CORRELATES OF ELEVATED INFLAMMATORY AND IMMUNE ACTIVATION MARKER IN THE AIDS LINKED TO INTRAVENOUS EXPERIENCE (ALIVE) COHORT: INSIGHT FOR PUBLIC HEALTH INTERVENTIONS AGAINST CHRONIC INFLAMMATION

by
Byran Dai

A thesis submitted to the Johns Hopkins University in conformity with the requirements for the degree of Master of Science in Epidemiology

Baltimore, Maryland
April 2014

Abstract

Background: Though extensive literature on the relationship between chronic inflammation and health outcomes has been published, the same level of attention has not been directed towards the identification of clinical and behavioral risk factors on chronic inflammation as an outcome in marginalized populations. These populations include intravenous drug users (IVDU) and individuals with HIV/Hepatitis C (HCV), whose underlying conditions may amplify the effects of chronic inflammation on the body. The pathophysiology of chronic inflammation on cardiovascular health and mortality has been well established, yet the identification of modifiable risk factors that can be targeted for public health interventions is of utmost importance to reduce disease burden associated with chronic inflammation. Data on inflammatory biomarkers TNFR1, TNFR2, and neopterin have recently become available through the AIDS Linked to IntraVenous Experience (ALIVE) study, and provide a potential surrogate measure that may be used to ascertain contributing factors for chronic inflammation.

Methods: We conducted a cross-sectional study of ALIVE participants to investigate correlates of elevated inflammatory biomarker levels. ALIVE is a prospective cohort that is comprised of current and former intravenous drug users in Baltimore, Maryland. In total, there were 1,191 participants in this analysis, and demographic information was collected through the use of questionnaires administered by trained interviewers. Self-report of drug use and risk behaviors were obtained through standardized computer-aided questionnaire. For inflammatory biomarker data collection, two duplicate measurements in subjects were taken for each biomarker to derive an average value, with average measurements

subsequently log-transformed. Multivariable linear regressions were conducted comparing biomarker level to potential correlates. Additional analysis with HIV and HCV-specific covariates on biomarker concentration was carried out in HIV and HCV positive populations respectively to elucidate further trends within these at-risk groups.

Results: Of the 1,191 ALIVE participants, 322 (27%) were HIV seropositive and 1025 (86%) were HCV seropositive. The mean age was 46.8 years (SD: 7.9 years) and among the participants, 420 (35%) were female and 1043 (88%) were African American. 252 (21%) reported using intravenous drugs more than once a day, and the median number of comorbidities in the population was 1 (IQR: 1, 2). ***In the overall multivariate model,*** three key covariates were found to be strongly positively associated with TNFR1, TNFR2, and neopterin. These variables were number of non-AIDS-related comorbidities, daily intravenous drug use, and HCV/HIV status. Notably, age was only significantly associated in TNFR1 ($\beta = 0.05$, 95% CI: 0.021 - 0.079). ***HCV and HIV-positive patients were stratified*** and analyzed further to better understand covariates of interest that are of unique relevance to these populations. ***In the HCV subgroup***, Fibroscan score showed a strong, positive association with neopterin ($\beta = 0.541$, 95% CI: 0.095 - 0.987), TNFR1 ($\beta = 0.458$, 95% CI: 0.172 - 0.743), and TNFR2 ($\beta = 0.884$, 95% CI: 0.538 - 1.229) after adjustment while HCV viral load, past hepatitis treatment, and ALT level were insignificant with inflammatory biomarker level. ***In the HIV subgroup***, HIV viral load was statistically significant for neopterin ($\beta = 0.236$, 95% CI: 0.147 - 0.324) and TNFR2 ($\beta = 0.123$, 95% CI: 0.049 - 0.196), while HAART treatment in the past six months and CD4 nadir were not observed to possess any meaningful association with biomarker level.

Conclusions: The association between chronic inflammation and negative health outcomes has been discussed extensively throughout the scientific literature, but there is a vital need to recognize risk factors that contribute to elevated inflammation. The determination of modifiable risk factors is a public health imperative, and our results suggest several behavioral and clinical areas of focus that may be suitable for further intervention efforts. In our analysis, we identified several variables that show significant relationships with the inflammatory biomarkers of interest. In particular, our findings suggest that intravenous drug usage, non-AIDS-defining comorbidities, and HCV/HIV status show consistent statistical significance in their associations with higher levels of TNFR1, TNFR2, and neopterin. We noticed that within the HCV and HIV subgroups, additional strong positive relationships were present. Liver fibrosis was found to be closely linked with increased biomarker concentrations among HCV-positive subjects, while HIV viral load saw similarly significant associations with increased neopterin and TNFR2 levels that bring to light the additional impact of HIV-specific factors on chronic inflammation. This study served to identify potential modifiable lifestyle factors that may be targeted through public health intervention, in particular within at-risk IVDU and HIV/HCV-infected populations. Efforts to reduce injection drug use, treat underlying comorbidities such as hypertension, and increase access to antiretroviral treatments will be beneficial, and together may moderate cardiovascular disease, mortality, and other chronic inflammation-related conditions.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am extremely grateful for the wonderful mentorship offered by my academic and thesis advisor, Dr. Gregory Kirk. What began as a project for a fourth term Biostatistics course flourished into a thesis that has provided me the opportunity to delve deeply into epidemiology and establish a powerful foundation that will serve me well in my future pursuits. He has been an incredible resource, one that helped to provide an oasis of calm during the frenetic times that accompanied my data analysis and thesis writing. A special thanks to Jill Kessler and Samantha Burns for their assistance in the administrative functions that have gone towards making this thesis a reality.

I would also like to thank Dr. Sean Leng for being instrumental in helping me structure and contextualize my thesis. His detailed comments were integral in understanding the biological framework and pathways involved, and I am thankful for his feedback and consideration during this process.

I would like to express my appreciation to Megan Salter for her assistance in clarifying the intricacies of the ALIVE data, along with Jacquie Astemborski for helping me compile the final dataset from which I conducted the entirety of my thesis. Without their support, I have no doubt this process would have been significantly more difficult, and I am humbled to stand on their shoulders and advance upon the groundwork that they have laid.

I am indebted to the investigators and study staff from the ALIVE study for their tireless work in maintaining the high quality and rigor of one of the most insightful and respected cohorts in the world.

I would like to thank the additional mentorship and research opportunities that were afforded to me by Dr. Justin Lessler, who always found time to speak with me about my

interests and professional aspirations as well as explore my interests via the FluScape study. His insightful guidance and careful oversight helped to bolster my competency in epidemiologic research methods. The opportunity to work alongside him, Dr. Derek Cummings, and the ID Dynamics group at JHSPH has served as the perfect complement to my thesis research, and I hope to continue that same level of engagement within the infectious disease modeling field for the future. Thank you also to Dr. Trish Perl and Jon Teter at the Johns Hopkins Health System for providing me further opportunities for research in the healthcare setting that have crafted my academic interests.

I am grateful for my friends in the Department of Epidemiology for being so willing to serve as sounding boards for any concerns that arose through the course of this thesis. The Epi Student Room has been an immeasurably wonderful place to bounce ideas off, and I hope that it will retain that same magic that I as a first year student discovered so many months ago.

Finally, I am thankful beyond words for my parents. I would dedicate entire pages if I could to express my love, but I hope this final thesis product will encapsulate how lucky I am to have you as my family.

TABLES AND FIGURES

Introduction

Recent literature has evaluated commonly employed markers of systemic inflammation and immune activation in the context of HIV and Hepatitis C infected populations.[1–4] Of interest with chronic inflammation has been its pathological contributions among elderly adults, and attempts have been made to expand that insight into additional subgroups. From Salter et al.,[4] elevated interleukin-6 (IL6) levels were found in HIV and Hepatitis C infected individuals, yet C-reactive protein (CRP) was observed to have the opposite effect. Furthermore, injection drug use intensity was associated with higher levels of both IL6 and CRP. Though much has been discussed of the relationship between chronic inflammation and health outcomes, particularly in aging populations,[5–7] the same attention has not been directed towards the identification of clinical and behavioral risk factors on chronic inflammation as an outcome.

The pathophysiology of chronic inflammation on cardiovascular health and mortality has been well established,[8–12] yet it is important now for the focus to shift towards identifying modifiable risk factors that can be targeted for public health interventions. In doing so, we look to expand the insight provided by IL6 and CRP through better understanding more proximate inflammatory biomarkers with TNFR1/TNFR2 and upstream immune activation markers with neopterin. TNFR1/TNFR2 are tumor necrosis factor (TNF) receptors that are responsible for propagating signals responsible for a number of cellular responses,[13] in particular those associated with inflammation and infectious disease.[14,15] TNFR2 in particular has commonly been used as a clinically relevant biomarker within the HIV literature, and together with neopterin and TNFR1 has been employed in many preceding cohort studies.[16–20]

The recent availability of these additional markers has provided a novel opportunity to further our understanding of the contributing factors for chronic inflammation. The utility of inflammatory biomarkers and immune activation markers is due to their potential in serving as rational endpoints within clinical trials focusing on chronic inflammation, and we have based our analysis upon this premise. To investigate correlates of elevated inflammatory biomarker levels, we conducted a cross-sectional study of intravenous drug users from the ongoing AIDS Linked to IntraVenous Experience (ALIVE) cohort whose exposure and biomarker information had been systematically collected for this analysis.

Methods

Study Population

The ALIVE study is a prospective cohort that is comprised of current and former intravenous drug users in Baltimore, Maryland.[21] Initial recruitment for ALIVE commenced in 1988, and since then there have been four additional recruitment waves (1994, 1998, 2000, 2005), with an overall retention rate of >95%. Subjects are >17 years old and recruited from clinical treatment settings, and they participate in semi-annual visits comprising of medical examination and interview, behavioral assessments for risk behaviors including drug use, and collection of blood samples and other specimens. In total, there were 1,191 participants in this analysis; exclusion criteria for this study included CRP > 100 mg/L (n = 3), infection within 28 days of biomarker testing (n = 8), and missing data on 2 out of 6 measured comorbidities defined further in Supplemental Table 1. This research was approved by Johns

Hopkins Institution Review Boards, and all participants provided written consent for their participation in the study.

Data Collection

Demographic information was collected through the use of questionnaires administered by trained interviewers. Additional self-report data for HAART use, defined as taking three or more antiretroviral drugs, was provided through similar means. Self-report of drug use and risk behaviors were obtained through standardized computer-aided questionnaire.

Neopterin, TNFR1, and TNFR2 were collected using ELISA kits and stored at 80 degrees Celsius throughout the duration of the study. Two duplicate measurements in subjects were taken for each biomarker to find an average value, and the measurements were repeated in the event that the two measurements differed greater than 15%.

Standard laboratory procedures were carried out for determination of HIV and HCV serostatus, and the ascertainment of HIV and HCV RNA was conducted using commercially available tests. For HIV RNA measurements, ELISA, T-Cell subset assays, and real-time PCR were utilized. HCV infection was initially ascertained with HCV 3.0 enzyme immunoassay, and further confirmatory testing of HCV RNA was analyzed through confirmatory testing with reverse transcriptase PCR in a sub-sample of the study population (n = 999). CD4 cell counts and liver enzyme testing were additionally examined through routine laboratory assays.

Guidelines for comorbidity measurement were established from a prior analysis.[1] We selected six non-AIDS-defining comorbidities including anemia, chronic kidney disease, diabetes, fibrosis, hypertension, and obstructive lung disease, and created a variable for the number of comorbidities within each subject. Further measurements were done for liver stiffness, provided by Fibroscan machine through transient elastography, and alanine aminotransferase testing, utilizing the Olympus 5200 Multichannel Chemistry Analyzer with an upper limit of 30 U/L in males and 19 U/L for females.

Statistical Analysis

The inflammatory biomarkers TNFR1, TNFR2, and neopterin were log transformed and assessed continuously to account for influential effects of outlying values and assist with the interpretation of our findings. Similar log transformations were conducted for HIV and HCV viral load. Through literature review and other empirical methods, potential variables believed to be correlates of biomarker level were identified. Univariate regression was performed initially to determine potentially significant factors for inclusion in the multivariate model. Linear regression models were used in the univariate and multivariate regressions, and bootstrapping was implemented in the final models to account for non-normally distributed residuals and estimate 95% confidence intervals. The final multivariate model was constructed using Akaike's Information Criterion (AIC) during model selection, while fixing demographic characteristics of interest such as age, sex, and race.

Looking within the HIV and HCV positive populations, additional analysis with HIV and HCV-specific variables on biomarker level was carried out to elucidate further trends within

these at-risk groups. Univariate regressions were conducted in a similar fashion to the overall model, with initial selection of HIV and HCV subgroup variables informed by review of corresponding literature. For the HIV subgroup, HIV viral load and CD4 count were included while for the HCV subgroup, HCV viral load, ALT, and Fibroscan values were analyzed. To address the influence of past versus current exposures, CD4 nadir was included in the multivariate models for the HIV subgroup alongside the aforementioned variables.

Marginal ($p < .1$) and statistical significance ($p < .05$) were assessed on the bootstrapped models to identify risk factors. All analyses were conducted using STATA 12.1 (StataCorp, College Station, Texas) and R version 2.15.2.

Results

Summary Statistics

Of the 1,191 ALIVE participants, 322 (27%) were HIV seropositive and 1025 (86%) were HCV seropositive (Table 1). The mean age was 46.8 years (SD: 7.9 years), and among the participants 420 (35%) were female and 1043 (88%) were African American, with 187 (16%) reporting themselves as homeless in the past 6 months. 252 (21%) reported using intravenous drugs more than once a day, with 636 (53%) reporting alcohol use more than once a day. The median number of comorbidities in the population was 1 (IQR: 1, 2), and 249 (22%) had a BMI greater than 30 indicating obesity. For the outcome biomaker variables, neopterin had a mean of 16.54 (95% CI: 10.88, 27.09), TNFR1 had a mean of 1494.55 (95% CI: 1261.93, 1848.23), and TNFR2 had a mean of 4983.62 (95% CI: 3839.34, 6978.90).

Univariate Associations

From Table 2, we identified several categories of potential correlates. These were divided into four categories: demographic, drug use, clinical outcomes, and HCV/HIV variable. Age was found to be positively associated for each biomarker, with less consistent results for sex and race. Greater than daily use of intravenous drugs was found to carry consistent levels of significance, with various degrees of significance in alcohol and cigarette use. ALT, comorbidities, Fibroscan, and many other clinical measurements were found to have significance across the biomarkers. In neopterin and TNFR2, CD4 count and CD4 nadir were found to be strongly significant, though that significance was not replicated in TNFR1. This trend stays consistent in the HCV/HIV category, with HIV viral load positively associated in continuous and categorical format. Notably, HCV serostatus was significantly positively associated in each biomarker, along with similar trends in significance for the continuous and categorical HCV viral load variables.

Multivariate Associations

In the multivariate model, three key covariates were found to be strongly associated with neopterin, TNFR1, and TNFR2. These variables were number of non-AIDS-related comorbidities, daily intravenous drug use, and HCV/HIV status. According to Table 3, each additional non-AIDS-related comorbidity present within the body was linked to higher mean $\log_e$ concentrations of neopterin (0.072, 95% CI: 0.039 - 0.105), TNFR1 (0.077, 95% CI: 0.057 - 0.097), and TNFR2 (0.109, 95% CI: 0.083 - 0.134). Greater than daily use of intravenous drugs was found to be associated with increased mean $\log_e$ concentrations of each biomarker, with larger effect sizes observed in neopterin (0.134, 95% CI: 0.051 - 0.217)

and TNFR2 (0.123, 95% CI: 0.059 - 0.187) as compared to the marginally significant relationship in TNFR1 (0.046, 95% CI: -0.001 - 0.094). HCV-infected subjects were reported to have higher mean concentration of all three biomarkers when compared with uninfected subjects, while HIV-infected patients were observed to have heightened neopterin (0.431, 95% CI: 0.345 - 0.517) and TNFR2 (0.327, 95% CI: 0.263 - 0.390) levels as compared to the HIV-uninfected.

Associations between biomarker concentration and other covariates were found to be more heterogeneous. Age was only significantly associated in TNFR1 with an increase of 0.050 mean $\log_e$ concentrations per year (95% CI: 0.021 - 0.079), while African Americans were shown to have lower levels of TNFR1 (-0.152, 95% CI: -0.205 - -0.098) and TNFR2 (-0.132, 95% CI: -0.199 - -0.065) but not neopterin (-0.067, 95% CI: -0.172 - 0.039). No consistent trends were observed in other demographic and clinical variables such as sex and BMI. Additional drug variables such as alcohol and cigarette use were similarly observed to be discordant across the biomarkers under investigation. Greater than daily alcohol intake was found to be negatively associated with TNFR1 (-0.045, 95% CI: -0.083 - -0.008) while sharing no relationship with neopterin and TNFR2. Similarly, cigarette use in the past six months was negatively linked with neopterin (-0.224, 95% CI: -0.316 - -0.133), yet was not significantly associated with TNFR1 (0.003, 95% CI: -0.059 - 0.064) and TNFR2 (0.010, 95% CI: -0.066 - 0.087).

HCV and HIV Subgroup Analysis

HCV and HIV-positive patients were stratified and analyzed further to better understand covariates of interest that are of unique relevance to these populations. The HCV subgroup consisted of 1025 HCV-seropositive subjects, while the HIV subgroup was made up of 322 HIV-positive subjects (a characteristic to note is that each HIV-positive subject was simultaneously co-infected with HCV in the ALIVE cohort, due to the inclusion/exclusion criteria for this analysis and high HCV prevalence within the study population).

In the HCV subgroup, univariate associations were found between HCV viral load, ALT level and liver stiffness as indicated by Fibroscan (Table 4a). Fibroscan score was determined to have the strongest relationship with inflammatory biomarker levels, indicating that greater liver stiffness was linked to increased levels of neopterin (0.893, 95% CI: 0.500 - 1.287), TNFR1 (0.776, 95% CI: 0.564 - 0.988), and TNRF2 (1.476, 95% CI: 1.188 - 1.764). Furthermore, a $\log_{10}$ increase in HCV viral load was positively correlated with neopterin (0.029, 95% CI: 0.013 - 0.046), TNFR1 (0.009, 95% CI: 0.000 - 0.018), and TNFR2 (0.029, 95% CI: 0.016 - 0.041), while subjects with ALT levels greater than 2.5 times the upper normal limit were observed to have heightened biomarker concentration. Through multivariate analysis, many of the significant relationships were mitigated (Table 4b). After adjustment for other covariates, Fibroscan score continued to show a strong, positive association with neopterin (0.541, 95% CI: 0.095 - 0.987), TNFR1 (0.458, 95% CI: 0.172 - 0.743), and TNFR2 (0.884, 95% CI: 0.538 - 1.229), while the remaining associations in HCV viral load, past hepatitis treatment, and ALT level were insignificant.

The covariates for the HIV subgroup analysis included HIV viral load in $\log_{10}$, CD4 count per 100, and whether HAART treatment was received in the past 6 months. Consistent with the results from Table 3, the univariate analysis found that HIV viral load and CD4 count had strong associations with neopterin and TNFR2, while no statistically significant results were observed between HIV-specific variables and TNFR1 (Table 5a). HIV viral load was positively associated with the two biomarkers, while increased CD4 count was found to have a negative association. Upon adjustment in a multivariate model, HIV viral load remained statistically significant for neopterin (0.236, 95% CI: 0.147 - 0.324) and TNFR2 (0.123, 95% CI: 0.049 - 0.196), while the significant relationships between CD4 count and the biomarkers were eliminated (Table 5b). In addition, HAART treatment was not observed to possess any meaningful association with biomarker level, and CD4 nadir was similarly insignificant.

Sensitivity Analysis

To assess the accuracy of our results, we looked to replicate our findings by employing proxy variables for covariates included in our analysis. HCV and HIV serostatus were replaced with HCV and HIV viral load respectively, and our analyses were re-run to determine whether significant differences would arise when replacing with an alternate variable. The substitution of HCV viral load in place of HCV serostatus revealed few substantial changes in effect size, though IV drug use was found to be statistically significant (0.061, 95% CI: 0.011 - 0.111) for TNFR1 when originally marginally significant (0.046, 95% CI: -0.001 - 0.094). Similar results were seen when replacing HIV status with HIV viral load, with most covariates showing similar magnitude and variability regardless of HCV/HIV status variable utilized. In addition, heroin and cocaine use in the past six months were included in a

separate model alongside the intravenous drug use variable. This was performed to determine whether drug type influenced the relationship between intravenous drug use and the inflammatory biomarkers. Our results found that neither heroin or cocaine use was significantly associated with any of the three biomarkers, nor their inclusion did not noticeably affect the relationships between other covariates in the model.

Discussion

The association between chronic inflammation and negative health outcomes has been discussed extensively throughout the scientific literature, but there is a vital need to recognize risk factors that contribute to elevated levels of inflammation. The determination of modifiable risk factors is a public health imperative, and our results suggest several behavioral and clinical areas of focus that may be suitable for further intervention efforts. In our analysis, we identified several variables that show significant relationships with the inflammatory biomarkers of interest. In particular, our findings suggest that intravenous drug usage, non-AIDS-defining comorbidities, and HCV/HIV status show consistent marginal and statistical significance in their associations with neopterin, TNFR1, and TNFR2. The strength of association for neopterin and TNFR2 with IV drug use suggests that increased frequency of injection may activate an inflammatory mechanism with biological plausibility as elucidated in Salter et al.[4] We noticed that within the HCV and HIV subgroups, additional strong positive relationships were present. Liver fibrosis demonstrated a strong positive relationship with neopterin, TNFR1, and TNFR2 mean $\log_{10}$ concentrations among HCV-positive subjects, with a large effect size in each after adjustment for treatment and other clinical variables, suggesting an inflammatory pathway that may be accentuated by non-

AIDS-related comorbidities. HIV viral load saw similarly significant associations with increased neopterin and TNFR2 levels that bring to light the added contributory effect of HIV-specific factors on chronic inflammation. Past investigations have advanced that the mechanisms for these risk factors on chronic inflammation are hypothesized to be virally-mediated. Our findings appear to corroborate those suggestions in our findings with TNFR2; the close linkage of TNFR2 with immune cells in the body provides a biological basis for the prior assertion.

Upon adjustment for other covariates, the non-significance of age with neopterin and TNFR1 concentration was of interest. The current body of knowledge has linked aging with chronic inflammation via other clinical biomarkers such as C-reactive protein,[9] yet the expected relationships did not manifest in our analysis. Our univariate regressions showed higher age being strongly significant with elevated concentration of all three biomarkers. However, the lack of significance with two of our biomarkers in the multivariate model was believed to be due to the inclusion of associated covariates possessing explanatory power for the role of age on chronic inflammation.

In our subgroup analysis, we noticed that temporally proximate covariates appeared to show stronger associations with inflammatory biomarker concentrations than more distal variables. It is suggestible that inflammatory status is influenced more by an individual's current health status than their past exposures. For example, CD4 nadir was found to be inconsequential in affecting neopterin and TNFR1 in the univariate regression for HIV-positive subjects, and in the multivariate regression was not significantly associated with any biomarker (data not

shown). However, HIV viral load at the most recent visit was linked to significantly increased concentrations of neopterin and TNFR2 after adjustment for other factors. Though current CD4 count was similarly not significant across the three biomarkers, it is accounted for with the inclusion of the HIV viral load variable. Furthermore, among HCV-positive participants, current fibrosis level as indicated by Fibroscan was uniformly significant with all three biomarkers, providing additional evidence of stronger relationships with recent conditions. The larger effect of current status on inflammatory biomarker levels indicates that recent events may be more influential than those that have occurred in the past. Nevertheless, additional inquiry will be necessary on other distal variables to determine the extent to which temporality may affect a covariate's association with biomarker concentration.

Of note, we find that TNFR2 has been shown to be a consistent biomarker in the ALIVE cohort, reflecting systemic inflammation with respect to HIV and HCV as opposed to TNFR1 which has demonstrated greater variability in its associations. TNFR2 has been found to be primarily expressed upon monocytes and macrophages along with other immune cells, whereas TNFR1 is generally observed across all nucleated cell types.[22] From there, we anticipate a higher magnitude of effect to be observed with increasing HIV and HCV load on TNFR2 level due to the immune response from the body. The role of neopterin in immune activation is similarly relevant as its production by monocytes and macrophages in response to interferon-γ (IFN-γ) suggest a biologically consistent pathway to TNFR2 that was corroborated by our analysis.[23] Previous reports have suggested a relationship between detectable levels of HIV and HCV RNA with chronic inflammation, hypothesized through activation of tissue factor pathways and other inflammatory changes.[3,24] We anticipate that

injection intensity and non-AIDS-related comorbid diseases may function partly through mediating increased immune activation through a similar pathway, though further investigation will be necessary to better elucidate these biological mechanisms.

There are several limitations that arose over the course of our study to be addressed in future analyses. By design, the cross-sectional nature of our investigation does not provide temporality to allow for more substantive determinations of causality. Biomarkers concentrations were collected with one visit, which precluded us from investigating changes based on baseline characteristics and may not be representative of a subject's true status. Past investigations have suggested that variability of biomarker level may exist over time,[25] yet previous research has found that the extent of reproducibility with other markers is high.[1,2] Future inquiry may benefit from additional follow-up to mitigate and account for individual variation. We are further limited by the low number of subjects reporting to as non-African American and female, as well as the older ages captured in our cohort, limiting the extent to which we may be able to generalize our results towards the IV drug using population. Few distal variables were available to investigate the effect of recent vs. past exposures on biomarker levels, and a more comprehensive set of covariates encapsulating each category would provide the basis for further study.

In all, we have found several factors that may impact the level of neopterin, TNFR1, and TNFR2 inflammatory biomarker in the body. We cited intensity of IV drug use, number of non-AIDS-related comorbidities, and HCV/HIV status as the primary contributors of elevated inflammation within our study. Our subgroup analysis has suggested that clinical

variables such as liver stiffness and HIV viral load in HCV and HIV-specific populations, respectively, contribute additionally to the burden of chronic inflammation in these marginal groups. The impact of chronic inflammation on negative health outcomes is of great importance as HCV and HIV-infected populations continue to age. However, insights into risk factors affecting chronic inflammation have not been as forthcoming. This study was conducted to shed additional light on this realm and give further impetus on potential modifiable lifestyle factors that may be targeted through public health intervention in an effort to address the effects of systemic inflammation in the population. Efforts to reduce IV drug use, treat underlying comorbidities such as hypertension, and increase access to antiretroviral treatments will be beneficial, and together carry the potential to mitigate cardiovascular disease, mortality, and other chronic inflammation-related conditions.

REFERENCES

1. Salter, M. L., Lau, B., Go, V. F., Mehta, S. H. & Kirk, G. D. HIV Infection, Immune Suppression, and Uncontrolled Viremia Are Associated With Increased Multimorbidity Among Aging Injection Drug Users. *Clin Infect Dis.* **53,** 1256–1264 (2011).
2. Neuhaus, J. *et al.* Markers of Inflammation, Coagulation, and Renal Function Are Elevated in Adults with HIV Infection. *J Infect Dis.* **201,** 1788–1795 (2010).
3. Kuller, L. H. *et al.* Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection. *PLoS Med* **5,** e203 (2008).
4. Salter, M. L. *et al.* Correlates of elevated interleukin-6 and C-reactive protein in persons with or at high-risk for HCV and HIV infections: *JAIDS Journal of Acquired Immune Deficiency Syndromes* 1 (2013). doi:10.1097/QAI.0b013e3182a7ee2e
5. Aberg, J. A. Aging, inflammation, and HIV infection. *Top Antivir Med* **20,** 101–105 (2012).
6. Provinciali, M., Cardelli, M. & Marchegiani, F. Inflammation, chronic obstructive pulmonary disease and aging. *Curr Opin Pulm Med* **17 Suppl 1,** S3–10 (2011).
7. Prasad, S., Sung, B. & Aggarwal, B. B. Age-associated chronic diseases require age-old medicine: role of chronic inflammation. *Prev Med* **54 Suppl,** S29–37 (2012).
8. Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P. & Hennekens, C. H. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. *New England Journal of Medicine* **336,** 973–979 (1997).
9. Tracy, R. P. Emerging relationships of inflammation, cardiovascular disease and chronic diseases of aging. *Int J Obes Relat Metab Disord* **27,** S29–S34 (2003).
10. Ridker, P. M., Hennekens, C. H., Buring, J. E. & Rifai, N. C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. *New England Journal of Medicine* **342,** 836–843 (2000).
11. Tien, P. C. *et al.* Inflammation and Mortality in HIV-infected Adults: Analysis of the FRAM Study Cohort. *J Acquir Immune Defic Syndr* **55,** 316–322 (2010).
12. Lindahl, B., Toss, H., Siegbahn, A., Venge, P. & Wallentin, L. Markers of Myocardial Damage and Inflammation in Relation to Long-Term Mortality in Unstable Coronary Artery Disease. *New England Journal of Medicine* **343,** 1139–1147 (2000).
13. Bradley, J. TNF-mediated inflammatory disease. *J. Pathol.* **214,** 149–160 (2008).
14. Robak, T., Gladalska, A. & Stepień, H. The tumour necrosis factor family of receptors/ligands in the serum of patients with rheumatoid arthritis. *Eur. Cytokine Netw.* **9,** 145–154 (1998).
15. Nürnberger, W. *et al.* Definition of a new score for severity of generalized Neisseria meningitidis infection. *Eur. J. Pediatr.* **154,** 896–900 (1995).
16. Herbein, G. & Khan, K. A. Is HIV infection a TNF receptor signalling-driven disease? *Trends in Immunology* **29,** 61–67 (2008).
17. Nixon, D. E. & Landay, A. L. Biomarkers of immune dysfunction in HIV. *Curr Opin HIV AIDS* **5,** 498–503 (2010).
18. Keating, S. M. *et al.* The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of US women. *AIDS* **25,** 1823–1832 (2011).
19. Ross, A. C. *et al.* Relationship between Inflammatory Markers, Endothelial Activation Markers, and Carotid Intima-Media Thickness in HIV-Infected Patients Receiving Antiretroviral Therapy. *Clin Infect Dis.* **49,** 1119–1127 (2009).

20. Ross, A. C. *et al.* Endothelial Activation Markers Are Linked to HIV Status and Are Independent of Antiretroviral Therapy and Lipoatrophy. *J Acquir Immune Defic Syndr* **49,** 499–506 (2008).
21. Vlahov, D. *et al.* The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res. Monogr.* **109,** 75–100 (1991).
22. Hijdra, D., Vorselaars, A. D., Grutters, J. C., Claessen, A. M. & Rijkers, G. T. Differential expression of TNFR1 (CD120a) and TNFR2 (CD120b) on subpopulations of human monocytes. *Journal of Inflammation* **9,** 38 (2012).
23. Wirleitner, B. *et al.* Monocyte-derived dendritic cells release neopterin. *J. Leukoc. Biol.* **72,** 1148–1153 (2002).
24. Armah, K. A. *et al.* Human immunodeficiency virus, hepatitis C, and inflammatory biomarkers in individuals with alcohol problems: a cross-sectional study. *BMC Infect Dis* **13,** 399 (2013).
25. Maloney, E. M. *et al.* Temporal variability in immunological parameters: Peripheral blood mononuclear cell subsets, serum immunoglobulins, and soluble markers of immune system activation. *Journal of Clinical Laboratory Analysis* **11,** 190–195 (1997).

APPENDIX

Table 1: Participant characteristics (discuss whether to add additional columns for comparison)

	Overall (N=1191)
Demographics	
Age, mean [SD]	46.76 [7.9]
Female	0.35
African American	0.88
Married, ever	0.35
Employed	0.26
Homeless	0.16
Drug Use	
Cigarette Use	0.84
Alcohol Use, >= 1 day/week	0.53
Injection Drug Use Frequency	
<Daily	0.79
>Daily	0.21
Clinical Outcomes	
BMI	
<25	0.47
25-30	0.31
>30	0.22
Number of Comorbidities, median [IQR]	1 [1,2]
Chronic Kidney Disease	0.27
Anemia	0.24
Diabetes	0.08
Hypertension	0.40
Fibrosis	0.30
Obstructive Lung Disease	0.16
HCV/HIV Status	
HCV Positive	0.86
HIV Positive	0.27
Inflammatory Biomarkers	
Neopterin, median [IQR] (pg/ml)	16.54 [10.88,27.09]
TNFR1	1494.55 [1261.93,1848.23]
TNFR2	4983.62 [3839.34,6978.90]

Table 2: Univariate associations with covariates of interest

	Neopterin (pg/ml in log scale)	TNFR1 (pg/ml in log scale)	TNFR2 (pg/ml in log scale)
Demographic			
Age	**0.005502****	**0.00591****	**0.00483****
Female	**-0.09875****	0.021693	0.025011
African American	0.027174	**-0.09342****	-0.05116
Employed	-0.05469	**-0.07172****	**-0.14273****
Drug Use			
Alcohol Frequency (> Daily)	**-0.0786****	**-0.0425****	-0.03387
Cigarette Use (>Daily)	**-0.22091****	-0.00111	0.022524
Crack Use	**-0.10398****	**-0.05109****	**-0.07494****
IV Drug Use (>daily)	**0.08942***	**0.0446***	**0.1138****
Clinical Outcomes			
ALT	**0.002141****	**0.000669****	**0.002173****
AST	**0.00269****	**0.001017****	**0.003231****
CD4 Nadir	**-0.00063****	-5.40E-05	**-0.00055****
CD4 Cell Count	**-0.00054****	1.30E-05	**-0.00041****
Comorbidities	**0.12304****	**0.085097****	**0.15188****
Fibroscan (log)	**0.22096****	**0.1701****	**0.3284****
Obese	-0.0787	**0.049****	-0.03553
HCV/HIV Variables			
HCV Positive	**0.525263****	**0.195743****	**0.438942****
HCV VL	**.0810505****	.00008	**.0581367****
HCV VL Category (>40)	**.3473****	**.1235****	**.30152****
HIV Positive	**0.52207****	0.025385	**0.426742****
HIV VL	**0.2483****	0.01151	**0.13811****
HIV (undetectable)	**0.23664****	0.02947	**0.32777****
HIV (detectable)	**0.75009****	0.02213	**0.50581****

HIV (CD4<350)	**0.6395****	0.0226	**0.4779****
HIV (CD4>350)	**0.3573****	0.0293	**0.355****
Ever HAART Use	**-0.23252****	0.070924	-0.08086

Table 3: Risk factor associations with inflammatory biomarkers

	Neopterin (pg/ml in log scale) β coefficient (95% CI)[1]	**TNFR1 (pg/ml in log scale) β coefficient (95% CI)[1]**	**TNFR2 (pg/ml in log scale) β coefficient (95% CI)[1]**
Demographics			
Age	-0.002 (-0.047 - 0.043)	0.050 (0.021 - 0.079)**	0.021 (-0.014 - 0.055)
Sex			
Male	REF	REF	REF
Female	-0.058 (-0.132 - 0.015)	0.035 (-0.009 - 0.080)	0.037 (-0.020 - 0.093)
Race			
White/other	REF	REF	REF
African American	-0.067 (-0.172 - 0.039)	-0.152 (-0.205 - -0.098)**	-0.132 (-0.199 - -0.065)**
Drug Use			
IV Drug Use			
<Daily	REF	REF	REF
>Daily	0.134 (0.051 - 0.217)**	0.046 (-0.001 - 0.094)*	0.123 (0.059 - 0.187)**
Daily Alcohol Intake			
<1 day/week	REF	REF	REF
>1 day/week	-0.028 (-0.098 - 0.042)	-0.045 (-0.083 - -0.008)**	-0.015 (-0.065 - 0.034)
Current Cigarette Use			
No	REF	REF	REF
Yes	-0.224 (-0.316 - -0.133)**	0.003 (-0.059 - 0.064)	0.010 (-0.066 - 0.087)
Clinical Outcomes			
BMI			
<25	REF	REF	REF
25-30	-0.044 (-0.121 - 0.032)	0.013 (-0.033 - 0.059)	-0.029 (-0.088 - 0.029)
>30	-0.087 (-0.179 - 0.004)*	0.046 (-0.007 - 0.099)*	0.001 (-0.065 - 0.066)
Comorbidities	0.072 (0.039 - 0.105)**	0.077 (0.057 - 0.097)**	0.109 (0.083 - 0.134)**
HCV Status			
Uninfected	REF	REF	REF
HCV Positive	0.347 (0.263 - 0.431)**	0.144 (0.097 - 0.191)**	0.266 (0.206 - 0.327)**
HIV Status			
Uninfected	REF	REF	REF
HIV Positive	0.431 (0.345 - 0.517)**	-0.034 (-0.081 - 0.014)	0.327 (0.263 - 0.390)**

*Marginal significance: $p<0.1$

**Statistical significance: $p<0.05$

[1]β coefficients estimated using linear regression, and 95% CIs estimated from bootstrapping using 1000 replications to account for non-Gaussian residuals.

Table 4a: HCV subgroup analysis among 1025 HCV-seropositive participants (Univariate)

	Neopterin (pg/ml in log scale) β coefficient (95% CI)[1]	**TNFR1 (pg/ml in log scale) β coefficient (95% CI)[1]**	**TNFR2 (pg/ml in log scale) β coefficient (95% CI)[1]**
HCV VL[2]	0.029 (0.013 - 0.046)**	0.009 (0.000 - 0.018)**	0.029 (0.016 - 0.041)**
Hepatitis C Treatment			
Never Received	REF	REF	REF
Has Received	0.016 (-0.187 - 0.219)	0.004 (-0.107 - 0.116)	0.007 (-0.146 - 0.160)
ALT (ULN)			
<1.5	REF	REF	REF
1.5-2.5	0.150 (0.054 - 0.246)**	0.041 (-0.011 - 0.005)	0.098 (0.026 - 0.171)**
>2.5	0.148 (0.040 - 0.255)**	0.063 (0.005 - 0.120)**	0.196 (0.115 - 0.276)**
Fibroscan[2]	0.893 (0.500 - 1.287)**	0.776 (0.564 - 0.988)**	1.476 (1.188 - 1.764)**

*Statistically significant at $p<0.05$

[1]β coefficients estimated using linear regression, and 95% CIs estimated from bootstrapping using 1000 replications to account for non-Gaussian residuals.

[2]Per log base 10

Table 4b: HCV subgroup analysis among 1025 HCV-seropositive participants (Multivariate)

	Neopterin (pg/ml in log scale) β coefficient (95% CI)[1]	**TNFR1 (pg/ml in log scale) β coefficient (95% CI)[1]**	**TNFR2 (pg/ml in log scale) β coefficient (95% CI)[1]**
HCV VL[2]	0.014 (-0.003 - 0.032)	0.008 (-0.003 - 0.018)	0.015 (0.002 - 0.028)**
Hepatitis C Treatment			
Never Received	REF	REF	REF
Has Received	-0.047 (-0.254 - 0.159)	0.002 (-0.109 - 0.113)	-0.021 (-0.168 - 0.126)
ALT (ULN)			
<1.5	REF	REF	REF
1.5-2.5	0.054 (-0.055 - 0.164)	-0.036 (-0.093 - 0.022)	-0.031 (-0.109 - 0.047)
>2.5	0.023 (-0.099 - 0.145)	-0.022 (-0.086 - 0.042)	0.033 (-0.050 - 0.117)
Fibroscan[2]	0.541 (0.095 - 0.987)**	0.458 (0.172 - 0.743)**	0.884 (0.538 - 1.229)**

Adjusted for age, sex, race, BMI, comorbidity, IV drug use, alcohol, cigarette, HIV status

*Marginal significance: $p<0.1$
**Statistical significance: $p<0.05$
[1]β coefficients estimated using linear regression, and 95% CIs estimated from bootstrapping using 1000 replications to account for non-Gaussian residuals.
[2]Per log base 10

Table 5a: HIV subgroup analysis among 322 HIV-positive participants (Univariate)

	Neopterin (pg/ml in log scale) β coefficient (95% CI)[1]	TNFR1 (pg/ml in log scale) β coefficient (95% CI)[1]	TNFR2 (pg/ml in log scale) β coefficient (95% CI)[1]
HIV VL[2]	0.251 (0.185 - 0.316)**	0.013 (-0.026 - 0.053)	0.138 (0.085 - 0.191)**
CD4 Count (per 100)	-0.056 (-0.088 - -0.024)**	-0.010 (-0.028 - 0.008)	-0.045 (-0.069 - -0.020)**
Received HAART within last 6 months			
No	REF	REF	REF
Yes	-0.233 (-0.377 - -0.088)**	0.071 (-0.011 - 0.153)*	-0.081 (-0.193 - 0.032)

*Marginal significance: $p<0.1$

**Statistical significance: $p<0.05$

[1]β coefficients estimated using linear regression, and 95% CIs estimated from bootstrapping using 1000 replications to account for non-Gaussian residuals.

[2]Per log base 10

Table 5b: HIV subgroup analysis among 322 HIV-positive participants (Multivariate)

	Neopterin (pg/ml in log scale) β coefficient (95% CI)[1]	TNFR1 (pg/ml in log scale) β coefficient (95% CI)[1]	TNFR2 (pg/ml in log scale) β coefficient (95% CI)[1]
HIV VL[2]	0.236 (0.147 - 0.324)**	0.029 (-0.022 - 0.081)	0.123 (0.049 - 0.196)**
CD4 Count (per 100)	0.003 (-0.033 - 0.039)	-0.001 (-0.023 - 0.022)	-0.012 (-0.043 - 0.019)
Received HAART within last 6 months			
No	REF	REF	REF
Yes	-0.035 (-0.204 - 0.134)	0.097 (-0.004 - 0.199)*	0.029 (-0.107 - 0.166)

Adjusted for age, sex, race, BMI, comorbidity, IV drug use, alcohol, cigarette, HIV status

*Marginal significance: $p<0.1$
**Statistical significance: $p<0.05$
[1]β coefficients estimated using linear regression, and 95% CIs estimated from bootstrapping using 1000 replications to account for non-Gaussian residuals.
[2]Per log base 10

BYRAN N. DAI

School Address
Department of Epidemiology
Johns Hopkins Bloomberg School of Public Health
Baltimore, MD 21231

Permanent Address
1800 E Baltimore St
Baltimore, MD 21231
(845) 596-1424

OBJECTIVE

Obtain funding for training within infectious disease epidemiology and emerging infections, where I can develop my research abilities and gain public health expertise to influence policy at the population level.

EDUCATION

Master of Science (ScM), Infectious Disease Epidemiology — Expected May 2014
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Thesis: Correlates of Elevated Inflammatory and Immune Activation Marker Levels in the ALIVE Cohort

Bachelor of Arts, High Honors, History and Science — May 2011
Harvard University, Cambridge, MA 02138
Secondary Field in Global Health and Health Policy
Thesis: Vaccinating Against Fear: Framing the Salk/Sabin Poliomyelitis Vaccines

RESEARCH EXPERIENCE

Johns Hopkins Health System — January 2013 - Present
Infection Control Research Assistant — Baltimore, MD 21231

- Collected data from C-section line listings of five United Arab Emirates JHHS-affiliated hospitals to identify common risk factors and possible interventions by developing regression models to ascertain risk using STATA.
- Presented findings of line listing review to JHHS-infection preventionists and other stakeholders to inform on areas of emphasis for improving patient outcome.
- Conducted computations of standardized incidence ratios for surgical site infections and central line associated bloodstream infections of six domestic JHHS-affiliated hospitals for comparison to expected values provided by National Healthcare Safety Network.
- Created gap analysis questionnaire for distribution to Johns Hopkins Health System collaborating facilities.

Johns Hopkins Bloomberg School of Public Health — December 2012 - Present
FluScape Research Assistant, Justin Lessler and Derek Cummings Group — Baltimore, MD 21231

- Developed multivariate linear regression models for determining factors associated with elevated influenza antibody titer level using R statistical software for FluScape, a cohort study in Guangzhou, China exploring connectivity of influenza infection.
- Investigated potential association between presence of seroconverting household children and seroconversion of adults within the same household setting.
- Conducted substantive literature reviews on associations between influenza and childhood contacts to inform analyses.

ALIVE Research Assistant, Gregory Kirk Group — August 2012 - Present

- Provided statistical analysis for AIDS Linked to IntraVenous Experience (ALIVE), a prospective cohort study characterizing incidence and natural history of HIV infection among injection drug users, through R and STATA.
- Investigated potential inflammatory risk factors in HIV and Hepatitis C positive sub-populations with stratified multi-linear regression analysis.

- Drafted manuscript on findings from inflammation risk factor analysis for submission to BMC Infectious Disease.

Maryland Department of Health and Mental Hygiene October 2012 - April 2013
PHASE Intern Baltimore, MD 21201

- Advised DHMH healthcare-associated infections (HAI) officials on HAI outbreak and reporting criteria from other health departments.
- Conducted comprehensive literature reviews of reporting systems within other states for multidrug-resistant organisms including Carbapenem-Resistant Enterobacteriaceae.
- Prepared final report and presentation for submission to PHASE symposium to share findings from reviews with DHMH leadership and JHSPH faculty, along with poster and abstract for ID Week conference in San Francisco.

Massachusetts General Hospital August 2011 - August 2012
Clinical Research Assistant Boston, MA 02114

- Drafted and submitted significance and safety sections of grant applications for NIH and other funding agencies.
- Conducted literature reviews and research analysis for submission of FDA pre-IND and IND applications.
- Conducted qualitative interviews with subjects enrolled in MGH probiotics vaccine trials
- Drafted commentary on necessity for thermostable vaccines during instances of natural and manmade disasters.

PUBLICATIONS AND PRESENTATIONS

- Dai B, Kirk G. "Correlates of Elevated Inflammatory Biomarker Levels in the AIDS Linked to Intra-Venous Experience (ALIVE) Cohort: Insight for Public Health Interventions Against Chronic Inflammation." Delta Omega Poster Competition, JHSPH, Baltimore, MD. 20 February 2014.
- Dai B, Givan M, Wilson L, Richards K. "Review of Carbapenem-Resistant Enterobacteriaceae (CRE) and Multi-Drug Resistant Organism (MDRO) Reporting Requirements in the United States." ID Week, San Francisco, CA. 2013 October 3.
- Dai B. "Lessons Learned: Healthcare-Associated Infection (HAI) and Multidrug-Resistant Organism (MDRO) Reporting Requirements in the United States." Public Health Applications for Student Experience Symposium, Baltimore, MD. 2013 May 17.
- Dai B. "Honors Thesis Presentation: Vaccinating Against Fear: The Framing of the Poliomyelitis Vaccine in Post-War America." Currier House Thesis Symposium, Harvard University, Cambridge, MA 02138. 2011 May 12.

TEACHING EXPERIENCE

Johns Hopkins School of Public Health January 2013 - Present
Teaching Assistant Baltimore, MD 21231

- Epidemiologic Inference in Outbreak Investigation (Spring 2014) - Taha
- Epidemiologic Methods III (Spring 2014) - Althoff, Dowdy, Mehta
- Concepts and Methods in Infectious Disease Epidemiology (Spring 2014) - Cummings, Lessler, Moss
- Infectious Disease Epidemiology (Fall 2013) - Nelson
- Epidemiologic Methods I (Fall 2013) - Gange, Selvin, Sutcliffe
- Fundamentals of Epidemiology (Spring 2013, Fall 2013) - Phelan
- Principles of Epidemiology (Summer 2013) - Crum, Kirk
- Outbreak Investigation (Summer 2013) - Taha

COMMUNITY AND VOLUNTEER EXPERIENCE

Student Outreach Resource Center (SOURCE) May 2013 - September 2013
Volunteer Baltimore, MD 21205

- Educated Baltimore residents at East Baltimore Health Fair (CCI) on healthy nutrition and dietary habits.
- Coordinated indoor recreational activities for Baltimore-area children at Israel Baptist Church.

Baltimore City Community College March 2013 - Present
Citizenship Tutor Baltimore, MD 21202

- Provided one-on-one citizenship tutoring for permanent residents seeking American citizenship.
- Assisted in reviewing civics, reading, and writing comprehension within classroom environment.

Public Health Applications for Student Experience November 2012 - April 2013
Outreach Assistant Baltimore, MD 21201

- Instructed Baltimore residents on West Nile Virus awareness and prevention efforts through direct calling.
- Assisted in data collection for DHMH West Nile Virus prevalence survey through phone interviews with elderly residents in the Baltimore metro area.

SKILLS AND INTERESTS

Proficiency with STATA, R, SAS, ArcGIS, and Excel software packages. Extensive research and travels throughout East Africa and Asia. Strong literature review and writing skills. Significant interest in emerging infections, healthcare epidemiology, and infectious disease modeling. Contemporary piano, bicycling, travel.