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Title: Murine Bilateral Renal Lymphadenectomy

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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? **YES**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   Leica Mz9.5 stereo head operating microscope

   SCOPE shots: Step 2.3 to 2.10 and steps 3.3 to 3.9

Videographer: Please film the above-mentioned steps using the scope kit

Videographer's NOTE: iPhone was attached to scope. Could not center phone on scope, so recordings are slightly off center.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 20
Number of Shots: 39 (25 Scope)

Introduction

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

Videographer's NOTE: Names listed may not be who actually answered interview question. Had them say their name in beginning to be certain

1.1. **Jafar Al Souz:** We study trafficking of immune cells from the renal lymph node to the kidney and of kidney antigens to the draining lymph node.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Holly Nicole Blackburn:** Our surgical approach allows for the unique advantage of *selectively* disrupting renal lymph nodes, while leaving all other nodes intact, in a safe and well-tolerated long-term survival model.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1*

What new scientific questions have your results paved the way for?

1.3. **Shun-Fat Lau:** Now that this model has been developed, we can explore questions about the contribution of renal lymph nodes in immune responses, and whether removal of the nodes protects from or further drives immune-mediated kidney injury.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

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

Ethics Title Card

This research has been approved by Yale University's Institutional Animal Care and Use Committee

Protocol

Videographer's NOTE: All audio cues for entire video.

Author's NOTE: I talked through the procedures as much as I could to offer verbal cues to the video editors for which steps we were at. I hope that is helpful.

2. Surgical Preparation and Left Renal Lymphadenectomy

Demonstrator: Holly Nicole Blackburn

2.1. To begin, place the anesthetized animal on the operating table **[1-TXT]**. Use tape to secure the animal's left arm in a superior direction **[2]** and the tail in an inferior direction to create a relaxed but elongated posture **[3]**.

2.1.1. WIDE: Talent placing the anesthetized animal on the operating table. **TXT: 2 - 3% Isoflurane + suitable local anesthetic**

2.1.2. Talent gently taping the animal's left arm superiorly on the surgical platform.

2.1.3. Talent taping the tail inferiorly on the surgical platform.

Author's NOTE: Show step 2.2 after the step 2.11.

2.2. Optionally, prepare 10 to 20 microliters of sterile injection containing filtered 5 percent Evans Blue dye in PBS **[1]** and inject into both hindfoot pads to assist with visualization of the renal lymph nodes through drainage along the iliac lymphatic tract **[2]**.

2.2.1. Talent loading the dye into a syringe.

2.2.2. Talent injecting a solution into a hindfoot pad.

2.3. Now, make a 1-centimeter oblique incision through the skin just below the spleen, approximately 1 centimeter inferior to the last rib **[1]**. Using blunt dissection, separate the skin from the abdominal and chest wall **[2]** and identify the spleen within the abdomen **[3]**.

2.3.1. SCOPE: making a 1 centimeter oblique skin incision with a scalpel.

2.3.2. SCOPE: using blunt forceps to gently dissect the skin.

2.3.3. SCOPE: pointing to the revealed spleen.

Videographer's NOTE: Scope shots were repeated in a few scenes. Ended up restarting and using different mouse several times. There is one long take of this entire procedure with audio Cues the whole time.

2.4. Then, make a 1-centimeter incision through the abdominal wall musculature just inferior to the spleen to enter the peritoneal cavity **[1]**.

2.4.1. SCOPE: incising the abdominal wall musculature with a scalpel.

2.5. Identify the left kidney as a smooth organ located along the posterior abdominal wall, just beneath the spleen **[1]**.

2.5.1. SCOPE: identifying and pointing to the left kidney.

2.6. Using two angled forceps, bluntly dissect behind the left kidney to access the retroperitoneum **[1]** and gently reflect the left kidney in an anteromedial direction **[2]**.

2.6.1. SCOPE: using angled forceps to carefully dissect posterior to the kidney.

2.6.2. SCOPE: Shot of the left kidney reflected anteromedially.

2.7. Locate the adipose tissue pad surrounding the left renal lymph node, which lies posterior to the kidney and superior to the renal vessels **[1]**. Identify and avoid the adrenal gland located superiorly, the aorta posteriorly, and the cisterna chyli anteromedially **[2]**.

2.7.1. SCOPE: pointing to the adipose pad surrounding the lymph node beneath the microscope.

2.7.2. SCOPE: pointing to adrenal gland, aorta, and cisterna chyli near the lymph node.

2.8. Using a fine-tipped forceps, bluntly dissect around the lymph node capsule to separate it from adjacent vessels while providing gentle counter tension with an angled forceps **[1]**.

2.8.1. SCOPE: Fine-tipped and angled forceps manipulating the lymph node and surrounding tissue.

2.9. Use fine-tipped straight forceps to retract the lymph node superiorly **[1]**, then use fine-point scissors to sharply cut the posteromedial attachments **[2]**.

2.9.1. SCOPE: fine-tipped straight forceps retracting the lymph node upward.

2.9.2. SCOPE: Fine-point scissors incising posteromedial attachments.

2.10. Examine the area to confirm hemostasis **[1]**.

2.10.1. SCOPE: Clear surgical field showing absence of bleeding.

2.11. Next, return the kidney to its normal anatomical position **[1]**. Close the abdominal wall and then the skin using 6-0 *(6-oh)* monofilament polypropylene suture in a running stitch **[2]**. Apply sterile surgical glue to seal the wound **[3]**.

2.11.1. Talent repositioning the kidney back into place.

2.11.2. Talent suturing the abdominal wall and skin layers sequentially.

2.11.3. Talent applying surgical glue along the closed incision line.

NOTE: Show step 2.2 here, after the step 2.11.

3. Right Renal Lymphadenectomy

3.1. Reposition the animal into the left lateral decubitus position with the left side facing down **[1]**. Use tape to secure the animal's right arm in a superior direction **[2]** and the tail in an inferior direction to maintain a relaxed but elongated posture **[3]**.

3.1.1. Talent gently turning the animal onto its left side on the surgical platform.

3.1.2. Talent taping the right arm into the surgical board.

3.1.3. Talent taping the tail into position.

3.2. Now, make a 1-centimeter oblique skin incision just beneath the last rib, which will be positioned slightly higher than on the left side **[1]**. Using blunt dissection, separate the skin from the abdominal and chest wall **[2]**. Then, make a 1-centimeter incision through the abdominal wall musculature to access the peritoneal cavity **[3]**.

3.2.1. SCOPE: performing the oblique skin incision using a scalpel.

3.2.2. SCOPE: using blunt forceps to dissect the skin from the underlying wall.

3.2.3. SCOPE: incising the abdominal musculature with scissors.

3.3. Identify the right kidney, which is a smooth organ located along the posterior abdominal wall and just beneath the liver **[1]**.

3.3.1. SCOPE: identifying and pointing to the right kidney.

3.4. Using two angled forceps, bluntly dissect behind the right kidney to gain entry to the retroperitoneal space **[1]** and reflect the right kidney in an anteromedial direction **[2]**.

3.4.1. SCOPE: using angled forceps to dissect posterior to the right kidney.

3.4.2. SCOPE: Shot of the reflected right kidney.

3.5. Identify the adipose tissue pad surrounding the right renal lymph node, which lies posterior to the kidney and superior to the renal vessels **[1]**. Identify and avoid the adrenal gland located superior to the lymph node **[2]**.

3.5.1. SCOPE: pointing to the adipose tissue enveloping the lymph node under the microscope.

3.5.2. SCOPE: pointing to the adrenal gland relative to the lymph node.

3.6. Now, using a fine-tipped forceps, bluntly dissect around the capsule of the lymph node to separate it from nearby vessels, while applying gentle countertraction with an angled forceps **[1]**.

3.6.1. SCOPE: Fine-tipped forceps bluntly dissecting around the capsule of the lymph node to separate it from nearby vessels. **TXT: Alternatively, use fine-point scissors**

3.7. Then, with fine-tipped straight forceps, pull the lymph node superiorly **[1]** and sharply cut the posteromedial attachments with fine-point scissors **[2]**. Optionally, use the Evans blue dye to visualize each renal lymph node during the procedure **[3]**. **NOTE**: VO added for the extra shot

3.7.1. SCOPE: Lymph node being elevated by fine-tipped straight forceps.

3.7.2. SCOPE: attachments being incised cleanly with fine-point scissors.

Added shots (2 shots): SCOPE: Shot of the lymph node stained with Evans blue under the scope. **NOTE**: This was shot at the end of the procedure, after 3.9, as bonus shots

3.8. Inspect the area to ensure hemostasis **[1]**.

3.8.1. SCOPE: Bloodless surgical field shown under the microscope.

3.9. Finally, return the right kidney to its anatomical position **[1]**. Close the abdominal wall and skin in two layers using 6-0 monofilament polypropylene suture in a running pattern **[2]** and apply sterile surgical glue over the incision site **[3]**.

3.9.1. SCOPE: placing the kidney back in position inside the abdomen.

3.9.2. SCOPE: suturing the muscle and skin layers sequentially.

3.9.3. SCOPE: applying surgical glue to the closed incision.

Results

4. Results

4.1. Flow cytometry confirmed successful lymph node extraction in all 5 left and 5 right renal lymphadenectomy samples, each showing over 50% CD45-positive live cells **[1]**, compared to less than 2% in visceral adipose controls **[2]**.

4.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the black boxes inside panels labeled "L rLAD" and "R rLAD"*

4.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the black box inside panel "Visc Adip"*.

4.2. Quantification of CD45-positive cells showed a highly significant difference between both left and right renal lymphadenectomy **[1]** samples compared to visceral adipose tissue **[2]**, with no significant difference between the left and right sides **[3]**.

4.2.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the two leftmost bars labeled "L rLAD" and "R rLAD"*

4.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the rightmost bar labeled "Visc Adip" showing a large drop*.

4.2.3. LAB MEDIA: Figure 4B. *Video editor: Emphasize the statistical comparison markers above "L rLAD" and "R rLAD" labeled **"ns"***.

- lymphadenectomy

Pronunciation link: https://www.merriam-webster.com/medical/lymphadenectomy merriam-webster.com+2Cleveland Clinic+2
IPA: /ˌlɪmf.ə.dəˈnɛk.tə.mi/
Phonetic Spelling: limf-uh-dih-NEK-tuh-mee

- cisterna chyli

Pronunciation link: https://www.merriam-webster.com/medical/cisterna%20chyli merriam-webster.com+1
IPA: /sɪˈstɜːr.nə ˈkaɪ.laɪ/
Phonetic Spelling: sih-STUR-nuh KY-lye

- anteromedial

Pronunciation link: https://www.merriam-webster.com/medical/anteromedial merriam-

webster.com+1
IPA: /ˌæn.tə.roʊˈmiː.di.əl/
Phonetic Spelling: AN-tuh-roh-MEE-dee-uhl

- adipose

Pronunciation link: https://www.merriam-webster.com/dictionary/adipose merriam-webster.com
IPA: /ˈæd.ə.poʊs/
Phonetic Spelling: AD-uh-pohs

- isoflurane

Pronunciation link: https://www.howtopronounce.com/isoflurane-1 How To Pronounce+1
IPA: /ˈaɪ.səˌflʊəˈreɪn/
Phonetic Spelling: EYE-suh-FLU-rayn

- retroperitoneum

Pronunciation link: No confirmed link found
IPA: /ˌrɛt.rəʊˌpɛr.ɪˈtoʊ.ni.əm/
Phonetic Spelling: RET-roh-per-ih-TOH-nee-uhm

- anteromedially

Pronunciation link: No confirmed link found
IPA: /ˌæn.tə.roʊˈmiː.di.əli/
Phonetic Spelling: AN-tuh-roh-MEE-dee-uh-lee