Materials and Methods

Study design

The Mr. Bean study is a cohort in Baltimore, Maryland designed to assess demographic, substance use, and viral factors in the progression of kidney and cardiovascular disease (CVD).[1, 2] We recruited HIV-positive and HIV-negative participants in a 2:1 ratio, with the goal of achieving substantial representation of HCV infection and cocaine use in both groups. We recruited HIV-positive subjects from the Johns Hopkins HIV Clinic, and HIV-negative volunteers from a community-based cohort of persons with a history of injection drug use[3] and through city newspaper advertisements. Inclusion criteria included age 18 years or older and estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2. Exclusion criteria included history of radiocontrast allergy, pregnancy, diabetes mellitus, uncontrolled hypertension, or life threatening comorbidity. Participants provided written informed consent and the Johns Hopkins Medicine Institutional Review Board approved the study. Additionally, this study was granted a Certificate of Confidentiality by the National Institute on Drug Abuse to provide further protections for sensitive data collected.

Participants completed a baseline study visit and up to six semi-annual follow-up visits. Major study visits were conducted annually (baseline, 12, 24, and 36 months) and included a behavioral and medication survey, blood pressure measurement, height and weight measurement, phlebotomy, and urine samples (Supplementary Table 1).

Table. Data Collection Protocol for the Mr. Bean Study

Assessment	Study visit (months)						
	0	6	12	18	24	30	36
Interview							
Demographics	●						
Behavioral survey	●	●	●	●	●	●	●
Medication review	●	●	●	●	●	●	●
Anthropomorphic							
Height, weight	●		●		●		●
Blood pressure	●		●		●		●
Blood tests							
Creatinine	●		●		●		●
Lipid panel	●		●		●		●
Glycosylated hemoglobin	●		●		●		●
High-sensitivity CRP	●		●		●		●
Activated CD8 lymphocytes	●		●		●		●
Phosphorus	●		●		●		●
HIV RNA[1]	●		●		●		●
CD4 cell count[1]	●		●		●		●
Parathyroid hormone	●						
HCV antibody	●						
FGF23	●						
Urine tests							
Creatinine	●	●	●	●	●	●	●
Albumin	●	●	●	●	●	●	●
Protein	●	●	●	●	●	●	●
Dipstick	●		●		●		●
Phosphorus	●		●		●		●
Urine drug screen	●	●	●	●	●	●	●

Pulse wave velocity	●		●		●		●
Carotid ultrasound and IMT	●				●		

IMT, intima-media thickness

[1] Testing done in HIV-positive participants

Laboratory testing included plasma creatinine concentration, total cholesterol and subfractions, urine albumin-creatinine ratio (ACR), and urine drug testing (Rapid Tox Cup II, American Bio Medica Corp., Kinderhook, NY). We also measured pulse wave velocity (PWV) at each major visit as described below. GFR at baseline was estimated from serum creatinine with the Chronic Kidney Disease Epidemiology (CKD-EPI) estimating equation.[4] We conducted carotid imaging at the baseline and 24-month visits. Finally, minor visits (at 6, 18, and 30 months) included only the behavioral/medication survey, urine ACR, and urine drug testing.

Measurements and definitions

HIV testing was performed during study screening in persons without a documented negative HIV test in the prior 6 months. We categorized cocaine use status for each participant on the basis all self-report and urine drug test data collected during follow-up: 1) never users denied historical and recent (prior 6 months) cocaine use at all visits and had all negative urine cocaine tests; 2) past users reported prior cocaine use at the baseline survey, but denied recent use in all surveys and had all negative urine cocaine tests; 3) current users either reported recent cocaine use or had cocaine detected by urine drug testing at one or more visits. A positive urine drug test for cocaine reflects use in the previous 3 days. We defined active HCV as a detectable HCV RNA at baseline. We categorized smoking status as non-smoker (<100 cigarettes in lifetime), prior smoker (>6 months since last cigarette), and current smoker according to self-report at baseline. We defined hypertension as history of hypertension (high blood pressure) diagnosis or being prescribed antihypertensive mediation. Mean arterial pressure was defined as diastolic blood pressure (measured at the brachial artery) + (0.4*(systolic blood pressure – diastolic blood pressure)).

Trained ultrasonographers obtained electrocardiography-gated, end-diastolic images of the right and left distal common and proximal internal carotid arteries at the baseline and 24-month study visits. A comprehensive ultrasound scan of all extracranial carotid arteries was performed bilaterally, including transverse and then longitudinal scans to identify all plaques, as was performed in the Multi-Ethnic Study of Atherosclerosis (MESA).[5] The presence or absence of carotid plaque – defined as a focal area of intima-media thickness (IMT) greater than 1.5 mm or greater than 50% thicker than the neighboring wall – was scored in 12 anatomic segments of the extracranial carotid arteries (i.e., near and far walls of the right and left common, bifurcation, and internal carotid arteries). Plaque progression was defined as an increase in the number of anatomic segments with plaque at 24 months compared with baseline. Ultrasonographers also obtained detailed images of the distal centimeter of the right common carotid and the proximal centimeter of the right internal carotid arteries for IMT measurements. Carotid images were assessed at the University of Wisconsin Atherosclerosis Imaging Research Program (Madison, WI).[6] Baseline and 24-month images from an individual subject were read by a single technician who was masked to temporal image sequence and participant characteristics. These scans were obtained simultaneously with MESA Exam 5, the details and reproducibility data of which have been reported previously.[5] Briefly, for carotid plaque presence and score, intra-reader reproducibility was excellent, (kappa=0.83, 95% confidence interval [CI] 0.70-0.96) as was inter-reader reproducibility (kappa=0.89, 95% CI 0.72-1.00). Technicians measured IMT in triplicate in the right common and internal carotid arteries using a semi-automated edge detection software platform (Siemens syngo, Malvern, PA).[5, 7] The intra-class correlation coefficient (ICC) for intra-reader reproducibility for mean common carotid artery IMT was 0.99. The ICC for inter-reader common carotid artery IMT reproducibility was 0.95. For mean internal

carotid artery, intra-reader ICC was between 0.98-0.99 and inter-reader ICC was 0.93. To assess scan-rescan reproducibility, 44 scans were repeated by 3 sonographers. The Pearson correlation coefficient was 0.94. Mean (SD) differences were 0.006 (0.036-0.760) mm. Averages of the mean-maximum right common and internal carotid artery were used in analyses. Change in carotid IMT was calculated as the annualized difference.

We defined albuminuria as ACR >30mg/g [8] and progressive albuminuria as an ACR value during follow-up that was >30 mg/g and at least 2-fold higher than the baseline value. Two manufacturer-trained technicians measured PWV at major visits using applanation tonometry (SphygmoCor CvMA, West Ryde, Australia).[9, 10] Consistent with manufacturer guidelines, acceptable PWV measures were required to have beat-to-beat coefficient of variation <6% at both the carotid and femoral sites, and a pulse transit time standard deviation <15%. The within- and between-technician coefficients of variation for PWV measurements were 6.6% and 8.8%, respectively. We analyzed PWV as a continuous measure and as a binary marker with a cutoff at 9.6 m/s, which has been proposed as an indicator of abnormal arterial stiffness.[10]

Statistical analysis

We used Fisher's exact test and the Kruskal-Wallis test to compare categorical and continuous variables, respectively. We used Spearman's rho to quantify correlations between subclinical CVD markers. To provide an overview of the potential role of non-traditional risk factors (HIV, cocaine use [past or current], and HCV) in CVD, we stratified the study sample according to whether participants had none, 1 or 2, or all 3 of the risk factors and assessed the distribution of binary CVD indicators (presence of carotid plaque, albuminuria, and PWV> 9.6 m/s detected at any visit). We used logistic regression models to assess the associations of HIV status, cocaine use, and HCV status with dichotomous outcomes (carotid plaque at baseline, carotid plaque progression, albuminuria at baseline, and albuminuria progression).

We used linear regression to assess the associations of risk factors with annualized change in carotid IMT. Finally, we used linear generalized estimating equation models to assess associations with PWV, accounting for correlations in repeated measures using an exchangeable correlation matrix. We assessed adjusted estimates in models that included the American College of Cardiology/American Heart Association (ACC/AHA) CVD risk score [11], and all 3 non-traditional risk factors (HIV status, cocaine use, and HCV status) simultaneously. ACC/AHA CVD risk score is the predicted 10-year risk of CVD derived from an equation that includes age, sex, race, total cholesterol, high-density lipoprotein (HDL) cholesterol, diabetes mellitus, systolic blood pressure, smoking status, and use of antihypertensive medication. In supplementary analyses, we adjusted associations of interest with individual components of the ACC/AHA CVD risk score, rather than the score itself. Specifically, in component-adjusted models, we included covariates for age, sex, race, current smoking status, total to HDL cholesterol ratio, use of an antihypertensive medication, and systolic blood pressure (diabetic individuals were excluded from the study). We assessed for the presence of statistical interactions between pairs of independent variables of interest (HIV, cocaine use, and HCV) by combining term in adjusted models. Associations were considered statistically significant if P value<0.05. Statistical analyses were performed using Stata version 13.0 (Stata Corp., College Station, Texas, USA).

References

1. Bhasin B, Lau B, Atta MG, Fine DM, Estrella MM, Schwartz GJ, Lucas GM. Hiv viremia and t-cell activation differentially affect the performance of glomerular filtration rate equations based on creatinine and cystatin c. *PLoS One*. 2013;8:e82028
2. Lucas GM, Atta MG, Zook K, McFall AM, Mehta SH, Fine DM, Stein JH, Schwartz GJ. Factors associated with iohexol-based glomerular filtration rate slope over 36 months in hiv-negative and hiv-positive individuals. *AIDS*. 2016;30:619-626
3. Vlahov D, Anthony JC, Munoz A, Margolick J, Nelson KE, Celentano DD, Solomon L, Polk BF. The alive study, a longitudinal study of hiv-1 infection in intravenous drug users: Description of methods and characteristics of participants. *NIDA Res.Monogr*. 1991;109:75-100.:75-100
4. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS, Investigators C-E. Estimating glomerular filtration rate from serum creatinine and cystatin c. *N Engl J Med*. 2012;367:20-29
5. Tattersall MC, Gassett A, Korcarz CE, Gepner AD, Kaufman JD, Liu KJ, Astor BC, Sheppard L, Kronmal RA, Stein JH. Predictors of carotid thickness and plaque progression during a decade: The multi-ethnic study of atherosclerosis. *Stroke*. 2014;45:3257-3262
6. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the american society of echocardiography carotid intima-media thickness task force. Endorsed by the society for vascular medicine. *J.Am.Soc.Echocardiogr.* 2008;21:93-111
7. Stein JH, Korcarz CE, Mays ME, Douglas PS, Palta M, Zhang H, Lecaire T, Paine D, Gustafson D, Fan L. A semiautomated ultrasound border detection program that facilitates clinical measurement of ultrasound carotid intima-media thickness. *J Am Soc Echocardiogr*. 2005;18:244-251
8. Lucas GM, Ross MJ, Stock PG, Shlipak MG, Wyatt CM, Gupta SK, Atta MG, Wools-Kaloustian KK, Pham PA, Bruggeman LA, Lennox JL, Ray PE, Kalayjian RC. Clinical practice guideline for the management of chronic kidney disease in patients infected with hiv: 2014 update by the hiv medicine association of the infectious diseases society of america. *Clin Infect Dis*. 2014;59:e96-e138
9. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H, European Network for Non-invasive Investigation of Large A. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur Heart J*. 2006;27:2588-2605
10. Reference Values for Arterial Stiffness C. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'Establishing normal and reference values'. *Eur Heart J*. 2010;31:2338-2350
11. Goff DC, Jr., Lloyd-Jones DM, Bennett G, et. al. 2013 acc/aha guideline on the assessment of cardiovascular risk: A report of the american college of cardiology/american heart association task force on practice guidelines. *J Am Coll Cardiol*. 2014;63:2935-2959