DAUGHERTY LAB

Saha Cardiovascular Research Center

University of Kentucky

Quantification of Elastin Fragmentation in the Ascending Aorta

Equipment:

- Wide field microscope
- 40x objective lens

Samples:

- Cut the ascending aorta into 10 µm serial sections from the aortic root to the aortic arch.
  See “Protocol for Harvesting and Sectioning the Ascending Aorta”
  http://cvrc.med.uky.edu/sites/default/files/Harvesting_and_Sectioning_the_Ascending_Aorta_UK.pdf
- Perform Movat’s or Verhoeff-Van Gieson (VVG) staining using standard protocols. These sections are used for the quantification of elastin fragmentation.
- Since elastin fibers have auto-fluorescence illuminated by FITC channel, FITC image of unstained sections could be used instead of Movat’s and VVG stained sections (Figure 1). H&E or X-gal stained sections could also be used.
- Advantages and disadvantages of Movat’s and VVG stained and FITC images are shown in Table 1.

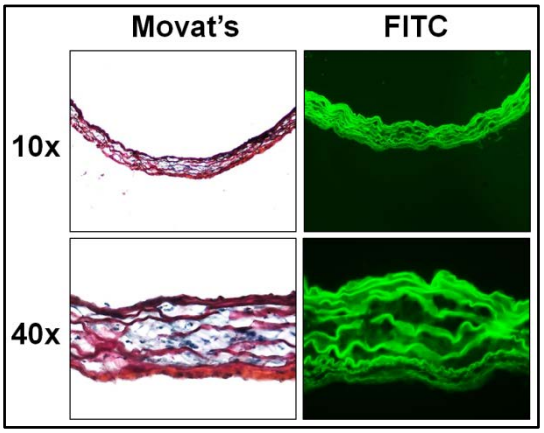

Figure 1. Representative low and high magnification Movat’s staining and FITC illuminated images.

	Advantages	Disadvantages
Movat’s and VVG	- Good contrast	- Complicated procedure - Time consuming
FITC	- Easy procedure	- Unclear in low magnification image

Table 1. Advantages and disadvantages of Movat’s stained and FITC images for the quantification of elastin fragmentation

Investigators:

- Elastin fragmentation is independently examined by 2 investigators.
- Investigators should be blind to study groups.
- If the section has more than 5 breaks and the counting between the two investigators is more than 30% different, elastin fragmentation in the section should be recounted by both investigators together.

Microscopy:

1. Select three sections (100 µm interval) from the region of interest.
   Since angiotensin II-induced elastin fragmentation is generally detected in the middle of the ascending aorta (Rateri DL, et al. AJP. 2014), the middle three ascending aortic sections should be used for quantification in studies using angiotensin II infusion (Figure 2a, b).

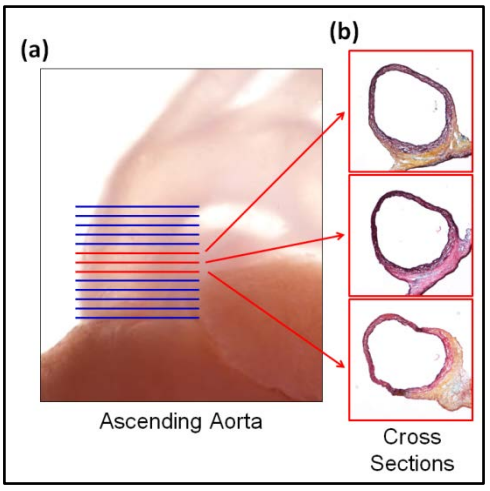

Figure 2. Representative gross appearance image of the ascending aorta **(a)**. Blue lines indicate sectioning levels and red lines are the middle three sections. Representative images of Movat's staining **(b)**. Sections are from the middle three of the ascending aorta.

2. Observe sections using 40x objective lens (Figure 3a, b).

3. Fragmentation is defined as the presence of free ends in what seems to be an otherwise continuous elastin fiber. Representative image of elastin fragmentation is shown in Figure 3b.

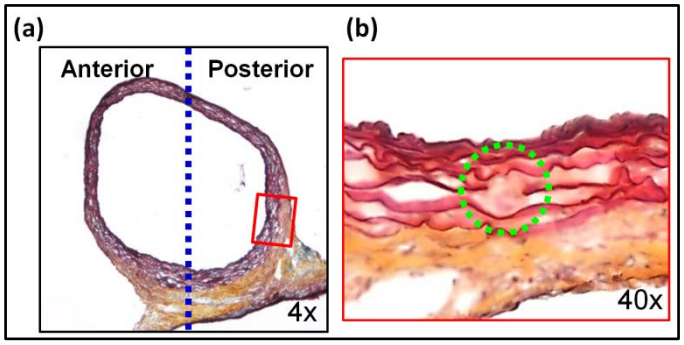

Figure 3. Representative aortic Movat's staining image **(a)**. Blue dotted line is the center line dividing the anterior and posterior portions of the aorta. Highly magnified (x40) image of Movat's staining **(b)**. Green dotted circle indicates representative elastin fragmentation.

4. Count elastin fragmentations in the whole aorta of three serial sections.

   Option: For investigating regionality of elastin fragmentation, aortic sections could be divided into anterior and posterior portions. Elastin fragmentation is examined in each region (Figure 3a). The descending aorta can be used to orient the ascending aorta so anterior and posterior is accurately identified.

5. In order to show fragmentation clearly, the level of the microscope stage may need to be adjusted finely.

6. Fragmentation should be counted in three serial sections (Table 2).

Section	Anterior	Posterior	Total
#1	5	2	7
#2	4	3	7
#3	6	4	10
Mean	5	3	8

Table 2. Example for collated data sheet

Data analysis:

1. For data analysis, the number of elastin breaks counted by two investigators should be calculated for the mean.
   Table 3 shows an example for data sheet.

Study	Mouse #	Slide	Section	Count by observer A			Count by observer B			Elastin Break Count = (A + B) / 2			Note
				Anterior	Posterior	Total count	Anterior	Porterior	Total count	Anterior	Porterior	Total count	
S1a	1	11	4	0	1	1.0	0	0	0.0				
			5	1	2	3.0	1	1	2.0				
			6	2	1	3.0	2	1	3.0				
			Mean	1	1	2.3	1	1	1.7	1	1	2.0	
	2	11	5	6	2	8.0	7	3	10.0				
			6	5	2	7.0	6	3	9.0				
			7	4	0	4.0	4	0	4.0				
			Mean	5	1	6.3	6	2	7.7	5	2	7.0	

Table 3. Example for data sheet

2. Appropriate statistical analysis should be performed using total mean numbers.

Protocol created: Hisashi Sawada on 1/5/18
Verified: Alan Daugherty on 1/8/18