# Effect of Antiretroviral Therapy on Damage-Associated Molecular Patterns (DAMPs), Lipopolysacharide (LPS),and Immune Reconstitution in HIV-Infected Individuals

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**Abstract**

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BACKGROUND: Even with successful antiretroviral therapy (ART), HIV infection is still accompanied by ongoing chronic immune activation and inflammation that may impact ART-mediated immune reconstitution. The mechanisms of this immune activation are not completely defined. Damage-associated molecular patterns (DAMPs) are endogenous innate immune activators that have not been well studied in HIV-infected persons.

METHODS: We conducted a quasi-experimental pre-post observational study of two DAMPs (HMGB1 and S100A9) and a marker of microbial translocation (LPS) in samples collected from research participants before and at least 2 years after initiation of continuously suppressive ART. Differences in mean biomarker levels were assessed using paired t-tests. Correlation between biomarker levels were assessed using Pearson correlation coefficients for normal data and Spearman's rho for non-normal data. Multivariate linear regression was used to assess association between biomarker values and clinical outcomes after suppressive ART.

RESULTS: Mean HMGB1 levels increased between pre- and post-ART samples (1.95 ng/mL vs. 3.02 ng/mL, p=0.01) and the proportion of individuals with detectable S100A9 increased significantly (p=0.01). We detected no change in mean LPS levels with effective ART (p=0.85). Neither LPS, HMGB1, nor S100A9 was associated with baseline CD4 or viral load or degree of CD4 reconstitution with effective ART-mediated viral suppression.

CONCLUSIONS: DAMPs do not appear to be significantly associated with CD4 count, viral load, or degree of CD4 reconstitution after virologic suppression. Increased HMGB1 levels after suppressive ART may be a non-specific marker of inflammation and hence subject to confounding by other conditions.

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# Chapter 1

## Introduction and Background

### *HIV Pathogenesis and Immune Activation*

Persistent T cell activation, as measured by increased levels of CD38+ and HLA-DR+ T cells is a hallmark of untreated HIV infection. It has been associated with rate of T cell decline, time to AIDS, and death independent of viral load (1, 2). The later suggests that viral replication alone is not responsible for HIV pathogenesis. The role of chronic inflammation in HIV pathogenesis has been an area of intense interest over the past decade (3, 4). This is highlighted by studies showing improvement, but generally not normalization, of markers of innate immune and T cell activation and systemic inflammation in individuals with ART-mediated viral suppression compared to HIV-uninfected controls in case control studies (5-8). More recently, innate immune activation has been shown to be involved in HIV pathogenesis and related, at least partially to gut epithelial barrier dysfunction and microbial translocation (9-11). This persistent immune activation and systemic inflammation, despite ART-mediated viral suppression, is associated with impaired immune reconstitution and death (12-15).

### *Damage-Associated Molecular Patterns*

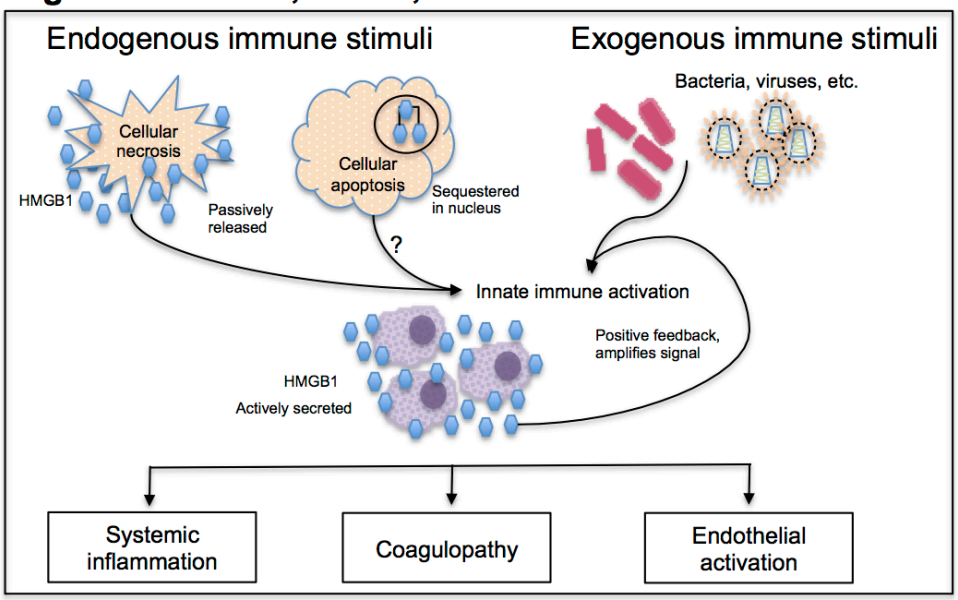
Damage-associated molecular patterns (DAMP) or alarmins are a diverse class of endogenous innate immune activation molecules released by dead or dying cells. DAMPs are capable of activating the same immune pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and the receptor for glycosylated end-products (RAGE), as more traditional exogenous pathogen-associated molecular patterns (PAMP) (e.g. lipopolysaccharide (LPS)) (16). In either case, the signals are recognized by cells of the innate immune system and result in the activation of downstream pro-inflammatory signaling pathways. DAMPs may work alone or as effectors with PAMPs to mediate both sterile and infectious inflammatory responses. DAMPs have been shown to be involved in the pathogenesis of a range of inflammatory clinical conditions including sepsis (17-19), auto-immune conditions (20, 21), malignancies (22-24) and cardiovascular disease (25-27).

Although several small studies have reported elevations in the levels of specific DAMPs in HIV infection and decreases in these elevations after successful ART (28, 29), the contribution of DAMPs to HIV pathogenesis has not been thoroughly elucidated. No studies to date have examined the association of effective ART-associated viral suppression on the prototypical DAMPs HMGB1 and S100A9, their correlation to LPS levels as measured by LAL, and their association with ART-mediated CD4 reconstitution.

*High Mobility Group Box 1*

HMGB-1 is nuclear binding protein important in transcriptional regulation and is present in most human cell types. It is unique in that it can be released into systemic circulation by at least three distinct mechanisms: 1) it can be liberated during cellular necrosis or pyroptosis, which may be an important driver of HIV pathogenesis (30); 2) although HMGB-1 is not released in programmed apoptotic cell death due to its irreversible binding to chromatin, engulfing macrophages may secrete HMGB-1 in the setting of massive apoptosis (31); 3) it can be actively secreted, primarily by myeloid and NK cells, upon activation by inflammatory stimuli. Macrophages, neutrophils, and plasmacytoid dendritic cells (pDCs) that become activated by HMGB1 release various pro-inflammatory cytokines, including IL-1$\alpha$, IL-6, IL-8 and IL-12 (32).

**Figure 1. HMGB1, sterile, and infectious inflammation**



HMGB1 may be a common factor in multiple inflammatory pathways in HIV infection. One study showed that HMGB1 levels were elevated in untreated HIV-infected individuals compared to matched uninfected controls (33). Several subsequent in vitro studies have shown increased HIV-1 replication in T-cell, dendritic, and monocytic cells lines in the presence of HMGB1 (34-36), although one study showed HMGB1 to be an inhibitor of viral replication in a monocytic cell line (37). More recently, HMGB1 has been associated with higher viral loads and to be reduced with ART in a study of 32 HIV-infected patients in Sweden (38).

The same group subsequently presented data using an ex vivo cell model suggesting that LPS and HMGB1 may act synergistically to induce viral replication in vitro (38). HMGB1 and LPS may also work synergistically to increase monocyte activation through enhanced binding of soluble CD14, an LPS receptor (39). Lastly, activated macrophages may secrete HMGB1 in a feedback mechanism to enhance cellular activation. These combined data suggest that HIV-associated cell death, via pyroptosis or other mechanisms (30), leads to the release of HMGB1, which, either alone or in concert with translocated LPS, may drive a chronic inflammatory state in HIV infection. The degree to which this inflammatory state is normalized with effective ART remains a subject of active investigation.

*S100A9*

The S100 proteins (also known as calprotectin and myeloid-related proteins) are a diverse group of calcium regulatory proteins involved in a variety of intracellular and extracellular processes (40). Among the best characterized of the S100 family of proteins is S100A9, which is involved in the regulation of innate immune activation and inflammation through a variety of extracellular receptors, including TLR4 and the receptor for advanced glycation end-products (RAGE). The S100 proteins may induce inflammatory cytokine production in macrophages, including TNF-α, IL-1β, IL-6, and IL-8 (41, 42). Two studies in the pre-ART and early-ART eras showed that S100A9 was elevated in untreated HIV infection (43, 44). Elevated levels have been shown to induce viral transcription in in vitro female genital tract (45, 46) and CD4 cell (47) models. The role of S100A9 in HIV pathogenesis and the impact of ART on S100A9 levels is unknown.

*Lipopolysaccharide in HIV Pathogenesis*

Numerous studies in pathogenic primate simian immunodeficiency virus (SIV) and in HIV infection have shown evidence of impaired gut mucosal integrity, massive depletion of gut-associated lymphoid tissue (GALT) T cells, and microbial translocation of gut products, primarily LPS, across the impaired gut into systemic circulation (3, 48-51). LPS is a potent exogenous activator of the innate immune system (3, 52), particularly monocytes, via pattern recognition receptors (PRRs), particularly toll-like receptor 4 (TLR4).

LPS may drive T cell activation in chronically HIV-infected individuals (3, 53), although one study did not detect a correlation between LPS levels and T cell activation during short-term ART interruption (54). LPS levels have been shown to be associated with HIV disease progression (55, 56), markers of innate immune activation (11), and mortality (11). One longitudinal study conducted in Uganda observed no association between LPS levels and disease progression(57). Surrogate markers of monocyte activation, including soluble CD14 (sCD14) have been associated with mortality in treated HIV infection (58, 59). Although sCD14 binds LPS and facilitates TLR4 activation, it does not reflect only LPS-induced activation, but may also reflect monocyte activation through additional pathways (60).

Discordant results from published studies are likely explained by a combination of differing patient populations; the balance of treated, as opposed to untreated HIV-infection in a given cohort; and difficulties in performing many of these assays. In particular, the LAL assay used to measure plasma LPS concentration has poor reproducibility, can be affected by plasma inhibitors, and is known to generate false positive results due to some non-LPS antigens, including $\beta$ D glucan (61). Although the hypothesis that microbial translocation drives innate immune activation and pathogenesis in HIV infection is widespread, it is important to note that a mechanistic role has not been demonstrated for either LPS or sCD14 in HIV-1 disease progression or the pathogenesis of specific morbid events

**Table 1.** Biomarkers and functions

| BIOMARKER | FUNCTION |
|---|---|
| *DAMPs/Alarmins* | |
| HMGB1 | Normal function: nuclear protein involved in transcriptional regulation<br>Inflammatory function: secreted by immune cells, released passively during necrosis and pyroptosis and indirectly in apoptosis |
| S100 | Normal function: family of proteins that regulate phosphorylation and calcium homeostasis, constitutively expressed in neutrophils<br>Inflammatory function: induced in monocytes and smooth muscle cells |
| *Innate Immune Activation* | |
| Limulus Amebocyte Lysate | Indirect measure of lipopolysaccharide released from bacteria |

*Significance*

Even with successful HIV viral suppression with ART, life expectancy in treated HIV infection likely remains shorter than in the general population (62). There is an urgent need for therapies that may minimize ongoing inflammation in treated HIV infection. However, the underlying mechanisms of this inflammation remain a subject of debate. DAMPs are potent activators of the innate immune system, are likely released during pyroptosis, and have been shown to be involved in immune activation and inflammation in rheumatologic conditions and malignancies, though whether their role is causal or secondary is not clear. However, data on DAMPs in HIV infection, particularly HIV infection treated with modern ART regimens, are limited. Whether DAMPs are mechanistically involved in HIV-associated inflammation and as such may impact ART-mediated CD4 reconstitution or are merely bystanders remains unknown. Here, in this preliminary study, we examined levels of LPS and of two important DAMPs (HMGB1 and S100A9) in HIV-infected individuals before and after successful suppressive ART.

*Specific Aims*

Combination ART has dramatically decreased morbidity and mortality in HIV-infected individuals (63-65). However, even with ART-mediated viral suppression the average lifespan of HIV-infected persons may remain shorter than in HIV-uninfected persons (66, 67), and age and inflammation-associated conditions such as cardiovascular disease (68-70), malignancies (71-73), renal (74-77), and liver disease (78-80) likely occur more frequently and at a younger age in HIV-infected individuals. Much of this increased risk

is attributed to chronic inflammation and immune system activation even in treated HIV infection (4, 81). The mechanism(s) of persistent immune activation in treated HIV have not been fully elucidated. DAMPs are a heterogeneous class of endogenous immune activators that have not been extensively studied in HIV infected individuals. These molecules, if present due to ongoing cell death or microbial translocation, could be a cause of persistent immune activation and inflammation in HIV-infected individuals with virologic suppression and as such could hinder ART-mediated immune reconstitution.

Our study's specific aims were as follows:

**SPECIFIC AIM 1: To determine whether ART-mediated HIV viral suppression decreases levels of DAMPs (HMGB1, S100A9) and LPS.**

*Hypothesis addressed by this aim:*

a) Levels of LPS, HMGB1, and S100A9 will decrease with effective ART-mediated viral suppression.

**SPECIFIC AIM 2: To assess the correlation between DAMPs and LPS levels.**

*Hypothesis addressed by this aim:*

a) DAMP (HMGB1 and S100A9) levels pre- and post-ART will be not be correlated with LPS levels.

**SPECIFIC AIM 3: To determine if DAMPs and LPS levels are correlated with pre-ART HIV viral load, immunosuppression (pre-ART), or immune reconstitution (post-ART)**.

*Hypotheses addressed by this aim:*

a) LPS and DAMP levels will be associated with HIV viral load in untreated (pre-ART) HIV infection.

b) Elevated levels of all markers will be associated with HIV-associated immunosuppression (pre-ART) as reflected in pre-ART CD4 counts.

c) Elevated levels of all markers and change from pre to post-ART values will be associated with degree of immune reconstitution post-ART as reflected in the difference between individuals' pre and post-ART CD4 counts.

## Chapter 2

## Methods

*Study Design*

The study was designed as a quasi-experimental pre-post observational study of biomarker levels in samples collected from research participants before and after initiation of suppressive ART.

*Study Setting*

The study was conducted using de-identified data and specimens obtained from HIV-infected individuals enrolled in the University of Washington (UW) HIV Cohort and who consented to specimen collection as part of the University of Washington Center for AIDS Research (CFAR) Specimen Repository. Specimens were collected in the UW Harborview Medical Center Madison Clinic by trained study nurses using established protocols. The UW Human Subjects Division issued a non-human subjects determination for this project (#48199).

*Study Subjects*

All patients enrolled in the UW HIV cohort were eligible to participate in this study. Cohort inclusion criteria include: ≥18 years of age, attendance at ≥2 primary care visits at an affiliated clinic, and provision of informed consent both for inclusion of clinical data and for biologic specimen donation for research. Fifty-one HIV-infected individuals with both of the following plasma specimens available were selected for this sub-study: 1) a specimen prior to starting the initial HAART regimen; 2) a specimen at least 2 years after documented viral suppression. In order to minimize the likelihood that productive HIV replication might be responsible for variation in our biomarker values, cohort participants were excluded from this study if they had any documented HIV viral load >400 copies/mL in between the two specimen times or if they developed an AIDS-defining illness or any malignancy (excluding non-melanoma skin cancer). We examined the mean viral loads per year for each participant to ensure that we were not selecting for individuals with poor engagement in care (Appendix 1).

*Data Collection*

Cryopreserved plasma specimens were obtained from the associated specimen repository. Data from HIV-infected individuals were extracted from the UW HIV Information System (UWHIS). The UWHIS captures a broad range of clinical information through prospective data collection in routine clinical care. It has established standards for terminology, format, data verification, and quality assurance (82). Data validation and integrity checks occur in multiple stages at the collection site and at the repository. Data are maintained on 9 domains: 1) diagnoses, 2) laboratory data, 3) medications, 4) demographics, 5) health care utilization, 6) vital status, 7) patient reported outcomes (PROs), 8) genotypic resistance, and 9) biologic specimens.

*Specimen Collection and Storage*

Specimens for the UW CFAR specimen repository are collected, processed, and transported according to current Department of Allergy and Infectious Diseases AIDS Clinical Trial Group (ACTG) protocols. All specimens are processed and maintained by the UW CFAR Virology Laboratory led by Dr. Robert Coombs and accessioned into the standard Laboratory Data Management System (LDMS).

In brief, whole blood specimens are collected in 10 mL purple-top (EDTA) tubes. All specimens are transported to the CFAR Virology Laboratory on ice using leak-proof, crush resistant, biohazard-labeled containers and are processed as soon as possible (within 30 hours of collection). Upon receipt, sample labels, paperwork, and specimen condition are verified. Specimens are logged in LDMS and processed according to the ACTG Specimen Processing Guide. Specimens are centrifuged at 400xg for 10 minutes to separate cells and plasma, then the plasma is transferred to a sterile centrifuge tube before being centrifuged again at 800xg for 10 minutes to remove any contaminating debris, cells, and platelets. The plasma is then aliquoted into cryovials and placed in labeled storage boxes at -70°C.

Specimens were obtained from the UW CFAR repository and transported on dry ice to the Liles Laboratory where they were verified and placed back into ultra-low temperature freezers until processing.

*Data Storage and Management*

Clinical data for the UW HIV cohort are maintained and managed by the Clinical Epidemiology and Health Services Research Core of the UW Center for AIDS Research, led by Dr. Mari Kitahata.

*Variable Definitions*

Variable definitions to be used in this study were based on validated published definitions (Table 2).

**Table 2. Operational definitions**

| **Covariate** | **Definition/Notes** |
|---|---|
| Age | Modeled as continuous variable |
| Sex | Modeled as binary male/female |
| Pre and post-ART CD4 | Measures of CD4 count were modeled as both a continuous variable and using clinically meaningful categories (<100, 100-199, 200-349, 350-499, >=500; <350, >=350 cells/ml) |
| Pre-ART HIV viral load | Measures of viral load were modeled as log transformed continuous variables and using clinically meaningful categories (<400, 400-9,999, >10,000 copies/ml) |
| Hepatitis C infection | Defined as the presence of any of the following: a positive HCV antibody, a detectable HCV RNA measurement, or an HCV genotype |
| Hepatitis B Infection | Defined as a positive HBsAg or detectable HBV DNA |
| HIV transmission risk factor | Men who have sex with men, injection drug use, heterosexual, and other. Men who have sex with men and are injection drug users are classified as injection drug users for analysis. |
| First ART medication class | Non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase strand transfer inhibitor (InSTI) |

*Laboratory Assays*

HMGB1 was measured by commercially available enzyme-linked immunosorbent assay (ELISA) available from the Shino-Test Corporation (Tokyo, Japan). S100A9 was measured by the commercially available ELISA assay from R & D Systems (Minneapolis, MN). The LAL assay is commercially available from the Lonza Corporation (Basel, Switzerland). All assays were performed according to package insert instructions. Assays were run in the Liles Lab by Dowon An and were overseen by Dr. Liles.

**Data Analysis**

*Specific Aim 1*

In order to determine if effective ART reduces levels of inflammation as measured by HMGB1, S100A9, and LPS, we compared HMGB1, S100A9, and LPS levels before and after ART-mediated viral suppression. Biomarker assay values were assessed for normality using the Shapiro-Wilk test and visually using scatterplots. Non-normal absolute results for either pre or post-ART or the difference between an individual's pre and post-ART values were log transformed. We assessed for outliers using both visual inspection and studentized residuals. Data were explored using either continuous or dichotomous (detectable vs. below the limit of quantification) measures. Dichotomous measures were used when >50% of results were below the limit of detection of the assay. Pre- and post-ART paired samples were compared using a paired t-test for continuous variables and a McNemar's test for dichotomous variables. Two-sided p values less than 0.05 were considered significant.

*Specific Aim 2*

In order to determine if HMGB1 and S100A9 levels were correlated with LPS levels, we assessed correlations between HMGB1, S100A9, and LPS levels before and after ART using either Spearman's rho or Pearson's correlation coefficients (when log transformation of biomarker values led to linearity). Two-sided p values less than 0.05 were considered significant.

*Specific Aim 3*

We used linear regression analysis with robust confidence intervals to determine if any of the biomarkers predicted: 1) baseline CD4 count 2) baseline HIV viral load; and 3) change in CD4 count pre and post-ART adjusted for baseline CD4. Bivariable associations were determined prior to multivariable modeling. We planned a priori that if either of the two DAMPs was associated with any outcome, we would add LPS to the model to determine if the effect of DAMPs on this outcome was mediated by LPS levels.

*Study Power*

The study was designed to detect a difference in mean (pre-ART($m_1$), post-ART($m_2$)) values of pre and post-ART values for the biomarkers studied. Based on paired two-sample means test comparing $H_0$: $m_2 = m_1$ versus $m_2$ != $m_1$. Using mean HMGB1 levels ($m_1$ = 3.5 ng/ml, $m_2$ = 1.5 ng/ml) and associated standard deviations ($SD_1$ = 2.9 ng/ml, $SD_2$ = 1.1 ng/ml) in individuals pre and post-ART derived from published literature (83), assuming two-sided $\alpha = 0.05$, $\beta = 0.80$, the requisite sample size to detect a significant difference in means between the two groups is: N=18 if (r=0.3), N=15 if (r=0.5). Using mean LPS levels ($m_1$ = 62.5 pg/ml, $m_2$ = 45 pg/ml) and associated standard deviations ($SD_1$ = 35 pg/ml, $SD_2$ = 37 pg/ml) in individuals pre and post-ART derived from published literature (29), assuming two-sided $\alpha = 0.05$, $\beta = 0.80$, the requisite sample size to detect a significant difference in means between the two groups is: N=49 if (r=0.3), N=36 if (r=0.5). No published data exists on the impact of ART on S100A9 levels. We have paired pre and post-ART samples available from 51 HIV-infected individuals. Sample size calculations were performed in Stata 13.1 (College Station, Texas).

## Chapter 3

## Results

*Population*

Our sub-study population included 51 individuals who started ART between 2003 and 2011. Eighty-four percent of study participants were men, 73% were men who have sex with men, and 35% were injection drug users. The mean age at the date of initial sample collection was 42 years old. Mean pre and post-ART CD4 counts were 315 and 656 cells/ml, respectively, with a mean of three years of suppressive ART between samples. Additional baseline characteristics are outlined in Table 3.

| **Table 3. Baseline Characteristics** | |
|---|---|
| **Variable** | **n (%)** |
| Male | 43 (84) |
| HIV Transmission Risk | |
| Men who have sex with men | 25 (49) |
| Injection drug use | 18 (35) |
| Heterosexual | 8 (16) |
| Age at Pre-ART sample | |
| <40 | 21 (41) |
| 40-49 | 18 (35) |
| 50-59 | 11 (22) |
| >=60 | 1 (2) |
| Hepatitis C infection | 19 (37) |
| Hepatitis B infection | 2 (4) |
| ART Regimen | |
| PI | 22 (43) |
| NNRTI | 25 (49) |
| InSTI | 4 (8) |
| | **Median [IQR]** |
| Pre-ART CD4 | 315 [209, 378] |
| Pre-ART HIV RNA | 64200 [13900, 154000] |
| Post-ART CD4 | 656 [392, 785] |
| Delta CD4 | 326 [117, 508] |
| Days between pre & post-ART samples | 1105 [894, 1474] |

*Impact of ART-mediated Viral Suppression on DAMP and LPS Levels*

We detected no significant difference in mean LPS and S100A9 levels before and after successful ART-mediated viral suppression, but did detect a significant increase in mean HMGB1 levels

from 1.95 before to 3.02 ng/ml after ART (p=0.01) (Table 4, Figure 2). Because 80% of S100A9 levels prior to ART initiation were at or below the limit of detection of the assay (0.005 pg/ml), the variable was redefined as a dichotomous variable (i.e., above or below the limit of detection of the assay) for subsequent analysis. The proportion of individuals with undetectable S100A9 pre and post-ART was tested using McNemar's chi-square and values differed significantly pre and post-ART. The proportion of individuals with detectable S100A9 was 19.6% pre-ART and 43.1% post-ART (p=0.01 by McNemar's test).

**Table 4. Pre and Post ART Results**

| Biomarker | Pre-ART Mean (SD) | Post-ART Mean (SD) | p-value |
|---|---|---|---|
| LPS (EU/ml) | 0.20 (0.06) | 0.20 (0.04) | 0.85 |
| HMGB1 (ng/ml) | 1.95 (1.72) | 3.02 (1.98) | 0.01 |
| S100A9 (pg/ml) continuous | 3.54 (10.08) | 3.16 (8.23) | 0.80 |

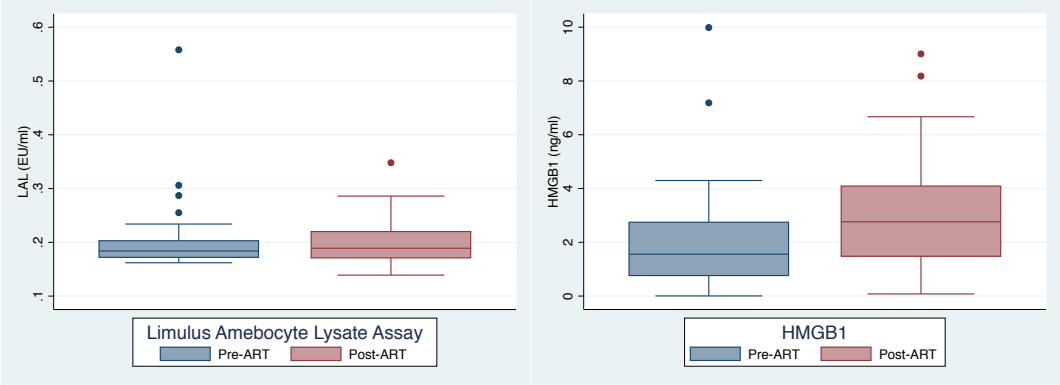



Figure 2. Mean Pre and Post-ART LPS and HMGB1 Levels

*Correlation Between DAMPs and LPS Levels*

We found no correlations between either pre- and post-ART levels of each individual biomarker or between DAMP and LPS levels (Table 5).

**Table 5. Biomarker Correlation**

| Biomarkers | Spearman's rho | p-value |
|---|---|---|
| Pre-ART LPS, Pre-ART HMGB1 | 0.15 | 0.28 |
| Pre-ART LPS, Pre-ART S100 | 0.11 | 0.45 |
| Post-ART LPS, Post-ART HMGB1 | -0.16 | 0.26 |
| Post-ART LPS, Post-ART S100 | -0.19 | 0.19 |
| Pre-ART LPS, Post-ART LPS | -0.08 | 0.56 |
| Pre-ART HMGB1, Post-ART HMGB1 | -0.07 | 0.65 |
| Pre-ART S100, Post-ART S100 | 0.14 | 0.32 |

*Correlation Between Pre-ART DAMP, CD4, and HIV RNA Levels*

The median pre-ART CD4 count was 315 cells/ml [IQR: 209, 378] and HIV RNA was 64,200 [IQR: 13900, 154000]. In bivariable analysis using linear regression looking at pre-ART CD4 count as the dependent variable, the following variables were assessed as independent predictors: LPS, HMGB1, S100A9, HIV RNA, age, sex, and hepatitis B and C infections. Only log HIV RNA (p=0.11), male sex (p=0.17), and detectable S100A9 (p=0.17) had associations with baseline CD4 count significant at p<0.20 in univariate analysis (Table 6). In a similar bivariable analysis examining log-transformed pre-ART HIV RNA levels as the dependent variable, LPS (p=0.07), CD4 (p=0.11), male sex (p=0.14), and HCV infection (p=0.10) had associations significant at p<0.20. In multivariable analysis exploring pre-ART HIV RNA levels using the aforementioned variables, no significant associations were identified.

| Table 6. Bivariate associations with baseline CD4 Count and log transformed HIV RNA | | | | |
|---|---|---|---|---|
| **Dependent variable** | **Covariate** | **β coefficient** | **95% CI** | **p-value** |
| **CD4 count** | | | | |
| | LPS | -97.76 | [-800.64, 605.12] | 0.78 |
| | HMGB1 | -9.29 | [-33.38, 14.80] | 0.44 |
| | Detectable S100A9 | -69.97 | [-171.78, 31.85] | 0.17 |
| | Log HIV RNA | -3.19 | [-29.03, 22.65] | 0.11 |
| | Age | 0.13 | [-4.34, 4.61] | 0.95 |
| | Male | 78.82 | [-34.30, 187.95] | 0.17 |
| | HCV | 1.49 | [-83.73, 86.70] | 0.97 |
| | HBV | 79.47 | [-131.56, 290.50] | 0.45 |
| | | | | |
| **Log HIV RNA** | | | | |
| | LPS | 5.85 | [-2.09, 13.78] | 0.15 |
| | HMGB1 | -0.08 | [-0.36, 0.21] | 0.59 |
| | S100A9 detectable | -0.29 | [-1.49, 0.91] | 0.63 |
| | CD4 (per 100) | -0.04 | [-0.37, 0.29] | 0.81 |
| | Age | -0.04 | [-0.09, 0.16] | 0.17 |
| | Male | **1.68** | **[0.47, 2.89]** | **0.01** |
| | HCV | 0.28 | [-0.72, 1.28] | 0.58 |
| | HBV | -0.53 | [-2.96, 1.91] | 0.67 |

*Relationship Between Pre-ART DAMP Levels, Changes in DAMP levels and CD4 Reconstitution*

The median post-ART CD4 count was 656 cells/ml [IQR: 392, 785]. Median ART-mediated increase in CD4 after ART start was 326 cells/ml [IQR: 117, 508]. A mean of 1236 days elapsed between pre and post-ART CD4 results (SD: 413 days; range 723-2560 days). In bivariate analysis time elapsed in years between ART start and post-ART sample collection and pre-ART CD4 were significantly associated with CD4 reconstitution (Table 7). Neither pre-ART biomarker values, nor changes between pre and post-ART values were associated with change in CD4 count on ART.

| Table 7. Univariate predictors of post-ART CD4 Reconstitution | | | |
|---|---|---|---|
| **Covariate** | **β coefficient** | **95% CI** | **p-value** |
| Pre-ART LPS | 194.88 | [-1110.13, 1499.89] | 0.77 |
| Change in LPS | -33.98 | [-1082.65, 1014.69] | 0.95 |
| Pre-ART HMGB1 | 4.67 | [-40.32, 49.66] | 0.84 |
| Change in HMGB1 | 1.12 | [-26.78, 29.02] | 0.94 |
| Pre-ART S100A9 Detectable | 5.90 | [-186.79, 198.60] | 0.95 |
| Change in S100A9 | -0.85 | [-7.93, 6.22] | 0.81 |
| Log Pre-ART HIV RNA | 6.74 | [-40.41, 53.88] | 0.77 |
| Pre-ART Age | -0.53 | [-8.84, 7.78] | 0.90 |
| Male | -114.78 | [-322.56, 93.00] | 0.27 |
| HCV | -78.40 | [-235.04, 78.23] | 0.32 |
| HBV | 36.06 | [-357.95, 430.08] | 0.86 |
| Per year of ART | **82.26** | **[18.14, 146.37]** | **0.01** |
| Pre-ART CD4 | **-0.52** | **[-1.03, -0.005]** | **0.05** |

## Chapter 4

## Discussion and Conclusions

To our knowledge, this is the first study to assess whether pre-ART DAMP levels or changes in DAMP levels on ART are associated with the degree of CD4 reconstitution on effective ART. In addition, we explored whether DAMP levels were correlated with levels of LPS, an indirect marker of microbial translocation, which has been proposed to be involved in HIV pathogenesis. Our results are largely negative, and we did not find any correlation between DAMP levels and LPS levels. Nor were any of these markers correlated with either pre-ART HIV RNA levels or pre-ART CD4 cell count – two markers clinically associated with disease progression.

Interestingly we did observe an increase in mean HMGB1 levels in the post-ART compared to pre-ART samples. This result is in contrast to the one published study that reported a decrease in HMGB1 levels after virologic suppression on ART (83). The results of the prior study were largely driven by participants who had lower CD4 and higher viral loads at ART initiation (83). The authors of the study point out that these individuals were largely recent immigrants raising the possibility that the decrease in HMGB1 levels may have been related to the treatment of co-infections rather than a direct effect of viral suppression. Whether this discrepancy is due to differences in the underlying patient populations studied, including differing CD4 counts or other unmeasured factors is unclear. One potential explanation for elevated HMGB1 levels post-ART is that HMGB1 exists in multiple redox states *in vivo (84, 85)*, only one of which is known to be pro-inflammatory. It is possible, though speculative, that as HIV viral replication is controlled by ART that over time on balance the redox state of HMGB1 changes to its non-inflammatory form through oxidation of three key cysteine residues. Unmeasured confounding could also account for these findings particularly in a cohort with high HCV prevalence, suggesting at least a history of injection drug use.

### *Limitations*

As with any non-randomized study, we are unable to make any statements about causality, and unmeasured or time-dependent confounders may account for differences seen in biomarker values.

These samples are taken from a specific population – mostly white men who have sex with men, who initiated ART with a moderate degree of immunosuppression and achieved and maintained successful virologic suppression with ART. As such, our findings may not be applicable to other populations, including individuals with severe immunosuppression and untreated HIV infection. Because we did not have multiple samples available before and after treatment, we are not able to comment on intra-subject variation and non-differential measurement error in the biomarkers studied. If we did have multiple samples, it is possible that more precise results could be obtained by averaging intra-subject measures over time. While we did not directly assess ART adherence, maintenance of persistently suppressed HIV viral loads is possible only with good ART adherence. By excluding individuals with detectable viral loads, we have focused on the subset of the population with good ART adherence.

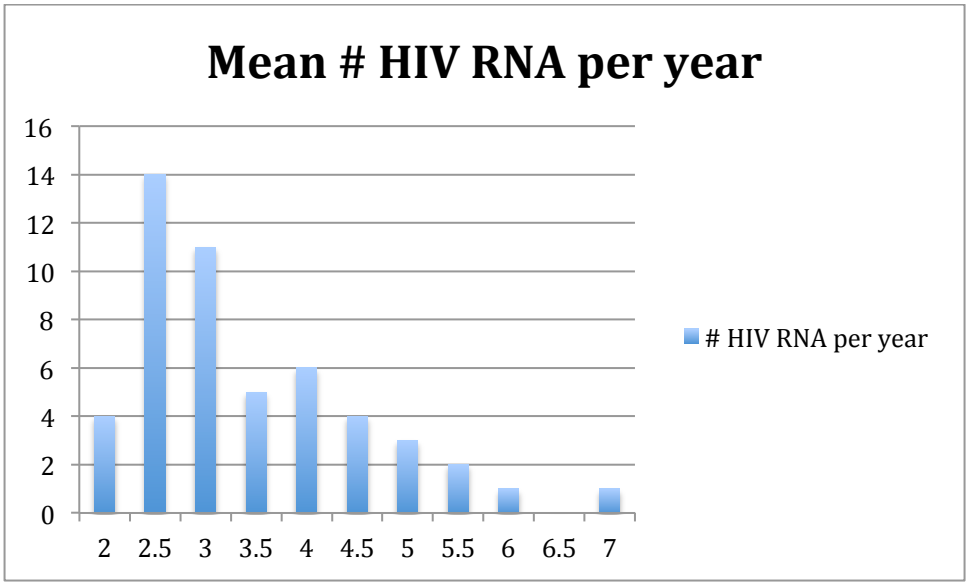
*Conclusions*

In contrast to many published studies, we did not detect a significant decline in LPS levels with effective ART. We did detect a significant increase in HMGB1 levels with effective ART and in the proportion of individuals with a detectable S100A9 level, although the significance of this finding remains unclear. In summary, we did not find any significant associations between CD4 reconstitution and either pre or post-ART DAMP or LPS levels nor any relation between pre and post-ART levels HMGB1, S100A9 or LPS levels.

## Chapter 5

## Appendix

**Appendix 1. Histogram of Mean HIV RNA Assays per Year for Study Participants**



**REFERENCES**


1. Giorgi JV, Lyles RH, Matud JL, et al. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *Journal of Acquired Immune Deficiency Syndrome* 2002;29(4):346-55.
2. Deeks SG. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 2004;104(4):942-7.
3. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365-71.
4. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. *Annual Review of Medicine* 2011;62:141-55.
5. French MA, King MS, Tschampa JM, et al. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. *J Infect Dis* 2009;200(8):1212-5.
6. Burdo TH, Lo J, Abbara S, et al. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J Infect Dis* 2011;204(8):1227-36.
7. Neuhaus J, Jacobs DR, Jr., Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis* 2010;201(12):1788-95.
8. Valdez H, Connick E, Smith KY, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS* 2002;16(14):1859-66.
9. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. *Clinical Immunology (Orlando, Fla)* 2008;126(3):235-42.
10. Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clinical Immunology (Orlando, Fla)* 2007;123(2):121-8.
11. Hunt PW, Sinclair E, Rodriguez B, et al. Gut Epithelial Barrier Dysfunction and Innate Immune Activation Predict Mortality in Treated HIV Infection. *J Infect Dis* 2014;210(8):1228-38.
12. Hunt PW, Martin JN, Sinclair E, et al. T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy. *J Infect Dis* 2003;187(10):1534-43.
13. Lederman MM, Calabrese L, Funderburg NT, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis* 2011;204(8):1217-26.
14. Hunt PW, Cao HL, Muzoora C, et al. Impact of CD8+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. *AIDS* 2011;25(17):2123-31.
15. Grund B, Baker J, Deeks SG, et al. Combined effect of interleukin-6 and D-dimer on the risk of serious non-AIDS conditions: data from 3 prospective cohorts. *Conference on Retroviruses and Opportunistic Infections*. Atlanta, GA, 2013.
16. Bianchi M. DAMPS, PAMPs and alarmins: all we need to know about danger. *Journal of Leukocyte Biology* 2007;81:1-5.
17. Diener KR, Al-Dasooqi N, Lousberg EL, et al. The multifunctional alarmin HMGB1 with roles in the pathophysiology of sepsis and cancer. *Immunology and Cell Biology* 2013;91(7):443-50.
18. Lamkanfi M, Sarkar A, Vande Walle L, et al. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J Immunol* 2010;185(7):4385-92.
19. Wang H, Ward MF, Sama AE. Targeting HMGB1 in the treatment of sepsis. *Expert Opinion on Therapeutic Targets* 2014;18(3):257-68.
20. Dubaniewicz A. Microbial and human heat shock proteins as 'danger signals' in sarcoidosis. *Human Immunology* 2013;74(12):1550-8.
21. Li J, Wang X, Zhang F, et al. Toll-like receptors as therapeutic targets for autoimmune connective tissue diseases. *Pharmacology & Therapeutics* 2013;138(3):441-51.
22. Boone BA, Lotze MT. Targeting damage-associated molecular pattern molecules (DAMPs) and DAMP receptors in melanoma. *Methods in Molecular Biology (Clifton, NJ)* 2014;1102:537-52.
23. Escamilla-Tilch M, Filio-Rodriguez G, Garcia-Rocha R, et al. The interplay between pathogen-associated and danger-associated molecular patterns: an inflammatory code in cancer? *Immunology and Cell Biology* 2013;91(10):601-10.

24. Garg AD, Dudek AM, Agostinis P. Cancer immunogenicity, danger signals, and DAMPs: what, when, and how? *BioFactors (Oxford, England)* 2013;39(4):355-67.
25. Christia P, Frangogiannis NG. Targeting inflammatory pathways in myocardial infarction. *European Journal of Clinical Investigation* 2013;43(9):986-95.
26. McCarthy CG, Goulopoulou S, Wenceslau CF, et al. Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension. *American journal of physiology Heart and Circulatory Physiology* 2014;306(2):H184-96.
27. Schiopu A, Cotoi OS. S100A8 and S100A9: DAMPs at the crossroads between innate immunity, traditional risk factors, and cardiovascular disease. *Mediators of Inflammation* 2013;2013:828354.
28. Trøseid M, Sönnerborg A, Nowak P. Highmobility group box protein-1 in HIV-1 infection: Connecting the microbial translocation, cell death, and immune activation. *Curr HIV Res* 2011;9:6-10.
29. Anraku I, Rajasuriar R, Dobbin C, et al. Circulating heat shock protein 60 levels are elevated in HIV patients and are reduced by anti-retroviral therapy. *PloS One* 2012;7(9):e45291.
30. Doitsh G, Galloway NL, Geng X, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014;505(7484):509-14.
31. Qin S WH, Yuan R, Li H, Ochani M, Ochani K, Rosas-Bellina M, Czura CJ, Huston JM, Miller E, et al. Role of HMGB1 in apoptosis-mediated sepsis lethality. *J Exp Med* 2006;203:1637-42.
32. Lotze MT ZH, Rubartelli A, Sparvero LJ, et al. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol Rev* 2007;220:60-81.
33. Nowak P, Barqasho B, Sonnerborg A. Elevated plasma levels of high mobility group box protein 1 in patients with HIV-1 infection. *AIDS* 2007;21(7):869-71.
34. Nowak P, Barqasho B, Treutiger CJ, et al. HMGB1 activates replication of latent HIV-1 in a monocytic cell-line, but inhibits HIV-1 replication in primary macrophages. *Cytokine* 2006;34(1-2):17-23.
35. Thierry S, Gozlan J, Jaulmes A, et al. High-mobility group box 1 protein induces HIV-1 expression from persistently infected cells. *AIDS (London, England)* 2007;21(3):283-92.
36. Saidi H, Melki MT, Gougeon ML. HMGB1-dependent triggering of HIV-1 replication and persistence in dendritic cells as a consequence of NK-DC cross-talk. *PloS One* 2008;3(10):e3601.
37. Cassetta L, Fortunato O, Adduce L, et al. Extracellular high mobility group box-1 inhibits R5 and X4 HIV-1 strains replication in mononuclear phagocytes without induction of chemokines and cytokines. *AIDS* 2009;23(5):567-77.
38. Lindkvist A NP, Troseid M, Abdurahman S, Nystrom J, Sonnerborg A. HMGB1 and bacterial products synergistically induce HIV-1 replication in vitro. *17th Conference on Retroviruses and Opportunistic Infections, San Fransisco, CA* 2010:Abstract No 245.
39. Youn JH OY, Kim ES, Choi JE, Shin JS. High mobility group box 1protein binding to lipopolysaccharide facilitates transfer of lipopolysaccharide to CD14 and enhances lipopolysaccharide-mediated TNF-alpha production in human monocytes. *J Immunol* 2008;180(7):5067-74.
40. Donato R, Cannon BR, Sorci G, et al. Functions of S100 Proteins. *Curr Mol Med* 2013;13(1):24-57.
41. Sunahori K, Yamamura M, Yamana J, et al. The S100A8/A9 heterodimer amplifies proinflammatory cytokine production by macrophages via activation of nuclear factor kappa B and p38 mitogen-activated protein kinase in rheumatoid arthritis. *Arthritis Research & Therapy* 2006;8(3):R69.
42. Vogl T, Tenbrock K, Ludwig S, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nat Med* 2007;13(9):1042-9.
43. Muller F, Froland SS, Aukrust P, et al. Elevated serum calprotectin levels in HIV-infected patients: the calprotectin response during ZDV treatment is associated with clinical events. *Journal of Acquired Immune Deficiency Syndromes* 1994;7(9):931-9.
44. Strasser F, Gowland PL, Ruef C. Elevated serum macrophage inhibitory factor-related protein (MRP) 8/14 levels in advanced HIV infection and during disease exacerbation. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology : official publication of the International Retrovirology Association* 1997;16(4):230-8.

45. Hashemi FB, Mollenhauer J, Madsen LD, et al. Myeloid-related protein (MRP)-8 from cervico-vaginal secretions activates HIV replication. *AIDS (London, England)* 2001;15(4):441-9.
46. Cummins JE, Christensen L, Lennox JL, et al. Mucosal innate immune factors in the female genital tract are associated with vaginal HIV-1 shedding independent of plasma viral load. *AIDS Research and Human Retroviruses* 2006;22(8):788-95.
47. Ryckman C RG, Roy J, Cantin R, Tremblay MJ, Tessier PA. HIV-1 transcription and virus production are both accentuated by the proinflammatory myeloid-related proteins in human CD4+ T lymphocytes. *J Immunol* 2002;169(6):3307-13.
48. Jiang JW LM, Hunt P, Sieg SF, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis* 2009;199(1177-85).
49. Funderburg NT, Mayne E, Sieg SF, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood* 2010;115(2):161-7.
50. Brenchley JM DD. HIV disease: fallout from a mucosal catastophe. *Nature Immunology* 2006;7(3):235-9.
51. Brenchley JM PD, Schacker TW, Asher TE, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365-71.
52. Wallet MA, Rodriguez CA, Yin L, et al. Microbial translocation induces persistent macrophage activation unrelated to HIV-1 levels or T-cell activation following therapy. *AIDS (London, England)* 2010;24(9):1281-90.
53. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis* 2008;197(1):126-33.
54. Papasavvas E, Pistilli M, Reynolds G, et al. Delayed loss of control of plasma lipopolysaccharide levels after therapy interruption in chronically HIV-1-infected patients. *AIDS (London, England)* 2009;23(3):369-75.
55. Marchetti G, Cozzi-Lepri A, Merlini E, et al. Microbial translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. *AIDS (London, England)* 2011;25(11):1385-94.
56. Nowroozalizadeh S, Mansson F, da Silva Z, et al. Microbial translocation correlates with the severity of both HIV-1 and HIV-2 infections. *The Journal of Infectious Diseases* 2010;201(8):1150-4.
57. Redd AD, Dabitao D, Bream JH, et al. Microbial translocation, the innate cytokine response, and HIV-1 disease progression in Africa. *Proceedings of the National Academy of Sciences of the United States of America* 2009;106(16):6718-23.
58. Sandler NG WH, Roque A, Law M, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis* 2011;203(6):780-90.
59. Justice AC, Freiberg MS, Tracy R, et al. Does an index composed of clinical data reflect effects of inflammation, coagulation, and monocyte activation on mortality among those aging with HIV? *Clinical Infectious Diseases : an Official Publication of the Infectious Diseases Society of America* 2012;54(7):984-94.
60. Ranoa DR, Kelley SL, Tapping RI. Human lipopolysaccharide-binding protein (LBP) and CD14 independently deliver triacylated lipoproteins to Toll-like receptor 1 (TLR1) and TLR2 and enhance formation of the ternary signaling complex. *The Journal of Biological Chemistry* 2013;288(14):9729-41.
61. Brandenburg K HJ, Gutsman T, Garidel P. The expression of endotoxic activity in the Limulus test as compared to cytokine production in immune cells. *Curr Med Chem* 2009;16(21):2653-60.
62. Samji H, Cescon A, Hogg RS, et al. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. *PloS One* 2013;8(12):e81355.
63. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998;338(13):853-60.
64. Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. *Ann Intern Med* 2001;135(1):17-26.

65. Hogg RS, Heath KV, Yip B, et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *JAMA* 1998;279(6):450-4.
66. Hemophilia and von Willebrand's disease: 2. Management. Association of Hemophilia Clinic Directors of Canada. *CMAJ* 1995;153(2):147-57.
67. Lohse N, Hansen AB, Pedersen G, et al. Survival of persons with and without HIV infection in Denmark, 1995-2005. *Ann Intern Med* 2007;146(2):87-95.
68. Silverberg MJ, Leyden WA, Xu L, et al. Immunodeficiency and risk of myocardial infarction among HIV-positive individuals with access to care. *Journal of Acquired Immune Deficiency Syndromes* 2014;65(2):160-6.
69. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. *JAMA Internal Medicine* 2013;173(8):614-22.
70. Triant VA, Lee H, Hadigan C, et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* 2007;92(7):2506-12.
71. Franceschi S, Dal Maso L, Pezzotti P, et al. Incidence of AIDS-defining cancers after AIDS diagnosis among people with AIDS in Italy, 1986-1998. *Journal of Acquired Immune Deficiency Syndromes* 2003;34(1):84-90.
72. Powles T, Robinson D, Stebbing J, et al. Highly active antiretroviral therapy and the incidence of non-AIDS-defining cancers in people with HIV infection. *Journal of Clinical Oncology* 2009;27(6):884-90.
73. Worm SW, Bower M, Reiss P, et al. Non-AIDS defining cancers in the D:A:D Study--time trends and predictors of survival: a cohort study. *BMC Infectious Diseases* 2013;13:471.
74. Ryom L, Kirk O, Lundgren J, et al. Advanced chronic kidney disease, end-stage renal disease and renal death among HIV-positive individuals in Europe. *HIV Medicine*, 2013.
75. Choi AI, Rodriguez RA, Bacchetti P, et al. The impact of HIV on chronic kidney disease outcomes. *Kidney International* 2007;72(11):1380-7.
76. Schwartz EJ, Szczech LA, Ross MJ, et al. Highly active antiretroviral therapy and the epidemic of HIV+ end-stage renal disease. *J Am Soc Nephrol* 2005;16(8):2412-20.
77. Lucas GM, Lau B, Atta MG, et al. Chronic Kidney Disease Incidence, and Progression to End Stage Renal Disease, in HIV‐Infected Individuals: A Tale of Two Races. *J Infect Dis*, 2008:1548-57.
78. Rosenthal E, Pialoux G, Rey D, et al. Liver-related mortality in human immunodeficiency virus-infected patients in France (GERMIVIC cohort study, 1995-2003). . Presented at 55th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts2004; Abstract 572. .
79. Ragni MV, Belle SH. Impact of human immunodeficiency virus infection on progression to end-stage liver disease in individuals with hemophilia and hepatitis C virus infection. *J Infect Dis*, 2001:1112-5.
80. Limketkai B, Mehta S, Sutcliffe C, et al. Relationship of liver disease stage and antiviral therapy with liver-related events and death in adults coinfected with HIV/HCV. 2012;308(4):370-8.
81. Mogensen TH, Melchjorsen J, Larsen CS, et al. Innate immune recognition and activation during HIV infection. *Retrovirology* 2010;7:54.
82. Kitahata MM, Dillingham PW, Chaiyakunapruk N, et al. Electronic human immunodeficiency virus (HIV) clinical reminder system improves adherence to practice guidelines among the University of Washington HIV Study Cohort. *Clinical Infectious Diseases* 2003;36(6):803-11.
83. Troseid M, Nowak P, Nystrom J, et al. Elevated plasma levels of lipopolysaccharide and high mobility group box-1 protein are associated with high viral load in HIV-1 infection: reduction by 2-year antiretroviral therapy. *AIDS* 2010;24(11):1733-7.
84. Yang H, Antoine DJ, Andersson U, et al. The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. *J Leukoc Biol* 2013;93(6):865-73.
85. Venereau E, Casalgrandi M, Schiraldi M, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J Exp Med* 2012;209(9):1519-28.