Quantification of Ascending Aortic Aneurysms

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Abstract

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Protocol

DEFINITION and PRINCIPLE

Step 1.

Ascending aortic region - is the portion of the aorta commencing at the junction of the myocardium to the left subclavian artery. Dilation of the ascending portion is quantified by measuring the intimal area of ascending aortic region.

DISSECT, FIX and CLEAN

Step 2.

1. Cut open the mouse ventrally, perfuse with saline, and dissect the aorta.
2. Place dissected aortas in 4% paraformaldehyde or 10% neutrally buffered formalin for 24 - 48 hours. Once fixed, aortas are kept in saline at room temperature (or 2-8 °C)
3. Before cleaning, allow aortas to soak in saline for a few hours. Clean aortas by removing adventitial tissues. Be careful to not tear or nick the aorta and important branches. Use saline to keep the tissue moist during cleaning

CUT

Step 3.

- Leave 1 mm of innominate and left common carotid artery, and cut off the entire left subclavian artery. Cut open the outer curvature through the innominate artery, then to left common carotid artery, and then to the left subclavian artery. Cut open along the inner curvature of the ascending portion to the bottom of the abdominal portion

PIN

Step 4.

1. Pin aorta flat on black wax with pins (Fine Science Tools item # 26002-20).
2. Apply saline to keep aortas from drying.
3. Label mouse number on both lid and box of the black wax. Imaging and analysis should be done within 3 days after pinning

IMAGE

Step 5.

- Take pictures of en face aorta with a Nikon digital camera. A mm ruler must be included in the images to calibrate measurements.

CALIBRATION

Step 6.

1. Open Image Pro program 5.0 or 7.0 and open the image to be measured.
2. Go to the “Measure” menu 6 “Calibrate” 6 “spacial calibration”
3. Select “spacial calibration wizard” and follow directions, or click “new” and name your calibration.
4. Click “image”, position line over the ruler, and change reference units to mm. Click “ok” and then “apply”.

LABELING

Step 7.

1. Open Image Pro program 5.0 or 7.0, and open the image to be labeled.
2. Label the image by clicking on the annotation button (O) click on “Aa”, then click on the picture and fill in text (study name, mouse #, date), and click “ok”.
3. Burn the label to the image by pressing the double arrows (bb).
4. Save the picture.
5. Label remaining images.

MEASURING

Step 8.

1. Select the correct calibration for each image opened. “Measure” 6 “Calibration” 6 “set system” 6 “Apply”.
2. Click “measure” menu and select “measurements”.
3. Select the polygon button (-) on the Measurement Toolbar and trace the intimal area of the ascending region

EXPORTING DATA

Step 9.

1. Click on “input/output’. Chose measurements and click “export now”.
2. Paste into spreadsheet
3. Save image: Input/output tab, save as “mouse #....msr”. Image may be printed with overlay at this time.

NOTES

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Do not close the image until the image has been printed and saved, and measurements are recorded in the spreadsheet

VERIFICATION and UNPIN

Step 10.

1. Quantification is verified by a second observer who is blind to study groups.
2. After verification, aortas should be unpinned, put into properly labeled tubes with saline containing 0.02% sodium azide and stored at room temperature.