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# Brief Report: Plasma Homocysteine is Not Associated with HIV Serostatus or Antiretroviral Therapy in Women

**Farbod Raiszadeh, MD, PhD**[1], **Donald R. Hoover, PhD**[2], **Irene Lee, MPH**[3], **Qiuhu Shi, PhD**[3,4], **Kathryn Anastos, MD**[5], **Wei Gao, MS MPH**[5], **Robert Kaplan, PhD**[5], and **Marshall J. Glesby, MD, PhD**[6]

[1]Department of Medicine, St. Luke's-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, NY

[2]Department of Statistics and Biostatistics, Rutgers University, Piscataway, NJ

[3]Data Solutions, LLC, Bronx, NY

[4]School of Public Health, New York Medical College, Valhalla, NY

[5]Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY

[6]Division of International Medicine and Infectious Diseases, Department of Medicine, Weill Cornell Medical College, New York, NY

## Abstract

**Background—**The effects of HIV serostatus and combination antiretroviral therapy (cART) on plasma homocysteine (Hcy) are uncertain.

**Methods—**Plasma Hcy was assayed in a cross-sectional study of 249 HIV-infected and 127 HIV-uninfected women at the Bronx Women's Interagency HIV Study site.

**Results—**Mean plasma Hcy was 7.42 ± 2.68 in HIV-infected and 7.18 ± 2.66 μmol/L in HIV-uninfected women (P = 0.40). Hyperhomocysteinemia (defined as Hcy > 10 μmol/L) was seen in 16.9% and 13.4 % of HIV-infected and HIV-uninfected women, respectively (P=0.45). Among HIV-infected women, cART use was not associated with Hcy level. Compared to the lowest quartile, women with Hcy in the highest quartile had lower mean serum vitamin B12 and RBC folate levels. In multivariate analysis that did not include micronutrient levels, age, serum creatinine and lower CD4% were significantly associated with plasma Hcy level in HIV-infected women.

**Conclusions—**Plasma Hcy was not associated with HIV serostatus or use of cART in this cross-sectional study. Reduced availability of folate cofactors for Hcy remethylation in HIV-infected women with lower folate intake and decreased health status may influence Hcy levels.

### Keywords

Homocysteine; HIV; women; vitamin B12; folate

Correspondence to: Dr. Marshall J. Glesby, Division of International Medicine and Infectious Diseases, Weill Cornell Medical College, 525 E. 68th St., Box 566, New York, NY 10065 (e-mail: mag2005@med.cornell.edu).

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## Introduction

In the era of combination antiretroviral therapy (cART), epidemiological studies have documented higher rates of coronary heart disease (CHD) or myocardial infarction (MI) among HIV-infected patients relative to the general population [1,2,3,4,5]. Traditional risk factors for cardiovascular disease (CVD) such as hypercholesterolaemia, hypertriglyceridaemia, and low plasma HDL-cholesterol [6,7,8,9], may account for some of this increased risk. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study found that among HIV-infected individuals, even after adjustment for changes in lipids, there was increased risk of myocardial infarction with protease inhibitor (PI) use[10]. HIV-infection or cART may also change nontraditional or emerging risk factors for CHD, including inflammatory markers, clotting factors, apolipoproteins, lipoprotein (a), oxidative stress, non-esterified fatty acids, and homocysteine (Hcy)[11].

Some evidence suggests that the prevalence of mild to moderate hyperhomocysteinemia may be more common in HIV-infected individuals, especially those using cART[12]. The pathogenesis of hyperhomocysteinemia in HIV infection, if present, has not clearly been identified. A variety of pathophysiologic processes could contribute, including the effects of HIV infection and replication on host metabolism, antiretroviral drugs on Hcy metabolism and other drugs administered to HIV-infected patients; folate deficiency due to lack of dietary intake; and/or deficiency in vitamins B6 or B12 due to insufficient intake or altered metabolism. Since the determinants of Hcy level in HIV-infected individuals have not been well described, we conducted a cross-sectional study to determine the associations of plasma Hcy levels with HIV-serostatus, cART use, and other host and disease factors.

## Materials and Methods

The Women's Interagency HIV Study (WIHS) is a prospective cohort study with recruitment of primarily minority women with or at risk of HIV infection in six urban sites in the United States (Bronx, Brooklyn, DC, Los Angeles, San Francisco, and Chicago). At the Bronx, New York site, 537 women were enrolled in the study beginning in 1993, with an additional 233 women enrolled in 2001. About 39% of the women are Hispanic-Latina, 52% African American, and the rest white or Asian. At the Bronx WIHS site, HIV-infected and HIV-uninfected women in active follow-up were offered participation in this substudy at Visit 24 (April-September 2006). The only exclusion criterion was acute illness at the time of the visit as determined by the interviewer at the WIHS site.

For this substudy, blood samples were collected by strict blood collection protocol with immediate processing and storage at −70° centigrade. Homocysteine was assayed by fluorescence polarization immunoassay with an interassay coefficient of variation (CV) of 8.8% (Quest Diagnostics, Baltimore, MD). Red blood cell (RBC) folate and serum vitamin B12 levels were assayed by competitive immunoassay using direct chemiluminescent technology in HIV infected women in the highest and lowest quartiles of Hcy values using 5.5 and 8.6 μmol/L with an interassay CV of 5% and 4.8% respectively (Quest Diagnostics, Baltimore, MD). Fasting lipid panels, insulin, and glucose values obtained from WIHS visits conducted within the 12 months prior to the sub-study visit were used, and the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as (fasting insulin μU/mL × fasting glucose mg/dL) / 405.

Hyperhomocysteinemia was defined as Hcy >10 μm/L. Data were descriptively analyzed using plots, histograms, means, medians, standard deviation, skewness and kurtosis to identify erroneous values, outliers and optimal transformations. Due to the presence of extreme outliers, three values of serum creatinine greater than 2.0 mg/dL were set to missing and fasting insulin

and HOMA index were log transformed. Unadjusted comparisons were made using t-tests and chi-square tests. Adjusted associations between Hcy as a continuous variable and other variables of interest were assessed by multivariate linear regression modeling using forward stepwise variable section with a P-value of 0.05 to enter and to remain in the model. Multivariate logistic regression was used to model adjusted associations with hyperhomocysteinemia. Initial analyses adjusted for age; subsequent models assessed the effects of adjusting for variables with significant univariate associations with the outcome variables. Since data on vitamin B12 and RBC folate were only available in 50% of the HIV-infected women by design, these covariates were not included in the multivariate models. A P-value < 0.05 was considered statistically significant. Statistical analysis was performed using SAS version 9.3 (SAS Institute Inc, Cary, NC)

## Results

249 HIV-infected and 127 HIV-uninfected women provided specimens for this study. HIV-infected women were older (mean ± standard deviation) (age 45 ± 7.3 vs. 42 ± 9.9 years, p<0.0001) and had a lower body mass index (BMI) (29.0 ± 6.7 vs. 30.8 ± 8.0, p=0.03). The mean CD4 cell count of the HIV-infected women at the sub-study visit was 488 ± 324 cells/$mm^3$ and 54% had HIV RNA levels below the level of quantification (< 80 copies/ml). Fifty-five percent of the HIV-infected women had a clinical diagnosis of AIDS at or before the sub-study visit.

Mean plasma Hcy was 7.42 ± 2.68 and 7.18 ± 2.66 μmol/L in HIV-infected and uninfected women, respectively (P = 0.4). Hyperhomocysteinemia was present in 16.9 % and 13.4% of HIV-infected and uninfected women, respectively (P = 0.45). Among HIV-infected women, receiving cART was not associated with increased Hcy levels (mean 7.32 ± 2.39 vs. 7.46 ± 2.78 μmol/L in women currently taking cART vs. not taking cART, P=0.70). Hcy levels were also not associated with current or ever use of either nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs), or with either current use of protease inhibitors (PIs) or trimethoprim-sulfamethoxasole (TMP-SMX) (data not shown). Hcy levels were, however, higher in women who had ever used the following: a dideoxynucleoside analog (ddI, d4T, or ddC) (mean 7.66± 2.81 vs. 6.97 ± 2.37 in ever vs. never users; P = 0.04), TMP-SMX (7.76 ± 2.93 vs. 6.88 ± 2.14; P = 0.007) or a PI (7.60 ± 2.71 μmol/L vs. 6.92 ± 2.56 μmol/L; P = 0.07). But none of these associations remained statistically significant after adjustment for age in bivariate linear regression models.

In linear regression analysis significant univariate predictors of Hcy level included age, serum creatinine, CD4 percentage and ever having taken TMP-SMX (Table 1). After adjustment for age, TMP-SMX use was no longer a significant predictor. In a multivariate model, age, serum creatinine and CD4 % each remained significantly associated with plasma Hcy level (Table 1). Multivariate logistic regression models found that age (OR=2.66 per 10 years, 95% confidence interval (CI): [1.53, 4.64]), serum creatinine (OR=1.45 per mg/dL 95% CI: [0.98, 2.16]), CD4 percentage (OR=0.83 per 5% increase 95% CI: [0.70, 0.98]), and ever having taken TMP-SMX (OR=2.67 95% CI: [1.07, 6.67]) were independent predictors of hyperhomocysteinemia (Hcy > 10 umol/L ).

Serum vitamin B12 and RBC folate levels were measured in HIV-infected women in the highest and lowest quartiles of the Hcy distribution (Hcy <5.5 μmol/L and > 8.6 μmol/L). Serum levels of both vitamin B12 and RBC folate were each lower in women in the highest vs. the lowest Hcy quartile.(487 ± 215 pg/ml vs. 617 ± 364 pg/ml; P=0.02, and 353 ± 139 nmol/L vs. 432 ± 114 nmol/L; P < 0.001, respectively). In logistic regression models these associations remained significant after adjustment for age, CD4, count, and creatinine level (data not shown).

## Discussion

In this cross-sectional study, we did not find an association of either HIV serostatus or cART with Hcy concentrations in women. While higher Hcy levels were associated with ever using a dideoxynucleoside analog or TMP-SMX, the associations did not remain significant after adjustment for age. In multivariate analysis, age, serum creatinine, and lower CD4 percentage were each independently associated with higher Hcy levels. We also observed strong inverse associations of both RBC folate and vitamin B12 with Hcy levels.

Our results help clarify the previously published, conflicting data on the potential relationships between plasma HCy concentrations and both HIV infection and cART. In an evaluation of 73 participants from the Swiss HIV Cohort Study, a significantly higher mean total plasma Hcy concentration was observed in HIV-infected men receiving PI-based cART compared with healthy controls (9.1 vs. 7.8 μmol/L, P= 0.02) [12]. The subgroup of 26 HIV-infected women on PI regimens, however, did not have statistically different Hcy levels compared to healthy controls (8.25 vs 7.4 μmol/L, P=0.19)[12]. The HIV-infected subjects were older than the controls in this study, and the analysis was not adjusted for age, which we have shown to be an important potential confounder. Furthermore, the potential contribution of cART versus HIV disease severity could not be assessed given the design. In contrast, our results are similar to those of Bongiovanni and colleagues, who measured plasma HCy levels in 83 treatment naïve HIV-infected subjects, 161 on cART, and 54 healthy controls[13]. Although mean Hcy levels were higher in the subjects on cART compared to the other two groups in an unadjusted analysis, only older age and low serum folate concentration (and not patient group) were associated with hyperhomocysteinemia in a multivariate analysis. In another cross-sectional study of 98 patients who were on stable cART regimens for at least 6 months, duration of cART and PI exposure were not significantly associated with elevated Hcy level[14]. Taken together, these data suggest that hyperhomocysteinemia is not a direct of HIV or cART.

Elevated plasma Hcy is known to occur in association with nutritional deficiencies of vitamin B12 and folate in the general population[15;16]. Two prior studies found that serum folate concentrations were independently associated with hyperhomocysteinemia in HIV-infected patients [13,14]. Folate or vitamin B12 insufficiency due to poor intake or malabsorption could be a function of or marker of severity of HIV disease. In our study, both RBC folate – a more robust measure of folate stores than serum levels – and vitamin B12 levels remained lower in women with HCy in the highest versus lowest quartile even after adjustment for CD4 cell count, suggesting that HIV disease severity is not the primary underlying cause of the micronutrient deficiencies.

The observed associations in our study of Hcy level with RBC folate and vitamin B12 if causal, suggest a potential to lower Hcy levels by inexpensive and feasible methods such as folate supplementation and therefore to decrease cardiovascular disease risk in HIV-infected populations. However, lack of association between Hcy level and HIV infection or cART in HIV-infected subjects makes hyperhomocysteinemia an unlikely explanation for increased risk of cardiovascular disease in the HIV-infected population. In addition, two recent randomized clinical trials of Hcy lowering that have shown vitamin supplementation can effectively lower Hcy levels have nevertheless failed to identify a beneficial effect on cardiovascular disease outcomes in general populations[17;18].

Plasma Hcy is known to be elevated in chronic renal insufficiency[19]. In our analyses, we found a positive relationship between plasma Hcy and serum creatinine in HIV-infected women with creatinine values in the 0.2 to 1.5 mg/dL range in multivariate analysis. These findings are consistent with data from the general population and diabetics showing a strong inverse

relationship between plasma Hcy and glomerular filtration rate, even within the normal range of the latter parameter[20].

This study is limited by its cross sectional design and therefore no inferences on temporal relationship or causation between different variables can be made. Another potential limitation is the lack of dietary intake data on micronutrients of interest such as on folate, vitamin B6, and vitamin B12, though we found no association between multivitamin use and plasma HCy. While serum levels of these nutrients have been shown to be reliable surrogates of dietary intake in prior studies of general populations[15], data in this regard are limited in HIV-infected populations. It is possible that unmeasured metabolic parameters associated with HIV-infection or its treatment may also contribute to low folate or B12 levels. We were also not able to determine if homocysteine level was associated with future cardiovascular events as too few events have occurred during subsequent follow-up of the cohort.

In summary, this cross-sectional study of women with and at risk of HIV infection found no association between HIV serostatus or cART with Hcy levels. In HIV-infected women, RBC folate, vitamin B12, and CD4 % were significantly associated with Hcy level, which may in part be explained by reduced availability of folate cofactors for Hcy remethylation in women with lower folate intake secondary to the decreased health status that occurs with lower CD4 counts.

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**Table 1**

Univariate, bivariate, and multivariate linear regression of homocysteine in HIV-infected women.

| | Univariate unadjusted model | | Age-adjusted bivariate model | | Multivariate model | |
|---|---|---|---|---|---|---|
| | **Beta (μmol/L)*** | **P value** | **Beta(μmol/L)** | **P value** | **Beta (μmol/L)** | **P value** |
| Age (per 10 years) | 1.48 | <0.0001 | | | 1.41 | <0.0001 |
| BMI (kg/m$^2$) | −0.01 | 0.80 | 0.00 | 0.90 | | |
| Current smoking | 0.26 | 0.46 | 0.42 | 0.19 | | |
| Diabetes | 0.01 | 0.99 | −0.19 | 0.62 | | |
| Serum creatinine (mg/dl) | 0.66 | 0.005 | 0.47 | 0.03 | 0.48 | 0.03 |
| CD4 Number (per 100) | −0.06 | 0.22 | −0.09 | 0.07 | | |
| CD4 percent (per 5%) | −0.19 | 0.01 | −0.19 | 0.007 | −0.19 | 0.006 |
| cART since last visit | 0.14 | 0.72 | 0.08 | 0.83 | | |
| D-drug** use ever | 0.69 | 0.05 | 0.32 | 0.34 | | |
| D-drug*** use since last visit | 0.14 | 0.78 | 0.04 | 0.93 | | |
| TMP-SMX use ever | 0.88 | 0.01 | 0.36 | 0.28 | | |
| Dapsone use ever | −0.28 | 0.62 | −0.16 | 0.76 | | |
| Multivitamin use since last visit | −0.26 | 0.54 | −0.55 | 0.15 | | |
| AIDS diagnosis | 0.24 | 0.48 | −0.24 | 0.45 | | |

* The beta coefficients are interpreted as in the following example: For every 10 year increase in age, the Hcy level is expected to be 1.48 μmol/L higher in the unadjusted model and 1.41 μmol/L higher in the multivariate model after adjusting for serum creatinine and CD4 percent.

** D-Drug = Any of the dideoxynucleoside analogs. 111 women reported ever using ddI, 144 using d4T, and 34 using ddC.

*** 21 women reported use of ddI since the last study visit, 17 use of d4T, and 1 use of ddC.

Abbreviations: BMI, body mass index; cART, combination antiretroviral therapy; D-drug,dideoxynucleoside analog; TMP-SMX, trimethoprim-sulfamethoxasole.

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