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## Inaccuracy of haemoglobin A1c among HIV-infected men: effects of CD4 cell count, antiretroviral therapies and haematological parameters

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**Background:** There is limited evidence that among HIV-infected patients haemoglobin A1c (HbA1c) values may not accurately reflect glycaemia. We assessed HbA1c discordance (observed HbA1c – expected HbA1c) and associated factors among HIV-infected participants in the Multicenter AIDS Cohort Study (MACS).

**Methods:** Fasting glucose (FG) and HbA1c were measured at each semi-annual MACS visit since 1999. All HIV-infected and HIV-uninfected men for whom at least one FG and HbA1c pair measurement was available were evaluated. Univariate median regression determined the association between HbA1c and FG by HIV serostatus. The relationship between HbA1c and FG in HIV-uninfected men was used to determine the expected HbA1c. Generalized estimating equations determined factors associated with the HbA1c discordance among HIV-infected men. Clinically significant discordance was defined as observed HbA1c – expected HbA1c  $\leq$  –0.5%.

**Results:** Over 13 years, 1500 HIV-uninfected and 1357 HIV-infected men were included, with a median of 11 visits for each participant. At an FG of 125 mg/dL, the median HbA1c among HIV-infected men was 0.21% lower than among HIV-uninfected men and the magnitude of this effect increased with FG >126 mg/dL. Sixty-three percent of HIV-infected men had at least one visit with clinically significant HbA1c discordance, which was independently associated with: low CD4 cell count (<500 cells/mm<sup>3</sup>); a regimen containing a protease inhibitor, a non-nucleoside reverse transcriptase inhibitor or zidovudine; high mean corpuscular volume; and abnormal corpuscular haemoglobin.

**Conclusion:** HbA1c underestimates glycaemia in HIV-infected patients and its use in patients with risk factors for HbA1c discordance may lead to under-diagnosis and to under-treatment of established diabetes mellitus.

**Keywords:** HbA1c, diabetes, mean corpuscular volume, glycosylated haemoglobin, HIV

### Introduction

Use of combined antiretroviral therapy (cART) has dramatically improved survival and life expectancy among HIV-infected persons over time.<sup>1–3</sup> In this context, the management of chronic comorbidities, including metabolic complications, has become a major healthcare issue. Diabetes mellitus (DM) is common in HIV-infected persons and, as in the general population, is associated with hypertension, dyslipidaemia, cardiovascular disease and renal impairment.<sup>4–8</sup> In order to reduce the risk of macrovascular and microvascular complications of DM, adequate glycaemic

control is essential.<sup>9</sup> Haemoglobin A1c (HbA1c), which measures the percentage of haemoglobin A that is glycosylated, is the primary index of glycaemic control in patients with DM and has been recommended for the diagnosis of DM.<sup>10</sup>

Among HIV-infected persons, HbA1c values may not accurately reflect glycaemia. Two recent studies have suggested that HbA1c underestimates mean fasting glucose (FG) among HIV-infected patients, possibly as a result of low-grade haemolysis,<sup>11,12</sup> although the clinical significance of this effect is uncertain.<sup>13</sup> In these studies, factors associated with HbA1c inaccuracy were higher mean corpuscular volume (MCV) as well as low serum haptoglobin, use of

certain antiretroviral drugs (especially zidovudine or abacavir) or medication to treat diabetes.

Using data from the Multicenter AIDS Cohort Study (MACS) collected between 1 April 1999 and 31 March 2012, we sought to determine whether HbA1c underestimated FG in HIV-infected persons compared with HIV-uninfected persons, whether the magnitude of HbA1c discordance was clinically significant and what factors were associated with HbA1c discordance.

## Methods

### Study population

The MACS is an ongoing prospective study of HIV-1 infection among men who have sex with men (MSM) at four centres in the USA: Chicago, Baltimore/Washington DC, Pittsburgh and Los Angeles. Institutional review boards at each site approved the MACS protocol and forms, and each participant gave written informed consent. Details of the study design and follow-up methods have been published.<sup>14</sup> Briefly, participants attend semi-annual study visits, which include a detailed interview, physical examination and collection of biological specimens.

Beginning in April 1999, a fasting ( $\geq 8$  h) serum sample was obtained, from which glucose and HbA1c were measured. Our study population consisted of all MACS participants for whom at least one FG and HbA1c pair measured at the same visit was available. The first visit at which an FG-HbA1c pair was available was defined as the baseline visit and all subsequent available FG-HbA1c pairs from a participant were used in analyses. We excluded from our analysis those visits at which non-FG levels were obtained.

### Laboratory methods

FG levels were measured by the combined hexokinase/glucose-6-phosphate dehydrogenase method at a central laboratory (Heinz Laboratory, Pittsburgh, PA), (coefficient of variation 1.8%). HbA1c and complete blood counts (CBCs) were measured by Quest Diagnostics (Baltimore, MD) using standard clinical assays. HbA1c was measured by immunoassay using the Roche Cobas Integra 800 analytical system (Indianapolis, IN; coefficient of variation  $<3.3\%$ ). CBC was measured using a Coulter LH 750 (Beckman Coulter, Inc., Brea, CA). Haemoglobin, MCV and mean corpuscular haemoglobin (MCH) were examined since recent studies have identified relationships with HbA1c discordance.<sup>11,12,15</sup> Standardized protocols were used to measure T lymphocyte subsets.<sup>16</sup> Plasma HIV-1 RNA was assessed using either the Roche standard assay or the Roche ultrasensitive assay.

### Statistical analysis

Univariate median regression with HbA1c as the dependent variable was used to explore the association between FG and HbA1c by HIV status. The regression was done separately for FG values below and above 126 to assess differences in associations with HbA1c across the glycaemic spectrum. This was done by including interaction terms between indicators for  $FG \geq 126$  mg/dL,  $FG < 126$  mg/dL and HIV in the model. Because participants contributed multiple FG and HbA1c measurements to analyses, results were bootstrapped to assure appropriate 95% CIs.

Multivariate median regression was then used among the HIV-uninfected person-visits to predict the expected HbA1c values among the HIV-infected participants at each person-visit. The relationship between HbA1c and FG (centred at 125 mg/dL) was adjusted for age (centred at 50 years) and race [white (reference group), black, Hispanic or other race]. Then the expected HbA1c was subtracted from the observed HbA1c value for each HIV-infected person-visit to obtain the HbA1c discordance. This discordance

value was used as the primary outcome in a linear mixed effects regression analysis to determine factors associated with HbA1c discordance, with a random intercept to account for the repeated outcome measurements. Potential explanatory variables examined included age, race, BMI [ $<18.5$ ,  $18.5-24.9$  (reference group),  $25-29$ ,  $\geq 30$ ], CD4 cell count [ $<200$ ,  $200-349$ ,  $350-499$ ,  $\geq 500$  cells/mm<sup>3</sup> (reference group)], current ART use and plasma HIV-1 RNA level [no ART (reference group), on ART with or without RNA  $\leq 400$  copies/mL], hepatitis C virus (HCV) infection (defined by positive antibody or HCV RNA, yes/no), a history of clinical AIDS (yes/no),<sup>17</sup> use of individual classes of ARTs [protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), use of selected individual nucleoside reverse transcriptase inhibitors (NRTIs), including zidovudine, lamivudine, emtricitabine, abacavir or tenofovir, in the past 6 months], as well as haemoglobin level [stratified in categories of  $<10$ ,  $10-11$ ,  $12-13$ ,  $\geq 14$  mg/dL (reference group)], MCV [ $<85$  (reference group),  $85-89$ ,  $90-94$ ,  $95-99$ ,  $100-104$ ,  $\geq 105$  fL] and MCH [ $<27$ ,  $27-31$  (reference group),  $>31$  pg].

A second outcome was based upon whether the HbA1c discordance was  $\leq -0.5\%$  (yes versus no), which is considered clinically significant,<sup>18</sup> and analysed by logistic regression models, with a generalized estimating equation to account for within-subject correlation.

Missing data for BMI (9.2% of 15 161 HIV-infected person-visits), CD4 cell count (1.6%), HIV-1 RNA (0.6%), haemoglobin (1.4%), MCV (1.1%) and MCH (1.2%) were extrapolated based on the longitudinal data for each participant. All statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

## Results

### Baseline characteristics

Of the 3244 men with a visit between 1 April 1999 and 31 March 2012, 2857 (1500 HIV-uninfected and 1357 HIV-infected participants) had at least one visit with both FG and HbA1c measurements (Table 1). The mean of number of visits for each participant was 11 (range 1-26). Compared with the HIV-uninfected men, HIV-infected men were younger, were more likely to be Caucasian and have a lower level of education and BMI and were more likely to have DM.

The median FG was higher in the HIV-infected group at the index visit than in the HIV-uninfected group (91 and 90 mg/dL, respectively;  $P=0.013$ ), whereas the HbA1c level was lower (5.0% and 5.2%, respectively;  $P<0.001$ ). The HIV-infected men had a higher median MCV ( $P<0.001$ ), higher median MCH ( $P<0.001$ ) and lower median haemoglobin ( $P<0.001$ ) than the HIV-uninfected men.

### Relationship between HbA1c and FG in HIV-infected and HIV-uninfected men

The median HbA1c at  $FG=125$  mg/dL was 5.82% in the HIV-uninfected group compared with 5.61% in the HIV-infected group (median HbA1c discordance  $-0.21$ ;  $P<0.001$ ) (Figure 1). The magnitude of the difference in the slopes of HbA1c versus FG in HIV-infected and uninfected men was statistically significant for  $FG \geq 126$  mg/dL (difference in slope  $-0.0044\%$  for each additional mg/dL;  $P=0.018$ ). The slopes, however, were not significantly different for  $FG < 125$  mg/dL ( $P=0.094$ ).

### Factors associated with HbA1c discordance among HIV-infected men

Among the HIV-infected men, the median HbA1c discordance (difference between observed and expected HbA1c values) was

**Table 1.** Baseline characteristics of 2857 MACS participants<sup>a</sup>

	HIV-uninfected (n=1500)	HIV-infected (n=1357)
Age (years), median (IQR)	46 (40–53)	43 (37–49)
Race, n (%)		
Caucasian	1019 (68)	737 (54)
African American	334 (22)	400 (30)
Hispanic or other race	147 (10)	220 (16)
Education, n (%)		
college degree or above	852 (57)	569 (42)
BMI, median (IQR)	26 (23–29)	25 (23–27)
Current diabetes, n (%) <sup>b</sup>	75 (5)	102 (8)
Fasting glucose (mg/dL), median (IQR)	90 (82–98)	91 (83–99)
HbA1c, (%), median (IQR)	5.2 (4.9–5.5)	5.0 (4.6–5.4)
Haemoglobin (g/dL), median (IQR)	15 (14.4–15.7)	14.7 (13.9–15.6)
MCV (fL), median (IQR)	91 (88–94)	100 (91–111)
MCH (pg), median (IQR)	31 (30–32)	34 (31–38)
CD4 cell count (cells/mm <sup>3</sup> ), median (IQR)	—	486 (320–679)
ART treated, with HIV RNA <400 copies/mL, n (%)	—	770 (76%)
HCV infected, n (%) <sup>c</sup>	73 (5)	148 (11)
HBV infected, n (%) <sup>d</sup>	31 (2)	88 (7)
Treatment exposure, n (%)		
ART naive		254 (19)
past ART		95 (7)
current ART (in last 6 months)	—	1008 (74)
PI-containing regimen (%)	—	59
NNRTI-containing regimen (%)	—	47
ZDV-containing regimen (%)	—	41
ABC-containing regimen (%)	—	25
3TC-containing regimen (%)	—	72
TDF-containing regimen (%)	—	18

ZDV, zidovudine; ABC, abacavir; 3TC, lamivudine; TDF, tenofovir.

<sup>a</sup>Baseline was the first visit with both fasting glucose and HbA1c data from visit 31 onwards.

<sup>b</sup>History of diabetes was defined as a fasting glucose  $\geq 126$  mg/dL or (diagnosed with diabetes and use of medications) at any visit before or at baseline visit.

<sup>c</sup>HCV infection was defined as having a positive serum antibody to HCV or positive HCV RNA at baseline visit.

<sup>d</sup>Hepatitis B virus (HBV) infection was defined as having a positive serum HBV surface antigen test at the first visit or during MACS follow-up visits.

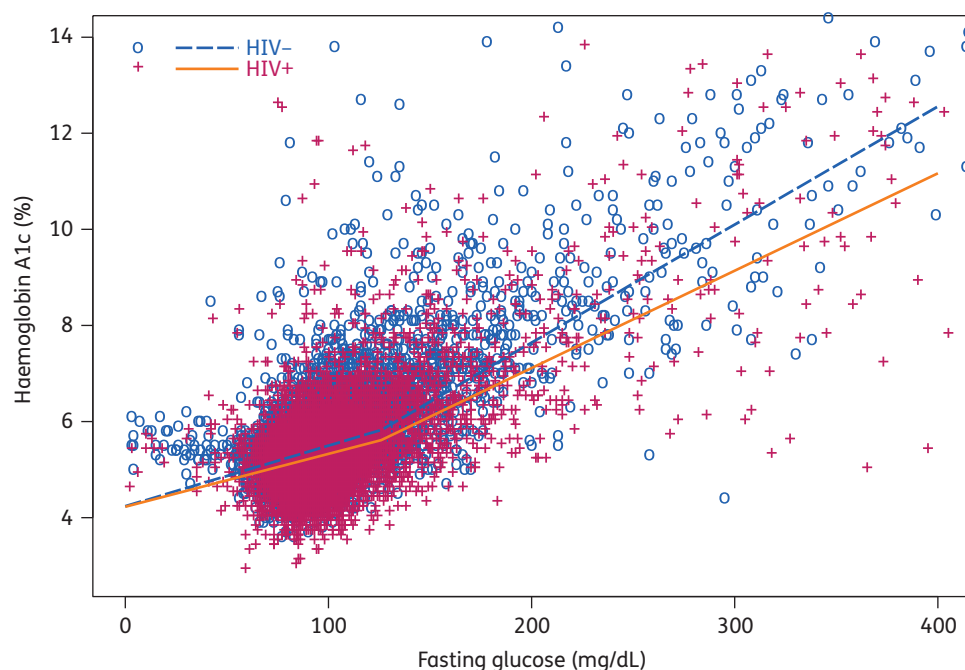
–0.17%, with the 25th percentile of –0.49 and the 75th percentile of 0.11. Of these person-visits ( $n=14\,860$ ), 24.5% had an HbA1c discordance  $\leq -0.5\%$ , occurring in 871/1378 (63%) men.

In univariate models (Table 2), a lower than expected HbA1c value (observed – expected  $< 0$ ) was associated with a CD4 cell count  $< 500$  cells/mm<sup>3</sup>, use of a PI, zidovudine and/or a lamivudine-containing regimen and higher MCV and MCH. Older age, non-Caucasian race and obesity were associated with a higher than expected HbA1c (observed – expected  $> 0$ ).

We used a series of nested multivariable models to determine the independence of each of these factors in their relationship with HbA1c discordance. In a model that contained demographic and HIV-related parameters (Table 2, Model 2), lower CD4 cell count, ART treatment (with or without a suppressed HIV-1 RNA level) and HCV co-infection were each independently associated with a lower observed than expected HbA1c value. After the addition of haematological parameters (Model 3), lower CD4 cell count, but not current ART or HCV co-infection, remained

associated with HbA1c discordance. In addition, HbA1c discordance was associated with abnormal MCH (either above or below the normal range) and higher MCV, with each 5 fL increase in MCV associated with a progressively greater degree of HbA1c discordance. In a final model (Model 4), which included ART variables, we found that ART regimens in the previous 6 months containing a PI, an NNRTI or zidovudine were associated with a lower observed than expected HbA1c value. In contrast, abacavir and emtricitabine were associated with a higher observed HbA1c than expected.

Figure 2 shows the relationship between demographic, HIV-related and haematological factors and the presence of clinically significant HbA1c discordance defined as observed – expected HbA1c  $\leq -0.5\%$ . In the multivariable logistic model, the odds ratios for the CD4 cell count were similar at 1.57 (95% CI 1.20, 2.07) for CD4 cell count  $< 200$  cells/mm<sup>3</sup>, 1.61 (95% CI 1.31, 1.97) for CD4 209–349 cells/mm<sup>3</sup> and 1.45 (95% CI 1.23, 1.70) for CD4 350–499 cells/mm<sup>3</sup> versus CD4  $> 500$  cells/mm<sup>3</sup> ( $P < 0.001$ ). Current



**Figure 1.** Relationship between haemoglobin A1c and fasting glucose in HIV-infected and HIV-uninfected men using median regression with a knot at 125 mg/dL.

ART (with or without HIV-1 RNA viral suppression) was not associated with clinically significant HbA1c discordance. The HbA1c discordance increased markedly with each 5 fL increase in MCV, from 2.09 (95% CI 1.31, 3.36 for MCV 85–89 fL) to 15.39 (95% CI 9.19, 25.79 for MCV >105 fL) and was also related to abnormal MCH [1.95 (95% CI 0.95, 4.0) for MCH <27 pg ( $P=0.086$ ) versus 1.32 (95% CI 1.05, 1.65) for MCH >31 pg;  $P=0.018$ ].

When use of specific ARTs was added to the model, PI use [OR 1.39 (95% CI 1.08, 1.78);  $P=0.01$ ] and zidovudine use [OR 1.38 (95% CI 1.06, 1.81);  $P=0.019$ ] were independently associated with clinically significant discordance (data not shown). In contrast, abacavir [OR 0.73 (95% CI 0.58, 0.91);  $P=0.002$ ], emtricitabine [OR 0.36 (95% CI 0.26, 0.49);  $P<0.001$ ] and lamivudine [OR 0.74 (95% CI 0.56, 0.99);  $P=0.041$ ] appeared to be protective against clinically significant HbA1c discordance.

## Discussion

This analysis of MACS data that span a 13 year period and include 1357 HIV-infected men constitutes the largest study to date that has evaluated whether HbA1c accurately predicts glycaemia among HIV-infected persons. We found that, at an FG of 125 mg/dL, median HbA1c values were 0.21% lower in HIV-infected men than in HIV-uninfected men and that the magnitude of this difference increased at higher glucose values. We also found that HbA1c discordance (observed – expected HbA1c) was associated with lower CD4 cell counts, haematological parameters including high MCV and MCH, and exposure to certain ART agents including PIs, NNRTIs and zidovudine. Our findings suggest that HbA1c may not be an adequate marker of glycaemic control in certain HIV-infected persons and that its use may lead to under-treatment

of established DM or under-diagnosis of DM when used as a diagnostic criterion.

Following an initial case report,<sup>19</sup> three controlled studies have been published to date that addressed the relationship between glycaemia and HbA1c levels in HIV-infected persons. In a retrospective cross-sectional study, Diop *et al.*<sup>11</sup> found that HbA1c underestimated mean FG in HIV-infected patients ( $n=112$ ) by ~12% compared with HIV-uninfected persons. A prospective cross-sectional study<sup>12</sup> compared observed and expected HbA1c levels in 100 HIV-infected patients with DM (77% of patients) or fasting hyperglycaemia (23%) and 200 matched HIV-uninfected participants and found that HbA1c underestimated glycaemia (mean of one fasting and one non-fasting sample) among HIV-infected persons by an average of 29 mg/dL. Most recently, Glesby *et al.*<sup>13</sup> compared FG values and HbA1c values among 315 HIV-infected and 109 HIV-uninfected women with DM and found that HbA1c values were modestly lower among HIV-infected women compared with HIV-uninfected women (6.4% and 6.8%, respectively,  $P=0.023$ ) at the same level of FG. In our study, we found that, at a glucose level of 125 mg/dL, HbA1c values were a median of 0.21% lower in HIV-infected than in HIV-uninfected men and that the magnitude of this difference increased at higher glucose values. When we examined the HbA1c discordance as a categorical variable we found that 63.2% of men (24.5% observations) had an HbA1c discordance  $\leq -0.5\%$ , defined as clinically significant.

The mechanisms underlying the underestimation of glycaemia by HbA1c among HIV-infected persons are unclear. Previous reports have suggested that HIV infection and/or its treatment is associated with a low-grade haemolysis,<sup>11</sup> thereby leading to a shorter period of time during which haemoglobin within erythrocytes can become glycosylated. This hypothesis was supported by

**Table 2.** Parameter estimates (95% CIs) from linear mixed models of discordance (observed – expected HbA1c) among HIV-infected patients

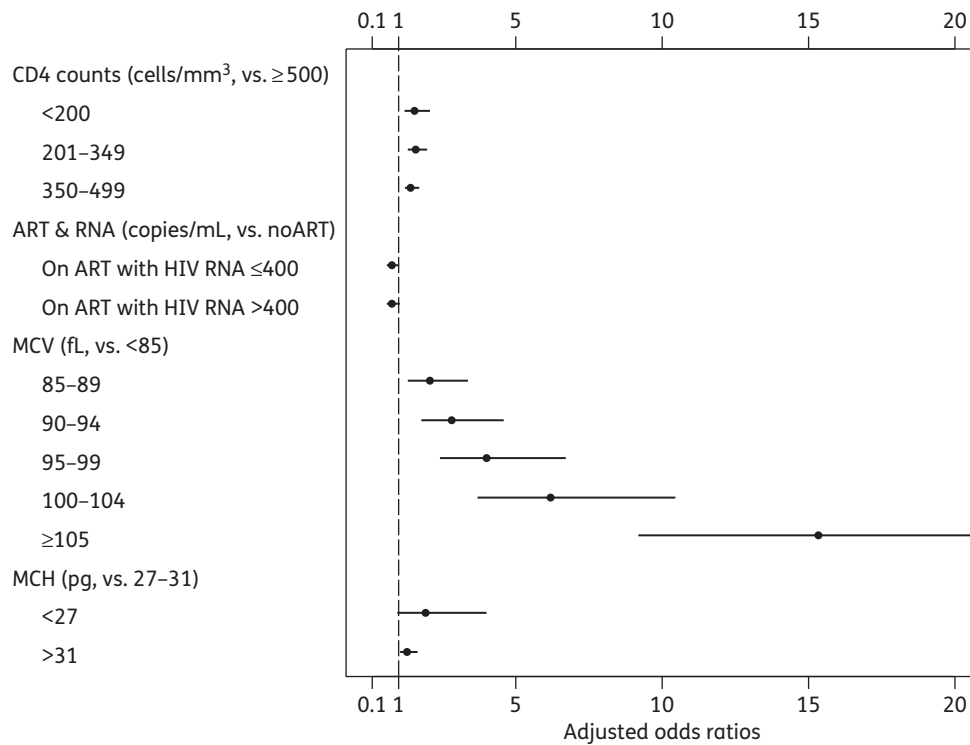
Factors	Univariate model	P value	Multivariate Model 1	P value	Multivariate Model 2	P value	Multivariate Model 3	P value	Multivariate Model 4	P value
Intercept	-0.123 (-0.149, -0.096)	<0.001	-0.295 (-0.33, -0.259)	<0.001	-0.114 (-0.161, -0.067)	<0.001	0.176 (0.112, 0.24)	<0.001	0.072 (-0.033, 0.177)	0.178
Age (per 1 year)	0.024 (0.022, 0.026)	<0.001	0.025 (0.023, 0.027)	<0.001	0.027 (0.025, 0.029)	<0.001	0.017 (0.015, 0.019)	<0.001	0.015 (0.012, 0.017)	0.000
Race (versus white)										
black	0.145 (0.095, 0.194)	<0.001	0.256 (0.199, 0.313)	<0.001	0.268 (0.209, 0.327)	<0.001	0.131 (0.078, 0.183)	<0.001	0.091 (0.032, 0.15)	0.003
Hispanic or other	0.122 (0.059, 0.184)	<0.001	0.364 (0.292, 0.436)	<0.001	0.384 (0.311, 0.457)	<0.001	0.234 (0.169, 0.298)	<0.001	0.197 (0.126, 0.268)	0.000
BMI (kg/m <sup>2</sup> , versus 18.5–24.9)										
<18.5	0.05 (-0.04, 0.139)	0.275	0.014 (-0.074, 0.102)	0.757	0.012 (-0.076, 0.1)	0.791	0.012 (-0.072, 0.096)	0.782	0.047 (-0.044, 0.138)	0.312
25–29	0.044 (0.016, 0.072)	0.002	0.034 (0.006, 0.062)	0.018	0.027 (-0.001, 0.055)	0.058	0.007 (-0.02, 0.033)	0.611	0.011 (-0.02, 0.042)	0.481
≥30	0.284 (0.239, 0.329)	<0.001	0.246 (0.2, 0.292)	<0.001	0.239 (0.193, 0.285)	<0.001	0.173 (0.13, 0.216)	<0.001	0.181 (0.131, 0.232)	0.000
Current CD4 cell count (cells/mm <sup>3</sup> , versus ≥500)										
<200	-0.11 (-0.154, -0.065)	<0.001			-0.115 (-0.161, -0.07)	<0.001	-0.142 (-0.185, -0.098)	<0.001	-0.308 (-0.371, -0.245)	0.000
200–349	-0.101 (-0.132, -0.07)	<0.001			-0.093 (-0.124, -0.062)	<0.001	-0.108 (-0.137, -0.078)	<0.001	-0.137 (-0.175, -0.099)	0.000
350–499	-0.061 (-0.085, -0.037)	<0.001			-0.06 (-0.084, -0.036)	<0.001	-0.071 (-0.094, -0.048)	<0.001	-0.079 (-0.106, -0.052)	0.000
Current ART and HIV RNA level (copies/mL, versus no ART)										
on ART with HIV RNA ≤400	-0.077 (-0.107, -0.047)	0.000			-0.174 (-0.205, -0.144)	<0.001	0.012 (-0.02, 0.043)	0.471	0.148 (0.064, 0.232)	0.001
on ART with HIV RNA >400	-0.13 (-0.168, -0.093)	0.000			-0.138 (-0.174, -0.101)	<0.001	0.021 (-0.016, 0.057)	0.263	NA	NA
HCV-infected, yes versus no	-0.005 (-0.068, 0.059)	0.882			-0.082 (-0.151, -0.013)	<0.001	-0.029 (-0.092, 0.034)	0.363	0.016 (-0.056, 0.088)	0.665
AIDS, yes versus no	0.026 (-0.028, 0.079)	0.346			0.021 (-0.036, 0.079)	0.466	0.02 (-0.033, 0.072)	0.466	0.048 (-0.014, 0.11)	0.133
Haemoglobin (mg/dL, versus ≥14)										
<10	0.045 (-0.071, 0.162)	0.444					-0.053 (-0.164, 0.057)	0.343	0.192 (0.054, 0.33)	0.006
10–11	-0.041 (-0.097, 0.015)	0.148					-0.095 (-0.148, -0.041)	0.001	-0.102 (-0.17, -0.035)	0.003
12–13	0.013 (-0.011, 0.037)	0.301					-0.014 (-0.037, 0.009)	0.245	-0.01 (-0.038, 0.018)	0.499
MCV (fL, versus <85)										
85–89	-0.138 (-0.186, -0.091)	<0.001					-0.154 (-0.205, -0.103)	<0.001	-0.113 (-0.188, -0.037)	0.003
90–94	-0.176 (-0.224, -0.127)	<0.001					-0.193 (-0.248, -0.138)	<0.001	-0.157 (-0.236, -0.078)	0.000
95–99	-0.273 (-0.323, -0.223)	<0.001					-0.283 (-0.344, -0.222)	<0.001	-0.228 (-0.312, -0.143)	0.000
100–104	-0.432 (-0.484, -0.379)	<0.001					-0.422 (-0.486, -0.358)	<0.001	-0.349 (-0.436, -0.261)	0.000
≥105	-0.673 (-0.723, -0.624)	<0.001					-0.616 (-0.679, -0.553)	<0.001	-0.518 (-0.608, -0.428)	0.000
MCH (pg, versus 27–31)										
<27	0.073 (-0.007, 0.154)	0.074					-0.077 (-0.163, 0.01)	0.084	-0.091 (-0.201, 0.02)	0.108
>31	-0.256 (-0.28, -0.232)	0.000					-0.064 (-0.095, -0.032)	<0.001	-0.084 (-0.123, -0.044)	0.000
PI, yes versus no	-0.095 (-0.126, -0.064)	<0.001							-0.12 (-0.156, -0.084)	0.000
NNRTI, yes versus no	0.033 (0.003, 0.063)	0.033							-0.071 (-0.105, -0.037)	0.000
ZDV, yes versus no	-0.404 (-0.436, -0.372)	<0.001							-0.108 (-0.148, -0.068)	0.000
3TC, yes versus no	-0.263 (-0.289, -0.238)	<0.001							0.03 (-0.006, 0.066)	0.106
FTC, yes versus no	0.301 (0.276, 0.326)	<0.001							0.045 (0.006, 0.085)	0.025
ABC, yes versus no	0.028 (-0.006, 0.062)	0.109							0.04 (0.005, 0.075)	0.027
TDF, yes versus no	0.299 (0.274, 0.324)	<0.001							0.023 (-0.011, 0.058)	0.188

ZDV, zidovudine; 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; TDF, tenofovir.

The estimate of intercept was for those who were 50 years old in the univariate model and for those who were 50 years old and also in the reference group for other factors in multivariate models.

Factors in multivariate Model 1 included age, race and BMI; factors in multivariate Model 2 included factors in Model 1 and CD4 cell count, current ART and HIV RNA level, HCV and AIDS; factors in multivariate Model 3 included factors in Model 2 and haemoglobin, MCV and MCH; factors in multivariate Model 4 (restricting to those with HIV RNA ≤400 copies/mL) included factors in Model 3 and all ART variables of interest.

HCV infection was defined as having a positive serum antibody to HCV or positive HCV RNA at baseline visit.



**Figure 2.** Factors associated with HbA1c discordance (observed – expected HbA1c) ≤−0.5% among HIV-infected patients in the MACS. Other factors included age, race, BMI, HCV infection, AIDS and haemoglobin. The upper 95% CI for the OR of MCV ≥105 fL was 25.8.

Diop,<sup>11</sup> who found that the difference between measured and predicted HbA1c based on FG was correlated with low serum levels of haptoglobin, a plasma protein that binds to free haemoglobin and decreases during haemolysis.<sup>20</sup> In contrast, Kim *et al.*<sup>12</sup> showed no association between haptoglobin and HbA1c discordance in HIV-infected persons. It should be noted that haptoglobin levels might be less reliable indicators of haemolytic anaemia in the setting of inflammation or low-grade extravascular haemolysis. In the current study we did not have measurements of serum haptoglobin, but plan to assess this in future MACS investigations.

As in previous studies, we found that higher MCV was a strong predictor of HbA1c discordance among HIV-infected persons.<sup>11,12</sup> Macrocytosis is common among HIV-infected persons and has been attributed to certain antiretroviral agents, including zidovudine, stavudine and lamivudine. Macrocytosis has also been associated with lower HIV-1 RNA levels, independently of specific ART.<sup>21-23</sup> Our data suggest that clinicians should be particularly cautious about using HbA1c in the management or diagnosis of DM among HIV-infected persons who have an MCV ≥95 fL, for whom the odds of HbA1c discordance is 4–15-fold higher than among persons with MCV <95 fL.

MCH, defined as the average mass of haemoglobin per erythrocyte, was also an important predictor of HbA1c discordance in our study. Low levels are characteristic of iron deficiency anaemia and may also be related to HbA1c. In a study involving 423 women without DM in the general population,<sup>15</sup> an increase of 1 pg in MCH corresponded to a decrease of ~0.03% in HbA1c, independently of haemoglobin levels or other red cell parameters. Since MCH values are readily available from complete blood cell count reports, identification of an HIV-infected person with a high MCH (>31 pg) might

serve as an alert that HbA1c levels need to be interpreted with caution.

As in prior studies, we also found that use of certain ART drugs was associated with HbA1c discordance. Many of these effects, including those associated with zidovudine and PI use, remained significantly associated with HbA1c discordance in models adjusted for MCV and other haematological variables, suggesting that ART effects are not fully mediated by their impact upon erythrocyte parameters. In contrast to the study of Kim *et al.*,<sup>12</sup> we did not detect an association between abacavir use and HbA1c discordance.

A novel finding in our analysis is that HbA1c discordance was strongly associated with CD4 counts <500 cells/mm<sup>3</sup>. We hypothesize that lower CD4 cell counts among HIV-infected patients are associated with chronic monocyte activation, leading to increased activation of the reticulo-endothelial system, erythrocyte phagocytosis, decreased red cell lifespan and consequent lowering of HbA1c levels.<sup>24</sup> Of note, the monocyte activation marker sCD163, which is commonly evaluated in studies of HIV-related immune activation, is a haemoglobin–haptoglobin scavenger receptor and a marker specific for the reactive haemophagocytic syndrome.<sup>25</sup> Future work in the MACS will investigate associations between HbA1c discordance and sCD163 levels.

We also found that several demographic factors were related to differences between observed and expected HbA1c, including older age and higher BMI. The finding regarding age has also been observed in NHANES III, in which HbA1c rose by ~0.10% with each decade after age 40,<sup>26</sup> which has been attributed to the acceleration of haemoglobin glycation with advancing age.<sup>27</sup> In addition, in our study we used FG as our primary

measure of glycaemia and hence did not capture post-prandial fluctuations in blood glucose. Since glucose intolerance increases with ageing, older individuals may have greater post-prandial glucose excursions, which would not impact the expected HbA1c levels that we calculated using FG values. Similarly, obese individuals may also experience larger post-prandial glucose excursions, which could lead to higher observed HbA1c levels than expected. Lastly, as in previous studies in the general population, the observed HbA1c was greater than expected among black and Hispanic compared with white participants.<sup>26,28,29</sup> Whether these differences are due to differences in glycaemia or non-glycaemic genetic factors is debated.<sup>28</sup>

Our study has several limitations. We used FG to assess glycaemia rather than seven-point glucose monitoring or glycaemia as assessed by a continuous glucose monitor, which is the gold standard.<sup>30</sup> Our study population included relatively few persons with impaired FG or overt diabetes, for whom inaccuracy of HbA1c would have the highest clinical relevance. In addition, without measurements of other indices of haemolysis we were unable to assess the mechanism(s) accounting for HbA1c discordance. Similarly, we did not have other laboratory-based methods to assess glycaemia, including fructosamine or 1,5-anhydroglucitol, which have been proposed as alternatives to HbA1c among HIV-infected persons.<sup>12</sup> Finally, our study included only men and cannot be extrapolated to women.

In conclusion, we found that HbA1c underestimated glycaemia in certain HIV-infected men. This effect was particularly pronounced in men with a CD4 cell count <500 cells/mm<sup>3</sup>, a higher MCV and a high MCH, and in persons receiving ART regimens that included PIs, NNRTIs or zidovudine. In these sub-populations, it would be prudent for clinicians to use direct measures of glycaemia (i.e. fasting glucose or an oral glucose tolerance test) to diagnose diabetes and, for persons with diabetes, to consider a lower HbA1c level treatment target in order to prevent long-term diabetes complications.

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## Multicenter AIDS Cohort Study members

The Multicenter AIDS Cohort Study (MACS) includes the following: Baltimore: The Johns Hopkins University Bloomberg School of Public Health: Joseph B. Margolick (PI), Barbara Crain, Adrian Dobs, Homayoon Farzadegan, Joel Gallant, Lisette Johnson-Hill, Michael Plankey, Ned Sacktor, Ola Selnes, James Shepard, Chloe Thio. Chicago: Feinberg School of Medicine, Northwestern University and Cook County Bureau of Health Services: Steven M. Wolinsky (PI), John P. Phair, Sheila Badri, Maurice O'Gorman, David Ostrow, Frank Palella, Ann Ragin. Los Angeles: University of California, UCLA Schools of Public Health and Medicine: Roger Detels (PI), Otoniel Martinez-Maza (Co-P I), Aaron Aronow, Robert Bolan, Elizabeth Breen, Anthony Butch, Beth Jamieson, Eric N. Miller, John Oishi, Harry Vinters, Dorothy Wiley, Mallory Witt, Otto Yang, Stephen Young, Zuo Feng Zhang. Pittsburgh: University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (PI), Lawrence A. Kingsley (Co-PI), James T. Becker, Ross D. Cranston, Jeremy J. Martinson,

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## Transparency declarations

Data in this manuscript were collected by the Multicenter AIDS Cohort Study (MACS). MACS (Principal Investigators): Johns Hopkins University Bloomberg School of Public Health (Joseph Margolick); Northwestern University (Steven Wolinsky); University of California, Los Angeles (Roger Detels); University of Pittsburgh (Charles Rinaldo); the Center for Analysis and Management of MACS, Johns Hopkins University Bloomberg School of Public Health (Lisa Jacobson).

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