STANDARD OPERATING PROCEDURE

STAGING OF ATHEROSCLEROTIC LESIONS

Vascular Biology and Atherosclerosis Lab
University of Ottawa Heart Institute, Rm H259

1. PURPOSE:
The purpose of this document is to outline the protocols and procedures to be followed when staging atherosclerotic lesions in mice, as well as define the landmarks and features that are characteristic of each lesion stage.

2. MATERIALS:
   i. Gomori Trichrome stained serial sections: stain to detect collagen
   ii. Sudan IV stained serial sections: neutral lipid stain, Sudan IV, stains lipid-rich lesion red
   If necessary, the following are also used:
   iii. Serial sections immunostained for macrophages: MOMA2 stains macrophage brownish colour
   iv. Microscope slides of stained serial sections

3. ANATOMY OF THE ARTERY:

   ![Artery Diagram]
   - **Artery**
   - **Tunica intima**: endothelium that lines the lumen of all vessels
   - **Tunica media**: smooth muscle cells and elastic fibers
   - **Tunica adventitia**: collagen fibers
4. **LANDMARKS:**

Staging of atherosclerotic lesions is restricted to the aortic root. In serial sectioning of the heart, the coronary ostia are readily identifiable, and for this reason are used as a “landmark” for the starting point of lesion staging.

**NOTE:** when the lesion extends into the coronary ostia, the area extending into the ostia is NOT included in the lesion staging.

5. **FEATURES OF LESION STAGES:**

**Early Stage Atherosclerotic Lesions (Stages I-III):**

Note: morphological feature of early stage lesions is the accumulation of numerous cholesterol-ester enriched foam cells that come together to form a mass of cells termed the “fatty streak”.

i. **Stage I:**
   - The lesion contains mainly macrophage derived foam cells, 1-3 cell layers thick.

ii. **Stage II:**
   - The lesion contains mainly macrophage derived foam cells, 3-7 cell layers thick.

iii. **Stage III:**
   - The lesion contains mainly macrophage derived foam cells, 7+ layers thick.

**Late Stage Atherosclerotic Lesions (Stages IV-V):**

**NOTE:** Both stage IV and stage V lesions are capable of developing fissures, haematomas, and/or thrombi.

iv. **Stage IV:** (characterized by the acellular neutral lipid core)
   - First stage to be considered advanced by histological criteria, due to the accumulation of extracellular lipid, known as the lipid core.
   - This lipid core occupies an extensive but well-defined region of the tunica intima.
• As the lipid core develops there is a thickening of the tunica intima, which can cause deformity in the arterial wall (generally do not cause narrowing of the vascular lumen).

v. Stage V: (characterized by the fibrous cap)
• When the intimal area on the lumen side of the lipid core undergoes an increase in fibrous tissue, a cap is formed, converting the lesion from stage IV into stage V.
• Gomori Trichrome staining is helpful in differentiating stage IV lesions from stage V lesions, as this allows for better visualization of the fibrous cap.
6. REPRESENTATIVE IMAGES:

Stage I
(Sudan IV)

Stage II
(Sudan IV)

Stage III
(Sudan IV)
Stage IV (Sudan IV)  
Stage IV (Gomori Trichrome)

Stage V (Sudan IV)  
Stage V (Gomori Trichrome)

7. REFERENCE: