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


