielsen:** The key consideration is stable ventricular geometry; this acute-only method becomes unreliable with remodeling, dilation, edema, or major thoracic-pressure changes.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Oskar Hørsdal:** This protocol may benefit other acute disease models and intervention studies requiring serial contractility assessment when repeated preload reduction is unsafe.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

NOTE: Talent didn't want to shoot the testimonial - They were not prepared

~~What problem in your field of research does your JoVE article address?~~

~~1.6 **Oskar Kjærgaard Hørsdal, Medical Doctor, PhD student [Aarhus University]:** (authors will present their testimonial statements live)~~

~~1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.~~

~~How does the video format of your article help you to share your work with the broader scientific community?~~

~~1.7 **Roni Nielsen, Medical Doctor, Associate Professor [Aarhus University]:** (authors will present their testimonial statements live)~~

~~1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.~~

Ethics Title Card

This research was approved by the Danish Animal Research Inspectorate

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Instrumentation and Catheter Placement for Hemodynamic Monitoring

Demonstrators: Oskar Hørsdal and Mark Stoltenberg Ellegaard

2.1. To begin, obtain a fully anesthetized pig connected to a ventilator support and fully instrumented for further procedures [1-TXT]. Insert an 8 French sheath into the common carotid artery [2], an 8 French sheath into the external jugular vein [3], and a 12 French sheath into the femoral vein [4].

2.1.1. WIDE: Show the experimental setup with the anesthetized pig lying on the operating table, connected to ventilator support, and fully instrumented for the upcoming procedures. **TXT: General anesthesia: 3.5 - 4.5 mg/kg/h Propofol and 12.5 - 15.5 µg/kg/h Fentanyl** *Videographer: Please obtain CU shot as well for this shot* After 2.1.1 the author requested an extra shot to be used (C0004)

2.1.2. Talent inserting an 8 Fr sheath into the common carotid artery.

2.1.3. Talent inserting a 12 Fr sheath into the femoral vein. **Author's NOTE: Upon cath of femoral vein, the membrane in 12 Fr sheath broke - hence it started bleeding through the sheath. I suggest editing this part out. Also - every insertion of sheaths are very much the same procedure. I would consider not including every single shot.**

2.1.4. Talent inserting an 8 Fr sheath into the external jugular vein. **NOTE: 2.1.5 One extra shot is filmed**

~~2.2. After inserting the catheter into the opposite common carotid artery, use a 10-milliliter syringe to draw blood from each sheath to confirm proper placement [1]. Perform saline flushing to confirm that there is no resistance [2]. NOTE: The VO is edited for the deleted shot. NOTE: This information is included in 2.1~~

~~2.2.1. Talent inserting an 8 French sheath into the opposite common carotid artery. **TXT: For coronary catheterization and embolization NOTE: Not filmed**~~

~~2.2.2. Talent aspirating blood from a sheath with a 10-mL syringe.~~

~~2.2.3. Talent flushing saline through the sheath.~~

2.3. Suture all sheaths to the skin [1]. Connect them to the relevant monitoring systems [2]. Perform pressure calibration before in vivo insertion of the left ventricular pressure-volume or LV PV (L-V-P-V) catheter [3].

- 2.3.1. Talent suturing the sheaths to the skin.
- 2.3.2. Talent connecting the sheaths to the relevant monitoring systems.
- 2.3.3. Talent performing pressure calibration before in vivo insertion of the left ventricular pressure-volume catheter

Added Step

Insert a pulmonary artery catheter through the jugular sheath to the pulmonary artery, which can be used for volume calibration [1]

Shot: Placement of PA cath.mp4: : 00:00-00:20. Author's NOTE: There is both a video from the videographer and screen recording. Make it fit with the hemodynamics monitor

- 2.4. Insert the LV PV catheter through an 8 French carotid sheath. Follow the pigtail of the LV PV catheter to advance through the aortic valve during systole under fluoroscopic guidance. Place the tip of the LV PV catheter as close to the left ventricular apex as possible [1].

2.4.1. SCREEN: SCREEN_PV_Cath_placement. NOTE: The shots are merged together

~~2.4.2. SCREEN: The catheter advancing under fluoroscopic guidance to and through the aortic valve during systole.~~

~~2.4.3. SCREEN: Talent positioning the tip of the LV PV catheter near the left ventricular apex.~~

NOTE: 2.4.4 as requested extra shot, take 2 should be used

- 2.5. Follow the manufacturer's protocol to choose the relevant number of recording volume segments [1]. Optimize LV PV catheter positioning based on the recorded phase and magnitude signals [2]. Insert the inferior vena cava balloon through the 12 French femoral vein sheath. Advance the balloon to the diaphragm level under fluoroscopic guidance [3].

~~2.5.1. SCREEN: SCREEN_PV_SEGMENT_CHANGES: 00:01-00:21 Talent optimizing positioning based on the recorded phase and magnitude signals.~~

2.5.2. Talent inserting the inferior vena cava balloon through the 12 Fr femoral vein sheath.

2.5.3. Talent advancing the balloon to the diaphragm level under fluoroscopy. NOTE: Use "SCREEN_PLACEMENT_OF_IVC_BALOON.mp4" along with videographer' footage for 2.5.3

3. Baseline Calibration and ESPVR Analysis

- 3.1. At a healthy baseline after instrumentation, calibrate all equipment according to the manufacturer's instructions [1]. Ensure stable sinus rhythm [2]. Use stroke volume

derived from the pulmonary artery catheter for volume calibration [3]. Perform a transient end-expiratory breath hold for 10 to 15 cardiac cycles to obtain baseline values [4].

- 3.1.1. Shot of the talent calibrating all equipment at baseline. **NOTE: 3.1.1 and 3.1.2 are combined in a single shot**
- 3.1.2. Shot of stable sinus rhythm on the monitoring display.
- 3.1.3. Talent using stroke volume derived from the pulmonary artery catheter for volume calibration.
- 3.1.4. Talent performing a transient end-expiratory breath hold while baseline values are recorded.

NOTE: 3.1.5 extra shot, use C0022

- 3.2. To determine volume-axis intercept, perform another end-expiratory breath, hold and wait a few heartbeats [1] Slowly inflate the inferior vena cava balloon until the left ventricular pressure is below 50 millimeters of mercury [2]. Deflate the balloon quickly and resume ventilation [3].

- 3.2.1. Talent performing an end-expiratory breath, holding and waiting for a few heartbeats.
- 3.2.2. Talent slowly inflating the inferior vena cava balloon until the left ventricular pressure is below 50 mm of mercury.
- 3.2.3. Talent quickly deflating the balloon and resuming ventilation.

- 3.3. Allow the animal to recover after each inferior vena cava occlusion and breath hold [1]. After recovery, perform 2 additional rounds of inferior vena cava occlusion [2]. Ensure stable sinus rhythm [3].

- 3.3.1. Talent allowing recovery after the inferior vena cava occlusion and breath hold.
- 3.3.2. Representative shot of the talent repeating the inferior vena cava occlusion procedure and
- 3.3.3. Talent confirming stable sinus rhythm.

- 3.4. In LabChart 8 Pro, open the built-in pressure-volume loop module [1]. In **Analyze**, select **Occlusion**, manually select sequential loops during preload reduction, exclude ectopic beats and adjacent cycles, and apply a linear or quadratic fit from the dropdown menu. Repeat three times and use the average for analysis [2].

- 3.4.1. SCREEN: LABCHART OCCLUSION_NEW: 00:01-00:07
- 3.4.2. SCREEN: LABCHART OCCLUSION_NEW: 00:07-00:22

~~3.5. Use the built-in software function to analyze the selected cardiac cycles [1]. Analyze 10-20 loops during progressive preload reduction. Screen all heartbeats to exclude ectopic beats or artifacts while retaining monotonic, smoothly evolving loops [3]. Fit the end-systolic pressure-volume relationship or ESPVR (E-S-P-V-R) line. Compute V_0 as the volume-axis intercept of the fitted curve or line [5].~~ NOTE: The shots are similar to 3.4, hence the authors requested to not include them

~~3.5.1. SCREEN: LABCHART OCCLUSION_NEW_2.mp4; 00:00-00:07~~

~~3.5.2. SCREEN: LABCHART OCCLUSION_NEW_2.mp4; 00:08-00:15~~

~~3.5.3. SCREEN: LABCHART OCCLUSION_NEW_2.mp4; 00:17-00:23. TXT: Quadratic fit $V_0 = 10$ mL~~ NOTE: Authors: Requested to add this onscreen text

~~3.6. Repeat this approach across the 3 independent occlusions and take the mean of the 3 V_0 measurements [1]. Calculate the end-systolic elastance as the slope between V_0 and the precalculated mean end-systolic pressure-volume point. The end-systolic pressure-volume point can be derived from the hemodynamics table or read manually [2].~~

~~3.6.1. SCREEN: Show the V_0 measurements from 3 independent occlusions and the mean value being calculated.~~

~~3.6.2. SCREEN: BASE Ees.mp4; 00:13-00:35~~

4. Induction of Cardiogenic Shock and Pressure-Volume Analysis

~~4.1. Prepare 10 milliliters of embolization particles using microspheres with iodinated contrast and saline [1]. Place a coronary guide catheter through an arterial sheath in a carotid artery into the ostium of the left coronary artery [2]. Induce cardiogenic shock by stepwise intracoronary microembolization of the left main coronary artery using polyvinyl alcohol particles, allowing hemodynamics to stabilize for 2-5 minutes after each injection [3].~~ NOTE: The VO is edited for the additional shot

Added shot: Shot of the 3 items needed for production of the emboli solution

~~4.1.1. Talent placing a 5 to 7 French coronary guide catheter through the arterial sheath into the ostium of the left coronary artery.~~

~~4.1.2. Talent injecting a few milliliters of contrast into the left coronary artery (simulating the microemboli).~~

~~4.1.3. SCREEN: Injection-of-microspheres.mp4; 00:04-00:15~~

~~4.1.4. Shot of the talent repeating injections. Video editor: Please add LAB MEDIA 1 as an inset~~ NOTE: Merged in the previous shot

~~4.2. At each time point, perform 3 consecutive end-expiratory breath holds for 10 to 20 cardiac cycles while recording pressure-volume loops [1]. Define the mean end-systolic pressure and volume point for the time point, either manually or using the hemodynamics table. Ensure stable sinus rhythm during analysis [2].~~

- 4.2.1. SCREEN: 421-JOVE.mp4 NOTE: videographer also recorded a video "flipping a switch" on the ventilator, which induces apnea, use that also here
- 4.2.2. SCREEN: 423-JOVE.png
- 4.3. Use the presented equation [1] to calculate end-systolic elastance for each time point, ensuring all loops are controlled, and note the end-systolic pressure–volume point [2].
 - 4.3.1. TEXT ON A PLAIN BACKGROUND:
Use
$$E_{es} = ESP / (ESV - V_0)$$

where:
 E_{es} = end-systolic elastance
ESP = end-systolic pressure
ESV = end-systolic volume
 V_0 = volume-axis intercept
 - 4.3.2. SCREEN: LABCHART-BASELINE-APNEA.mp4: 00:17-00:31.

Results

5. Results

5.1. Healthy baseline V_0 was 8 milliliters [1], and the healthy single-beat estimated end-systolic elastance was 1.38 millimeters of mercury per milliliter [2].

5.1.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the label " $V_0 = 8$ "*

5.1.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the label " $E_{es} = 1.38 \text{ mmHg/mL}$ " on the healthy loop panel*

5.2. Following ischemia, left ventricular end-systolic volume increased [1] while left ventricular end-systolic pressure decreased [2], resulting in a rightward and downward shift of the left ventricular pressure-volume loop compared with healthy [3].

5.2.1. LAB MEDIA: Figure 6B. *Video editor: Show that the red loop ends farther to the right than the black loop*

5.2.2. LAB MEDIA: Figure 6B. *Video editor: Show that the top of the red loop is lower than the top of the black loop*

5.2.3. LAB MEDIA: Figure 6B. *Video editor: Highlight the full red loop and compare it with the black loop beside it*

5.3. Stroke volume and left ventricular ejection fraction were markedly reduced, as reflected by a narrowing of the left ventricular pressure-volume loop [1].

5.3.1. LAB MEDIA: Figure 6B. *Video editor: Emphasize that the red loop is narrower than the black loop*

5.4. With V_0 kept constant [1], end-systolic elastance decreased to 0.87 millimeter of mercury per milliliter following ischemia [2].

5.4.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the shared starting point marked " $V_0 = 8$ "*

5.4.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the red label " $E_{es} = 0.87 \text{ mmHg/mL}$ "*

1. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnes-thə-ˌtīzd/
Phonetic Spelling: uh·nes·thuh·tyzd
2. Ventilator
Pronunciation link: <https://www.merriam-webster.com/dictionary/ventilator>
IPA: /ˈven-tə-ˌlā-tər/
Phonetic Spelling: ven·tuh·lay·ter
3. Carotid
Pronunciation link: <https://www.merriam-webster.com/dictionary/carotid>
IPA: /kəˈrā-təd/
Phonetic Spelling: kuh·raa·tid
4. Jugular
Pronunciation link: <https://www.merriam-webster.com/dictionary/jugular>
IPA: /ˈjü-gyə-lər/
Phonetic Spelling: joo·gyuh·ler
5. Femoral
Pronunciation link: <https://www.merriam-webster.com/dictionary/femoral>
IPA: /ˈfe-m(ə-)rəl/
Phonetic Spelling: fem·uh·rul
6. Catheterization
Pronunciation link: <https://www.merriam-webster.com/dictionary/catheterization>
IPA: /kə-ˌthē-tə-rə-ˈzā-shən/
Phonetic Spelling: kuh·thee·tuh·ruh·zay·shun
7. Embolization
Pronunciation link: <https://www.merriam-webster.com/dictionary/embolization>
IPA: /ˌem-bə-lə-ˈzā-shən/
Phonetic Spelling: em·buh·luh·zay·shun
8. Fluoroscopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluoroscopic>
IPA: /ˌflūr-ə-ˈskä-pik/
Phonetic Spelling: floor·uh·skah·pik
9. Systole
Pronunciation link: <https://www.merriam-webster.com/dictionary/systole>
IPA: /ˈsis-tə-(,)lē/
Phonetic Spelling: sis·tuh·lee
10. Aortic
Pronunciation link: <https://www.merriam-webster.com/dictionary/aortic>
IPA: /ā-ˈör-tik/
Phonetic Spelling: ay·or·tik
11. Pulmonary
Pronunciation link: <https://www.merriam-webster.com/dictionary/pulmonary>
IPA: /ˈpül-mə-ˌner-ē/
Phonetic Spelling: pull·muh·nair·ee

12. Hemodynamics
 Pronunciation link: <https://www.merriam-webster.com/dictionary/hemodynamics>
 IPA: /ˌhɛ-mō-dī-'na-miks/
 Phonetic Spelling: hee·moh·dy·nam·iks
13. Inferior Vena Cava
 Pronunciation link: <https://www.merriam-webster.com/dictionary/vena%20cava>
 IPA: /in-'fir-ē-ər ˌvɛ-nə 'kā-və/
 Phonetic Spelling: in·feer·ee·er vee·nuh kay·vuh
14. Preload
 Pronunciation link: <https://www.merriam-webster.com/dictionary/preload>
 IPA: /ˌprē-'lōd/
 Phonetic Spelling: pree·loh
15. Ectopic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ectopic>
 IPA: /ek-'tä-pik/
 Phonetic Spelling: ek·tah·pik
16. Elastance
 Pronunciation link: <https://www.merriam-webster.com/dictionary/elastance>
 IPA: /i-'la-stən(t)s/
 Phonetic Spelling: ih·las·tuhns
17. Cardiogenic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cardiogenic>
 IPA: /ˌkär-dē-ō-'je-nik/
 Phonetic Spelling: kar·dee·oh·jen·ik
18. Microembolization
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microembolization>
 IPA: /ˌmī-krō-ˌem-bə-lə-'zā-shən/
 Phonetic Spelling: my·kroh·em·buh·luh·zay·shun
19. Polyvinyl
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polyvinyl>
 IPA: /ˌpā-lē-'vī-nəl/
 Phonetic Spelling: pah·lee·vy·nul
20. Ischemia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>
 IPA: /is-'kē-mē-ə/
 Phonetic Spelling: iss·kee·mee·uh
21. Ejection Fraction
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ejection>
 IPA: /i-'jek-shən 'frak-shən/
 Phonetic Spelling: ee·jek·shun frak·shun

