

***High Sensitivity IL-6 assay:*** All blood samples were collected according to the HABC protocol for the year 10 collection. Serum samples used previously for measurements associated with ancillary study AS05-73AM at the University of Pittsburg, where they underwent 1 freeze/ thaw cycle, unless otherwise stated . These serum samples were sent on dry ice by priority overnight shipment to the Pepper Center Biological Specimen Repository at Wake Forest Baptist Health and stored at -80°C until testing.

The samples were used to analyze high sensitivity IL-6 assay by absorbance ELISA (enzyme-linked immunosorbant assay) methods using the R&D Systems (Minneapolis, MN) Quantikine kits. R&D Systems reports the following information per kit lot number: 1) sensitivities (using the average minimum detection dose), 2) the standard concentration ranges, and 3) inter- and 4)intra-assay coefficients of variation. These values for the R & D Systems high sensitivity IL-6 kit (pg/mL) for kit lot # 294142, are as follows: 1) sensitivity-0.04, 2) standard range-0.156-10, 3) inter-assay cv-7.8%, and intra-assay cv-7.4%. All samples were measured in duplicate, and the average of the two values was used for data analyses. Duplicate samples that did not provide a CV of less than 25% were reanalyzed.