ASSESSING THE PROGNOSTIC SIGNIFICANCE OF $CD8^+$ T-CELL COUNTS IN DETERMINING THE RISK OF MYOCARDIAL INFARCTION IN THE SETTING OF HIV INFECTION

by

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ABSTRACT

There is a growing body of research to suggest that Human Immunodeficiency Virus (HIV) infection is associated with an increased risk for myocardial infarction (MI) although the underlying processes remain unclear. An assessment of MI risk using a commonly-available measure of the immune status is therefore of Public Health importance. $CD8^+$ and $CD4^+$ T-cell counts are periodically measured in the routine management of HIV-infected patients. However, $CD8^+$ T-cell counts are often not reported or are simply incorporated into the calculation of a $CD4^+/CD8^+$ ratio. Total $CD8^+$ T-cell counts have been shown to be associated with an increased risk for MI in at least one recent study. A few other studies have examined this association indirectly by using the $CD4^+/CD8^+$ ratio, but only considered MI surrogates (e.g. subclinical coronary atherosclerosis) as an outcome. Also, measuring cell-surface markers of $CD8^+$ T-cell activation and HIV-specific $CD8^+$ T cell counts is costly and often not requested in the routine management of HIV infection.

This study investigated the association between total $CD8^+$ T-cell counts and MI risk among a large cohort of HIV-uninfected and HIV-infected Veterans. Using Cox proportional

hazard regression models, the results suggest that MI risk is associated with a high $CD8^+$ T-cell count of ≥1066 cells/ mm^3 (Adjusted HR = 1.82, *P* <0.001, 95% CI: 1.46 to 2.28). They also suggest that the risk for MI posed by total $CD8^+$ T-cell counts should be interpreted in the context of $CD4^+$ T-cell clinical cut-points, or the overall immune status. The degree of MI risk in the cohort differed depending on the level of the immunosuppression. Total $CD8^+$ T cell-counts seemed to modestly improve the risk stratification provided by $CD4^+$ T-cell clinical cut-points, though the mechanisms are still unclear. Future studies will be instrumental in understanding the role of the immune system in MI risk prediction.

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1.0 INTRODUCTION

1.1 BACKGROUND

It has been observed that HIV-infected persons are at a higher risk for the development of myocardial infarction (MI) and other cardiovascular diseases compared to HIV-uninfected persons [1-5]. In the natural history of Human Immunodeficiency Virus (HIV) infection, $CD4^+$ and $CD8^+$ T-cell counts follow a characteristic pattern (Figure 1) [6-8]. Within the first two weeks following HIV infection, there is a large drop in the $CD4^+$ T-cell count while the $CD8^+$ T-cell count rises, but these levels soon regain near-normalcy. In the ensuing period, a strong cytotoxic immune response then occurs to achieve viremic control – large numbers of HIV viral particles are produced yet destroyed each day [6, 9]. Since most of the viral particles come from recently infected $CD4^+$ T cells, the $CD4^+$ T-cell count gradually declines as these cells are destroyed both by the virus and the immune system in the course of the infection. Spontaneous apoptosis of uninfected $CD4^+$ and $CD8^+$ T cells also occurs [6]. $CD8^+$ T-cell levels, however, remain persistently elevated.

Immune activation, in of itself, does not appear to be solely responsible for the high $CD8^+$ T-cell count. Rather, it has been demonstrated to be possibly due to homeostatic mechanisms to maintain the circulating T-cell pool [10]. Studies suggest that eventually, the persistent immune activation (of $CD4^+$ and $CD8^+$ T cells) from viral particles that are released

from latent reservoirs of continuous HIV replication, such as lymph nodes, leads to exhaustion of quiescent naïve T cells available to replace them [11].

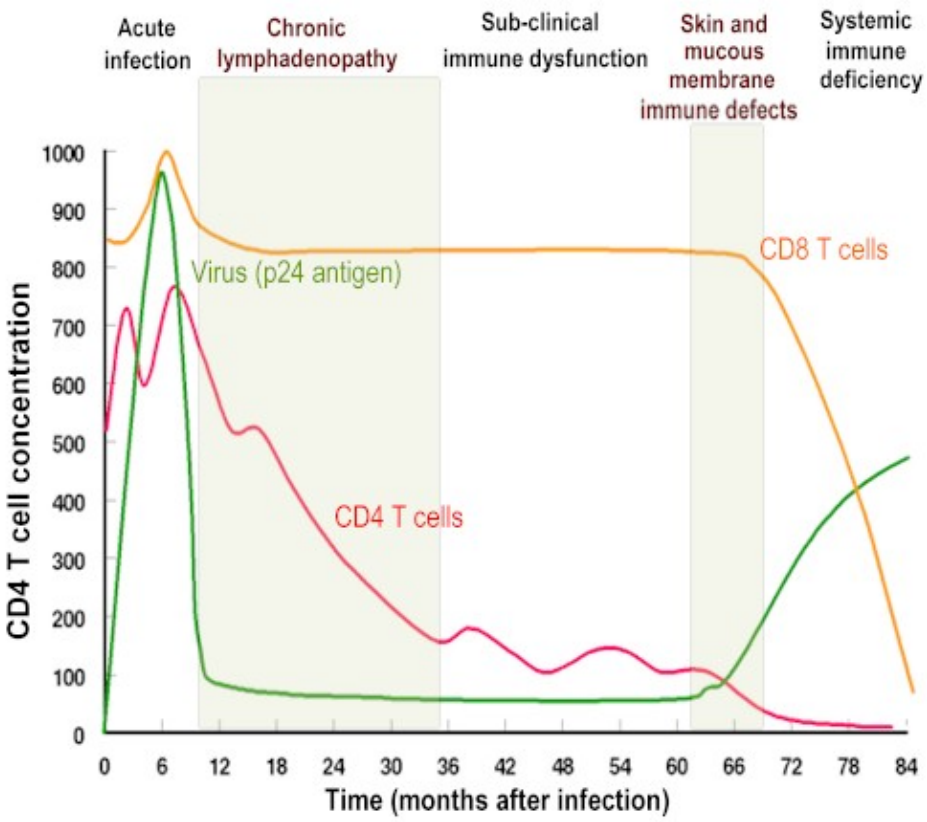

Figure 1. Natural History of HIV

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Richard Hunt http://pathmicro.med.sc.edu/lecture/hiv_time_course2.jpg

Thus, at this stage $CD8^+$ T-cell levels drop dramatically, viremic control is lost and the individual becomes more prone to co-morbid disease, opportunistic infections and eventually AIDS. At late stages of HIV infection, mutant HIV virus particles are also able to directly infect $CD8^+$ T cells and destroy them, further contributing to the low $CD8^+$ T-cell count and immunodeficiency [6].

1.2 MOTIVATION FOR THE STUDY

Such findings have led to the question of whether a HIV-infected person's immune status could be indicative of the risk of MI [2]. Indeed, changes in the levels of $CD4^+$ and $CD8^+$ T cells have already been used as predictors for the progression to Acquired Immune Deficiency Syndrome (AIDS) [12-15]. It is also of interest whether a clinically-relevant measure of immune status could be demonstrated to consistently describe MI risk.

Previous studies have enumerated the usefulness of $CD4^+$ T-cell counts (and traditional risk factors such as hypertension, smoking, and diabetes, for example) in determining the risk of MI (or its surrogates) in HIV [2, 16, 17]. However, a MEDLINE®/PubMed® search of available literature revealed that as yet only one study had investigated the association between $CD8^+$ T-cell counts with MI risk in HIV [2]. The paucity of research into the predictive role of total $CD8^+$ T-cell counts associated with MI risk may be related to inconsistent results from studies attempting to associate these counts with HIV progression to AIDS: both significant [18, 19] and non-significant [20, 21] relationships have been reported. Some studies have demonstrated that immune activation may be associated with an increased risk of MI [16, 17], by focusing on biomarkers of immune activation and the $CD4^+/CD8^+$ ratio [2]. These latter studies had some limitations in that they investigated surrogates of MI (e.g. subclinical carotid artery disease) and had relatively small sample sizes. Hence, the exact prognostic role of the $CD8^+$ T-cell count in this process has not been clearly shown.

1.3 PURPOSE OF THE STUDY

This study sought to investigate if any potential association exists between the total $CD8^+$ T-cell count and the risk of MI in a large cohort of HIV-infected and HIV-uninfected persons, adjusting for common traditional cardiovascular risk factors as well as HIV-specific parameters. Cases of fatal MI were also included in this study as they have sometimes been excluded in studies associating HIV with MI risk [22-24]. The implications of this research are of Public Health significance: Total $CD8^+$ T-cell counts are often obtained during routine care of HIV-infected persons but are usually used in the calculation of a $CD4^+/CD8^+$ T-cell ratio [25]. This may not be wholly informative as the natural history of HIV (Figure 1) shows that the ratio could be very similar at different stages of disease. Hence, use of both total $CD4^+$ and $CD8^+$ T-cell counts in characterizing the immune status' association with MI risk could provide a measure that is of immediate relevance to clinical practice settings and could lead to an improvement in care of HIV-infected persons. The results of this study may also offer a better understanding of the immune system's role in the risk of MI in HIV-infected persons.

2.0 METHODS

2.1 STUDY POPULATION

A sample of 73,398 Veterans was derived from the U.S. Veterans Aging Cohort Study – Virtual Cohort (VACS-VC) [26, 27] for the study period between April 1, 2003 to December 31, 2009. This virtual cohort was created from VACS, an ongoing prospective, longitudinal cohort study group of HIV-infected Veterans matched (1:2) with HIV-uninfected Veterans based on demographic factors (including age and race) and Veterans Affairs Medical Center (VAMC) clinic site at which they were enrolled [27]. The VACS-VC has been in existence since 1998, utilizing a national electronic medical system from the U.S. Department of Veterans Affairs, such that matched Veterans are those enrolled in the same calendar year. The cohort comprises of data from at least five databases including the National Patient Care Database, the Immunology Case Registry, the Health Factor Dataset, the National Pharmacy Benefits Management Database, and the Decision Support System.

For better event ascertainment in this study, these data were combined with information from the Veterans-specific Ischemic Heart Disease Quality Enhancement Research Initiative (IHD-QUERI) database [28], and Medicare and Medicaid databases. Using the Veterans Health Administration's Medical SAS® inpatient datasets [29] as well as the Veterans Affairs vital status electronic file, any MI-related deaths within the VACS-VC were identified. Information

from the Social Security Administration Death Master file and the Beneficiary Identification and Records Locator subsystem were also utilized in this identification process. The National Death Index was used to obtain information on the probable cause of death.

All participants provided written, informed consent. Institutional Review Board (IRB) approval for this study was obtained from the IRBs of the West Haven VAMC, Yale University and the University of Pittsburgh.

The selected sample from the VACS-VC consisted of 73,398 persons who were enrolled in VACS on or after April 1, 2003 and were free of pre-existing cardiovascular disease, including MI (based on clinical diagnosis and/ or International Classification of Diseases, 9th revision (ICD-9) codes). The baseline date was thus a participant's first clinic visit on or after April 1, 2003. Participants were then followed until the development of MI, death or the last observed follow-up date selected for this study (December 31, 2009).

2.2 DEPENDENT VARIABLE

This was incident MI, i.e. all new cases of *definite* or *probable* MI [30] in the VACS-VC for the follow-up period aforementioned. MI events that were completely managed in non-VA hospitals were identified using inpatient ICD-9 code 410 data in the Medicare database, as has been utilized previously [1]. MI associated with death was of two types, using criteria by Ives et al – mortality within thirty days of MI diagnosis was classified as *definite fatal MI*, while death certificates noting mortality to be primarily a result of MI resulted in another class termed *probable fatal MI* [30].

Adjudicated MI events were used to minimize ascertainment bias. Following the Universal Definition of Myocardial Infarction guidelines [31], proper adjudication for MI included the existence of serially raised cardiac enzymes, Troponin I (or Troponin T) and Creatine Kinase MB fraction (CK MB), deemed significant for MI based on the laboratory assay used. Equally utilized was the presence of characteristic electrocardiogram (EKG) findings, especially ST segment elevation of ≥0.1mV in any two or more contiguous EKG leads (except V_2 and V_3 leads), with or without the presence of left bundle branch heart block – an interruption in the electrical conduction pathway of the heart muscle that makes the left ventricle contract slower than the right ventricle instead of synchronously. Adjudication of MI events that were managed solely in hospitals other than the VAMC without transfer to a VA facility required the use of Medicare inpatient ICD-9 code 410 data, as has been employed in a previous study [1]. Using the Compensation and Pension Records Interchange (CAPRI) information service access [32], MI diagnosis was sought from electronic medical records for VACS-VC participants, with due consideration of discharge summary documentation, laboratory test results and inpatient/ outpatient ward and/or clinic reviews.

2.3 PREDICTOR VARIABLES

2.3.1 Primary Independent Variable

The main independent variable of interest was the total $CD8^+$ T-cell count recorded at baseline. As $CD8^+$ T-cell counts were available only for Veterans that were HIV-seropositive, study participants were classified as being either HIV-uninfected, or HIV-infected with $CD8^+$ T-cell

tertiles of ≤666, 667–1065 and ≥1066 cells/ mm^3 at baseline. In separate analyses, the total $CD8^+$ T-cell count variable was stratified by baseline $CD4^+$ T-cell level categories of clinical importance [33]. Baseline $CD8^+$ T-cell count was also assessed as a continuous variable for HIV-infected participants.

2.3.2 Covariates

Data on demographic variables, traditional cardiovascular risk factors [34, 35] of high blood pressure, high lipid levels, obesity, diabetes, and smoking were obtained from medical records and ICD-9 codes. Other covariates identified were renal disease, substance abuse (cocaine and alcohol), Hepatitis C virus infection, anemia, and use of statins. Data on HIV viral load and antiretroviral therapy were also included for HIV-infected participants.

2.3.2.1 Blood pressure

Blood pressure measurement at baseline was the average of three routine outpatient observations closest to the date of enrollment. Using the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) definition for hypertension [36], participants were stratified as being either non-hypertensive (i.e. <140/90 mmHg and not on anti-hypertensive medication) or controlled-hypertensive (i.e. <140/90 mmHg and on anti-hypertensive medication) or uncontrolled-hypertensive (i.e. ≥140/90 mmHg).

2.3.2.2 Lipid levels

Lipid level measurements were of high density lipoprotein (HDL) cholesterol levels, low density lipoprotein (LDL) cholesterol levels and triglyceride (TG) levels and classified following the

National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III) clinical guidelines [37]. LDL cholesterol levels were optimal (<100 mg/dL), above optimal (100-129 mg/dL), borderline high (130-159 mg/dL), and high to very high (160 mg/dL and above). HDL cholesterol levels were high (60 mg/dL and above), or low (< 40mg/dL), TG levels were optimal to normal (<150 mg/dL) or borderline high to high and very high – broadly grouped as being 'elevated' – (150 mg/dL and above).

2.3.2.3 Obesity

Obesity was defined as a body mass index (BMI) of 30 kg/m^2 and above [38].

2.3.2.4 Renal Disease

Renal disease was classified using clinical practice guidelines [39], based on estimated glomerular filtration rate (eGFR), as normal kidney function to mild chronic kidney disease (≥60 $mL/min/1.73m^2$), moderate chronic kidney disease (30-59 $mL/min/1.73m^2$), or severe kidney disease to kidney failure (<30 $mL/min/1.73m^2$) .

2.3.2.5 Diabetes

Diabetes was identified as being either present or absent in as per medical record documentation and ICD-9 codes using an algorithm that has been previously employed [1, 40, 41].

2.3.2.6 Smoking History

Participants' smoking history was classified as either 'never smoked', 'previously smoked' or 'currently smoking' [1].

2.3.2.7 Substance Abuse

Cocaine and alcohol abuse were defined as being present or absent using ICD-9 codes [1, 42].

2.3.2.8 Hepatitis C Virus Infection

Hepatitis C virus infection was deemed present if a laboratory diagnosis or ICD-9 codes for one or more inpatient records or two or more outpatient records identified this infection [1, 43].

2.3.2.9 Hemoglobin Concentration

For persons older than 15 years, the World Health Organization [44] defines the threshold for anemia in males and in non-pregnant females as 13 g/dL and 12 g/dL respectively. Thus hemoglobin concentration was categorized in 0.1 unit decrements as ≥14.0 g/dL (normal), 12.0-13.9 g/dL, 10-11.9 g/dL and ≤10g/dL (the concentration at which transfusion may begin to be indicated).

2.3.2.10 Covariates Specific to HIV-Infected Veterans

For HIV-infected Veterans, HIV-1 RNA viral load and $CD4^+$ T cell count were also obtained at baseline. Antiretroviral therapy (ART) use was categorized as either: (1) none, (2) a combination of Nucleoside Reverse Transcriptase Inhibitors with a Non-Nucleoside Reverse Transcriptase inhibitor (NRTI + NNRTI), (3) a combination of NRTI with a Protease Inhibitor (PI), or (4) some other drug combination. It has been reported that most Veterans obtain ART within the VA system [27].

2.4 STATISTICAL METHODS AND ANALYSES

As the primary aim of the study was to investigate the prognostic value of $CD8^+$ T-cell levels in the setting of HIV, the study participants were first stratified by HIV status and then HIV-infected Veterans were further stratified by baseline $CD8^+$ T-cell counts (in tertiles). Based on this categorization, differences in the independent variables were compared. Pearson's Chi-squared tests of independence were used to assess categorical variables. The Pearson's Chi-squared test assumes that the observations are a simple random sample from the population where each participant had an equal probability of selection, assumes that the overall sample is 'large' (often greater than 30), assumes that expected 'table cell' counts are not less than five in 80% of the cells, and assumes that observations are independent of each other. Continuous variables were assessed using either Analysis of Variance (ANOVA) tests or the Kruskal-Wallis One way ANOVA test if necessary. ANOVA is a parametric test, meaning it is used for data that follow a normal or asymptotically-normal distribution, used to compare the equality of means of several different groups. The Kruskal-Wallis test is a non-parametric test, meaning it is used for data that do not have a normal or asymptotically-normal distribution assumption. However it does assume that the distributions of the variables have a similar shape and scale.

Using the following general formula:

$$\text{Incidence rate} = \frac{Number\ of\ MI\ events}{Person-years\ for\ specific\ category}$$

incidence rates of MI per 10,000 person-years of follow-up were calculated for each of the HIV-$CD8^+$ T-cell categories. Rates for mortality from any cause per 10,000 person years were also calculated. Additionally, the $CD8^+$ T-cell tertiles were stratified by baseline $CD4^+$ T-cell levels of clinical importance and rates were calculated for these new categories as well. Ninety-five per

cent confidence intervals for each of the rates were estimated using a quadratic approximation to the Poisson log likelihood for the log rate parameter [45].

2.5 REGRESSION MODELS

Cox proportional hazard regression models [46] were then used to determine the association between baseline $CD8^+$ T-cell counts and MI in terms of hazard ratios, 95% confidence intervals and *P* values, adjusting for all of the covariates. A proportional hazards model is a regression model used in time-to-event analyses whereby the intensity with which the event occurs (its hazard or risk) is multiplicatively proportional to some function of the hazard which all members in the cohort face (the baseline hazard rate, $h_0(t)$).

Mathematically,

$$h(t|x_j) = h_0(t)\, exp(x_j\beta_x)$$

and

$$\frac{h(t|x_j)}{h(t|x_m)} = \frac{exp(x_j\beta_x)}{exp(x_m\beta_x)}$$

where $h(t|x_j)$ and $h(t|x_m)$ are the hazard rates of the *j*th and *m*th persons in a dataset, with x_j and x_m as their respective covariates, β_x as the respective beta-coefficients of these covariates, and with the caveat that x_j and x_m are assumed to remain constant over time [47].

The baseline $CD8^+$ T-cell count was assessed in separate models as a categorical and continuous variable. Another proportional hazards model was also created in which the

categorical $CD8^+$ T-cell count was stratified by clinically-important $CD4^+$ T-cell count categories. A separate proportional hazards model for HIV-infected participants was also constructed to include the effects of HIV viral load, baseline $CD4^+$ T-cell measurements and antiretroviral therapy as covariates. Separate models with and without baseline $CD4^+$ T-cell measurements were compared to eliminate possible collinearity. Assumptions of proportional hazards were also checked using scaled Schoenfeld residuals tests. Kaplan-Meier survival estimates were calculated to describe survival time to MI for the $CD8^+$ T-cell categories.

Stata® software package, version 12, was used for all statistical analyses.

2.6 MISSING DATA

Proper statistical inference may be hindered in the presence of missing data, especially in large datasets [48]. Multiple imputation is a widely-accepted statistical technique for handling missing data and involves the replacement of a missing value by two or more imputed, plausible values, thereby creating simulated datasets containing each of these values [48]. The imputed datasets are analyzed separately by standard procedures and then their results are averaged. In this study, missing data was assumed to be missing at random and multiple imputation techniques were used to generate five datasets in order to handle the missing data for the covariates. The choice of five sets of imputations follows the recommendation by Rubin [48].

3.0 RESULTS

3.1 BASELINE CHARACTERISTICS

A total of 73,398 study participants were included in the study and followed for a total period of about 6.8 years. The majority was male with a median age of 48 years (mean ± SD = 48.5 ± 9.3, Table 1). Participants were categorized as being either HIV-uninfected, or HIV-infected within the lowest, intermediate or highest baseline $CD8^+$ T-cell tertile (i.e. ≤666 cells/mm^3, 667–1065 cells/mm^3 and ≥1066 cells/mm^3 respectively). Despite prior matching at enrollment into the VACS, members of the sample from the virtual cohort still differed by HIV-$CD8^+$ T-cell tertile status for almost all covariates (P ≤0.004 for substance abuse, P ≤0.001 for others). No significant differences were seen in the use of Non-Nucleoside Reverse Transcriptase Inhibitor medication (NNRTI) among HIV-infected participants (P = 0.423). Additionally, low HDL cholesterol and elevated Triglycerides were highest among HIV-infected participants in the highest $CD8^+$ tertile.

Table 1. Baseline Characteristics of HIV-Infected and Matched HIV-Uninfected Veterans, Stratified by $CD8^+$ T-cell Level[a]

Baseline Characteristics	HIV-uninfected (n = 55,109)	HIV-infected (n = 18,289)		
		$CD8^+$ lowest tertile ($\leq$666 cells/mm^3) (n = 5,987)	$CD8^+$ intermediate tertile (667 – 1065 cells/mm^3) (n = 6,185)	$CD8^+$ highest tertile (>1066 cells/mm^3) (n = 6,117)
Age (years), n (median ± IQR, mean ± SD)	55,109 (49.00 ± 11.00, 48.76 ± 9.24)	5,987 (48.00 ± 12.00, 48.02 ± 9.28)	6,185 (48.00 ± 13.00, 47.54 ± 9.37)	6,117 (48.00 ± 12.00, 47.90 ± 9.27)
Males (n, %)	53, 572 (97.21%)	5,813 (97.09%)	6,050 (97.82%)	5,988 (97.89%)
Race/ethnicity (n, %)				
African American	26,358 (47.83%)	2,999 (50.09%)	2,869 (46.39%)	2,887 (47.20%)
Hispanic	4,294 (7.79%)	524 (8.75%)	459 (7.42%)	423 (6.92%)
White	20,827 (37.79%)	2,077 (34.69%)	2,441 (39.47%)	2,434 (39.79%)
Other	3,630 (6.59%)	387 (6.46%)	416 (6.73%)	373 (6.10%)
Traditional CHD risk factors				
Blood pressure (BP) (n, %)				
Non-hypertensive	31,522 (58.41%)	4,098 (69.05%)	4,133 (67.38%)	4,035 (66.55%)
Controlled-hypertensive	5,210 (9.65%)	443 (7.46%)	432 (7.04%)	404 (6.66%)
Uncontrolled-hypertensive	17,236 (31.94%)	1,394 (23.49%)	1,569 (25.58%)	1,624 (26.79%)
Diabetes (n, %)	11,385 (20.66%)	724 (12.09%)	806 (13.03%)	954 (15.60%)
Lipid profile (n, %)				
Triglycerides $\geq$ 150 mg/dL	16,247 (38.24%)	2,186 (43.41%)	2,587 (47.89%)	2,918 (54.47%)
HDL cholesterol $\geq$ 60 mg/dL	5,980 (14.76%)	621 (13.90%)	504 (10.54%)	372 (7.91%)
HDL cholesterol 40 - 59 mg/dL	19,161 (47.28%)	1,692 (37.84%)	1,832 (38.33%)	1,682 (35.77%)
HDL cholesterol < 40 mg/dL	15,382 (37.96%)	2,157 (48.27%)	2,444 (51.13%)	2,648 (56.32%)
LDL cholesterol <100 mg/dL	12,223 (31.70%)	2,152 (50.80%)	2,071 (44.93%)	2,046 (44.87%)
LDL cholesterol 100-129 mg/dL	12,842 (33.30%)	1,155 (27.27%)	1,376 (29.84%)	1,391 (30.50%)
LDL cholesterol 130-159 mg/dL	8,783 (22.78%)	598 (14.12%)	762 (16.52%)	739 (16.21%)
LDL cholesterol $\geq$ 160 mg/dL	4,715 (12.23%)	331 (7.81%)	401 (8.72%)	384 (8.42%)
Body Mass Index $\geq$ 30kg/m^2 (n, %)	20,762 (38.78%)	632 (10.67%)	836 (13.63%)	1,006 (16.57%)
Smoking history (n, %)				
Never	15,235 (29.95%)	1,582 (28.19%)	1,615 (27.41%)	1,630 (28.12%)
Past	8,154 (16.03%)	744 (13.26%)	767 (13.02%)	803 (13.85%)
Current	27,487 (54.03%)	3,286 (58.55%)	3,509 (59.57%)	3,363 (58.02%)

(continued)

Table 1 (continued)

Baseline Characteristics	HIV-uninfected (n = 55,109)	HIV-infected (n = 18,289)		
		$CD8^+$ lowest tertile (≤666 cells/mm^3) (n = 5,987)	$CD8^+$ intermediate tertile (667 – 1065 cells/mm^3) (n = 6,185)	$CD8^+$ highest tertile (>1066 cells/mm^3) (n = 6,117)
Other risk factors				
Hepatitis C virus infection (n, %)	8,591 (15.59%)	2,154 (35.98%)	2,062 (33.34%)	1,954 (31.94%)
Estimated Glomerular Filtration Rate (eGFR) (n, %)				
eGFR ≥ 60 mL/min/1.73m^2	45,837 (95.19%)	5,321 (92.64%)	5,567 (94.12%)	5,490 (93.53%)
eGFR 30 - 59 mL/min/1.73m^2	2,037 (4.23%)	318 (5.54%)	282 (4.77%)	313 (5.33%)
eGFR < 30 mL/min/1.73m^2	281 (0.58%)	105 (1.83%)	66 (1.12%)	67 (1.14%)
Use of Statins (n, %)	5,393 (9.79%)	311 (5.19%)	433 (7.00%)	479 (7.83%)
Hemoglobin (Hb) (n, %)				
Hb ≥ 14.0 g/dL	33,788 (72.46%)	2,543 (46.41%)	3,436 (60.50%)	3,537 (62.09%)
Hb 12.0 – 13.9 g/dL	10,906 (23.39%)	1,894 (34.56%)	1,731 (30.48%)	1,702 (29.88%)
Hb 10.0 – 11.9 g/dlL	1,554 (3.33%)	729 (13.30%)	406 (7.15%)	363 (6.37%)
Hb < 10.0 g/dL	383 (0.82%)	314 (5.73%)	106 (1.87%)	95 (1.67%)
History of Substance Use				
Cocaine abuse or dependence	3,968 (7.20%)	705 (11.78%)	654 (10.57%)	586 (9.58%)
Alcohol abuse or dependence	7,265 (13.18%)	851 (14.21%)	792 (12.81%)	736 (12.03%)
Baseline Laboratory Analysis				
$CD4^+$ T-cell level				
≥500 cells/ mm^3	…	1,097 (20.36%)	1,971 (35.27%)	2,354 (41.86%)
200 – 499 cells/ mm^3	…	1,901 (35.29%)	2,447 (43.78%)	2,382 (42.36%)
<200 cells/ mm^3	…	2,389 (44.35%)	1,171 (20.95%)	887 (15.77%)
HIV-1 RNA copies/ mL				
<500 copies/ mL	…	2,388 (43.66%)	2,680 (47.33%)	2,428 (42.52%)
≥500 copies/ mL	…	3,081 (56.34%)	2,982 (52.67%)	3,282 (57.48%)
Antiretroviral therapy use (n, %)				
None	…	2,901 (48.46%)	2,827 (45.71%)	2,645 (43.24%)
NRTI + NNRTI	…	1,437 (24.00%)	1,523 (24.62%)	1,446 (23.64%)
NRTI + PI	…	1,219 (20.36%)	1,393 (22.52%)	1,547 (25.29%)
Other combination	…	430 (7.18%)	442 (7.15%)	479 (7.83%)

[a] The variables with missing data were blood pressure, HDL level, LDL level, TG level, Smoking history, eGFR category, Body Mass Index ≥30kg/m^2, Hemoglobin concentration, baseline $CD4^+$ count and baseline viral load. Please see Appendix for details.

Abbreviations used: SD, standard deviation; IQR, interquartile range; CHD, coronary heart disease; HDL high density lipoprotein; LDL, low density lipoprotein; HIV, Human Immunodeficiency Virus; RNA, Ribonucleic Acid; NRTI, Nucleoside Reverse Transcriptase Inhibitor, NNRTI, Non-nucleoside Reverse Transcriptase Inhibitor; PI, Protease Inhibitor.

3.2 RATES OF MI

There were 766 cases of MI observed during the entire follow-up period, giving a rate of 18.49 per 10,000 person years (95% CI: 16.95 to 20.17) among HIV-uninfected Veterans and 28.52 per 10,000 person years (95% CI: 25.24 to 32.22) among HIV-infected Veterans. As shown in Tables 2 and 3, the rates of MI were consistently higher among HIV-infected Veterans, regardless of the $CD8^+$ T-cell count stratification, compared to HIV-uninfected Veterans. The highest rate of MI was seen in the HIV-infected group within the $CD8^+$ T-cell category $\geq$1066 cells/mm^3 with a rate of 32.20 per 10,000 person years (95% CI: 26.50 to 39.14), when participants were stratified based on only their HIV status and $CD8^+$ T-cell count (Table 2). MI rates were also compared with all-cause mortality rates for each category (Tables 2 and 3).

Table 2. MI Rates compared with Mortality Rates, by Baseline $CD8^+$ T-cell Level (per 10,000 person years)

	No. of MI Events	MI Rate [a] (95% CI)	No. of Deaths	Mortality Rate
HIV-uninfected	508	18.49 (16.95 – 20.17)	4,674	18.63 (18.10 – 19.17)
HIV-infected				
$CD8^+ \leq 666$ cells/mm^3	72	26.08 (20.70 – 32.86)	1,449	63.17 (60.00 – 66.51)
$CD8^+$ 667 – 1065 cells/mm^3	85	26.98 (21.81 – 33.37)	1,005	38.54 (36.23 – 41.00)
$CD8^+ \geq 1066$ cells/mm^3	101	32.20 (26.50 – 39.14)	1,045	40.89 (38.49 – 43.45)

[a] 1 missing record (0.0014% of the total)
Abbreviations used: HIV, Human Immunodeficiency Virus; MI, Myocardial infarction

When participants were stratified based on their HIV status and $CD8^+$ T-cell count in the context of their baseline $CD4^+$ T-cell count using clinical cut-points (Table 3, Figure 2), the rates of MI were again consistently higher among HIV-infected Veterans.

The highest MI rates were seen in the following instances:

1) within the highest tertile of $CD8^+$ T cells (for the $CD4^+$ T cells $\geq$ 500 cells/mm^3 category) with MI rate of 28.68 per 10,000 person years (95% CI: 20.96 to 39.26). This tertile also had the highest mortality rate within this $CD4^+$ T-cell category.

2) within the highest tertile of $CD8^+$ T cells (for the $CD4^+$ T cells 200–499 cells/mm^3 category) with MI rate of 37.38 per 10,000 person years (95% CI: 28.25 to 49.46). The mortality rate for this tertile was 0.36 per 10,000 person years below the highest mortality rate within this $CD4^+$ T-cell category; and

Table 3. MI Rates compared with Mortality Rates by Baseline $CD4^+$T-cell Clinical Cut-points and Baseline $CD8^+$ T-cell Level (per 10,000 person years)

	No. of MI Events	MI Rate [a]	No. of Deaths	Mortality Rate
HIV-uninfected	508	18.49 (16.95 – 20.17)	4,674	18.63 (18.10 – 19.17)
HIV-infected				
$CD4^+ \geq 500$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	16	24.00 (14.70 – 39.18)	160	28.08 (24.05 – 32.78)
$CD8^+$ 667 – 1065 cells/mm^3	31	26.68 (18.76 – 37.93)	242	24.83 (21.89 – 28.17)
$CD8^+ \geq 1066$ cells/mm^3	39	28.68 (20.96 – 39.26)	340	30.66 (27.57 – 34.10)
$CD4^+$ 200-499 cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	23	21.54 (14.32 – 32.42)	464	43.88 (39.72 – 48.48)
$CD8^+$ 667 – 1065 cells/mm^3	36	26.08 (18.81 – 36.16)	428	37.68 (34.27 – 41.42)
$CD8^+ \geq 1066$ cells/mm^3	49	37.38 (28.25 – 49.46)	387	43.52 (39.74 – 47.67)
$CD4^+ < 200$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	33	32.16 (22.86 – 45.23)	902	107.13 (100.36 – 114.35)
$CD8^+$ 667 – 1065 cells/mm^3	18	29.60 (18.65 – 46.97)	335	67.40 (60.56 – 75.02)
$CD8^+ \geq 1066$ cells/mm^3	13	27.89 (16.19 – 48.02)	241	63.32 (55.81 – 71.85)

[a] 1 missing record (0.0014% of the total)

Abbreviations used: HIV, Human Immunodeficiency Virus; MI, Myocardial infarction

3) within the lowest tertile of $CD8^+$ T cells (for the $CD4^+$ T cells < 200 cells/mm^3 category), with MI rate of 32.16 per 10,000 person years (95% CI: 22.86 – 45.23). The mortality rate was highest in this tertile for this $CD4^+$ T-cell category.

The MI rates among HIV-infected participants were observed to have overlapping 95% confidence intervals. All-cause mortality appeared to rise with decreasing $CD4^+$ T-cell levels.

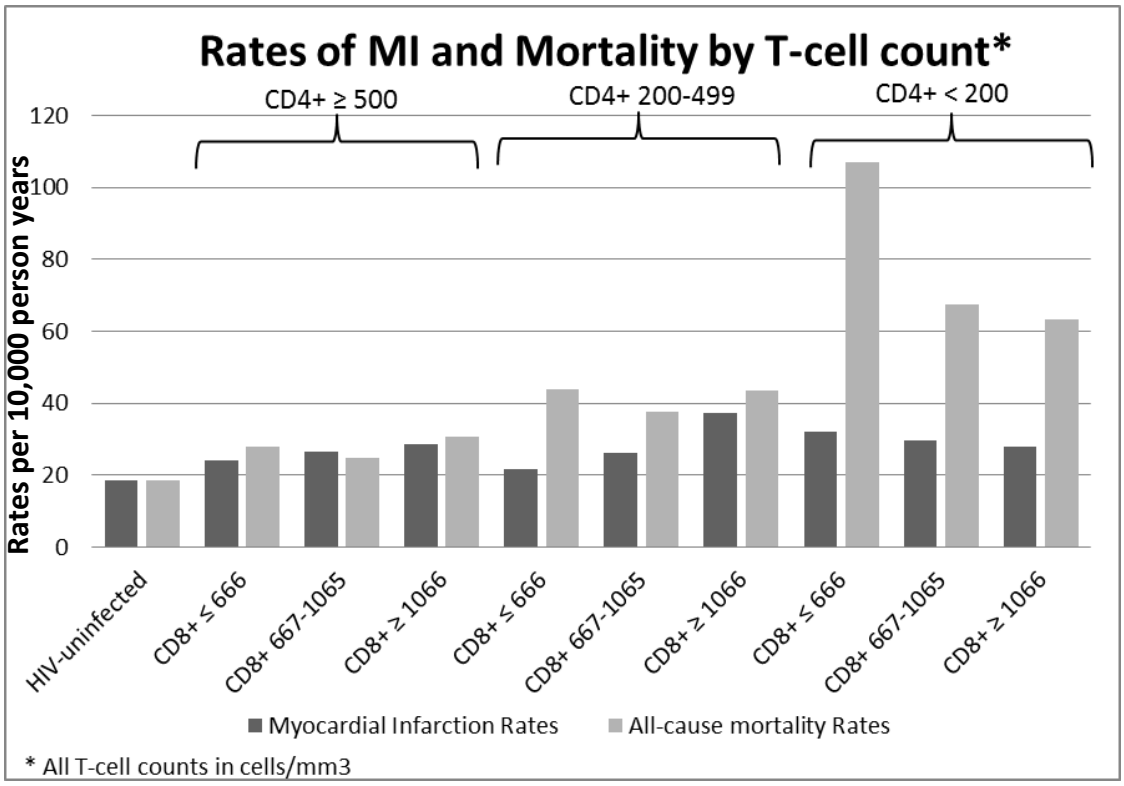

Figure 2. Rates of MI and Rates of All-cause mortality by T-cell count

3.3 ESTIMATES OF SURVIVAL FUNCTION

Kaplan-Meier estimates of survival time to MI were graphed to show the survival distribution of the different $CD8^+$ T-cell categories (see Appendix for Risk tables). When Veterans were stratified by HIV-$CD8^+$ T-cell status alone (Figure 3), the estimated survival functions for the

lowest and highest $CD8^+$ tertile (≤666 and >1065 cells/mm^3 respectively) were seen to be very similar to each other. In contrast, the curve for the middle $CD8^+$ tertile (666-1065 cells/mm^3) was similar to the HIV-uninfected participants for the most part until after about four years of follow-up time. Median survival time was not observed in any of the categories, indicating the rarity of MI events in this cohort.

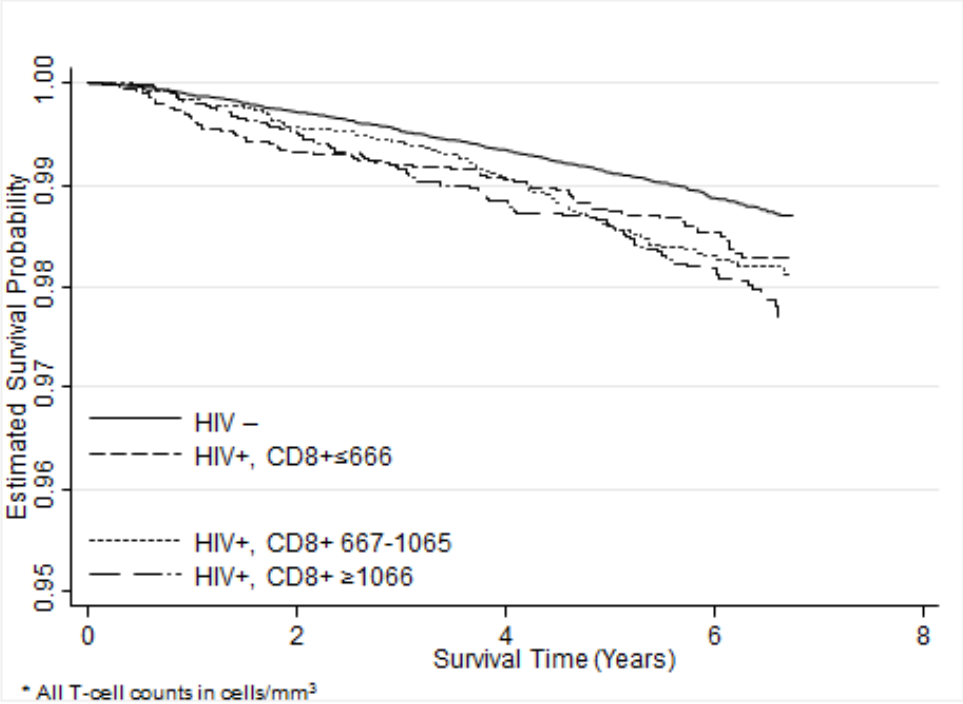

Figure 3. Graph of Kaplan-Meier survival estimates of MI development for different HIV/ $CD8^+$ T-cell strata

Abbreviations used: HIV- , HIV-uninfected; HIV+, HIV-infected.

When Veterans were stratified by both $CD8^+$ T-cell and $CD4^+$ T-cell levels according to HIV status (Figure 4), the Kaplan-Meier curves were less distinct due to the number of groups. However, formal tests of the equality of survival functions revealed that the survival experiences of different $CD8^+$ categories within each $CD4^+$ T-cell level were not statistically different from

one another (see Appendix). One category among the HIV-infected Veterans seemingly had a 'better' initial survival experience than the HIV-uninfected group (Figure 4). Subsequent investigation revealed this to be as a result of the relatively fewer number of participants in this category early in the follow-up period – hence fewer MI events were recorded during that period.

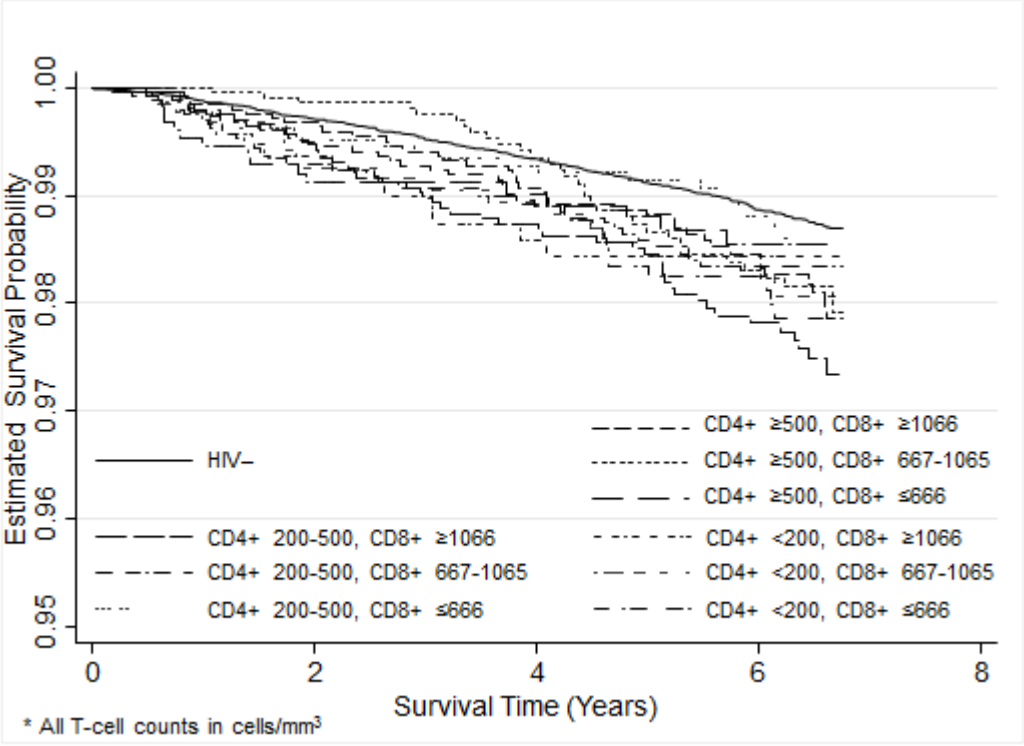

Figure 4. Graph of Kaplan-Meier survival estimates of MI development for different HIV/ $CD8^+$ T-cell strata by $CD4^+$ T-cell level

Abbreviations used: HIV- , HIV-uninfected; HIV+, HIV-infected.

3.4 ESTIMATED MI RISK

Proportional hazard models showed that MI risk was greatest among HIV-infected participants in the highest $CD8^+$ T-cell tertile compared with HIV-uninfected participants (Table 4), following adjustment for all covariates (Hazard ratio, HR= 1.82, 95% CI: 1.46 to 2.28, *P* <0.001).

With additional stratification by $CD4^+$ T-cell clinical cut-points (Table 4), MI risk was greatest among those within the highest $CD8^+$ T-cell tertile who were also in the $CD4^+$ T-cell category of either ≥500 cells/mm^3 (adjusted HR = 1.69, 95% CI: 1.21 to 2.36, P = 0.002) or 200–499 cells/mm^3 (adjusted HR = 2.08, 95% CI: 1.53 to 2.82, P <0.001). Persons who were in the $CD4^+$ T-cell category of <200 cells/mm^3 were at greatest risk for MI if they were correspondingly within the lowest $CD8^+$ T-cell tertile (adjusted HR = 1.82, 95% CI: 1.26 to 2.64, P = 0.001).

Table 4. Multivariable-adjusted Hazard Ratios (HR) for the association between $CD8^+$ T-cell levels and MI

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
HIV status				
HIV-uninfected	1.00 (reference)		1.00 (reference)	
HIV infected by $CD8^+$ count				
$CD8^+ \leq 666$ cells/mm^3	1.45 (1.12 – 1.88)	0.005	…	…
$CD8^+$ 667 – 1065 cells/mm^3	1.54 (1.21 – 1.96)	<0.001	…	…
$CD8^+ \geq 1066$ cells/mm^3	1.82 (1.46 – 2.28)	<0.001	…	…
HIV infected by $CD4^+$ & $CD8^+$ count				
$CD4^+ \geq 500$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	…	…	1.30 (0.76 – 2.20)	0.339
$CD8^+$ 667 – 1065 cells/mm^3	…	…	1.51 (1.03 – 2.21)	0.037
$CD8^+ \geq 1066$ cells/mm^3	…	…	1.69 (1.21 – 2.36)	0.002
$CD4^+$ 200-499 cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	…	…	1.22 (0.80 – 1.87)	0.360
$CD8^+$ 667 – 1065 cells/mm^3	…	…	1.47 (1.03 – 2.09)	0.034
$CD8^+ \geq 1066$ cells/mm^3	…	…	2.08 (1.53 – 2.82)	<0.001
$CD4^+ < 200$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	…	…	1.82 (1.26 – 2.64)	0.001
$CD8^+$ 667 – 1065 cells/mm^3	…	…	1.80 (1.10 – 2.94)	0.019
$CD8^+ \geq 1066$ cells/mm^3	…	…	1.51 (0.85 – 2.67)	0.158
Age (in 10-year increments)	1.80 (1.66 – 1.96)	<0.001	1.81 (1.66 – 1.96)	<0.001
Female	0.34 (0.14 – 0.82)	0.017	0.34 (0.14 – 0.82)	0.017
Race/ethnicity				
African American	0.74 (0.63 – 0.87)	<0.001	0.74 (0.63 – 0.87)	<0.001
Hispanic	1.07 (0.83 – 1.38)	0.602	1.07 (0.83 – 1.38)	0.593
White	1.00 (reference)		1.00 (reference)	
Other	0.59 (0.39 – 0.88)	0.009	0.59 (0.39 – 0.87)	0.009
Traditional CHD risk factors				
Blood pressure (BP)				
Non-hypertensive	1.00 (reference)		1.00 (reference)	
Controlled-hypertensive	1.36 (1.07 – 1.72)	0.013	1.36 (1.07 – 1.72)	0.013
Uncontrolled-hypertensive	1.66 (1.42 – 1.95)	<0.001	1.67 (1.42 – 1.96)	<0.001
Diabetes	1.74 (1.48 – 2.04)	<0.001	1.74 (1.48 – 2.04)	<0.001

(continued)

Table 4. (continued)

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
Lipid profile				
Triglycerides ≥ 150 mg/dl	1.17 (0.99 – 1.37)	0.058	1.17 (0.99 – 1.37)	0.059
HDL cholesterol ≥ 60 mg/dl	1.00 (reference)		1.00 (reference)	
HDL cholesterol 40 - 59 mg/dl	1.02 (0.79 – 1.33)	0.862	1.02 (0.79 – 1.33)	0.860
HDL cholesterol < 40 mg/dl	1.05 (0.81 – 1.36)	0.724	1.04 (0.80 – 1.36)	0.743
LDL cholesterol <100 mg/dl	1.00 (reference)		1.00 (reference)	
LDL cholesterol 100-129 mg/dl	1.15 (0.95 – 1.38)	0.145	1.15 (0.96 – 1.38)	0.139
LDL cholesterol 130-159 mg/dl	1.54 (1.23 – 1.93)	<0.001	1.55 (1.24 – 1.94)	<0.001
LDL cholesterol ≥ 160 mg/dl	1.71 (1.33 – 2.21)	<0.001	1.72 (1.34 – 2.22)	<0.001
Body Mass Index ≥ 30kg/m^2	0.99 (0.83 – 1.17)	0.895	0.99 (0.84 – 1.17)	0.914
Smoking history				
Never	1.00 (reference)		1.00 (reference)	
Past	1.07 (0.82 – 1.40)	0.591	1.08 (0.83 – 1.40)	0.575
Current	1.73 (1.43 – 2.09)	<0.001	1.72 (1.42 – 2.09)	<0.001
Other risk factors				
Hepatitis C virus infection	1.17 (0.98 – 1.40)	0.087	1.17 (0.98 – 1.40)	0.082
Estimated Glomerular Filtration Rate (eGFR)				
eGFR ≥ 60 ml/min/1.73m^2	1.00 (reference)		1.00 (reference)	
eGFR 30 - 59 ml/min/1.73m^2	1.50 (1.16 – 1.95)	0.002	1.50 (1.16 – 1.95)	0.003
eGFR < 30 ml/min/1.73m^2	3.23 (2.14 – 4.86)	<0.001	3.28 (2.17 – 4.95)	<0.001
Use of Statins	0.85 (0.68 – 1.06)	0.147	0.85 (0.68 – 1.06)	0.149
Hemoglobin (Hb)				
Hb ≥ 14.0 mg/dl	1.00 (reference)		1.00 (reference)	
Hb 12.0 – 13.9 mg/dl	1.23 (1.03 – 1.47)	0.024	1.22 (1.02 – 1.47)	0.028
Hb 10.0 – 11.9 mg/dl	2.13 (1.61 – 2.81)	<0.001	2.09 (1.58 – 2.77)	<0.001
Hb < 10.0 mg/dl	2.13 (1.29 – 3.52)	0.003	2.03 (1.22 – 3.38)	0.007
History of Substance Use				
Cocaine abuse or dependence	1.01 (0.74 – 1.38)	0.969	1.00 (0.73 – 1.37)	0.984
Alcohol abuse or dependence	1.11 (0.87 – 1.41)	0.403	1.11 (0.87 – 1.41)	0.405

[a] Model when CD8$^+$ T-cell level was not stratified by CD4$^+$ T-cell clinical cut-point

[b] Model when CD8$^+$ T-cell level was stratified by CD4$^+$ T-cell clinical cut-point

Abbreviations used: CHD, coronary heart disease; HDL high density lipoprotein; LDL, low density lipoprotein; HIV, Human Immunodeficiency Virus.

As the median age of members of the cohort was approximately 50 years (middle age) in all HIV-$CD8^+$ T-cell categories, proportional hazard models restricted to those either above or below this age were analyzed (Table 5), to examine if there was an age effect affecting the previous results. A median of 50 years indicated that approximately half of the study participants fell either above or below this age. Effects of $CD8^+$ T-cell counts lost statistical significance for most participant categories (likely due to reduced power). However, a consistent pattern of increasing MI risk with increasing $CD8^+$ T-cell levels was observed across all $CD4^+$ T-cell categories among those aged 50 years and above. The same pattern was seen in those below 50 years with the exception of the $CD4^+$ T-cell <200 cells/mm^3 category.

Table 5. Multivariable-adjusted Hazard Ratios (HR) for the association between $CD8^+$ T-cell levels and MI for persons below or above middle age (50 years)

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
HIV status				
HIV-uninfected	1.00 (reference)		1.00 (reference)	
HIV infected by $CD4^+$ & $CD8^+$ count				
$CD4^+ \geq 500$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	0.87 (0.27 – 2.86)	0.818	1.55 (0.84 – 2.89)	0.163
$CD8^+$ 667 – 1065 cells/mm^3	1.14 (0.56 – 2.34)	0.718	1.72 (1.09 – 2.71)	0.019
$CD8^+ \geq 1066$ cells/mm^3	1.52 (0.86 – 2.71)	0.147	1.77 (1.16 – 2.67)	0.007
$CD4^+$ 200-499 cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	1.26 (0.58 – 2.72)	0.554	1.20 (0.71 – 2.04)	0.495
$CD8^+$ 667 – 1065 cells/mm^3	1.29 (0.69 – 2.41)	0.426	1.57 (1.02 – 2.41)	0.041
$CD8^+ \geq 1066$ cells/mm^3	1.51 (0.82 – 2.75)	0.183	2.34 (1.63 – 3.34)	<0.001
$CD4^+ < 200$ cells/mm^3				
$CD8^+ \leq 666$ cells/mm^3	1.93 (1.08 – 3.45)	0.027	1.63 (1.00 – 2.66)	0.052
$CD8^+$ 667 – 1065 cells/mm^3	2.01 (0.96 – 4.18)	0.062	1.65 (0.85 – 3.19)	0.136
$CD8^+ \geq 1066$ cells/mm^3	0.64 (0.16 – 2.62)	0.540	1.87 (1.00 – 3.52)	0.050
Female	0.19 (0.05 – 0.79)	0.022	0.49 (0.16 – 1.53)	0.221
Race/ethnicity				
African American	0.75 (0.57 – 0.99)	0.044	0.71 (0.58 – 0.86)	0.003
Hispanic	1.03 (0.66 – 1.63)	0.887	1.08 (0.80 – 1.47)	0.605
White	1.00 (reference)		1.00 (reference)	
Other	0.39 (0.16 – 0.96)	0.041	0.67 (0.43 – 1.06)	0.086
Traditional CHD risk factors				
Blood pressure (BP)				
Non-hypertensive	1.00 (reference)		1.00 (reference)	
Controlled-hypertensive	1.94 (1.24 – 3.03)	0.003	1.40 (1.05 – 1.86)	0.020
Uncontrolled-hypertensive	1.81 (1.38 – 2.38)	<0.001	1.78 (1.46 – 2.17)	<0.001
Diabetes	2.04 (1.53 – 2.72)	<0.001	1.70 (1.40 – 2.06)	<0.001
Lipid profile				
Triglycerides ≥ 150 mg/dl	1.30 (0.96 – 1.75)	0.088	1.04 (0.85 – 1.26)	0.718
HDL cholesterol ≥ 60 mg/dl	1.00 (reference)		1.00 (reference)	
HDL cholesterol 40 - 59 mg/dl	0.86 (0.56 – 1.33)	0.499	1.12 (0.76 – 1.66)	0.542
HDL cholesterol < 40 mg/dl	0.88 (0.55 – 1.41)	0.596	1.13 (0.80 – 1.58)	0.486
LDL cholesterol <100 mg/dl	1.00 (reference)		1.00 (reference)	
LDL cholesterol 100-129 mg/dl	1.35 (0.94 – 1.96)	0.105	1.07 (0.85 – 1.35)	0.557
LDL cholesterol 130-159 mg/dl	1.94 (1.37 – 2.75)	<0.001	1.35 (0.99 – 1.85)	0.060
LDL cholesterol ≥ 160 mg/dl	2.11 (1.31 – 3.39)	0.003	1.46 (1.06 – 2.01)	0.020

(continued)

Table 5. (continued)

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
Body Mass Index ≥ 30kg/m^2	1.16 (0.87 – 1.54)	0.312	0.79 (0.64 – 0.98)	0.029
Smoking history				
Never	1.00 (reference)		1.00 (reference)	
Past	2.12 (1.44 – 3.11)	<0.001	1.40 (1.12 – 1.74)	0.003
Current	1.44 (0.84 – 2.46)	0.187	1.04 (0.79 – 1.38)	0.774
Other risk factors				
Hepatitis C virus infection	1.58 (1.19 – 2.11)	0.002	0.90 (0.72 – 1.13)	0.369
Estimated Glomerular Filtration Rate (eGFR)				
eGFR ≥ 60 ml/min/1.73m^2	1.00 (reference)		1.00 (reference)	
eGFR 30 - 59 ml/min/1.73m^2	1.55 (0.87 – 2.77)	0.134	1.83 (1.38 – 2.43)	<0.001
eGFR < 30 ml/min/1.73m^2	0.78 (0.19 – 3.24)	0.730	4.20 (2.71 – 6.51)	<0.001
Use of Statins	0.76 (0.48 – 1.21)	0.246	0.90 (0.70 – 1.15)	0.400
Hemoglobin (Hb)				
Hb ≥ 14.0 mg/dl	1.00 (reference)		1.00 (reference)	
Hb 12.0 – 13.9 mg/dl	1.24 (0.90 – 1.72)	0.189	1.32 (1.07 – 1.63)	0.010
Hb 10.0 – 11.9 mg/dl	2.38 (1.42 – 4.02)	0.001	2.23 (1.61 – 3.10)	<0.001
Hb < 10.0 mg/dl	2.36 (0.94 – 5.95)	0.068	2.17 (1.18 – 4.00)	0.012
History of Substance Use				
Cocaine abuse or dependence	1.15 (0.73 – 1.81)	0.536	0.81 (0.52 – 1.28)	0.370
Alcohol abuse or dependence	1.00 (0.67 – 1.48)	0.995	1.07 (0.79 – 1.45)	0.674

[a] Model restricted to persons below 50 years old

[b] Model restricted to persons 50 years old and above

Abbreviations used: CHD, coronary heart disease; HDL high density lipoprotein; LDL, low density lipoprotein; HIV, Human Immunodeficiency Virus.

Additionally, a proportional hazards model specific to HIV-infected Veterans (Table 6) revealed that MI risk was greatest among those within the highest and middle $CD8^+$ T-cell tertiles compared to the lowest $CD8^+$ T-cell tertile following adjustments for viral load and antiretroviral therapy, but this was not statistically significant (adjusted HR = 1.25 and 1.07 respectively, P = 0.164 and 0.690 respectively, Table 6). A proportional hazards model with $CD8^+$ T-cell count as a continuous variable, for HIV- infected Veterans only (Table 6), revealed that the MI risk increased by 0.03% for every additional cell/mm^3 of $CD8^+$ T-cell count (P=0.004). The exclusion of the baseline $CD4^+$ T-cells variable from these two proportional hazard models just described did not reveal any marked changes in hazard ratios or standard errors (analyses not shown).

Table 6. Multivariable-adjusted Hazard Ratios (HR) for the association between $CD8^+$ T-cell levels and MI in HIV-Infected Veterans only

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
HIV infected by $CD8^+$ count strata				
$CD8^+ \leq 666$ cells/mm^3	1.00 (reference)		…	…
$CD8^+$ 667 – 1065 cells/mm^3	1.07 (0.77 – 1.47)	0.690	…	…
$CD8^+ \geq 1066$ cells/mm^3	1.25 (0.91 – 1.72)	0.164	…	…
HIV infected with $CD8^+$ count as continuous variable	…	…	1.0003 (1.0001 – 1.0005)	0.004
Age (in 10-year increments)	1.74 (1.50 – 2.01)	<0.001	1.74 (1.50 – 2.01)	<0.001
Female	0.41 (0.10 – 1.68)	0.217	0.42 (0.10 – 1.71)	0.224
Race/ethnicity				
African American	0.70 (0.52 – 0.93)	0.014	0.69 (0.52 – 0.92)	0.013
Hispanic	0.92 (0.59 – 1.45)	0.727	0.93 (0.59 – 1.47)	0.756
White	1.00 (reference)		1.00 (reference)	
Other	0.63 (0.32 –1.24)	0.182	0.63 (0.32 –1.24)	0.181
Traditional CHD risk factors				
Blood pressure (BP)				
Non-hypertensive	1.00 (reference)		1.00 (reference)	
Controlled-hypertensive	1.72 (1.13 – 2.61)	0.011	1.73 (1.13 – 2.63)	0.011
Uncontrolled-hypertensive	1.96 (1.49 – 2.58)	<0.001	1.96 (1.49 – 2.58)	<0.001
Diabetes	1.54 (1.15 – 2.06)	0.004	1.53 (1.15 – 2.05)	0.004
Lipid profile				
Triglycerides ≥ 150 mg/dl	1.36 (1.03 – 1.81)	0.030	1.36 (1.02 – 1.80)	0.034
HDL cholesterol ≥ 60 mg/dl	1.00 (reference)		1.00 (reference)	
HDL cholesterol 40 - 59 mg/dl	1.08 (0.64 – 1.83)	0.773	1.08 (0.63 – 1.82)	0.785
HDL cholesterol < 40 mg/dl	1.04 (0.60 – 1.81)	0.889	1.02 (0.59 – 1.78)	0.931
LDL cholesterol <100 mg/dl	1.00 (reference)		1.00 (reference)	
LDL cholesterol 100-129 mg/dl	1.02 (0.73 – 1.44)	0.902	1.02 (0.72 – 1.44)	0.907
LDL cholesterol 130-159 mg/dl	1.69 (1.17 – 2.43)	0.005	1.68 (1.17 – 2.42)	0.005
LDL cholesterol ≥ 160 mg/dl	1.26 (0.74 – 2.15)	0.388	1.26 (0.74 – 2.14)	0.398

(continued)

Table 6. (continued)

Covariates	HR (95% CI)[a]	*P* value	HR (95% CI)[b]	*P* value
Body Mass Index ≥ 30kg/m^2	1.18 (0.84 – 1.66)	0.344	1.17 (0.83 – 1.64)	0.373
Smoking history				
Never	1.00 (reference)		1.00 (reference)	
Past	1.12 (0.75 – 1.67)	0.580	1.13 (0.76 – 1.68)	0.557
Current	1.47 (1.07 – 2.04)	0.019	1.48 (1.07 – 2.04)	0.018
Other risk factors				
Hepatitis C virus infection	1.29 (0.98 – 1.69)	0.065	1.30 (0.97 – 1.70)	0.062
Estimated Glomerular Filtration Rate (eGFR)				
eGFR ≥ 60 ml/min/1.73m^2	1.00 (reference)		1.00 (reference)	
eGFR 30 - 59 ml/min/1.73m^2	1.44 (0.96 – 2.17)	0.081	1.43 (0.95 – 2.16)	0.086
eGFR < 30 ml/min/1.73m^2	3.29 (1.25 – 4.57)	0.008	2.42 (1.27 – 4.62)	0.007
Use of Statins	1.03 (0.69 – 1.54)	0.876	1.04 (0.70 – 1.55)	0.856
Hemoglobin (Hb)				
Hb ≥ 14.0 mg/dl	1.00 (reference)		1.00 (reference)	
Hb 12.0 – 13.9 mg/dl	1.29 (0.95 – 1.75)	0.107	1.29 (0.95 – 1.76)	0.103
Hb 10.0 – 11.9 mg/dl	2.06 (1.35 – 3.16)	0.001	2.09 (1.36 – 3.20)	0.001
Hb < 10.0 mg/dl	1.57 (0.71 – 3.49)	0.264	1.62 (0.73 – 3.59)	0.236
Hx of Substance Use				
Cocaine abuse or dependence	0.97 (0.59 – 1.59)	0.900	0.98 (0.60 – 1.60)	0.938
Alcohol abuse or dependence	1.15 (0.76 – 1.76)	0.504	1.15 (0.75 – 1.75)	0.526
Baseline Laboratory Analysis				
CD4$^+$ T-cell level				
≥500 cells/ mm^3	1.00 (reference)		1.00 (reference)	
200 – 499 cells/ mm^3	1.06 (0.78 – 1.44)	0.706	1.08 (0.80 – 1.46)	0.621
<200 cells/ mm^3	1.22 (0.84 – 1.77)	0.302	1.27 (0.88 – 1.85)	0.200
HIV-1 RNA copies/ mL				
<500 copies/ mL	1.00 (reference)		1.00 (reference)	
≥500 copies/ mL	1.10 (0.82 – 1.48)	0.539	1.08 (0.80 – 1.45)	0.620
Antiretroviral therapy use				
None	1.00 (reference)		1.00 (reference)	
NRTI + NNRTI	0.87 (0.62 – 1.22)	0.428	0.88 (0.63 – 1.24)	0.465
NRTI + PI	1.01 (0.63 – 1.62)	0.962	1.02 (0.63 – 1.63)	0.942
Other combination	0.99 (0.71 – 1.39)	0.974	1.00 (0.72 – 1.40)	0.989

[a] Model when CD8$^+$ T-cell level is treated as a categorical variable

[b] Model when CD8$^+$ T-cell level is treated as a continuous variable

Abbreviations used: CHD, coronary heart disease; HDL high density lipoprotein; LDL, low density lipoprotein; HIV, Human Immunodeficiency Virus; RNA, Ribonucleic Acid; NRTI, Nucleoside Reverse Transcriptase Inhibitor, NNRTI, Non-nucleoside Reverse Transcriptase Inhibitor; PI, Protease Inhibitor.

The assumption of proportional hazards was not violated when participants were stratified by HIV status and $CD8^+$ T-cell level only (see Appendix). However when the HIV-infected participants were further stratified by $CD4^+$ T-cell level, the proportional hazards assumption was not met for the middle and low $CD8^+$ T-cell tertiles within the $CD4^+$ $\geq$500 cells/mm^3 category (i.e. 667-1065 and $\leq$666 cells/mm^3 groups).

4.0 DISCUSSION

This study examined whether an association exists between baseline total $CD8^+$ T-cell count and the risk of MI, in the setting of HIV, in a large, representative cohort. It is of Public Health importance in that the study findings may help improve the current management of HIV-infected persons, especially with regards to their MI risk.

In the sample of VACS-VC participants assessed, high $CD8^+$ T-cell counts ($\geq$1066 cells/mm^3) were associated with the greatest risk of MI. This was demonstrated in regression models that considered both HIV-infected and HIV-uninfected participants and in secondary analysis restricted to HIV-infected participants. These results are in agreement with an earlier study by Lang et al which showed that, as an independent predictor, high $CD8^+$ T-cell counts are associated with an increased risk for MI [2]. They also agree with studies that looked at surrogates for MI (subclinical carotid artery disease), which showed that the risk was increased in the presence of markers for immune activation, including a low $CD4^+/CD8^+$ T-cell ratio [16, 17]. However, $CD4^+/CD8^+$ ratios may be very similar despite the individual being at different spectrums of HIV disease progression and thus, perhaps, at a different risk for MI (Figure 1). The approach used in this current study attempts to overcome this obstacle by making use of the actual T-cell counts. And it goes a step further by taking into account risk stratification for $CD8^+$ T cells based on clinically-relevant $CD4^+$ T-cell cut-points.

$CD4^+$ T-cell counts have long been established into clinically relevant cut-points [33]. HIV-infected persons with values ≥500 cells/mm^3 are considered closer to a "normal" immune status and experience fewer co-morbid conditions while those with values <200 cells/mm^3 are generally at a higher risk of co-morbid disease and have a severely compromised immune status. $CD4^+$ T-cell counts have thus been used as a proxy for the status of the immune system [33]. Harnessing these facts, an association of $CD8^+$ T-cell counts with MI risk is probably best viewed in the context of the person's immune status. Indeed, these T-cell populations exhibit a dynamism that should be accounted for within the limits of clinical relevance and resources available to research.

When viewed in the context of baseline $CD4^+$ T-cell clinical cut-points, the MI risk associated with the $CD8^+$ T-cell count varied. Persons with a high $CD8^+$ T-cell count may be at most risk for MI events if with a $CD4^+$ T-cell count ≥500 cells/mm^3 or 200–499 cells/mm^3. In contrast, when the $CD4^+$ T-cell count was low (<200 cells/mm^3), the risk of MI appeared to be greatest among those persons with an equally low $CD8^+$ count (≤666 cells/mm^3).

The results from this present study do not disagree with the usefulness of $CD4^+$ T cells in determining the risk of comorbid disease in HIV. Rather they optimize its value in specifically determining the risk of MI in the setting of HIV. These findings were also consistent with the rates of MI observed in this cohort.

The reasons why MI risk associated with $CD8^+$ T-cell count differs depending on the status of the immune system are unclear at present, yet a potential explanation is presented here: HIV-infected individuals who do not have a low immune status (i.e. $CD4^+$ T cell counts ≥500 cells/mm^3 or 200–499 cells/mm^3) appeared to be at greatest risk for MI if their baseline $CD8^+$ T-cell count was also high (≥1066 cells/mm^3). This may be a reflection of the damage to

endothelial cells and arterial walls due to increased amounts of pro-inflammatory cytokines and oxidative stress brought about by such high numbers of this cell type. The immune mechanisms behind the pathogenesis of atherosclerosis (a common precedent for MI) have been well-described in the literature [49].

That HIV-infected Veterans with a low immune status ($CD4^+$ T-cell counts <200 cells/mm^3) appeared to be at greatest MI risk when their $CD8^+$ T-cell count was also low (≤666 cells/mm^3) may not be entirely true. Proportional hazard models restricted to persons above and below the median age of approximately 50 years showed the pattern of $CD8^+$ T-cell effect persisted in the older age group across all $CD4^+$ T-cell strata; i.e. those ≥50 years of age had an increasing MI risk with increasing levels of $CD8^+$ T cells. Some categories lacked statistical significance and this may be due to a reduction in statistical power from the stratification. Veterans below 50 years of age had a different $CD8^+$ T-cell effect in the $CD4^+$ T-cell <200 cells/mm^3 stratum, which did not appear to follow a distinct pattern. This may be due to a relatively lower sample size in these $CD8^+$ T-cell categories for persons in that $CD4^+$ T-cell stratum, leading to a reduction in statistical power. In addition, this $CD4^+$ T-cell stratum is associated with a severely compromised immune status: the competing risks of mortality from other comorbid conditions may have precluded the observance of MI events.

It should be noted that the effects of $CD8^+$ T-cell levels appeared to have a larger effect size for the older age group in all $CD4^+$ T-cell strata: With the exception of the $CD4^+$ T-cell 200-499, $CD8^+$ T-cell ≤666 cells/mm^3 group, more than 50% in each of the $CD8^+$ T-cell categories were at greater MI risk than HIV-uninfected participants across all $CD4^+$ T-cell strata compared to the younger age group. Whether a biological reason might be responsible for this is unclear at present.

In the regression model restricted to HIV-infected participants the trend of increasing MI risk with increasing $CD8^+$ T-cell level compared to the lowest $CD8^+$ tertile may have lacked statistical significance because of the reduced sample size (18,289) which potentially reduced statistical power. Less than half (258) of the 766 MI events observed were among HIV-infected Veterans. However, when $CD8^+$ T-cell count was treated as a continuous variable in the HIV-restricted model, it was observed that the risk of MI increased for every unit increase in $CD8^+$ T-cell count. This supports the findings from the previous results that MI risk does indeed appear to be greater with an increasing $CD8^+$ T-cell level. A linear relationship between $CD8^+$ T-cell counts and MI risk should not be necessarily assumed in this cohort however, as there was a wide range in the observed $CD8^+$ T-cell counts.

It should also be noted that the differences in the effect of $CD8^+$ T-cell counts appeared to be most marked in the group with $CD4^+$ T-cell levels of 200-499 cells/mm^3. This effect was observed in all the regression models and reflected in the observed MI rates as well. This may indicate that that even though a high $CD8^+$ level is associated with an increased MI risk compared to uninfected persons, this relationship may be most useful in persons within this particular $CD4^+$ T-cell level. The violation of proportional hazards for the middle and low $CD8^+$ T-cell tertiles within the $CD4^+ \geq 500$ cells/mm^3 category does not signify that the hazard ratios for these groups are non-informative. The ratios reveal the *average* effect for these groups, with the caveat that these ratios do not change proportionally with time. Moreover, proportional hazards regression models are often robust to slight changes in proportionality: it was observed that the non-proportionality appeared in the latter stages of the follow-up period (see log-log plot in Appendix).

This study confirmed that HIV-uninfected Veterans are at the least risk for MI as suggested by recent research [1]. It also showed that the risk of MI posed by traditional risk factors, demographic variables and other risk factors such as substance abuse, statin use and Hepatitis C virus infection virtually remained unchanged irrespective of whether the $CD8^+$ T-cell count was stratified by $CD4^+$ T-cell clinical cut-points or not. These factors should therefore be considered whenever assessing patients for MI risk.

In addition, this study compared rates of MI with HIV-uninfected persons as part of the study sample, utilized a large cohort and had clinically adjudicated MI outcomes. By making use of baseline $CD4^+$ and $CD8^+$ T-cell counts to determine MI risk, it also closely reproduced the setting in a physician clinic whereby reliable risk stratification for MI (or another comorbid disease) is often based on laboratory values obtained from that clinic visit.

The study had some limitations. It did not explore whether the changes in total $CD8^+$ T-cell counts were due to changes in HIV-specific $CD8^+$ T cells nor did it examine markers for $CD8^+$ T-cell immune activation, such as $CD38^+$. However, it should be noted that these measures are often not available for routine clinic visits and are expensive.

Also, the stratification of the $CD8^+$ T-cell count into tertiles was arbitrary, being based on the cohort and not on pre-existing clinical cut-points. Interestingly, these tertile levels are very similar to those reported in other studies [2, 50]. Stratification of the $CD8^+$ T-cell count into tertiles enabled inclusion of HIV-uninfected Veterans into most of the analyses, through the creation of a "HIV-negative, no $CD8^+$ count" variable. But, because these uninfected persons did not have $CD8^+$ T-cell count measurements, the effects of $CD8^+$ T cells as a continuous variable could not be explored for them. The drawbacks of categorizing data include reduced statistical

power, reduced information, concealed non-linearity relationships and possibly even an increase in Type I error rates.

Another limitation was that time-updated covariates for $CD8^+$ (and $CD4^+$ T cells) were not used to determine MI risk. A time-updated model may have reflected the longitudinal changes in these two cell types and may have shown their relation with MI risk more clearly. Again, it should be stressed that this study was designed to simply determine associations between $CD8^+$ T-cell counts and MI risk, especially associations that would be of immediate relevance when making a clinical decision. Hence, while more accurate prediction of MI risk might have been achievable with a time-updated model or by taking into account measures of $CD8^+$ or $CD4^+$ T-cell nadirs, or recent $CD8^+$ or $CD4^+$ T-cell counts shortly before an MI event, in most clinic situations these parameters are not foreseeable when deciding patient care.

Yet another limitation was the demographic-makeup of the cohort: over 90% of the participants were male, so the study results may not be generalizable to females. Age-related factors may have also confounded the results. The proportional hazard model restricted to persons below the age of 50 years did not show a particular pattern of $CD8^+$ T-cell effect in the $CD4^+$ <200 cells/mm^3 group (as discussed earlier). Future studies could address these problems.

Additionally, while attempts were made to adjust for several known risk factors for MI, some confounding might still persist, especially with an ever-rapidly increasing number of non-traditional risk factors being discovered. However, traditional risk factors for MI still play a major role in its determination [34] and it is believed that adjusting for them, as in this study, will have done much to offset the effects of any residual confounding.

The all-cause mortality rates in the cohort showed that mortality increased with declining immune status and this may have posed some measure of competing-risk for the occurrence of

MI events. Mortality rates were most marked in the $CD4^+$ T-cell <200 cells/mm^3 category which may have affected the study findings in this category. Further research could resolve this issue.

Being a large study cohort, there may also be a limitation due to the presence of missing data. However, the multiple imputation techniques instituted appear to have handled this appropriately, for the observed MI rates were very similar to the estimated hazard ratios for $CD8^+$ T-cell count with and without stratification by $CD4^+$ T-cell count. Although the descriptive rates and also estimated risks of MI were found to have overlapping 95% confidence intervals, this does not necessarily make them equivalent to each other. Indeed, overlapping confidence intervals do not indicate that two or more rates (or risks) are not significantly different [51, 52]. Prior work on the association between $CD8^+$ T-cell subsets and AIDS progression has also revealed some overlap in the confidence intervals of the estimated hazard ratios [15]. Thus a reliance on *P* values for statistical significance alongside formal tests to differentiate between the various hazard-ratios may serve as a useful guide. Such interpretations should also take the sample size and statistical power into account. Tests of equality of the survivor function using Kaplan-Meier estimates showed that there was no statistically significant difference in survival experience between $CD8^+$ T-cell categories among HIV-infected persons, for each level of $CD4^+$ T-cells (see Appendix). Similarly, comparisons of hazard ratios within each $CD4^+$ T-cell level revealed that there was no statistically significant difference in MI risk between the $CD8^+$ categories (analyses not shown). More definitive studies may explain the reasons for this.

5.0 CONCLUSION

In conclusion, data from this longitudinal cohort study of Veterans confirm that MI risk is greater among HIV-infected compared to HIV-uninfected individuals. The results suggest that the $CD8^+$ T-cell count may be associated with an increased risk of MI, but the data are unclear. More specifically, the data would suggest that the $CD8^+$ T-cell count may need to be interpreted in the context of the $CD4^+$ T-cell count or overall immune status. They appear to be most informative when the individual has an immune status that is "near normal" (i.e. $CD4^+$ T-cell count 200-499 cells/mm^3), with a high $CD8^+$ T-cell count being associated with greater MI risk. $CD8^+$ T-cell counts may help optimize $CD4^+$ T-cell clinical cut-points and may offer useful risk stratification for MI. They offer a potential means for improving HIV-patient care using resources already available in most clinic settings. As results from this study are not definitive, more research into the relationship between $CD8^+$ T-cell counts and MI is advised.

APPENDIX: SUPPLEMENTAL TABLES AND FIGURES

Table A.1. Missing Data for Baseline Variables of HIV-uninfected and HIV-infected Veterans

Covariate	Total (n, %)	HIV-uninfected (n, %)	HIV-infected (n, %)
Gender	0	0	0
Race	0	0	0
Diabetes	0	0	0
Blood pressure	1,298 (1.77%)	1,141 (2.07%)	157 (0.86%)
HDL level	18,924 (25.78%)	14,856 (26.96%)	4,338 (23.72%)
LDL level	21,427 (29.19%)	16,546 (30.02%)	4,881 (26.69%)
TG level	15,115 (20.59%)	12,621 (22.90%)	2,494 (13.64%)
Statins use	0	0	0
Smoking history	5,223 (7.12%)	4,233 (7.68%)	990 (5.41%)
Hepatitis C status	0	0	0
eGFR category	7,714 (10.51%)	6,954 (12.62%)	7,714 (4.16%)
BMI $\geq$ 30 kg/ m^2	1,733 (2.36%)	1,570 (2.85%)	163 (0.89%)
Cocaine abuse	0	0	0
Alcohol abuse	0	0	0
Hemoglobin	9,911 (13.50%)	8,478 (15.38%)	1,433 (7.84%)
Baseline $CD4^+$ TCL	1,690 (9.24%)	…	1,690 (9.24%)
Baseline VL	1,448 (7.92%)	…	1,448 (7.92%)
ART Regimen	0	…	0

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; TG, Triglyceride; eGFR, estimated glomerular filtration rate; BMI, body mass index; TCL, T-cell level; VL, Viral load; ART, antiretroviral therapy.

Table A.2. Number of Persons at Risk at Specific Time Points during the Follow-up Period (Risk Tables)

UNSTRATIFIED BY $CD4^+$ T-CELL LEVEL

Risk category	Time = 0 year	Time = 2 years	Time = 4 years	Time = 6 years	Time = 8 years
HIV –	55,124	48,988	40,005	26,613	0
HIV+, $CD8^+$ ≤666	5,988	4,965	3,797	2,564	0
HIV+, $CD8^+$ 667-1065	6,191	5,516	4,516	3,242	0
HIV+, $CD8^+$ ≥1066	6,121	5,498	4,486	3,264	0

STRATIFIED BY $CD4^+$ T-CELL LEVEL

Risk category	Time = 0 year	Time = 2 years	Time = 4 years	Time = 6 years	Time = 8 years
HIV –	55,124	48,988	40,005	26,613	0
$CD4^+$ ≥500					
HIV+, $CD8^+$ ≤666	1,297	1,152	954	707	0
HIV+, $CD8^+$ 667-1065	2,186	2,006	1,689	1,270	0
HIV+, $CD8^+$ ≥1066	2,548	2,330	1,979	1,492	0
$CD4^+$ 200-499					
HIV+, $CD8^+$ ≤666	2,137	1,883	1,523	1,081	0
HIV+, $CD8^+$ 667-1065	2,686	2,410	1,981	1,425	0
HIV+, $CD8^+$ ≥1066	2,580	2,313	1,856	1,366	0
$CD4^+$ <200					
HIV+, $CD8^+$ ≤666	2,554	1,930	1,320	776	0
HIV+, $CD8^+$ 667-1065	1,319	1,100	846	547	0
HIV+, $CD8^+$ ≥1066	993	855	651	406	0

*All T-cell counts in cells/ mm^3
Abbreviations used: HIV- , Human Immunodeficiency Virus uninfected; HIV+, Human Immunodeficiency Virus infected.

Table A.3. Test statistics, Degrees of Freedom and *P* values for the Equality of the Survival Functions for various participant groups

Comparison Groups	Statistic[a]	Value	df	*P* value
All 4 groups stratified by HIV status and $CD8^+$ tertile	Tarone-Ware	35.05	3	<0.001
	Peto-Peto-Prentice	35.27	3	<0.001
For HIV+, $CD8^+$ ≤666 cells/mm^3 compared with $CD8^+$ 667-1065 cells/mm^3	Tarone-Ware	0.00	1	0.9736 [b]
	Peto-Peto-Prentice	0.02	1	0.8793 [b]
For HIV+, $CD8^+$ ≤666 cells/mm^3 compared with $CD8^+$ ≥1065 cells/mm^3	Tarone-Ware	1.13	1	0.2874 [b]
	Peto-Peto-Prentice	1.66	1	0.1971 [b]
For HIV+, $CD8^+$ 667-1065 cells/mm^3 compared with $CD8^+$ ≥1065 cells/mm^3	Tarone-Ware	1.30	1	0.2549 [b]
	Peto-Peto-Prentice	1.42	1	0.2332 [b]
All 10 groups when participants were stratified by HIV status and $CD8^+$ tertile for each $CD4^+$ T-cell level	Tarone-Ware	42.22	9	<0.001
	Peto-Peto-Prentice	42.09	9	<0.001
All $CD8^+$ tertiles, for HIV+ with $CD4^+$ ≥ 500 cells/ mm^3	Tarone-Ware	0.15	2	0.9281 [b]
	Peto-Peto-Prentice	0.31	2	0.8565 [b]
All $CD8^+$ tertiles, for HIV+ with $CD4^+$ 200-499 cells/ mm^3	Tarone-Ware	5.04	2	0.0803 [b]
	Peto-Peto-Prentice	5.60	2	0.0609 [b]
All $CD8^+$ tertiles, for HIV+ with $CD4^+$ <200 cells/ mm^3	Tarone-Ware	0.10	2	0.9497 [b]
	Peto-Peto-Prentice	0.20	2	0.9056 [b]

[a] The Tarone-Ware and Peto-Peto-Prentice tests were chosen because the Kaplan Meier curves of estimated survival function (Figures 3 and 4) showed late differences in survival experience.
[b] Bonferroni correction for alpha level of 0.0167 (i.e. 0.05/ 3) was used for these post-hoc comparisons.
Abbreviations used: HIV- , Human Immunodeficiency Virus uninfected; HIV+, Human Immunodeficiency Virus infected.

Table A.4. Test statistics of Scaled Schoenfeld Residuals, Degrees of Freedom and *P* values for the tests of Proportional-Hazards assumptions for various proportional-hazards models using different functions of time

UNSTRATIFIED BY $CD4^+$ T-CELL LEVEL

		g(t) = t		g(t) = ln (t)		g(t) = $\hat{S}_{KM}$ (t)		g(t) = rank (t)	
$CD8^+$ category	**df**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**
HIV –	1	…	…	…	…	…	…	…	…
HIV+, $CD8^+$ ≤666	1	1.86	0.1731	2.54	0.1111	1.54	0.2140	2.15	0.1428
HIV+, $CD8^+$ 667-1065	1	0.37	0.5455	1.11	0.2923	0.23	0.6341	0.41	0.5196
HIV+, $CD8^+$ ≥1066	1	0.38	0.5391	0.75	0.3850	0.46	0.4970	0.36	0.5513

STRATIFIED BY $CD4^+$ T-CELL LEVEL

		g(t) = t		g(t) = ln (t)		g(t) = $\hat{S}_{KM}$ (t)		g(t) = rank (t)	
Category	**df**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**	**chi^2 value**	***P* value**
$CD4^+$ ≥500									
HIV+, $CD8^+$ ≤666	1	5.75	0.0165	5.72	0.0168	5.52	0.0188	6.28	0.0122
HIV+, $CD8^+$ 667-1065	1	6.46	0.0110	5.99	0.0144	5.90	0.0151	6.66	0.0098
HIV+, $CD8^+$ ≥1066	1	1.89	0.1696	1.00	0.3177	2.15	0.1422	1.80	0.1800
$CD4^+$ 200-499									
HIV+, $CD8^+$ ≤666	1	0.00	0.9773	0.19	0.6607	0.01	0.9285	0.01	0.6523
HIV+, $CD8^+$ 667-1065	1	1.10	0.2947	0.18	0.6722	1.34	0.2465	1.04	0.3081
HIV+, $CD8^+$ ≥1066	1	0.21	0.6463	0.60	0.4382	0.25	0.6168	0.20	0.6523
$CD4^+$ <200									
HIV+, $CD8^+$ ≤666	1	0.13	0.7157	0.13	0.7168	0.10	0.7567	0.15	0.6998
HIV+, $CD8^+$ 667-1065	1	0.24	0.6273	0.03	0.8674	0.23	0.6304	0.23	0.6287
HIV+, $CD8^+$ ≥1066	1	2.70	0.1006	0.61	0.4363	2.86	0.0909	2.62	0.1053

*All T-cell counts in cells/mm^3

Abbreviations used: t , time; ln (t) , natural logarithmic transformation of time; $\hat{S}_{KM}$ (t) , Kaplan-Meier transformation of time; rank (t) , rank transformation of time; HIV- , Human Immunodeficiency Virus uninfected; HIV+, Human Immunodeficiency Virus infected.

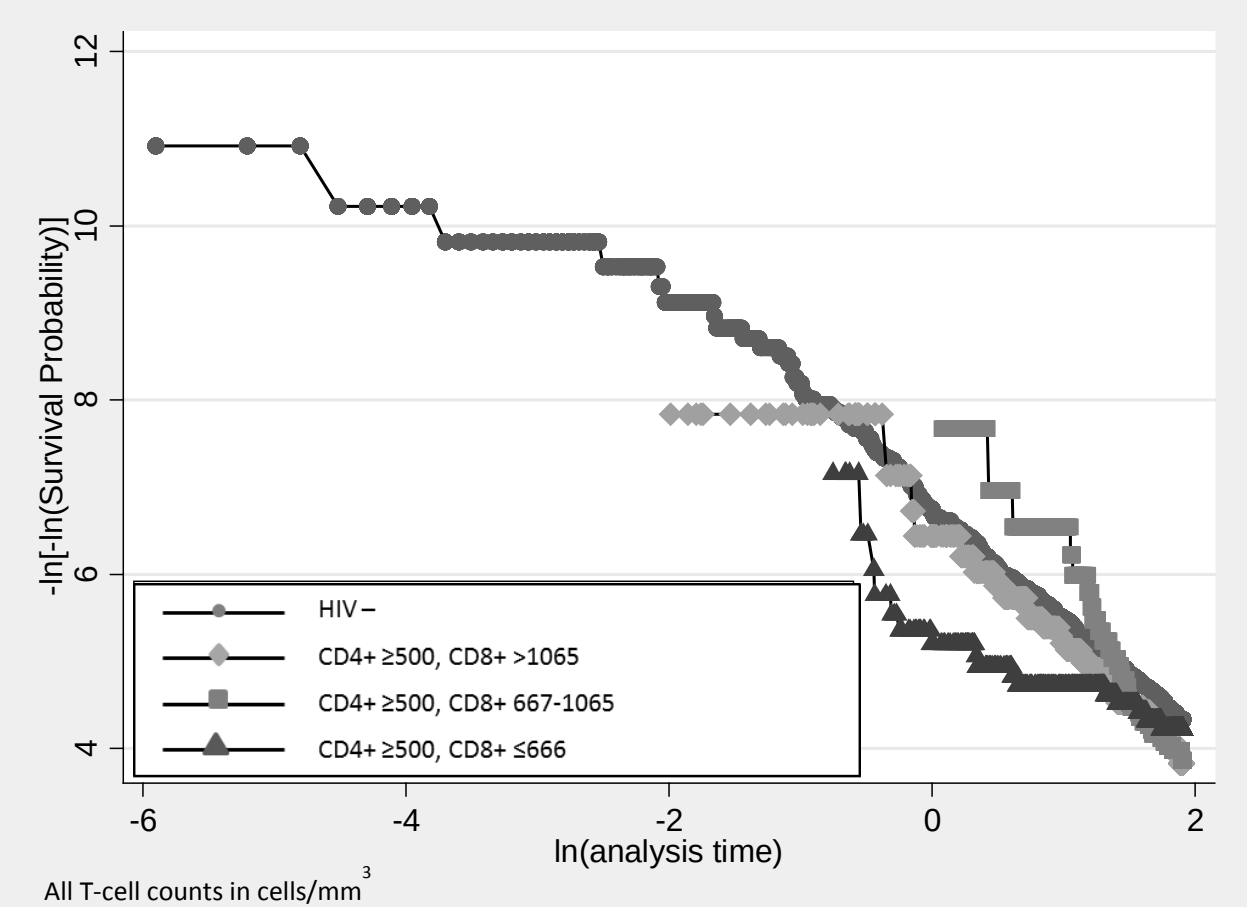

Figure A.1. Log-log plot testing Proportional Hazards Assumptions for three HIV+, CD8$^+$ T-cell categories compared to the HIV-uninfected group.

Abbreviations used: HIV- , HIV-uninfected; HIV+ , HIV-infected.

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