

## **Supplemental Digital Content**

Ho JE *et al.* The Effect of CD4+ T-Cell Count on Cardiovascular Risk in Treated HIV Disease

## **Supplemental Methods**

### **Laboratory Assays**

HIV RNA levels were measured using branched chain DNA method (Quantiplex HIV RNA, Chiron Version 3.0: Chiron Corporation, Emeryville, California, USA), and hs-CRP was measured using the CardioPhase hs-CRP assay [1]. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, and serum L-arginine levels were measured using high pressure liquid chromatography [2].

### **Assessment of Endothelial Function**

Participants were asked to refrain from drinking alcohol or caffeinated beverages for > 12 hours, and were studied lying supine in a dark and quiet room. High-resolution ultrasound of the right brachial artery was performed using a 10 MHz linear array probe and the GE VividSeven Imaging System (GE, Milwaukee, Wisconsin, USA) according to established guidelines [3]. To assess endothelium-dependent vasodilation (also referred to as flow-mediated dilation, FMD), the brachial artery diameter was measured under basal conditions and during reactive hyperemia following the inflation of a blood pressure cuff to suprasystolic pressures on the forearm for 5 minutes. Reactive hyperemia was measured one minute following cuff deflation as the increase in brachial artery diameter [4]. To assess endothelium-independent vasodilation (nitroglycerin mediated dilation, NMD), the baseline brachial artery diameter was determined after 20 minutes of rest, and maximal brachial artery dilation measured 3 minutes after the administration of 0.4 mg sublingual nitroglycerin. Acquisition and analysis of the digitized images was performed using dedicated software (Information Integrity, Inc., Iowa City, Iowa, USA) by a single technician blinded to the participant's HIV disease and treatment status. The vessel wall/lumen

interface was determined by derivative-based edge detection algorithm, and the maximum diameter of the vessel averaged over 3 consecutive cardiac cycles at end diastole [5, 6].

Repeated measurements of 10 scans in a blinded manner showing a correlation coefficient of 0.998. In addition, 10 patients underwent repeat scans within 14 days of study enrollment, with a difference in FMD of 0.005% (-0.06 to +0.04%,  $p = 0.99$ ).

### Supplemental References

1. Eda S, Kaufmann J, Roos W, Pohl S. Development of a new microparticle-enhanced turbidimetric assay for C-reactive protein with superior features in analytical sensitivity and dynamic range. *J Clin Lab Anal* 1998,**12**:137-144.
2. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992,**339**:572-575.
3. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, *et al*. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002,**39**:257-265.
4. Lieberman EH, Gerhard MD, Uehata A, Selwyn AP, Ganz P, Yeung AC, *et al*. Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. *Am J Cardiol* 1996,**78**:1210-1214.
5. Stadler RW, Karl WC, Lees RS. New methods for arterial diameter measurement from B-mode images. *Ultrasound Med Biol* 1996,**22**:25-34.
6. Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P, *et al*. Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. *Circ Res* 2006,**99**:1426-1432.