Published in final edited form as:

HIV Clin Trials. 2008 ; 9(3): 207–211. doi:10.1310/hct0903-207.

Higher Plasma Myeloperoxidase Levels Are Not Associated with an Increased Risk for Cardiovascular Events in HIV-Infected Adults

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Abstract

Objectives—Elevated myeloperoxidase (MPO) levels are predictive of high cardiovascular (CV) risk in the general population. The value of MPO as a CV marker in the HIV population has not been investigated.

Method—Medical records were reviewed to identify HIV+ patients with a documented CV event (myocardial ischemia/infarction) and stored plasma samples within 12 months prior to the event. HIV+ adults with no CV history and with similarly available stored plasma samples were site-, age-, and gender-matched 1:1 to cases.

Results—We identified 124 participants (62 case-control pairs): 94% male, median age 46 years. Median (IQR) MPO levels (pmoles/L) were lower in cases vs. controls: 292 (235–376) vs. 320 (249–467); $p = .004$. Cases were more likely to have other CV risk factors, including smoking, hypertension, and higher cholesterol and triglycerides. The observed MPO directional difference persisted after controlling for CV risk factors. In the reduced model, observed differences in MPO remained independently and negatively associated with CV event ($p = .03$) after adjusting for two positively associated risk factors, differences in cholesterol levels ($p = .01$), and differences in smoking history (ever smoked vs. never smoked; $p = .04$). Differences in triglyceride levels and hypertension were not statistically significant independent risk factors in this sample ($p > .05$). Within cases, MPO was negatively correlated with CD4 count ($r_s = -0.40$, $p = .0023$) and age ($r_s = -0.34$, $p = .01$). In contrast, age at blood draw was positively correlated with MPO in controls ($r_s = 0.28$, $p = .031$) and CD4 was uncorrelated ($r_s = -0.01$, $p > .9$). No other factors were significantly correlated with MPO within groups.

Conclusion—In contrast to the general population, higher MPO levels were not predictive of CV events in this study, underscoring the fact that pathways operative in HIV arteriopathy may be distinct from traditional CV disease pathogenesis.

Keywords

biomarkers; cardiovascular disease; myeloperoxidase; myocardial infarction

Epidemiological research has established that HIV-infected patients are at increased risk of cardiovascular (CV) events.^{1–3} Thus far, the pathogenesis of accelerated atherosclerosis in HIV patients remains unclear, and the relative contributions of classic CV risk factors and HIV-related factors (infection and/or treatment) are not known. HIV infection itself induces a state of chronic inflammation that may induce endothelial dysfunction and subsequent CV disease. Some antiretroviral therapy (ART) has been implicated as a cause of insulin resistance, dyslipidemias, and increased visceral adiposity,^{4,5} all of which are known risk factors for atherosclerosis.

Myeloperoxidase (MPO) is a proinflammatory enzyme released by activated neutrophils. Studies in the HIV-uninfected population have identified MPO-generated oxidants in ventricular remodeling after myocardial infarction.⁶ These oxidants also cause high-density lipoprotein (HDL) to lose its atheroprotective properties. Increased levels of MPO have been associated with risk of coronary artery disease and endothelial dysfunction,^{7–9} but these associations have not been examined in the HIV-infected population.

METHOD

We sought to investigate the value of MPO as a CV risk marker in HIV-infected patients. This study was designed to determine whether plasma MPO levels in the 6–12 months preceding CV events in HIV-infected participants differed from those in a matched HIV-infected group without CV disease. To identify case and control patients, we reviewed the electronic medical records of the John T. Carey Special Immunology Unit of the University Hospitals of Cleveland, Cleveland, Ohio, and the Comprehensive Care Center, Nashville, Tennessee (affiliated with the Vanderbilt University School of Medicine). We selected all HIV-infected patients with a documented CV event between February 1996 and March 2006 who had stored plasma samples (at -70°F) from the 12 months prior to the event as our case group. CV events were defined as a proven acute coronary syndrome, which included myocardial infarction, documented myocardial ischemia by angiographic procedure, and/or documentation of a coronary revascularization procedure. The control group included HIV-infected patients with no known CV events and with similarly available stored plasma samples. Case and control participants were matched within site 1:1 by gender and age at the time of the CV event. To avoid introducing unknown temporal effects associated with CV risk, age of control on date of CV event in case was used in matching. We reviewed patients' medical records for data on the following traditional CV risk factors prior to but proximal to the CV match date: smoking, weight, total cholesterol, HDL, low-density lipoprotein (LDL), triglycerides, and diagnosis of diabetes mellitus or hypertension. We also obtained data on patients' HIV status: current and nadir CD4 cell counts, known duration of HIV diagnosis, and cumulative duration and type of ART. White blood count (WBC) and absolute neutrophil count (ANC) determined in blood drawn at the time of the stored blood sample were also collected from participants' charts. MPO levels in stored plasma were determined by sandwich ELISA (Prognostix, Inc., Cleveland, Ohio, USA) at the Cleveland Clinic Foundation, Cleveland, Ohio.

STATISTICAL METHODS

Univariate analyses were performed using nonparametric statistical methods, the Wilcoxon signed rank and McNemar's tests. In addition to these methods, nonparametric Wilcoxon rank sum test and Spearman rank correlation coefficients (r_s) were used to identify statistically significant correlates of observed MPO differences in pairs as well as correlates of MPO levels within cases and controls. Multivariate conditional logistic regression modeling for matched pair data was used to evaluate observed differences in MPO after adjusting for other CV risk factors. Due to the non-Gaussian distribution of observed MPO differences, the conditional logistic regression was performed on a trimmed dataset. Six matched case-control pairs were

removed from the multivariate analysis for which differences in MPO values in the case-control pairs were determined to be outliers (absolute value of difference in MPO levels greater than 1000 pmoles/L). In five of the six pairs, MPO level in the matched control was greater than in CV case so that removal of the six outlier pairs favored attenuating the observed directional difference in MPO. Backward removal of statistically nonsignificant risk factors after adjustment for remaining factors was used to select the final model of CV correlates in this sample. Because only six case-control pairs had complete information for HDL or LDL, these CV risk factors were not included in the conditional logistic regression analysis.

RESULTS

A total of 124 participants (62 case-control pairs) were included; 34/62 (55%) case-control pairs were from the Cleveland site. The median age was 46 years (range 26–68); 116 (94%) participants were male, 79 (64%) were white, and 43 (35%) were African Americans. For two CV cases, race was not provided in the clinical record. Overall, median CD4+ cell count was 304 (range 3–947) cells/mm³ and median HIV-1 RNA was 2096 (16–578, 431) copies/mL; 39% (39/99) had HIV-1 RNA <400 copies/mL. Case-control participants were matched within site by gender and age on the date of CV event in case. For variables not included in matching, there was no statistically significant difference between cases and controls in terms of race, WBC, ANC, nadir CD4 cell count, proportion of patients with HIV-1 RNA <400 copies/mL, duration of ART, and duration of protease inhibitor therapy (see Table 1). Case participants had a higher prevalence of ever smoking (83% vs. 62%, $p = .023$) and a higher prevalence of hypertension (32% vs. 17%, $p = .063$) when compared to matched control participants. Case participants had significantly higher median (interquartile [IQR]) levels of total cholesterol (201 [173–238] vs. 175 [144–215] mg/dL; $p = .0022$) and triglycerides (215 [155–347] vs. 151 [93–243] mg/dL; $p = .026$) when compared to matched control participants. Contrary to expected, the median MPO levels (pmoles/L) were significantly lower in case compared to control participants (292.3 [235–376] vs. 320.4 [249–467]; $p = .0037$). The observed MPO directional difference persisted despite controlling for these other CV risk factors ($p = .03$).

Finally, within cases, MPO was negatively correlated with CD4 cell count ($r_s = -0.40$, $p = .0023$) and age at blood draw prior to CV event ($r_s = -0.34$, $p = .01$). In contrast, age at blood draw was positively correlated with MPO in controls ($r_s = 0.28$, $p = .031$) and CD4 was not correlated ($r_s = -0.01$, $p > .9$). No other factors were significantly correlated with MPO within groups.

DISCUSSION

In the general population, several studies demonstrated strong and independent relationships between MPO and CV disease risks.^{7–10} Increasing systemic levels of MPO have also been demonstrated to predict future CV events after controlling for risk factors and C-reactive protein levels.^{7–9} Individuals with elevated MPO levels are 15- to 20-fold more likely to demonstrate abnormal coronary angiograms compared with persons in the lowest quartile. It is important to note that this relationship remained significant after statistical adjustments for Framingham risk score and C-reactive protein.

To our knowledge, this study is the first to assess MPO levels in HIV-infected adults. In contrast to the findings in the general population, higher MPO levels did not seem to be associated with future CV events in this HIV-infected population from two North American ambulatory clinics. However, this may have been due to nonsystematic evaluation for underlying CV disease and risk factors in this retrospective study of clinical records. We do recognize that some of the “controls” may have had underlying atherosclerosis; however, the study aim was to assess whether MPO can predict short-term clinical events (and not underlying atherosclerosis) in

this population with overall high risk. We investigated whether site-specific issues, such as differences in processing and collection, confounded the results. Even though median reported MPO levels in both cases and controls were higher at the Cleveland site, and statistically significantly so in controls ($p = .03$), observed median differences in MPO within pairs were not statistically different between the sites ($p = .3$). At both the Cleveland and Nashville sites, MPO levels observed in cases were lower than in matched controls, $p = .009$ and $p = .17$, respectively.

Another possibility for our findings is that in HIV-infected participants several factors may influence MPO levels. We accounted for known factors, such as WBC and ANC, but other yet unknown factors may have influenced the results. Another possible explanation would be that arteriopathy seen in HIV may have different pathogenesis than that seen in the HIV-negative population. The relatively small sample size in this study limited the ability to investigate interactions between the various CV risk factors in this HIV-infected sample.

A potentially important finding in our study is the negative correlation between MPO levels and CD4 cell count. This is consistent with recent findings of a close relationship between inflammation due to uncontrolled viremia/advanced HIV disease and a higher risk of CV disease and endothelial dysfunction.^{11–13}

As ART prolongs the lives of HIV-infected adults, understanding the pathogenesis of CV disease in this population is essential. Further studies are needed to explore the role of MPO in HIV-infected adults and establish useful biochemical markers in this high-risk group. In particular, this marker should be investigated in large prospective studies of incident risks for myocardial infarction, development of CV disease requiring intervention/revascularization, and death.

Acknowledgments

This work was supported by The Center for AIDS Research at Case Western Reserve University (AI36219), the Vanderbilt-Meharry Center for AIDS Research (AI 54999), and by grant P01 HL 076491 (SH).

This work was presented at the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention; July 22–25, 2007; Sydney, Australia.

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Table 1

Demographic, cardiovascular risk factors, and metabolic results at the time of myeloperoxidase (MPO) evaluation

Characteristic	Cases (n = 62)	Controls (n = 62)	p
Median age, years (IQR)	46 (40–52)	45.5 (39–51)	
Male sex	94%	94%	
Race/ethnicity			>.9
White	65%	63%	
African American	32%	37%	
Not specified	3%	—	
Median weight, lbs (IQR)	170.5 (151–190)	166 (147–195)	.5
Smoking status			.057
No	17%	38%	
Past	19%	18%	
Current	64%	43%	
Diabetes	10%	3%	.5
Hypertension	32%	17%	.063
Median systolic blood pressure, mm Hg (IQR)	131 (116–145)	124 (116–136)	.1
Median diastolic blood pressure, mm Hg (IQR)	82 (76–88)	80 (76–86)	.5
Median nadir CD4+ cell count, cells/ μ L (IQR)	103 (30–213)	130 (36–310)	.12
Median known duration of HIV, months (IQR)	124 (85–165)	119.5 (82–163)	.6
Antiretrovirals at time of MPO			>.4
NRTI	97%	93%	
NNRTI	52%	42%	
PI	75%	73%	
Median duration of NRTI, months (IQR)	61.1 (30–92)	61.2 (23–92)	.9
Median duration of NNRTI, months (IQR)	1.3 (0–20)	0 (0–18)	.7
Median duration of PI, months (IQR)	37.6 (0–60)	20.2 (0–57)	.3
CD4+ cell count, cells/ μ L (IQR)	286.5 (197–442)	363.5 (142–481)	.9
Median HIV-1 RNA, log ₁₀ copies/mL (IQR)	3.0 (2–4.3)	3.8 (1.8–4.9)	.5
≤ 400	41%	38%	
Median cholesterol level, mg/dL (IQR)	201 (173–238)	175 (144–215)	.0022
Median triglycerides level, mg/dL (IQR)	215 (155–347)	151 (93–243)	.026
Median absolute neutrophil count, $\times 10^9$ cells/L (IQR)	2.6 (2.0–3.6)	2.6 (2.0–3.7)	.7
Median white blood cells, $\times 10^9$ cells/L (IQR)	5.0 (4.2–6.7)	4.7 (3.8–6.6)	.9
Median MPO, pmoles/L (IQR)	292.3 (235–376)	320.4 (249–467)	.0037

Note: IQR = interquartile range; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.