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EXAMINING HIV-ASSOCIATED SYMPTOM BURDEN AND MICROBIAL TRANSLOCATION IN THE VETERANS AGING COHORT STUDY: A SECONDARY DATA ANALYSIS

by

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A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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2016

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ABSTRACT

The pathophysiological effects of HIV infection, including inflammation, contribute to symptom burden and poor clinical outcomes. Within the gut associated lymph tissue, immune activation leads to dysfunction of the gastrointestinal epithelial barrier and subsequent movement of microbial products from the gut into the blood. The translocation of microbial products from the gut into the blood circulation has been identified as a key contributor to HIV disease progression and chronic inflammation. Chronic inflammation has been associated with various symptoms including symptoms commonly reported in HIV disease. However, it is unknown if these symptoms are associated with inflammation related to microbial translocation. The inflammatory effects of HIV infection on the gastrointestinal epithelial barrier and circulating microbial products may be associated with symptoms of the gastrointestinal tract and systemic symptoms in general.

The summation of symptoms or symptom burden has been associated with poor adherence to HIV medications. However, it is unknown whether symptom presence can support the identification of epithelial barrier dysfunction or chronic microbial translocation. Furthermore, it is unknown if interventions targeted toward reducing microbial translocation could possibly reduce or prevent symptom burden and thus

improve outcomes. The results of this study are intended to contribute to identifying possible strategies aimed at decreasing health disparities among people living with HIV.

Data from the Veterans Aging Cohort Study provides a unique opportunity to retrospectively examine the relationship among microbial translocation, inflammation, and symptoms among people on HIV antiretroviral therapy. As a first step of developing strategies to heal gastrointestinal epithelial barrier dysfunction and reduce microbial translocation, the aim of this study is to evaluate whether there is an association between gastrointestinal barrier dysfunction and symptoms, as well as, whether the microbial translocation is associated with symptoms. A secondary aim is to identify if there is an association between specific symptoms or symptom clusters within the context of microbial translocation.

Keywords: microbial translocation, symptoms, inflammation, HIV, adherence, retrospective analysis, gastrointestinal, epithelial barrier dysfunction

DEDICATION

I would like to dedicate this dissertation to my patients living with HIV who sent me here. I have enjoyed my career as a clinician partnering with you; I thank you for sending me to the high diving board at the deep end of the life pool. For those including my family members that have transitioned to the other side, thank you for inspiring me to make a difference in the world and field of HIV Nursing.

Namasté

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I would like to acknowledge my brother, David C. Wilson, PhD for being the trailblazer in the world of doctoral studies. Thank you for all of your support and tough talks over the past several years. You really got me through this program in my darkest times. To my dog Murphy, thanks for staying by my side and making me laugh. You hung in there for a lot of hours and were truly a best friend. To Kim and Jim, thank you for feeding me, and for being my friends and neighbors through all of this. I love you, honor you, and you have my heart. I would also like to acknowledge my dissertation chair and advisor who challenged me to go big but never go home; and to go by Cadillac and not by Honda Civic. I truly appreciate everything. Finally, thank you to my dissertation committee for your encouragement, hard work, and mentoring.

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LIST OF ABBREVIATIONS

µL	microliter
AAD	Antibiotic Associated Diarrhea
AIDS	Acquired Immune Deficiency Syndrome
ALT	alanine transaminase
AST	aspartate transaminase
cART	combined antiretroviral therapy
BMI	Body Mass Index
CD4	Cluster of differentiation – 4
cfu	colony forming units
CINAHL	Cumulative Index to Nursing and Allied Health Literature
DNA	Deoxyribonucleic acid
EndoCAb	Endotoxin core antibodies
FDA	Federal Drug Administration
FIB-4	Liver Fibrosis Index 4
GALT	Gut Associated Lymph Tissue
GI	Gastrointestinal
HCV	Hepatitis C
HIV	Human Immunodeficiency Virus
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Disease
Ig	Immunoglobulin
IL	Interleukin

IV	Intravenous
LPS	lipopolysaccharide
mm^3	millimeters cubed
NF-κβ	nuclear factor kappa-light-chain-enhancer of activated B cells
ng	nanogram
OR	Odds Ratio
PCA	principal components analysis
pg	pictogram
PLWH	people living with HIV
RNA	ribonucleic acid
ROS	reactive oxygen species
sCD14	soluble CD14
SMART	Strategies for Management of ART
Th17	T-helper 17
TNF	Tumor Necrosis Factor
US	United States
VA	Veterans Administration
VACS	Veterans Aging Cohort Study
VHA	Veterans Health Administration

CHAPTER 1: INTRODUCTION

Nearly 1.2 million people are living with human immunodeficiency virus (HIV) infection in the United States with close to 600,000 having died from acquired immune deficiency syndrome (AIDS) caused by this virus since 1981 (Center for Disease Control, 2011). Forty-one percent of people living with HIV (PLWH) disease are retained in medical care. Of the total number of PLWH, 36% are on combination antiretroviral therapy (cART); only 28% of those infected with HIV achieve clinical suppression of their virus (Center for Disease Control, 2011; Mugavero, Amico, Horn, & Thompson, 2013).

Advances in HIV therapy have advanced life expectancy and changed the focus of clinical management of a terminal illness to one of a chronic disease. However, despite effective HIV therapy, residual immune activation leads to chronic inflammation which is associated with the early development of conditions seen in otherwise uninfected aging adults (Deeks, 2011). Inflammation and failed immune reconstitution are predictors of devastating complications and HIV disease progression (Baroncelli et al., 2009; Douek, 2007; Marchetti et al., 2011). Persons with higher levels of soluble CD14 (sCD14), D-dimer, Interleukin (IL)-6, and C-reactive protein are more likely to progress to a diagnosis of AIDS and non-AIDS events in HIV disease (Duprez et al., 2012; El-Sadr et al., 2006; Nixon & Landay, 2010; Rodger et al., 2009; Sandler et al., 2011). Chronic inflammation from immune activation, and elevated levels of sCD14 and IL-6 have been linked to early aging, metabolic disease, cardiovascular disease, decline in renal function, cancer, bone disease, and other end-organ diseases (Ancuta et al., 2008; Armah et al., 2012; Baroncelli et al., 2009; Brenchley & Douek, 2008; Brenchley et al., 2006; Cassol,

Rossouw, Seebregts, & Cassol, 2011; Deeks, 2011; Douek, 2007; Duprez et al., 2012; El-Sadr et al., 2006; Erlandson et al., 2013; Jiang et al., 2009; Kamat, Lyons, et al., 2012; Kuller et al., 2008; Marchetti et al., 2011; Marks et al., 2013; Neuhaus et al., 2010; Nixon & Landay, 2010; Nowroozalizadeh et al., 2010; Pedersen et al., 2013; Rodger et al., 2009; Sandler et al., 2011; Tenorio et al., 2014)

The current research aimed to explore the association of symptoms with specific HIV-related biomarkers predictive of HIV-related morbidity, mortality and HIV disease progression. The long term goal of this research is to support the development of strategies to improve clinical outcomes of HIV disease which are targeted toward symptom management at the etiological level and reducing the effects of inflammation in the context of viral suppression.

Background and Significance

HIV-related Epithelial Barrier Dysfunction and Microbial Translocation

HIV infection has a profound effect on the gastrointestinal tract. The mechanisms underlying HIV transmission and pathogenesis initiate an inflammatory process in the gut. The gastrointestinal (GI) epithelial barrier has an integral role in maintaining homeostasis providing the interactive transition from the external environment and the internal body (Burgener, McGowan, & Klatt, 2015). This mucosal barrier provides an effective immune defense against pathogens with the main portion of the immune system, the gut-associated lymph tissue (GALT), on the host internal side and the microbiome on the external side (Burgener et al., 2015; Koboziev, Karlsson, & Grisham, 2010). HIV alters the microbial ecology in the GI tract resulting in a dysfunctional microbiome dysbiosis. Dysbiosis in conjunction with a mechanical dysfunction in the GI epithelial

barrier allow microbial products that normally reside in the GI lumen of the intestine to relocate across the monocellular barrier into the internal environment (Dillon et al., 2014). Several factors lead up to the impairment of the GALT which results in these microbial products accessing the blood circulatory system (Zevin, McKinnon, Burgener, & Klatt, 2016).

HIV antigenic properties activate local CD4+ cells. T helper 17 (Th17) cells in the GALT initiate the inflammatory process at the GI epithelial barrier. Once the Th17 cells are activated, HIV, which has an affinity for the CCR5 receptor expressed on the CD4+ Th17 cell surface, infects the cell (Estes et al., 2010; Pandrea et al., 2007). Th17 cells are responsible for producing three very important interleukin (IL) cytokines in this process: IL-17 which is pro-inflammatory, IL-22 which helps repair tight gap junctions, and IL-10, a regulatory cytokine that will regulate inflammation (Estes et al., 2010; Klatt et al., 2010). The pathogenesis of HIV significantly depletes CD4+ cells. The depletion of CD4+ cells weakens the immune defense of the GALT. The remaining CD4+ cells continue to be activated by HIV inducing inflammation at the local level (Gordon et al., 2010). Damaged enterocytes undergo apoptosis weakening the tight gap junctions and increasing the permeability of the barrier (Zheng et al., 2007). With low levels of IL-22 and ongoing activation from replicating HIV-1 virus, the tight gap junctions are impaired. Furthermore, there is limited IL-10 for anti-inflammatory immune modulation impairing the inflammatory regulatory process (Said et al., 2010). The changes in the microbiome become more pathogenic and the symbiotic relationship of the microbiome begins to shift (Zevin et al., 2016). Bacteria are able to translocate across the dysfunctional epithelial barrier, evade the protection at the GALT level due to depletion of the CD4+ cells, and

enter into circulation initiating systemic immune activation (Brenchley et al., 2008; Brenchley et al., 2004; Burgener et al., 2015; Zevin et al., 2016).

Microbial translocation, the movement of bacteria and/or microbial products from the gastrointestinal milieu into the blood, has been implicated as one of the key drivers of chronic inflammation and HIV disease progression (Brenchley & Douek, 2008; Brenchley et al., 2006; Funderburg et al., 2010; Marchetti et al., 2008; Marchetti et al., 2011; Sandler et al., 2011). Even with successful suppression of HIV-1 with effective cART, microbial translocation has been shown to result in inflammation from chronic immune activation, eventually contributing to increases in risk for morbidity and mortality among PLWH (Brenchley et al., 2006; Deeks, 2011; Kamat, Misra, et al., 2012; Marchetti et al., 2011; Marchetti, Tincati, & Silvestri, 2013).

Gastrointestinal epithelial barrier damage and microbial translocation are more prevalent at chronic stages of HIV disease than in acute/early stages of the disease. sCD14 is secreted by monocytes, liver hepatocytes, and dendritic cells in response to circulating the bacterial lipopolysaccharide (LPS) outer wall (Brenchley et al., 2006; Carotenuto et al., 2005). When left untreated, patients with HIV infection exhibit higher levels of sCD14 and LPS (Brenchley et al., 2006; Sandler et al., 2011). Levels of LPS, and sCD14 in response to LPS, decrease in treated HIV disease but not to levels of those uninfected with HIV (Brenchley et al., 2006). No correlation between sCD14 and plasma viral load has been documented in the literature (Brenchley et al., 2006). Interestingly, microbial translocation is a phenomenon also identified in Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS) (McGuckin, Eri, Simms, Florin, & Radford-Smith, 2009). It is also known there is residual viral replication in the GALT and HIV-1

reservoirs contributing to local immune activation and inflammation of the epithelial barrier (Chun et al., 2008). However, it is unknown whether the resulting inflammation of immune activation is related to HIV symptoms. IBD/IBS, along with leaky gut syndrome, are well studied disease models of inflammation and epithelial barrier dysfunction (Berkes, Viswanathan, Savkovic, & Hecht, 2003). Because of the similarities in epithelial barrier inflammation and dysfunction, the IBD/IBS pathologic model is used as the clinical conceptual framework for this study. IBD/IBS has a similar gut pathology that leads to microbial translocation although IBD/IBS has differing etiology. IBD and IBS are diagnoses that are accompanied by GI complaints such as nausea, diarrhea, bloating, abdominal pain, constipation, and irregularity of bowels (McGuckin et al., 2009); such symptoms are also commonly reported in HIV disease (McGuckin et al., 2009; Ohland & Macnaughton, 2010). Additionally, while there are similarities in microbial translocation and HIV disease symptoms reported, there are also similarities with loss of Th17 type CD4 cells in the GALT and gut inflammation in both HIV-uninfected IBD/IBS and in those with HIV disease. In contrast to the IBD/IBS model where there is immune-mediated clearance of microbial products, this mechanism is diminished in HIV disease (Yim, Li, Lau, & Lau, 2009). Therefore, in contrast to IBD/IBS in HIV-uninfected people, in HIV disease the body becomes accustomed to circulating levels of microbial products consequently developing chronic inflammation without sepsis in HIV disease (Brenchley et al., 2006; Estes et al., 2010; Klatt et al., 2010).

Given the similarities with IBD/IBS in pathophysiology, GI symptoms may be an indication of underlying acute and chronic HIV immune-mediated inflammation from activated Th17 CD4 cells. Furthermore, GI symptoms in HIV-infected patients resemble

symptoms experienced with IBD/IBS, but providers may attribute GI symptoms to other causes, such as depression or medications (Johnson, Stallworth, & Neilands, 2003). There are limited studies published on the investigation of IBD/IBS symptoms and the systemic inflammation associated with microbial translocation.

Although GI symptoms in HIV-infected patients resemble symptoms experienced with IBD/IBS, providers may attribute GI symptoms to depression or medication side effects (Johnson et al., 2003). Because IBD/IBS has similar GI symptoms as experienced in HIV disease, gut dysfunction and inflammation in HIV disease may be contributors to GI symptoms. Furthermore, because microbial translocation results in enhanced immune activation and chronic inflammation (Dillon et al., 2014), inflammatory-type systemic symptoms and/or disease conditions resulting from inflammation may develop or evolve. The relationship between microbial translocation and symptom development is unknown, although it is known that microbial translocation is broadly accepted as an indicator of GI barrier dysfunction and a source of inflammation (d'Ettorre, Paiardini, Ceccarelli, Silvestri, & Vullo, 2011; Estes et al., 2010). Multiple symptoms originating from underlying barrier dysfunction within the GI tract and from chronic inflammation in response to circulating microbial products may contribute to or be the source of commonly reported symptoms, poor clinical outcomes, and ultimately health disparities among PLWH.

Non-HIV Associated Conditions Contributing to Microbial Translocation

There are several confounders contributing to microbial translocation in HIV disease; hazardous drinking, smoking, and Hepatitis C co-infection (HCV) have each been associated with elevated levels of sCD14 in HIV disease (Armah et al., 2012;

Marchetti et al., 2014). Microbial translocation un-related to HIV disease is a source of immune activation and subsequent chronic inflammation.

Measuring Microbial Translocation in HIV Research

Microbial translocation can be measured in the blood by testing for the bacterial outer coating (LPS), bacterial DNA or RNA (16s RNA or DNA), or using indirect markers such as the release of sCD14 in response to bacterial products (Sandler et al., 2011). Each assay has its limitations; however, sCD14 has been the most widely used biomarker of microbial translocation, given that LPS produces inconsistent results and 16s rRNA and DNA testing has inherent technical processing risks resulting in false-positive results (Balagopal et al., 2012; Corless et al., 2000; Kramski et al., 2011; Marchetti et al., 2013). Additionally, since a significant correlation between plasma LPS and sCD14 has been shown, sCD14 is a better marker of microbial translocation (Brenchley et al., 2006).

sCD14 is an established and commonly used surrogate biomarker of microbial translocation that is strongly associated with severity of epithelial barrier dysfunction in HIV disease (Brenchley et al., 2006; Estes et al., 2010; Klatt et al., 2010). Higher levels of sCD14 have been associated with mortality rates up to 8 times greater than that of healthy controls (El-Sadr et al., 2006; Sandler et al., 2011). Furthermore, sCD14, as a measure of microbial translocation, has been shown to independently improve the predictive properties of the Veterans Aging Cohort Study (VACS) Index for mortality (Estes et al., 2010; Justice et al., 2013). Even with successful adherence to cART and HIV-1 viral suppression, inflammation from chronic immune activation increases the rates of morbidity and mortality among those living with HIV disease (Brenchley et al.,

2006; d'Ettorre et al., 2011; Deeks, 2011; Kamat, Misra, et al., 2012; Marchetti et al., 2011).

Measuring Inflammatory Biomarkers in HIV Research

One of the most impactful studies identifying the effect of inflammation on HIV disease progression was conducted by the Strategies for Management of Antiretroviral Therapy (SMART) study group. This large multicenter randomized clinical trial examined interruption and late-start of cART initiation based on CD4+ T cell count with a primary endpoint of new or recurrent opportunistic infection, or all-cause death. Secondary endpoints were a potentially life-threatening symptomatic event requiring medical intervention or death. The study was interrupted due to the high risk of morbidity and mortality in the treatment interruption group. Participants with higher levels of IL-6 were 2.4 (95% CI: 2.1 – 8.8) times more likely to develop opportunistic disease than those uninfected; likewise, participants with higher levels of hs-CRP were 7.6 (95% CI: 2.0 to 28.5) times more likely than those in the lower quartile to develop opportunistic disease than those uninfected (El-Sadr et al., 2006). Similarly, participants in the highest quartile of sCD14 levels had a 6-fold greater risk of mortality than those having levels of sCD14 in the lower quartile (Sandler et al., 2011).

In the IBD/IBS disease model among people who are not infected with HIV-1, levels of LPS are bound and cleared away by Endotoxin Core Antibodies (EndoCAb) (Brenchley et al., 2006). HIV disables the mechanism for clearing microbial products. Therefore, levels of EndoCAb are lower in PLWH subsequently augmenting chronic immune activation (Brenchley & Douek, 2008; Brenchley et al., 2006; Sandler et al., 2011) in response to circulating LPS. Levels of sCD14 have been correlated with IL-6 (*r*

= .35; p <.001) and D-dimer (r = .26; p =.006) (Brenchley & Douek, 2008; Brenchley et al., 2006; Sandler et al., 2011).

IL-6 is a classic marker of inflammation and has been associated with cardiovascular disease, AIDS, non-AIDS events, and sCD14 (Deeks, 2011; El-Sadr et al., 2006; Klatt et al., 2010; Rodger et al., 2009; Stehle et al., 2012). Circulating LPS stimulates the release of IL-6 (Stehle et al., 2012).

Another marker frequently measured in HIV research is D-dimer. D-dimer is a marker of coagulation and associated with sCD14. D-dimer is predictive of the development of cardiovascular disease and elevated levels associated with serious non-AIDS related cardiac events (Nixon & Landay, 2010; Redd et al., 2009).

Chronic inflammation from immune activation and elevated levels of sCD14 and IL-6 have been linked to early aging, metabolic disease, cardiovascular disease, decline in renal function, cancer, bone disease, and other end-organ diseases (Ancuta et al., 2008; Deeks, 2011; Duprez et al., 2012; Erlandson et al., 2013; Kamat, Lyons, et al., 2012; Marks et al., 2013; Neuhaus et al., 2010; Pedersen et al., 2013). Persons with higher levels of sCD14, D-dimer, IL-6, and C-reactive protein were more likely to progress to a diagnosis of AIDS and non-AIDS events in HIV disease (Duprez et al., 2012; El-Sadr et al., 2006; Nixon & Landay, 2010; Rodger et al., 2009; Sandler et al., 2011). Since microbial translocation is one of the key drivers of HIV disease progression and systemic inflammation (Brenchley & Douek, 2008; Brenchley et al., 2006; Cassol et al., 2011; Jiang et al., 2009; Marchetti et al., 2011; Nowroozalizadeh et al., 2010; Sandler et al., 2011), and GI epithelial barrier inflammation and systemic inflammation have been linked to symptoms, it may be that these biological processes may contribute to the

development of symptoms experienced in PLWH (Armah et al., 2012; Marchetti et al., 2014).

HIV-related Symptoms and Symptom Burden

HIV is associated with a high symptom burden that may be related to inflammation, as experienced in similar inflammatory conditions in uninfected population. The most commonly reported symptoms in HIV disease include: constitutional complaints, GI symptoms, peripheral neuropathy, myopathy, joint pain, cognitive decline and memory loss, and sleep problems (Cooper, Gellaitry, Hankins, Fisher, & Horne, 2009; Corless et al., 2013; Edelman, Gordon, & Justice, 2011; Gay et al., 2011; Johnson & Folkman, 2004). Whether the primary outcome is to examine adherence to cART, quality of life, or access to health care in HIV disease, symptoms serve as a barrier to achieving successful clinical outcomes. These symptoms often occur in clusters and accumulate to create burden on the person decreasing quality of life and/or affecting adherence to medications (Cooper et al., 2009; Corless et al., 2013).

In previous studies, symptoms such as functional decline (Erlandson et al., 2013; Stehle et al., 2012), cognitive decline (Ancuta et al., 2008; Kamat, Lyons, et al., 2012; Lyons et al., 2011; Vance, McDougall, Wilson, Debiasi, & Cody, 2014), obesity (Koethe et al., 2013), and anxiety and sadness (Liebregts et al., 2007) have been associated with microbial translocation and other inflammatory cytokines. Fatigue (Klimas, Broderick, & Fletcher, 2012), muscle aches, joint pain (Eriksson, Andersson, Ekerfelt, Ernerudh, & Skogh, 2004), poor sleep (Grandner, Sands-Lincoln, Pak, & Garland, 2013), fever/chills/sweats (Holtzclaw, 2013), night sweats (Mold, Holtzclaw, & McCarthy, 2012), peripheral neuropathy (Harezlak et al., 2011; Schifitto et al., 2005; Zheng et al.,

2011), diarrhea (Liebregts et al., 2007), anxiety and depression (Camacho, 2013), and weight loss/wasting (Stein et al., 1997) have been associated with inflammation. Inflammation leading to and resulting from microbial translocation may also play a significant role in commonly reported systemic symptoms, and be a barrier to successful clinical care. It is unclear if sCD14 is associated with GI symptoms prevalence specifically; however, sCD14 and often biomarkers of inflammation have been identified as a key predictor of disease progression.

While it is well documented that the inflammatory effects on the GI epithelial barrier in HIV-infected patients result in microbial products leaking from the gut lumen into the blood circulation (Brenchley & Douek, 2008; Brenchley et al., 2006; Estes et al., 2010), it is unknown whether these translocated microbial products are contributing to the development of symptoms or if the symptoms could provide providers with clues to the underlying pathogenesis of damaged or inflamed GI epithelium, microbial translocation, or chronic HIV-related inflammation.

Symptom Burden and HIV Medication Adherence

Symptom burden is the cumulative distress of symptoms experienced by a patient (Cleeland & Reyes-Gibby, 2002). Symptom burden is associated with decreased quality of life scores, poor health outcomes, and at times are often unreported or unrecognized by providers (Edelman et al., 2011). There are several validated scales to measure prevalence and magnitude of how bothersome symptoms are to patients, including the HIV Symptom Index developed for outpatient use (Justice, Holmes, et al., 2001). Over half of PLWH report that GI symptoms, such as diarrhea, bloating, and abdominal pain are frequent and bothersome (Johnson et al., 2003; Justice, Chang, Rabeneck, & Zackin,

2001). Over 30% of PLWH are likely to discontinue effective cART due to GI symptoms (Gay et al., 2011; Kempf et al., 2009). While many variables could contribute to symptom manifestation, some symptoms are associated with inflammation as are discussed in Chapter 3 of this thesis.

Adherence to cART is a strong predictor of successful HIV treatment (Wood et al., 2003). One study demonstrated that <92% cART coverage increased risk for virologic failure (OR = 1.47, 95% CI: 1.08-2.04) (Genberg et al., 2012). HIV-1 viral load and CD4+ cell count are key indicators of adherence and thus are predictors of clinical outcomes and disease progression (Glass et al., 2015; Palella et al., 2003). There have been significant improvements in the treatment of HIV disease and in the outcomes of treatment since the epidemic began over 30 years ago. Patients initiating cART in earlier days were at risk for experiencing serious adverse events and major side effects as a result of aggressive therapies. Current drug regimens are simpler, tolerable, more effective, and offer lower side effect profiles (Gay et al., 2011). However, symptoms persist and the burden of the symptom experience has been associated with poor adherence, either through postponing taking medication to avoid experiencing the symptom, or by forgetting, and/or sleeping through medications due to fatigue (Gay et al., 2011).

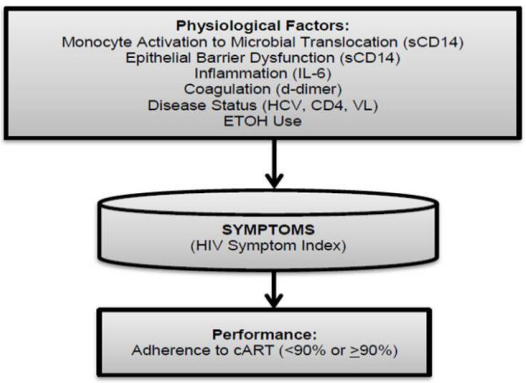
Symptoms, often attributed to cART or to HIV infection itself (Johnson et al., 2003) may influence adherence to successful HIV care (Gay et al., 2011; Heckman, Catz, Heckman, Miller, & Kalichman, 2004; Kempf et al., 2009; Kremer, Ironson, Schneiderman, & Hautzinger, 2006). It is estimated that only 25% of those living with HIV disease are virologically suppressed (Centers for Disease Control, 2012), which has

been attributed to non-adherence to medications and poor engagement in care. Patients perceive the management of co-morbidities and symptoms as a need, and influence their engagement in care which may impact symptom management and successful viral control if not addressed (Hall et al., 2013; Mugavero et al., 2013). Identifying whether HIV symptoms are associated with inflammation, and if such symptoms are associated with adherence to cART, are important questions to be addressed. This association has a potential impact on clinical outcomes.

In summary, this literature review provides a background outlining the foundation for investigating symptoms and inflammation. Following the rationale of HIV-1 initiating local immune activation of primarily the CD4+ Th17 cells in the GALT initiating a sequence of inflammatory events that led up to the dysfunction of the tight gap junctions and chronic inflammation of the GI epithelial barrier. The depletion of CD4+ T cells in the GALT and increased permeability of the GI epithelial barrier allows the translocation of microbial products past the depleted defense of the GALT into blood circulation initiating immune activation of the systemic innate immune system. Due to impairment of the immune function in the GALT and dysregulation of the host immune system in clearing microbial products, the body remains in immune activation and a chronic inflammatory condition. The chronic inflammatory state of the GI epithelial barrier is similar to IBD/IBS in which patients report a set of similar GI and systemic symptoms experienced in HIV disease. Furthermore, because of this underlying pathophysiology, we would expect the development of inflammatory-based symptoms in patients.

Nursing Research Framework

The background described follows the Middle Range Nursing Theory of Unpleasant Symptoms adapted in Figure 1 and provides a nursing-based conceptual model describing the foundation for the overall body of research conducted in fulfillment of this dissertation research. Symptoms develop from a variation of factors or antecedents: 1. physiological, 2. situational, and 3. psychological. These antecedent factors influence symptom timing, intensity, distress, and quality. Symptoms often work together to influence other symptoms and eventually have an influence on performance in activities or other outcomes (See Figure 1) (Lenz, Pugh, Milligan, Gift, & Suppe, 1997).



Adapted from Middle Range Theory of Unpleasant Symptoms. From "The middle-range theory of unpleasant symptoms: an update" by Lenz, E. R., Pugh, L. C., Milligan, R. A., Gift, A., & Suppe, F. (1997). *Advances in Nursing Science, 19*(3), 14-27. Permission obtained.

Figure 1: Nursing Theoretical Framework.

This background review of HIV pathogenesis at the site of the GI epithelial barrier and what is known of previous symptom work described as well as the unknown gaps, lays the foundation for the research questions proposed in this dissertation. What follows are the overall research purpose, and research questions and hypotheses addressed in the published manuscripts reprinted and prepared manuscript pre-publication in fulfillment of this research dissertation.

Overall Purpose

The goal of this dissertation research was to gain an understanding of commonly reported symptoms in the context of HIV-related epithelial barrier dysfunction and microbial translocation. This knowledge will support the improvement of clinical decision making and patient education. The results of this investigation will potentially support clinicians in their assessment of various symptoms and in modifying their treatment and education plans to accurately reflect underlying pathology and physiological abnormalities. The results from this work will contribute to the body of symptom research and to enhance clinical assessment by identifying symptoms associated with microbial translocation. In addition to the reframing of symptom assessment, the symptoms identified as a result of this research can be used as targeted variables of interest in future clinical trials to assess and improve clinical outcomes by reducing symptom burden and barriers to cART adherence.

Article Synthesis

PLWH have inflammation of the GI epithelial barrier initiated by HIV-1 activation of the CD4+ Th17 cells in the GALT (Brenchley et al., 2008; Klatt & Brenchley, 2010). Inflammation of the epithelial barrier and eventual dysfunction of the tight gap junctions allows the translocation of microbial products (Brenchley et al., 2006; Klatt et al., 2010). The pathway from inflammation to microbial translocation in PLWH is similar to the physiologic pathway seen in IBD/IBS (Kajander & Korpela, 2006; Kamat, Ancuta, Blumberg, & Gabuzda, 2010; McGuckin et al., 2009; Su, Judge, & Lichtenstein, 2002). Probiotics have been successful as a therapeutic option for reduction of inflammatory cytokines in people living with IBD/IBS (Furrie et al., 2005; Kajander,

Hatakka, Poussa, Farkkila, & Korpela, 2005; Steed et al., 2010). Probiotics have been successful in treating GI-related conditions and symptoms, such as, diarrhea, oral candida, bloating and gas, and abdominal adiposity (Gao, Mubasher, Fang, Reifer, & Miller, 2010; Hatakka et al., 2007; Kadooka et al., 2010; Kalman et al., 2009). Additionally, probiotics have also been used in healthy participants and demonstrated beneficial intestinal and immune effects with induction of regulatory cytokine IL-10, natural killer cells, monocytes, plasma levels of IgM, IgA, and IgG (Sierra et al., 2010). Therefore, it was hypothesized that probiotics could be used as supportive adjunct therapy in PLWH. We conducted a comprehensive review to assess whether this was a valid hypothesis.

Article 1: A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection

The first published article included in this dissertation is titled, *A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection* (Wilson, Moneyham, & Alexandrov, 2013) and describes the use of probiotics in certain GI conditions for uninfected populations. This article provides a comprehensive review on the investigation of probiotics and how they have been used to alleviate symptoms and demonstrated efficacy in reducing microbial translocation in patients not infected. This review provided the rationale for the use of probiotics in HIV disease to reduce microbial translocation and symptoms. Since its publication, several studies have demonstrated successful outcomes in using probiotics to reduce inflammatory cytokines (d'Ettorre et al., 2015; Stiksrud et al., 2015). We discussed how probiotics may be a consideration for

adjunct therapy in conjunction with cART in HIV disease management in Chapter 2 of this thesis.

Microbial translocation is the result of a dysfunctional GI epithelial barrier and CD4+ T cell depletion by HIV in the GALT (Estes et al., 2010; Klatt et al., 2010). Once microbial products from the gut lumen are in circulation, the innate immune system is activated causing systemic inflammation (Somsouk et al., 2015). We hypothesized that the inflammation or GI epithelial barrier dysfunction could lead to similar GI symptoms based on the IBD/IBS model and physiological changes. Symptoms such as abdominal pain, and functional symptoms of diarrhea, bloating, constipation, and a malabsorption of nutrients possibly affecting appetite have been associated with IBD/IBS (Berkes et al., 2003). Several symptoms such as memory loss, fatigue, pain, and lipodystrophy have been investigated and found to be associated with IL-6 and sCD14 (Ancuta et al., 2008; Eriksson et al., 2004; Klimas et al., 2012; Stein et al., 1997). We also hypothesized that systemic symptoms would be an outcome of elevations in pro-inflammatory cytokines resulting from microbial translocation induced systemic immune activation. Therefore, we developed a conceptual framework based on these pathways to describe this process.

Article 2: Connecting the Dots: Could Microbial Translocation Explain Commonly Reported Symptoms in HIV Disease?

The second published article entitled, *Connecting the Dots: Could Microbial Translocation Explain Commonly Reported Symptoms in HIV Disease?* (Wilson et al., 2014), provides a summary of what is known of the impact HIV infection has on inflammation of the GI barrier, which may lead to symptoms and leakage of microbial products from the gut into circulation, leading to systemic symptoms. This review

provides a conceptual framework for research on symptoms in the context of microbial translocation and inflammation. Additionally, an examination of the current prevalence and burden of symptoms in the HIV-infected population needed to be updated in the modern era of cART. In addition to updating the prevalence of HIV symptoms, it was necessary to determine if the pattern of symptoms prevalent in HIV disease supported the inflammatory-based framework presented in Chapter 3 of this thesis.

Article 3: Identifying Symptoms in HIV Disease

This article reports the findings of pilot research that was foundational to the dissertation research. The pilot study questions and hypotheses included:

Research Questions and Hypotheses

- Research question: What are the symptoms associated with HIV disease?

  Hypothesis 1: GI symptoms are prevalent in HIV disease.

- Research question: Are there symptom patterns inclusive of inflammatory related symptoms?

  Hypothesis 1: There are symptom clusters that form a pattern associated with inflammation.

We hypothesized that there was a reduction in the prevalence of HIV-related symptoms reported by PLWH due to the improvement of side effect profiles of antiretroviral drugs (Katlama et al., 2009; Lennox et al., 2009; Madruga, D. Berger, et al., 2007; Madruga, Cassetti, et al., 2007; Madruga et al., 2007). Based on our conceptual framework of symptoms being associated with chronic inflammation, we hypothesized that we should see an association between symptoms and inflammatory biomarkers, particularly those inflammatory biomarkers predictive of non-AIDS morbidity and

mortality. Based on this hypothesis, symptoms should form a pattern associated with inflammation.

Identifying Symptoms in HIV Disease (Wilson et al., 2015), the third published article, reported an up-to-date prevalence and burden of symptoms, and symptom clusters in PLWH. This article described the results a retrospective investigation of symptoms self-reported by patients attending an HIV outpatient care clinic in Birmingham, Alabama. The findings support that symptoms commonly reported were similar to symptoms reported in IBD/IBS and systemic symptoms were those that had previous studies demonstrating an association with inflammatory biomarkers that were predictive of HIV-related morbidity and mortality. (Ancuta et al., 2008; Camacho, 2013; Eriksson et al., 2004; Erlandson et al., 2013; Grandner et al., 2013; Harezlak et al., 2011; Holtzclaw, 2013; Kamat, Lyons, et al., 2012; Klimas et al., 2012; Koethe et al., 2013; Liebregts et al., 2007; Lyons et al., 2011; Mold et al., 2012; Sandler et al., 2011; Schifitto et al., 2005; Stehle et al., 2012; Stein et al., 1997; Vance et al., 2014; Zheng et al., 2011).

Article 4: A Retrospective Analysis of HIV-associated Symptoms and Microbial Translocation in the Veterans Aging Cohort Study

We address these questions in Chapter 5 of this thesis in a manuscript prepared for submission to the Journal of Acquired Immunodeficiency Syndromes, and titled, *A Retrospective Analysis of HIV-associated Symptoms and Microbial Translocation in the Veterans Aging Cohort Study (VACS). This chapter* describes the association of symptoms self-reported from 1,418 Veterans living with HIV disease and inflammatory biomarkers, including sCD14, IL-6, and D-dimer. The VACS is funded by the National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health. It is the

richness of the cohort and accessibility of this data that makes the VACS and the Veteran population a great resource to begin this research program.

The Veterans' Health Administration (VHA) is the largest single HIV care provider in the US. Within this system, there have been nearly 64,000 HIV-infected Veterans receiving any clinical care through this system with more than 26,000 receiving their HIV care service directly through the VHA (Center for Quality Management in Public Health, Office of Public Health and Environmental Hazards, & Veterans Health Administration, 2009). The VHA has a substantial electronic infrastructure with direct access to all electronic medical records that include clinical, mortality, and administrative data.

In 1999, the VACS began to examine Veterans living with and without HIV disease in a prospective, multisite observational study. By 2014, the VACS has expanded to 9 VHA sites in the US including Atlanta, Baltimore, Bronx, Manhattan, Houston, Dallas, Los Angeles, Pittsburgh, and Washington, D.C. with over 3,600 HIV-infected Veterans enrolled in the cohort. In addition, the cohort includes HIV-uninfected Veterans who are age-race-site group matched to their HIV-infected comparison group (Justice et al., 2006; Yale School of Medicine, 2014). Today, more than 7,000 Veterans HIV-infected and -uninfected have been consented and enrolled in the VACS (Yale School of Medicine, 2014). This cohort represents a nationally based snapshot of PLWH seeking outpatient care for HIV disease in the US.

This manuscript reports the main findings of the investigators dissertation research. Based on the symptoms reported in Chapter 4 and the conceptual framework of reported in Chapter 3 of this thesis, we focused our investigation specifically on GI

symptoms reported, as reported in *Identifying Symptoms in HIV Disease* (bloating/abdominal pain, nausea/vomiting, diarrhea, and loss of appetite). The research and hypotheses include:

Research Questions and Hypotheses

- Research question: Are epithelial barrier dysfunction (sCD14) and microbial translocation associated with the presence of GI symptoms (bloating/abdominal pain, diarrhea, nausea/vomiting, and loss of appetite)?

  Hypothesis 1: Epithelial barrier dysfunction measured by circulating gut microbial products (sCD14) is associated with the presence of GI symptoms.
- Research question: Is HIV-1 *viral suppression* associated with GI symptoms (bloating, abdominal pain, diarrhea, and nausea/ vomiting)?

  Hypothesis 2: GI symptoms are *independent of HIV-1 viral suppression.*
- Research question: Are inflammatory biomarkers such as IL-6, D-dimer, and sCD14 associated with GI symptom distress?

  Hypothesis 3: GI symptom distress is associated with higher levels of inflammation from IL-6, d-Dimer, and sCD14.

We hypothesized that functional GI symptoms would be associated with GI dysfunction, measured by monocytic response to circulating microbial products, sCD14. We also hypothesized that this would be independent of cART due to low side effect profiles. Because of the absence of information of cART adherence, we used viral load (<500 copies/mL and >500 copies/mL) as a surrogate marker of medication adherence. We sought to determine if sCD14 released from monocytes would be increased in the setting of microbial translocation. Furthermore, we hypothesized that microbial

translocation would result in non-specific immune activation, as measured by increases in IL-6 and D-dimer and therefore, elevated levels of sCD14, IL-6, and D-dimer would be associated with GI symptoms (bloating, nausea/vomiting, diarrhea, and loss of appetite).

In the outpatient setting, providers often rely on symptom assessment to guide diagnosis and education. This manuscript reports the findings of the dissertation identifying clinical factors that may contribute to GI symptom expression including sCD14, IL-6, and D-dimer. We also examined the association between elevated levels of circulating microbial products [sCD14] and GI symptoms to assess if GI symptoms can alert providers to epithelial barrier dysfunction and subsequent microbial translocation. We examined the difference between HIV-1 viral suppression, as a surrogate marker for adherence, and the presence GI symptoms. Differences between those suppressed and unsuppressed was assessed to provide evidence of whether GI symptoms could be related to persons taking or not taking their prescribed cART, and/or whether GI symptoms could be attributed to HIV-1 viral load.

This research is clinically important to nursing to better understand the source of the symptoms commonly reported by PLWH and reframe how providers consider symptom reported by patients during clinical visits. Symptoms are prevalent in HIV disease and moderately associated with inflammatory biomarkers that have been identified with key predictors of poor outcomes in HIV infection. This understanding is useful in exploring otherwise unexplained symptoms as a possible indication of inflammation of the epithelial barrier and take efforts to manage GI symptoms. Further studies would be required to investigate the use of probiotics to resolve these symptoms and reduce inflammation. In addition, substance abuse treatment and Hepatitis C

treatment should be investigated as a potential focus of reducing inflammation and improvement of HIV disease outcomes in this context.

SYSTEMATIC REVIEW OF PROBIOTICS AS A POTENTIAL INTERVENTION TO RESTORE GUT HEALTH IN HIV INFECTION

by

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A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection

Abstract

Probiotics have beneficial effects on the gut in numerous conditions. The purpose of this paper is to present a review of the current literature on probiotics used in chronic illnesses exhibiting similar pathology seen in HIV gut dysfunction in order to make recommendations for their use to promote and restore healing of the gut with subsequent reduction of ongoing inflammation caused by microbial translocation. A review of the literature was performed focusing on probiotics as an intervention to improve gut health. Key words were searched in PubMed and the Cumulative Index to Nursing and Allied Health Literature (CINAHL). The literature reviewed was limited to clinical trials, meta-analyses, and practice guidelines. The review provided evidence that probiotics were supportive in modulating aspects of gut physiology, barrier integrity, and immune function. Probiotic use is a supportive adjunct therapy, worthy of consideration and further research in persons infected with HIV.

Key Words: epithelial barrier, gut associated lymph tissue, gut inflammation, HIV, microbial translocation

A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection

Common complaints from patients infected with HIV include diarrhea, bloating, abdominal discomfort, and changes in body weight. In addition, providers and patients are frustrated with regard to patients failing to achieve immune reconstitution despite viral suppression with antiretroviral therapy (ART; Kelley et al., 2009; Torti et al., 2004). There is growing recognition that this immunologic failure may be due to changes in the gut mucosa. These changes have been attributed to HIV-associated inflammation in the gut (Paiardini, Frank, Pandrea, Apetrei, & Silvestri, 2008; Pandrea, Sodora, Silvestri, & Apetrei, 2008) and significant loss of Th17 cells, a certain type of CD4+ T cell exhibiting the CCR5 receptor that secretes a pro-inflammatory cytokine IL-17 (Brenchley et al., 2008). Probiotics have been effective in managing similar symptoms in other conditions with gut inflammatory etiology. Anecdotally, some providers are beginning to recommend probiotics to alleviate and manage these gastrointestinal (GI) symptoms. However, there is little empirical evidence supporting the effectiveness of probiotics with respect to populations infected with HIV.

There has been growing interest in examining microbial translocation caused by HIV immune activation within the gut-associated lymph tissue (GALT). Microbial translocation is associated with the failure of reconstitution of CD4+ T cells even in the presence of viral suppression with ART (Jiang et al., 2009; Marchetti et al., 2008). Translocation of bacterial products into peripheral circulation is partly in consequence of the degradation of protective mechanisms of epithelial components forming the gut barrier (Dandekar, George, & Baumler, 2010). Outcomes associated with this process

include ongoing inflammation in the gut and periphery. Serological markers and irritable bowel serological patterns have been studied and found to be present in HIV-infected patients with high levels of detectable translocated microbial products (Kamat, Ancuta, Blumberg, & Gabuzda, 2010). Patients with HIV infection experience a subsequent loss of CD4+ T cells and GI enteropathy with symptoms such as discomfort, diarrhea, bloating, and nutritional deficits described histologically by mucosal epithelial degeneration, intestinal microvilli loss, and inflammation (Bhaijee, Subramony, Tang, & Pepper, 2011). GI dysfunction has been a common companion to all stages of HIV disease (Knox, Spiegelman, Skinner, & Gorbach, 2000).

Complaints of GI discomfort, bloating, constipation, oral and esophageal candidiasis, and ailments such as diarrhea commonly seen in HIV might be well addressed by probiotic therapy. In addition, diarrhea is a side effect of antiretroviral medications and antibiotic use. Furthermore, patients with low albumin levels and CD4+ T cell counts below 50 cells/mm^3 are likely to develop a pathogenic cause of diarrhea (Bonacini, Skodras, Quiason, & Kragel, 1999). Chronic diarrhea increases the risk for mortality (Dillingham et al., 2009), and, in some cases, even after starting ART, survival is limited.

According to Gordon and colleagues (2010), HIV enteropathy affects absorption of ART leading to virologic failure and failure of immune reconstitution, leading to death. The immunopathogenesis of HIV is strongly associated with marked destruction of intestinal CD4+ T cell homeostasis, subsequent microbial translocation, and chronic immune activation (Gordon et al., 2010).

Probiotics have been effectively used as a prevention and therapeutic approach in

the arena of inflammatory bowel diseases. Probiotics inhibit pathogenic bacteria and toxins by adhering to the intestinal epithelium, induce anti-inflammatory cytokines, and promote intestinal epithelial cell homeostasis (Vanderpool, Yan, & Polk, 2008). The molecular mechanism of probiotics has provided support for their application as an adjunct and alternative treatment of gut inflammation.

Probiotics are commensal bacteria that have the beneficial effect of sustaining immune mediation and repair of the gut mucosa (Mazmanian & Kasper, 2006; Verhoeven, Sankaran, Silvey, & Dandekar, 2008). The gastroenteropathy seen in HIV is similar in pathology to that seen in inflammatory gut disease, and thus may be responsive to interventions used to treat inflammatory gut disease. Therefore, probiotics may be a cost-effective adjunct therapeutic intervention to reduce chronic GI symptoms seen in HIV-infected patients.

The purpose of this paper is to present a review of the current literature on probiotics used in chronic illnesses exhibiting similar pathology seen in HIV gut dysfunction in order to make recommendations for their use to promote and restore healing of the gut with subsequent reduction of ongoing inflammation caused by microbial translocation. The overall aim of this paper is to examine the evidence of the effect of probiotic therapy on epithelial function, symptoms, and gut immune response reported in other GI conditions, as an important first step to identify interventions that may be efficacious in treating GI symptoms in HIV disease and thus warrant further investigation.

Background

The success of HIV treatment is based on effective combination ART with

evidence of decreases in peripheral viral loads and increases in CD4+ T cell counts (Verhoeven et al., 2008). However, ART may have little effect on HIV-associated inflammation that has been linked to HIV-associated co-morbidities and abnormalities that occur despite the use of ART (Baker et al., 2011; Deeks, 2011; Harezlak et al., 2011; Mangili, Polak, Quach, Gerrior, & Wanke, 2011). The effects of inflammation on the cardiovascular, renal, neurologic, and gastroenterological systems are unchanged by the suppression of HIV by ART. Therefore, there is a need to clinically address the effects of such inflammation on the body to support health and wellness in addition to treating viral replication. An important research question is whether probiotics demonstrate the same response in HIV infection as they do in other conditions.

There is a complex relationship between the immune system, GI tract, and HIV pathogenesis. Figure 1 outlines the concepts of how HIV infects and causes the destruction of CD4+ T cells and describes HIV immunopathogenesis to AIDS. The replication capacity of HIV causes the progressive loss of the CD4+ T cell population, impairs the function of the GI mucosal surface, and is catastrophic. There are complex relationships and interactions among the gut ecology, the immune system, ongoing replication of HIV, microbial translocation causing endotoxemia, and the loss of T-cell function in the impaired recovery of the immune system (Douek, Roederer, & Koup, 2009). Given evidence of the beneficial effects of probiotics on recovery of gut function in a variety of conditions, it is possible that probiotics may be effective in restoration of gut health in patients with HIV. However, such evidence cannot be directly translated into HIV care without systematic exploration of their effectiveness and potential harmful effects within the context of HIV infection.

Commensal bacteria are benign microorganisms that benefit a healthy human physiology by serving the GI system and improving the health of the host in both digestive and immune function (Mazmanian & Kasper, 2006). Commensal bacteria secrete epithelial cytokines, serve as a barrier to the epithelial layer, and have antibacterial property secondary to colonization. An induction of regulatory T cells slows down or reverses inflammation (Boirivant & Strober, 2007). Wildt and colleagues (2006) reported that probiotics generated a nonspecific stimulation of proliferation of immune cells, an increased secretory IgA production associated with anti-inflammatory cytokines, and control of activation in dendritic cells. Initiation of CD4+ T cells stimulation in the event of CD4+ T cell loss was another benefit of this relationship (Mazmanian & Kasper, 2006).

The purpose of using probiotics is to restore the protective mechanism of gut function. Restoring the defense system involves repairing the homeostatic state of the mucosal lining. The gut has extrinsic and intrinsic protective barriers. The intrinsic barrier is made up of a semipermeable plasma membrane with tight junctions between epithelial cells, which prevent passage of bacteria and have various cellular defense mechanisms. Components of the GALT are part of the intrinsic barrier (Emami et al., 2009). This is part of the normal line of defense.

The microbial ecosystem has evolved to act in service to the human host. It derives its energy from indigestible food and plays a role in the stimulation of the immune system, regulation of cellular function, communication with cells, essential vitamin synthesis, metabolic regulation, and resistance of foreign pathogens (Possemiers et al., 2009).

Our aim in this paper is to identify scientific evidence that supports the use of probiotics in inflammatory gut disease, antibiotic-associated diarrhea, oral candidiasis, GI symptoms, and abdominal adiposity. In light of their efficacy in those conditions, we suggest their use may be beneficial to HIV-infected patients suffering similar pathologic conditions. We also provide the foundation for clinical trials that will evaluate efficacy for treatment of gut-related symptoms associated with HIV disease.

Search Methods

The primary search strategy entailed use of keywords listed in Table 1 to search the PubMed and CINAHL databases to identify relevant research evidence. A secondary review of references from key published papers was performed to ensure completeness of the literature search. Table 1 summarizes the search strategy and results. The search was limited to articles in the English language, adults, humans, clinical trials, meta-analyses, and practice guidelines. This strategy resulted in 100 research publications. Further, the decision was made to limit the review to only the most controlled high-quality research studies. The search strategies produced the reports of 17 double-blinded randomized controlled trials that are reported here. All but one of the 17 studies was placebo controlled.

The results of the search strategy included studies for inflammatory bowel disorders, irritable bowel syndrome, and acute diarrhea induced by antibiotics. Studies that included the use of a probiotic were included in the final group. In addition, trials in HIV-uninfected patients that studied probiotic use in health problems such as candida, weight issues, and GI symptoms caused by non-pathologic etiologies were also included. Articles were chosen based strictly on content.

Results

The evidence supported a conclusion that probiotics are effective in certain GI diseases such as acute infectious diarrhea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), pouchitis, small intestinal bacterial overgrowth, and lactose intolerance (Drouault-Holowacz et al., 2008; Hoveyda et al., 2009; Lin, 2004; McFarland & Dublin, 2008; Rolfe, 2000; Szajewska & Mrukowicz, 2001; Wildt et al., 2006). The articles selected for review are summarized in Table 2.

Effect of Probiotic Therapy

No studies have examined the use of probiotics in HIV infection. As noted in Table 2, three studies examined probiotics for IBD. All studies showed a reduction in clinical symptoms, with clinical improvement in intestinal inflammation and symptoms with the use of probiotics. Findings also included reductions of (a) polymerase chain reaction (PCR) bacterial 16S rRNA levels, (b) defensin expression, and (c) inflammatory cytokines with improvement in immune markers and bowel frequency and consistency. Probiotic strains were well tolerated and improved clinical symptoms (Furrie et al., 2005; Steed et al., 2010; Wildt et al., 2006).

An additional five studies examined probiotic use in IBS. Four of the five studies demonstrated improvement in symptoms with the use of probiotics. Overall, there were enhancements of anti-inflammatory markers and improvements in flatulence, bloating, bowel dysfunction, and quality of life (Kajander, Hatakka, Poussa, Farkkila, & Korpela, 2005; Kajander & Korpela, 2006; Kajander et al., 2008; Kim et al., 2005; Whorwell et al., 2006). In the one study that did not show improvement in symptoms of IBS, the authors attributed the outcome to lack of uniformity in the IBS subjects selected, a strong

placebo effect, and the fact that only 72% of participants completed the study (Niv, Naftali, Hallak, & Vaisman, 2005).

Three studies examined antibiotic associated diarrhea (AAD). One of the studies (Gao, Mubasher, Fang, Reifer, & Miller, 2010) was a pilot study that did not include a placebo group, but demonstrated a dose dependent response in effectiveness with superior outcomes in the 100 billion colony forming units (cfu) dosing as opposed to 50 billion cfu. It was also found that twice daily use was more effective than once daily use. All studies demonstrated tolerability of probiotics (Gao et al., 2010; Hickson et al., 2007; Song et al., 2010). The study by Song and colleagues (2010) reported no reduction in occurrence of AAD but found that Lacidofil® was effective at maintaining bowel habits. Both Gao and colleagues (2010) and Hickson and colleagues (2007) demonstrated a reduction of AAD and *C. difficile* in participants taking antibiotics.

Finally, studies looking at the effect of probiotics on other problems that also affect persons with HIV were examined. The findings from these five studies provided evidence of the effectiveness of probiotics in reducing GI symptoms exhibited post-prandial or induced by stress. Additionally, the prevalence in oral candida was reduced by 75% in a probiotic trial in the elderly (Hatakka et al., 2007). Another study looked at abdominal visceral and subcutaneous fat and provided evidence in support of the effectiveness of probiotics in reducing these parameters (Kadooka et al., 2010). A Phase II clinical trial of probiotics in healthy adults exhibited tolerability and improvement in both GI symptoms and immune response; there was also an induction of anti-inflammatory markers and the regulatory cytokine IL-10 (Sierra et al., 2010). In another study of stress-induced GI symptoms, reduced abdominal pain, nausea, and vomiting

were found with the use of *Bifidobacterium longum* and *Lactobacillus acidophilus* for 3 weeks (Diop, Guillou, & Durand, 2008).

We found few studies that highlighted concern for patients when probiotics were used for adjunct therapy. Besselink and colleagues (2008) conducted a study in patients with severe acute pancreatitis. In this well-designed study, intent-to-treat analysis of probiotics used to reduce infectious complications found a higher risk of complications and mortality in the probiotic group. This was contributed to small bowel ischemia and enteral feeding. Probiotics had been chosen for the study because infectious complications were thought to be caused by bacterial translocation (Besselink et al., 2008)

We found one case report, not included in the review, in which a 29-year-old female patient developed bacteremia with the probiotic strain *Lactobacillus rhamnosus*. This was the first known case of sepsis in a female aortic heart valve. The septicemia was a consequence of the weakened intestinal barrier and mesenteric ischemia resulting from her heart failure. This case report highlighted the concern to review contraindications and add warnings to widespread administration of probiotics to reduce gut symptoms (Kochan et al., 2011). *Lactobacillus rhamnosus* has been associated with severe infections and high pro-inflammatory responses compared to other species (Salminen et al., 2004). There is potential for septicemia in the critically ill and severely immune compromised.

In summary, the clinical trials we reviewed overwhelmingly supported the use of probiotics as an adjunct therapy for IBD, IBS, AAD, oral candidiasis, and other GI complaints, serving to lessen or eliminate the adverse GI symptoms associated with these

disorders. We found evidence supporting clinical, physiological, and symptomatic improvements in disease pathology affecting the GI tract with probiotic use. Additionally, the studies reported that probiotics were well tolerated and were beneficial to the reduction of symptoms and disease course. Variations in duration used did not alter the benefit.

Discussion

In our review, we found consistent evidence supporting the use of probiotic therapy in many conditions similar to those GI conditions found in HIV-infected patients. Probiotics were shown to be safe and effective in reducing GI symptoms and producing clinical improvements in symptoms involving the GI system in HIV-uninfected populations. Despite such evidence, it remains unclear whether their use in patients with HIV would demonstrate similar benefits. Anecdotal clinical evidence from providers who have recommended probiotics to their HIV-infected patients has been positive (W. Thompson, personal communication, March 14, 2011). The evidence reviewed supported the need to investigate probiotics for effectiveness in managing pathological gut changes seen in HIV. Based on similar clinical symptoms, immune markers, and the safety of this therapy, it may be worthwhile to translate this research to the clinical area of HIV care.

Probiotics provide a novel approach to treatment to improve health in populations infected with HIV. Although probiotics have been used in other conditions, there is limited evidence specific to their use in HIV-infected populations. Further research is needed to assess the efficacy and safety of probiotic use in HIV. Well-designed efficacy trials are warranted prior to recommendations for use in clinical practice. The use of probiotics may be a low-cost, non-invasive, and effective intervention for treating HIV-

related symptoms that impact quality of life.

Theoretically, it is possible to achieve homeostasis and wellness by the restoration of the natural GI flora and the flexible lines of defense in the gut with probiotics. It is also possible to prevent loss of this defense by maintaining the integrity of the defense mechanism, microenvironment, and control of stressors. Probiotics are likely to promote defense mechanisms in HIV-infected patients by the maintenance and promotion of homeostasis and balance in the gut, as well as by minimizing adverse reactions to stressors.

HIV causes significant damage to the lining of the GI mucosa. At this point, tertiary prevention would involve control and suppression of the virus with ART, along with repairing the lines of flexible defense and resistance with replacement of microbial products such as probiotics. ART stalls the replication of HIV and decreases antigen load, creating an opportunity for therapeutic reconstitution and repair of the gut. The promotion of strategies that repair and regenerate the mucosal lining, preserve the memory CD4+ T cells, and suppress or reduce inflammation are key therapeutic interventions (Verhoeven et al., 2008).

Repair and regeneration of the mucosal lining is the target for probiotic use. This is contrary to the target of microbial translocation as used in the study by Besselink and colleagues (2008). In light of these data, there is a justifiable concern about administering probiotics in the context of a patient in a fasting state and with pancreatitis. When designing studies for HIV-infected populations, probiotics should be excluded in patients with critical illness, receiving nutrition through parental or enteral routes, and with a diagnosis of pancreatitis (Barraud et al., 2010; Besselink et al., 2008).

Probiotics support immune regulation by controlling pro-inflammatory and anti-inflammatory cytokines. Recent studies have found that ingestion of probiotics by mice lowered inflammatory cytokines in the serum and had an indirectly positive effect on regulatory T cells (Petersen et al., 2011). In addition, the most commonly used strains of bacteria for probiotics have shown promising effects on inducing levels of IL-10 and suppressing the pro-inflammatory cytokine IL12p70 (Gad et al., 2011).

Because commensal bacteria serve different functional roles, it is imperative to understand their characteristics and use discretion in choice of probiotic strain and purpose. Specific imbalances of the gut flora are manifested in different ways and should be considered when making diagnostic decisions on appropriate strains to use to best optimize a healthy balance within the gut (Hanaway, 2006). In addition, a closer look at what causes the imbalance in gut flora needs to be examined.

Various strains of GI microflora have different functional effects. One strain versus another strain or combination cannot be generalized to all strains. It is important to understand the therapeutic context of different strains for their use in any specified population. Because probiotics are increasingly available in the marketplace, it is important for clinicians to know the risks and benefits in order to adequately advise patients about this treatment (Boyle, Robins-Browne, & Tang, 2006).

Currently, probiotics are widely marketed; they are safe and effective for healthy populations. Although not regulated by the Federal Drug Administration (FDA), quality assurance recommendations for safe production and consumption suggest that strains be human in origin and safe for human consumption. Products should be stable in bile and able to withstand the pH of the stomach and small intestines. There should also be

adherence sufficiently to the intestinal mucosa and mucus (Isolauri, Sutas, Kankaanpaa, Arvilommi, & Salminen, 2001; Tuomola, Crittenden, Playne, Isolauri, & Salminen, 2001). The guidance provided by the FDA should be based on scientifically sound research with well-designed clinical trials (Hibberd & Davidson, 2008).

The studies reviewed for our analysis had several limitations. Most of the studies had a small sample size and failed to set up appropriate methods for the measurement of cytokines. This was acknowledged by the authors (Kajander et al., 2008). Another limitation occurred when a microbial preparation that is not readily available on the market was used. We recommend that clinical trials be designed for people living with HIV to evaluate the efficacy of probiotics on indicators demonstrated by the randomized, double-blind, placebo-controlled trials presented in this paper. Efficacy studies of probiotics in persons infected with HIV should analyze strains, dosages, outcomes in terms of satisfaction, and biomarkers focusing on CD4+ T cell subset, body mass index, and viral load. In addition, plasma lipopolysaccharide and soluble CD14 levels should be monitored to analyze changes in microbial translocation resulting from gut inflammation and dysfunction.

Considering that patients with HIV infection experience bloating, diarrhea, flatulence, weight alterations, and candidiasis, probiotics may be an effective intervention for this population. Based on the evidence presented here, probiotics have been shown to reduce gut inflammation and microbial translocation and to improve symptoms. Probiotic use is a strategy to support healing and restoration of gut health. It is hoped that further research will demonstrate the ability of probiotics to alter HIV pathogenesis and delay progression to AIDS. Clinical guidelines and quality assurance criteria are needed for the

development and use of probiotics. Finally, further investigation of properties of specific bacterial strains is warranted to understand dynamics and properties of cytokine induction and suppression.

Clinical Considerations

- There is no evidence of contraindications for the use of probiotics in adults with HIV disease.
- There is evidence of contraindication for the use of probiotics in patients with acute pancreatitis; probiotics should not be used in patients with current pancreatic disease.
- If used, probiotics should be prescribed according to recommended daily doses as provided by the manufacturer.
- Users should be closely monitored for symptom improvement and documented changes.
- Patients should continue taking antiretroviral and prophylaxis medications as directed.

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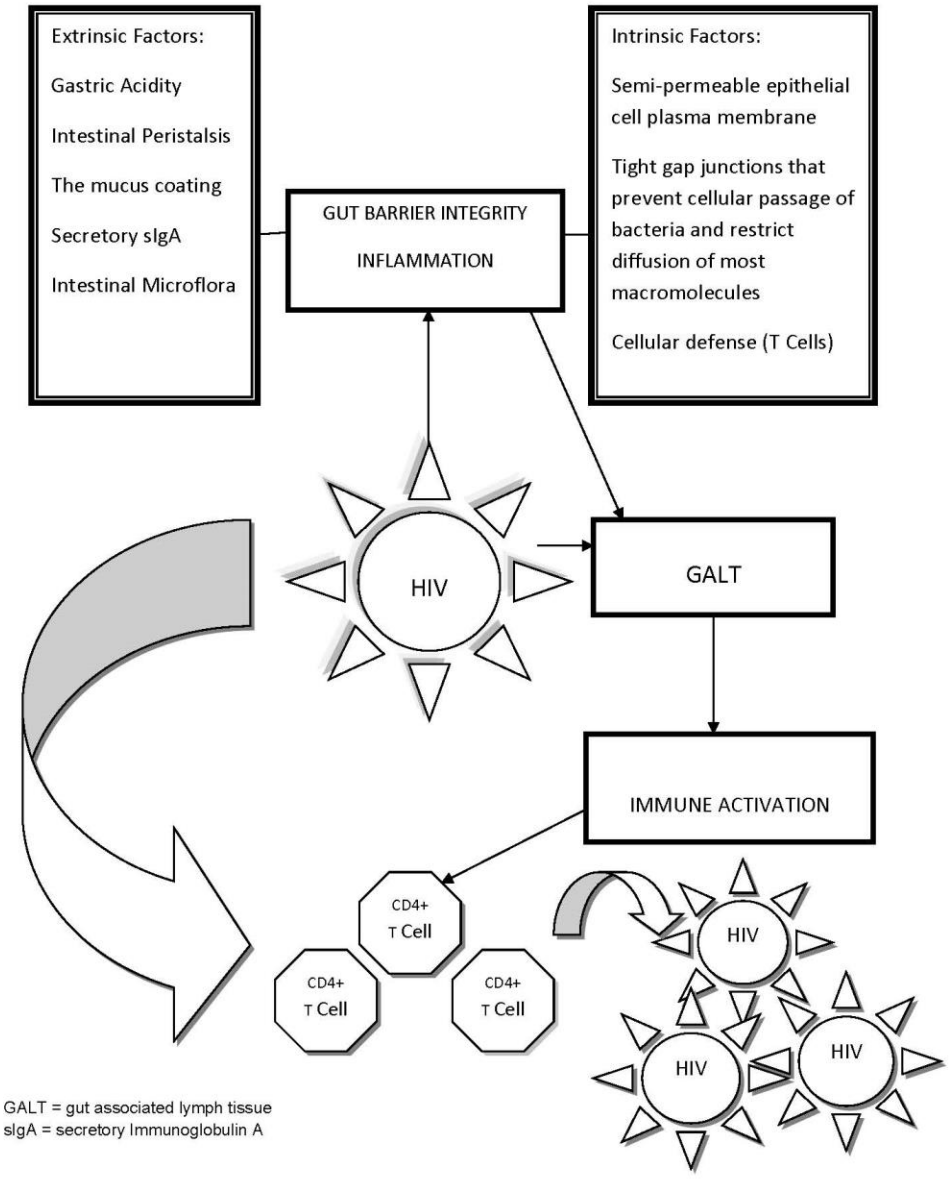

Figure 1. Model of gastrointestinal (GI) tract and HIV pathogenesis

Table 1. Search Strategy and Results

Search Terms	Articles Yielded	Limits	Reviewed	Retained	Discarded & Duplicated
Probiotics & HIV	52	CT, MA, & PG	12	7	5
Probiotics & GALT	1	CT, MA, & PG		1	
GALT & HIV	1	CT, MA, & PG		1	
Gastrointestinal immunity and HIV	3	CT, MA, & PG			3
GI Symptoms + Probiotics + HIV	3	CT, MA, & PG			3
Microbial Translocation	17	CT, MA, & PG			17
Gastrointestinal mucosa + HIV	73	H, A, CT, MA, PG, RCT, R, AIDS, CM, SR	73	8	65
Epithelial Barrier + HIV	17	H, A, CT, MA, PG, RCT, R, AIDS, CM, SR	17	3	14
HIV + Microbial Translocation	26	Humans, Adults	26	15	11
AIDS + Microbial Translocation	17	CT, MA, PG, RCT, R, AIDS, CM, SR	17	5	12
Th17 + Microbial Translocation	13	No limits	13	1	12

Probiotics + Treatment	4,472	CT, MA, PG, RCT, SR, Adults	16	6	10
Double-blind placebo controlled trial of probiotics	416	CT, MA, PG, RCT	374	*374**	0
Double-blind placebo controlled trial of probiotics*	374*	CT, MA, PG, RCT, SR,	6	40*	334
Double=blind placebo controlled trial of probiotics NOT skin	350	CT, MA, PG, RCT, SR,	5	3	2
Probiotics and gut Inflammation	22	CT, MA, PG, RCT, SR,	22	6	16
Bifidobacterium infantis	11	CT, MA, PG, RCT, SR,	11	3	8
Commensal bacteria and Human GALT	14	No Limits	14	11	3

Note. H = Humans; A = Animals; CT= Clinical Trial; GALT = gut associated lymph tissue; GI = gastrointestinal; MA = Meta-Analysis; PG = Practice Guideline; RCT= Randomized Controlled Trial; R = Review; Th17 = T helper 17 cell; CM = Complementary Medicine; SR = Systematic Reviews

*Combined search

Table 2. Articles Selected for Review: Double-Blind Controlled Trials

	Trial	DV/Probiotic	Outcome	Comments
Inflammatory Bowel Disease	Steed et al., 2010 R/DB/PC	Crohn's Disease 35 patients *Bifidobacterium longum*/Synergy 1 Twice a day for 6 months	Reduction in Crohn's Disease Mucosal *bifidobacteria* proliferated Measured PCR bacterial 16S rRNA levels and transcription levels immune markers.	Symbiotic consumption effective in improving clinical symptoms.
	Furrie et al., 2005 R/DB	Ulcerative colitis 18 patients *Bifidobacterium longum*/Synergy 1 30 days	Reduction in mRNA levels for human beta defensins 2, 3, and 4. Reduction in inflammatory cytokines, which drive inflammation and induce defensin expression.	Reduction of inflammation and regeneration of epithelial tissue with clinical improvement in chronic inflammation.
	Wildt et al., 2006 R/DB/PC	Collagenous Colitis 29 patients *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* 12 weeks	Reduction in bowel frequency ≥50%, time of liquid stools from 6-1 day(s), and increase in solid stools per week in post ad hoc analysis.	Probiotics may have a potential influence in the disease course of CC.

Irritable Bowel Syndrome (IBS)	Kajander, Hatakka, Poussa, Farkkila, & Korpela, 2005 Kajander & Korpela, 2006 R/DB/PC	IBS 103 patients *Lactobacillus rhamnosus* GG, *L. rhamnosus LC705,* *Bifidobacterium breve* Bb99 *and Propionibacterium freudenreichii* *ssp. shermanii* JS 6 months	Reduction in IBS symptoms of 42% versus 6% in placebo. Symptoms and bowel habits were recorded.	Probiotic may enhance anti-inflammatory effect, balance the microbial ecosystem, and improve motility. Effective at alleviating IBS symptoms.
	Niv, Naftali, Hallak, & Vaisman, 2005 R/DB/PC	IBS 54 patients *Lactobacillus reuteri* 6 months	IBS symptoms scored by Francis Severity score and IBS quality-of-life measured monthly 39 completed the study	IBS symptoms did **not** improve with *L. reuteri*. Authors contribute to lack of uniformity of IBS population and strong placebo effect.

	Kim et al., 2005 R/DB/PC	IBS with bloating 48 patients VSL #3® 4 weeks	Reduction in flatulence within 1st 4 weeks. Satisfactory relief of bloating and flatulence.	Retards colonic transit without altering bowel function in IBS. No adverse effects.
	Whorwell et al., 2006 Clinical trial R/DB/PC	IBS 362 patients (women) *Bifidobacterium infantis* 35624 4 weeks	Improvement in bloating, bowel dysfunction, incomplete evacuation, straining, and passage of gas. *B. infantis* 35624 exceeds placebo by 20% (p = < 0.02) at dose 1 x 10^8 cfu. Other doses were not significantly different.	Dose dependent probiotic relieves many IBS symptoms. No adverse effects
	Kajander et al., 2008 Clinical Trial R/DB/PC	IBS 86 patients *Lactobacillus rhamnous GG, L. rhamnosus LC705, Propionibacterium freudenreichii ssp. shermanii* JS and *Bifidobacterium animalis* ssp. *lactis* Bb12 5 months	Measured symptoms, quality of life, microbiota stability, serum cytokines, and CRP. Probiotic decrease in IBS composite score of 14 points vs. 3 points with placebo. Stabilization of microbiota was observed. No differences in CRP	This probiotic combination was effective and safe in alleviation of IBS symptoms and stabilization of microflora.

Antibiotic Associated Diarrhea (AAD)	Hickson et al., 2007 R/DB/PC	AAD 135 patients *Lactobacillus casei*, *L. bugaricus*, and *Streptococcus thermophilus* BID during course of antibiotics and 1 week post course	12% of probiotic group developed diarrhea versus 34% in placebo group. No *C. difficile* in the probiotic group. 17% developed diarrhea secondary to *C. difficile* in the placebo group.	Probiotic strain showed reduction of AAD and *C. difficile*.
	Gao, Mubasher, Fang, Reifer, & Miller, 2010 R/DB/PC	AAD 255 patients 50 billion versus 100 billion cfu of live *Lactobacillus acidophilus* CL1285 + *Lactobacillus casei* LBC80R Bio-K +CL1285 Started within 36 hours of antibiotic administration and continued for 5 days after last antibiotic dose.	Both probiotic groups had a lower incidence with the 15.5% versus 28.2% versus placebo (44.1%). Duration of AAD shortened to 2.8 and 4.1 days versus placebo (6.4days). There was a lower incidence in *C. difficile* in all groups but the Pro2 (1.2%), Pro1 (9.4%), and placebo (23.8%).	Probiotics given at 100 billion cfu yielded superior outcomes than 50 billion cfu. Probiotic blend used was well tolerated and effective at reducing risk of AAD and *C. difficile* with patients on antibiotics.
	Song et al., 2010 R/DB/PC	AAD 214 patients *Lactobacillus* (Lacidofil®) 14 days	AAD developed in 3.9% of the probiotic group versus 7.2% of the placebo group. Probiotic group showed lower change in bowel frequency and consistency.	Lacidofil® did not significantly reduce the rate of occurrence of AAD but was more effective at maintaining bowel habits than in placebo (48.5% vs. 31.5%).

GI Symptoms w/o diagnosis	Kalman et al., 2009 R/DB/PC	Post-prandial Functional Intestinal Gas Symptoms 61 patients *Bacillus coagulans* 4 weeks	Improvement seen in abdominal pain, abdominal distention, flatus, and dyspepsia, bloating, and gas with a strong placebo effect Marginal lack of statistical significance in some of the variables.	*Bacillus coagulans* based probiotic effective in improving the quality of life, reducing postprandial gas, and GI symptoms for persons without a GI diagnosis.
Candida	Hatakka et al., 2007 R/DB/PC	Oral Candida 276 elderly patients *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ssp. *Shermanii* JS 16 weeks	Prevalence of salivary yeast decreased in probiotic group. Reduction of yeast counts in probiotic group by 75% (OR = 0.25, p = 0.004) and risk of hypo-salivation (OR = .44, p = 0.05).	Probiotics effective in control and prevention of oral candidiasis and hypo-salivation in the elderly.
Weight	Kadooka et al., 2010 R/DB/PC	Abdominal adiposity 87 patients *Lactobacillus gasseri SBT 2055* 12 weeks	Significant decrease in abdominal visceral (4.6%) and subcutaneous fat areas (3.3%) and body weight (1.5%), BMI (1.5%), waist (1.8%), and hip (1.5%). No decrease in control group.	*Lactobacillus gasseri* SBT 2055 demonstrated benefits on influencing metabolic disorders of affecting abdominal adiposity and body weight.
Stress Induced GI symptoms	Diop, Guillou, & Durand,	Stress induced GI symptoms *Bifidobacterium longum* +	Reduced abdominal pain and nausea/vomiting in intent to treat or per protocol subjects.	Probiotic strain with beneficial effect on GI symptoms experienced in

	2008 R/DB/PC	*Lactobacillus acidophilus* 3 weeks	Did not modify other symptoms of stress.	chronic stress.
Healthy	Sierra et al., 2010 Phase II Clinical Trial R/DB/PC	Tolerance and beneficial intestinal and immune effects *Lactobacillus salivarius* CECT5713 4 weeks	Improvement in the frequency of defecation. Induction of NK cell, monocytes, plasma levels of IgM, IgA, and IgG, as well as the regulatory cytokine IL-10.	Well-tolerated and no adverse effects. Safe and effective in improving gut microbiota and parameters related to immune response and regulation.

Pancreatitis	Besselink et al., 2008 Clinical Trial R/DB/PC	Infectious complications with severe acute pancreatitis 298 patients Prophylaxis with six different strains: *Lactobacillus acidophilus, Lactobacilus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum,* and *Bifidobacterium infantis* Total daily dose of 10^{10} + prebiotics via nasojejunal feeding tube 28 days maximum	Infectious complications occurred in 30% of patients in probiotic group and 28% in the placebo group. 16% patients in probiotics group died with only 6% in the placebo group. Probiotics did **not** reduce the risk of infectious complications and had an association with an increased risk of mortality.	Probiotics in this group strongly discouraged until the mechanism of complication is investigated. Patients were on enteral feeding perhaps increasing demand for oxygen increasing ischemia. Concern is based on bowel ischemia caused by low flow state.

Note: CC = Collagenous Colitis; DB = Double Blinded; DV = Dependent Variable; OR = Odds Ratio; PC = Placebo Controlled; PCR = Polymerase Chain Reaction; R = Randomized; CRP = C-reactive protein; IgA = Immunoglobulin A; IgG = Immunoglobulin G; IgM = Immunoglobulin M; IL = Interleukin; mRNA = messenger ribonucleic acid; NK = Natural Killer; rRNA = ribosomal ribonucleic acid; AAD = Antibiotic Associated Diarrhea; BID = Twice a day; BMI= Body Mass Index; CFU = colony-forming unit; GI = Gastrointestinal; IBD = Inflammatory Bowel Disease; IBS = Irritable Bowel Syndrome

CONNECTING THE DOTS: COULD MICROBIAL TRANSLOCATION EXPLAIN COMMONLY REPORTED SYMPTOMS IN HIV DISEASE?

by

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Abstract

Microbial translocation within the context of HIV disease has been described as one of the contributing causes of inflammation and disease progression in HIV infection. HIV-associated symptoms have been related to inflammatory markers and sCD14, a surrogate marker for microbial translocation, suggesting a plausible link between microbial translocation and symptom burden in HIV disease. Similar pathophysiological responses and symptoms have been reported in inflammatory bowel disease (IBD). We provide a comprehensive review of microbial translocation, HIV-associated symptoms, and symptoms connected with inflammation. We identify studies showing a relationship among inflammatory markers, sCD14, and symptoms reported in HIV disease. A conceptual framework and rationale to investigate the link between microbial translocation and symptoms is presented. The impact of inflammation on symptoms supports recommendations to reduce inflammation as part of HIV symptom management. Research in reducing microbial translocation-induced inflammation is limited, but needed, to further promote positive health outcomes among HIV-infected patients.

Keywords: HIV, inflammation, microbial translocation, sCD14, symptom management, symptoms

Connecting the dots: Could microbial translocation explain commonly reported symptoms in HIV disease?

Insights into the pathogenesis of HIV infection have implicated microbial translocation as one of the key drivers of HIV disease progression and inflammation (Brenchley & Douek, 2008; Brenchley et al., 2006; Marchetti et al., 2011; Sandler et al., 2011). Microbial translocation is the movement of bacteria and/or microbial products from the gut to the bloodstream. Commonly reported gastrointestinal (GI) and systemic symptoms may have a relationship with chronic inflammation induced by circulating microbial products from the GI tract in patients with HIV disease. Even with effective combination antiretroviral therapy (cART) and viral suppression, inflammation from chronic immune activation increases the rates of morbidity and mortality among people living with HIV disease (PLWH; Brenchley et al., 2006; Deeks, 2011; Kamat, Misra, et al., 2012; Marchetti et al., 2011). It is critical for nurses to have a working understanding of the concepts of microbial translocation, inflammation, and symptom management in the clinical management of HIV disease.

Background and Significance

Chronic inflammation has been identified as a key predictor in the development of co-morbidities and mortality in HIV disease. One source of inflammation - the inflammation of the GI epithelial barrier - ultimately leads to dysfunction of the protective lining of the gut. Consequently, microbes naturally residing in the gut are able to pass through the gut associated lymph tissue (GALT) into the blood circulation (Estes et al., 2010; See Table 1 for definitions). The immune system responds to circulating microbes with systemic and often chronic inflammation (Brenchley et al., 2006).

Inflammation of the GI epithelial barrier in HIV disease resembles inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Inflammation of the GI epithelial barrier leads to symptoms including diarrhea, bloating, and abdominal pain (Berkes, Viswanathan, Savkovic, & Hecht, 2003; Epple & Zeitz, 2012). In IBD/IBS, inflammation leads to the translocation of microbes naturally residing in the gut into the bloodstream, as seen in HIV disease where microbial products residing in the gut translocate through the GALT into the bloodstream (Brenchley et al., 2006; Epple & Zeitz, 2012).

Systemic inflammation experienced chronically has been associated with systemic symptoms and conditions. Symptoms are often adverse experiences perceived from underlying changes in the bio-psychosocial function of an individual. Signs and symptoms provide key assessment information to support the formulation of diagnostic pathways for clinicians. Symptoms are usually measured by self-report as opposed to a sign, which is an abnormality that can be detected by the individual and by others observing the individual (Dodd et al., 2001). PLWH often experience and report symptoms to their providers. However, the subjectivity of symptoms can limit objective assessment by another individual, creating huge challenges for clinicians and scientific investigators as symptoms may not be objectively measured by another human being unless people report what they are experiencing.

Symptoms are often attributed to side effects of treatment with cART (Johnson, Stallworth, & Neilands, 2003). Patients initiating antiretroviral therapy in the early era of the HIV epidemic were at risk for serious adverse events and major side effects, but current cART regimens are simpler, better tolerated, more effective, and offer lower side

effect profiles than earlier regimens used in the treatment of HIV disease (Katlama et al., 2009; Lennox et al., 2009; Madruga et al., 2007). And yet, many symptoms persist in some individuals. In addition, the symptom burden experienced by PLWH has been associated with poor medication adherence, such as when people want to avoid symptoms, forget to take scheduled doses, and/or sleep through medications due to fatigue (Gay et al., 2011). Symptom burden is the summation of disease expression and/or the product of the treatment of that disease, usually referred to as the side effects of treatment (Cleeland & Reyes-Gibby, 2002).

Our purpose was to review how inflammation from HIV disease may lead to symptoms experienced by PLWH in the context of microbial translocation, as well as how this event may lead to treatment failure. We describe the process and consequences of microbial translocation and inflammation, and how this inflammatory process may be related to symptoms experienced (Figure 1). Furthermore, we address the gaps in knowledge and challenges in demonstrating a valid hypothesis linking microbial translocation and symptoms.

Inflammation of the Epithelial Barrier

HIV has an affinity for Th17 type CD4+ T cells in the GALT. These specific cells, once activated, are prime targets for HIV because of CCR5 receptors that HIV can bind to when entering CD4+ T cells. Th17 type CD4+ T cells are rapidly depleted during HIV infection, resulting in the release of signaling proteins called cytokines, which initiate the inflammatory process. Under natural physiological conditions, these cells would release cytokines that would regulate and control the inflammatory process. However, due to the rapid depletion of the Th17 type CD4+ T cells and ongoing

replication of HIV, inflammation continues and becomes chronic (Klatt et al., 2010; Pandrea et al., 2007).

Chronic inflammation eventually leads to damage of the tight gap junctions between the epithelial cells of the GI monolayer protective barrier. Under natural physiological circumstances, Th17 cells release a cytokine that can initiate the repair of these junctions. However, due to the depletion of Th17 cells in GALT, repair of the epithelial barrier is impaired (Estes et al., 2010; Klatt et al., 2010; Verhoeven, Sankaran, Silvey, & Dandekar, 2008).

Dysfunction of the Epithelial Barrier

GI barrier dysfunction has many consequences. Alterations to the cellular cytoskeleton and the function of tight gap junctions lead to disruption in epithelial permeability. Disruption of epithelial permeability may lead to malabsorption of nutrients and possibly even medications. In addition, alterations in fluid and electrolyte secretion, which may cause symptoms such as diarrhea, bloating, and constipation, may also lead to abdominal pain and functional symptoms (Berkes et al., 2003). GI symptoms are common in IBD/IBS disease as well as in HIV disease. Clinicians and PLWH have often attributed these symptoms to medication toxicities.

The microbial environment of the gut mucosal epithelium has an extraordinary ability to maintain protection against pathogenic invasion of harmful bacteria (Berkes et al., 2003). The microbiome competes for space and nutrients, while also providing a protective layer of mucous. The microbial environment maintains balance for the gut and interacts well with the immune system; the commensal flora operates synergistically with the human immune system. However, once the barrier becomes dysfunctional, microbes

are able to invade and pass through the barrier, evade immune intervention, and egress into circulation (Berkes et al., 2003). The pathway of microbial translocation is complex, with the main outcome being that microbial products are able to translocate from the gut to the bloodstream.

Microbial Translocation

Microbial translocation is not an exclusive feature of HIV disease. It has been well described in IBD/IBS (Spiller, 2009), graft versus host disease (Eriguchi et al., 2012), abdominal post-operative conditions (Sista et al., 2013), and liver disease (Wiest & Garcia-Tsao, 2005). Non-human primates are often used as models to understand the extent of damage caused by the immunodeficiency virus in humans because of similar pathology between non-human primates and humans. Simian models have similar structural and immunological responses to that of the human model and can be infected with the virus for investigation and results can be translated to understand the pathogenesis in humans (Klatt et al., 2010).

Brenchley et al. (2006) described microbial translocation as part of the pathogenic process in simian immunodeficiency virus (SIV) and HIV infection by detecting differences in microbial translocation in animal models using African Green Monkeys versus Rhesus Macaques, with higher levels of microbial translocation in the pathogenically-infected Rhesus Macaques. Prior to these findings, the inflammatory process and marked dysfunction of the immune response was attributed to HIV infection within GALT and of the GI epithelial barrier. Stein et al. (1997) described the chronic passage of bacteria leaking across a compromised epithelial wall in HIV disease.

GALT houses a rich supply of Th17 and memory CD4+ T cells, which express

high levels of CCR5 receptors, which facilitate entry into cells and result in rapid depletion of these immune cells during the acute phase of HIV/SIV infection (Estes et al., 2010; Pandrea et al., 2007). Immune activation in response to HIV/SIV begins an inflammatory process, secreting pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-17, IL-21, and IL-22, that ultimately impair the epithelial barrier (Estes et al., 2010; Klatt et al., 2010; See Table 2). The immune system, which is essential to survival, is subverted by HIV infection and, due to the inflammatory response, continues as a result of dysfunctional regulation. Although treatment with cART is unable to prevent acute loss of CD4+ T cells in GALT, it does support CD4+ T cells by suppressing HIV replication and allowing the body to restore these immune cells. In SIV models, preservation of memory CD4+ T cells, as well as the reduction and suppression of inflammation, promotes repair and regeneration of the mucosal epithelial barrier (Verhoeven et al., 2008).

Without successful cART, HIV infection goes on to deplete CD4+ T cells in the peripheral blood, lymph nodes, and effector tissues leading to AIDS (Klatt & Brenchley, 2010), and the continuous loss of CD4+ T memory cells inhibits reconstitution in spite of treatment (Mehandru et al., 2006). Additionally, cART fails to restore CD4+ T memory cells back to pre-infection levels and persistent immune activation continues in response to low levels of viremia in GALT, leading to chronic mucosal inflammation and microbial translocation (Estes et al., 2010). Even with undetectable levels of viral RNA, viral DNA can persist and contribute to ongoing immune activation (Chun et al., 2008), resulting in continued structural and immunologic damage (Brenchley & Douek, 2008).

Damage to the integrity of the mucosal epithelial barrier and loss of phagocytic

protection in GALT set the stage for microbial products to translocate to the lymph nodes and then to the plasma through the chronic phase of HIV disease. In addition, levels of lymphatic microbial products and circulating microbial products are associated with the extent of damage to the GI tract (Estes et al., 2010). Once in circulation, these products contribute to and amplify immune activation resulting in chronic inflammation (Klatt, Canary, et al., 2013; Klatt, Chomont, Douek, & Deeks, 2013). Even with long-term suppression of HIV replication in the blood, the epithelial barrier is only partially restored (Epple & Zeitz, 2012). The lack of restoration may be partially due to ongoing residual replication triggering the inflammatory process in the gut and, as a result, contributing to microbial translocation (Baroncelli et al., 2009; Reus et al., 2013). This inflammatory process can lead to systemic symptom development (See Figure 1).

Microbial Translocation and Immune Activation

Circulating microbial products from the gut result in immune inflammatory processes. Brenchley et al. (2006) reported microbial translocation as a hallmark predictor of immune activation and disease progression, describing the differences in pathogenic progressive HIV/SIV infections, long-term non-progressors, and non-pathogenic SIV models. The study established that the source of circulating microbial products, which were commensal and pathogenic bacteria, had passed through the damaged gut epithelial barrier. Levels of microbial products measured by the bacterial lipopolysaccharide (LPS) outer coat increased after the acute/early phase of HIV infection. Levels of LPS were linked to elevated levels sCD14 ($r = 0.3$, $p = 0.001$) and lower levels of naturally occurring endotoxin core antibodies (EndoCAb) to LPS ($r = -0.319$, $p = 0.0005$), which clears LPS from the system. In fact, significantly lower levels

of EndoCAb were detected in HIV/SIV-infected chronic progressors than in early/acute progressors ($p < 0.0001$) and those who were uninfected ($p = 0.0002$), meaning that over time the mechanism to remove circulating microbes decreases. HIV/SIV-infected non-progressors exhibit higher levels of LPS and sCD14 than uninfected participants, meaning that even elite controllers and long-term non-progressors with HIV disease have evidence of microbial translocation, which contributes to immune activation (Brenchley et al., 2006).

Microbial translocation has been well documented in the IBD/IBS literature. In fact, there are multiple similarities in the pathogenesis of IBD/IBS and HIV infection and its impact on the function of the GI system, including depletion of Th17 cells in GALT leading to inflammation and subsequent dysfunction of the epithelial barrier (Brenchley & Douek, 2008; McGuckin, Eri, Simms, Florin, & Radford-Smith, 2009). While the underlying reasons for low levels of EndoCAb in HIV disease are not clearly understood, the effect of HIV infection on the dysregulation of monocytes allows continuing circulation of microbial products, which results in chronic monocyte immune activation with sCD14 and a pro-inflammatory response (Yim, Li, Lau, & Lau, 2009). Even in the presence of high CD4+ T cell counts and suppressed viral loads, chronic inflammation exists with stable levels of sCD14 (Hattab et al., 2014).

Overall, LPS and the sCD14-LPS complex can stimulate the inflammatory pathway. In addition, the LPS-LBP bound complex stimulates sCD14, which initiates the inflammatory pathway. The LPS-sCD14 complex induces the production of IL-6; activation of the immune system by circulating microbes is a signature process in the inflammatory cycle (Brenchley et al., 2006; Cassol, Rossouw, Seebregts, & Cassol, 2011;

Kamat, Misra, et al., 2012). Therefore, sCD14 is a more reliable surrogate marker for microbial translocation than directly measuring bacterial LPS.

Symptoms in HIV Disease

Symptoms and symptom management are critical in HIV care. While symptom burden is an important issue for patients and providers, symptoms often go under-recognized. Edelman, Gordon, and Justice (2011) conducted a secondary data analysis of the Veterans Aging Cohort Study and found that providers demonstrated poor sensitivity to the report of symptoms, even with a symptom checklist completed by patients. Providers failed to recognize symptoms associated with disease progression. Symptoms reported were fatigue/loss of energy, cognitive decline, shortness of breath, loss of appetite, muscle aches/pain, and problems with weight loss (Edelman et al., 2011; Justice, Chang, Rabeneck, & Zackin, 2001). Among these symptoms, functional decline (Erlandson et al., 2013; Stehle et al., 2012), cognitive decline (Ancuta et al., 2008; Kamat, Lyons, et al., 2012), obesity (Koethe et al., 2013), anxiety, and sadness (Liebregts et al., 2007) have been associated with microbial translocation, indicated by elevated levels of sCD14 and/or gram-negative bacterial LPS along with other inflammatory cytokines. Fatigue (Klimas, Broderick, & Fletcher, 2012), muscle aches, joint pain (Eriksson, Andersson, Ekerfelt, Ernerudh, & Skogh, 2004), poor sleep (Grandner, Sands-Lincoln, Pak, & Garland, 2013), fever/chills/sweats (Holtzclaw, 2013), night sweats (Mold, Holtzclaw, & McCarthy, 2012), peripheral neuropathy (Harezlak et al., 2011; Zheng et al., 2011), diarrhea (Liebregts et al., 2007), anxiety, depression (Camacho, 2013), and weight loss/wasting (Stein et al., 1997) have been associated with chronic inflammation as indicated by the elevation of pro-inflammatory cytokines. Table 2

provides a list of HIV-associated symptoms consistently reported by PLWH and displays the gaps in knowledge in regard to whether each has been associated with inflammation. Abdominal pain, reduction in appetite, and sexual problems are commonly reported symptoms in HIV disease, however, there are no relevant data or publications citing an association or lack of one with inflammation. Therefore, because of the prevalence of these symptoms in HIV disease, it is worth an investigation to gain insight into the possible underlying problems contributing to their development.

While there are similarities in pathology and symptoms between IBD/IBS and HIV disease, some of the symptoms have not been widely investigated in the context of microbial translocation in HIV disease. For example, bloating and abdominal pain are commonly reported in both HIV disease and in IBD/IBS but have not been investigated in the context of inflammation and microbial translocation in HIV disease. Given the emphasis on patient-centered care over the past decade, it is important to understand and seek ways to validate patient symptoms and to work toward a model of care that is tuned in to the symptomatic experience of patients. Symptoms that may be associated with inflammation and disease progression in HIV disease warrant thorough investigation to support providers as they deliver symptom-focused interventions and care.

Inflammation, Immune Activation, and Disease Progression

The inflammatory process helps the host fight off foreign antigens. However, when unregulated inflammation causes damage, inflammation can also be harmful. In the same way, many strains of bacteria serve to protect and maintain functional abilities in a symbiotic relationship with the host. Killing all bacteria or even altering the normal flora is harmful. Some bacterial strains serve to boost and regulate the immune response.

However, when bacteria and inflammation become unregulated, disease develops in the body (Antoni, Nuding, Wehkamp, & Stange, 2014).

Immune activation is multifactorial and complex. In numerous clinical trials, mortality, disease progression, and opportunistic infections have been associated with elevations in inflammatory biomarkers, IL-6, C-reactive protein (CRP), and D-dimer (Nixon & Landay, 2010). One important study was conducted by the Strategies for Management of Antiretroviral Therapy (SMART) study group. This large, multicenter, randomized clinical trial examined interruption and late initiation of cART based on CD4+ T cell count with a primary endpoint of new or recurrent opportunistic infection or all causes of death. Secondary endpoints were (a) a potentially life-threatening symptomatic event requiring medical intervention or (b) death (El-Sadr et al., 2006). In this study, participants with higher versus lower levels of IL-6 were 2.4 (95% CI: 2.1-8.8) times more likely to develop opportunistic infections; participants with higher versus lower levels of CRP were 7.6 (95% CI: 2.0-28.5) times more likely to develop opportunistic disease. Baseline and latest IL-6 levels, and latest CRP were predictive of disease development (Rodger et al., 2009). Elevation of D-dimer was predictive of the development of cardiovascular disease but not opportunistic infection (Rodger et al., 2009). The adjusted risk of mortality was shown to be 8 times greater among participants with high sCD14 levels (95 % CI, 1.2-13.9; $p = .02$) versus low levels of sCD14. Participants with higher levels of sCD14 in the SMART study had increased enterocyte damage in comparison to participants with low levels of sCD14, even after treatment and adjusting for age (Sandler et al., 2011). Likewise, sCD14 has been shown to be a surrogate biomarker for immune activation in controlled and uncontrolled patients on

cART (Brenchley et al., 2006; Kamat, Misra, et al., 2012).

HIV-associated inflammation processes, as described above, have been shown to cause the early onset of non-AIDS related complications and early aging. Conditions normally associated with aging in uninfected populations manifest themselves prematurely in patients living with HIV disease (Deeks, 2011; Vance, McDougall, Wilson, Debiasi, & Cody, 2014). As such, aging is associated with cognitive decline, cardiovascular disease, cancer, bone disease, immunosenescence, and frailty (Deeks, 2011), which may be due to chronic inflammation caused by microbial translocation. Microbial translocation measured by sCD14 and LPS is associated with progression of the thickening of carotid arteries or subclinical atherosclerosis in HIV disease (Kelesidis, Kendall, Yang, Hodis, & Currier, 2012). Patients may experience symptoms such as shortness of breath, fatigue, lack of energy, and pain, due to hardened and thickened arterial walls. Atherosclerosis is normally associated with older patients and clinicians may not look for it in younger patients, especially if symptoms are attributed to medications, HIV, or depression.

Chronic inflammation has been associated with an array of symptoms, which have been commonly reported by PLWH (Edelman et al., 2011; Gay et al., 2011; Johnson et al., 2003). Because inflammation leads to epithelial barrier dysfunction and epithelial barrier dysfunction leads to microbial translocation, which results in inflammation, it is plausible that some symptoms can be linked to microbial translocation. In light of the association between inflammation and symptoms, we need to examine which HIV disease symptoms have an association with GI epithelial barrier dysfunction and which symptoms have an association with inflammation induced by microbial products

circulating in the blood. If we can understand the relationship of microbial translocation and symptoms reported by HIV-infected patients, we should be able to develop intervention studies to reduce the symptom burden in PLWH (Wilson, Moneyham, & Alexandrov, 2013).

Discussion

It is important for nurses to educate patients about the significance of discussing symptoms with their providers and not assuming the extent of their symptoms is related to cART. It is also important for nurses to familiarize themselves with microbial translocation, to encourage patients to ask their providers about microbial translocation, and/or to make recommendations on interventions to improve gut health. For example, various nutrition strategies can be discussed to support reduction of inflammation in the gut through dietary choices, such as decreasing sugar and alcohol intake, or taking over-the-counter probiotics.

Inflammatory-related symptoms may create a significant barrier to successful implementation of clinical care by affecting adherence to cART and engagement in care. Chronic inflammation from immune activation and elevated levels of soluble CD14 (sCD14), and interleukin (IL)-6 have been linked to early aging, decline in cognitive function, metabolic disease, cardiovascular disease, decline in renal function, cancer, bone disease, and other end-organ diseases (Deeks, 2011; Duprez et al., 2012; Erlandson et al., 2013; Kamat, Misra, et al., 2012; Marks et al., 2013; Pedersen et al., 2013; Vance et al., 2014). As noted in Table 2, many inflammatory-related symptoms are reported in HIV disease and there are even a few symptoms without any correlation data. Research studies should be conducted to determine if these symptoms are an indication of

underlying inflammation in HIV disease. If there is a correlation between various symptoms and inflammation, microbial translocation may be a target for interventions to prevent HIV disease progression and reduce symptoms experienced in chronic HIV disease.

Knowledge based on the association between epithelial barrier inflammation and GI symptoms, and the association between subsequent microbial translocation and systemic symptoms is limited. Interventions targeted toward improving gut health and microbial translocation still require rigorous research in PLWH. Research designs that address both the quality of life and the association and predictive perspectives of microbial translocation are warranted. Primary steps to improve symptom management strategies would be to conduct studies investigating the association between symptoms and microbial translocation. This would include examining the association between microbial translocation and reported GI and systemic symptoms commonly experienced in HIV disease. Characterizing the overall symptom experience in HIV disease in terms of prevalence, and the underlying influence of inflammation caused by immune activation in response to microbial translocation would support clinical trials to develop new treatment strategies.

We have addressed the relationship between symptoms of inflammation and microbial translocation in PLWH. As symptom burden has been associated with poor adherence to HIV medications, a possible target to improving symptoms may be to reduce symptom burden. If there is an association between symptom burden and microbial translocation, the reduction of microbial translocation may support adherence strategies. In addition, reducing inflammation of the epithelial barrier may reduce GI

symptoms and microbial translocation, as seen in the IBD/IBS disease model with probiotic use (Wilson et al., 2013).

There are many challenges to symptom research, including numerous confounders and the subjective nature of the symptom experience. Strategies targeting microbial translocation may become an objective supplement to measure improvement of outcomes with symptom management. Given the complexity of symptoms in HIV disease, an interdisciplinary approach from the perspectives of nursing, medicine, nutrition, clinical, and scientific communities would support a more holistic patient-centered model to symptom management. Adjunct treatment strategies designed to heal and reduce inflammation of the epithelial barrier have the potential to reduce the symptom experience, thereby improving adherence. New treatment strategies may also slow disease progression by reducing microbial translocation, one of the key predictors of HIV-associated morbidity and mortality.

Key Considerations

- Nurses need to have a working understanding of the key drivers of disease progression such as microbial translocation to support patient understanding of the HIV disease process.
- Symptoms should be regarded as important and addressed by providers in HIV disease.
- Nurses have the opportunity to educate patients about discussing symptoms with their providers and not assuming the symptoms are directly related to HIV medications.
- Nurses should educate patients on interventions to reduce inflammation.

- Symptom management research should begin to target microbial translocation.
- Nurses are in a critical position to make symptom management recommendations.

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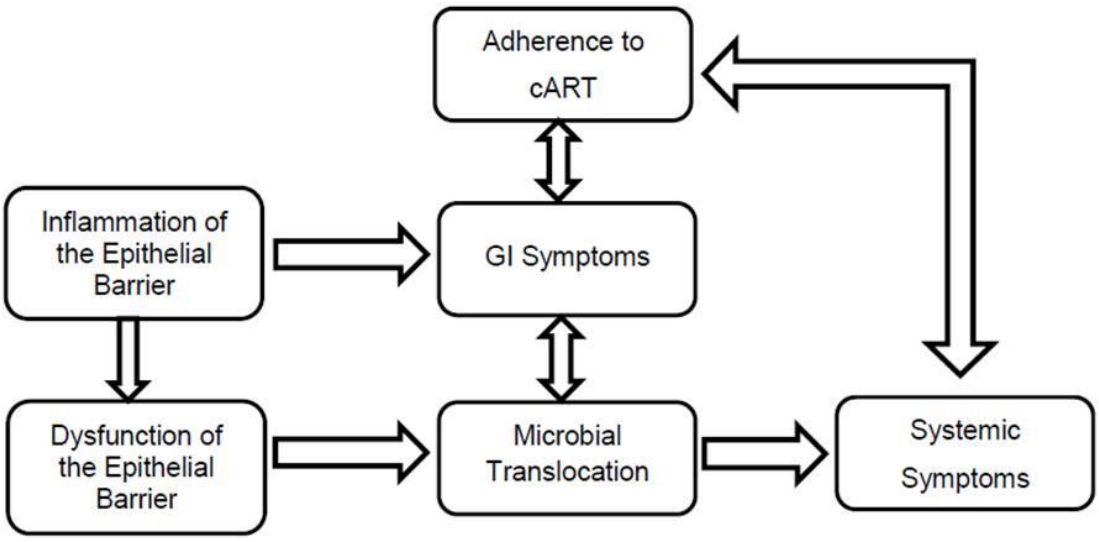

Figure 1. Conceptual framework: symptomatic consequences of inflammation. Inflammation of the epithelial barrier leads to possible GI symptoms and dysfunction of the epithelial barrier. Dysfunction of the epithelial barrier leads to microbial translocation. While microbial translocation has not been associated with GI symptoms, the double arrow signifies a possible association as outlined in this review. Microbial translocation and inflammation have been associated with some systemic symptoms. Symptom burden has been associated with adherence to cART.

Note. cART = combination antiretroviral therapy; GI = gastrointestinal.

Table 1: Key Definitions on Topic-Specific Abbreviations

Topic Specific Definitions	
Gut Associated Lymph Tissue (GALT)	The GALT is a large lymphatic area clustered behind the gut epithelial tissue that forms the mucosal lining of the gastrointestinal tract. It is organized into the lamina propria, Peyer's patches, and isolated lymphoid follicles. It is rich in Th17 cells (Estes et al., 2010).
T-Helper 17 (Th17)	Th17 white blood cells are a type of CD4+ T cell rich in the GALT. They express a CCR5 receptor on their surface and are a prime target for HIV attack once activated in response to HIV (Klatt & Brenchley, 2010).
Chemokine Receptor 5 (CCR5)	This gene is a receptor that is present on macrophages and T cells, like the CD4+ cell. HIV can attach to this receptor. (Klatt & Brenchley, 2010)
Commensal bacteria	Friendly bacteria living in the gut. These bacteria help to digest food, regulate immune function, and defend the gut barrier from harmful bacteria. Some strains can be ingested in the form of probiotics (Wilson et al., 2013).
Microbial translocation and epithelial barrier dysfunction	
Lipopolysaccharide (LPS)	Cell wall component of gram negative bacteria. Plasma LPS is directly associated with microbial translocation. Reduced by long term cART but not to health control levels (Kitchens & Thompson, 2005)
LPS binding protein (LBP)	LBP binds to LPS to form the LPS-LBP complex and presents to cells that produce sCD14, which is able to bind to this complex and present to lipoproteins that clear the LPS (Kitchens & Thompson, 2005).
Soluble CD14 (sCD14)	Released by monocytes and macrophages during immune activation in a pro-inflammatory innate response to circulating microbes in an LPS-LBP complex. sCD14 is a more accurate and relevant measure of microbial translocation (Sandler et al., 2011). It is recommended as a surrogate biomarker of microbial translocation and epithelial barrier dysfunction (Stehle et al., 2012).
Endotoxin core antibodies (EndoCab)	Released in response to LBP-LPS complex to clear LPS. Lower levels are found in HIV. Mechanism not fully understood but may be partially due to HIV-1 Tat protein downregulation impairment of immune responses to LPS (Yim et al., 2009).
Pro-inflammatory cytokines	
Interleukin (IL)	A type of cytokine, a protein substance released from activated white blood cells that communicates with other cells to stimulate or regulate responses.
Interleukin-1 (IL-1)	A family of pro-inflammatory cytokines secreted by eptihelial cells and leukocytes to induce an acute response and neutrophil

	production. Increased in HIV disease (Deeks, 2011).
Interleukin-6 (IL-6)	Classic marker of inflammation in HIV. Associated with CVD, advanced HIV disease , and non-AIDS events. Correlation with higher plasma viral loads especially with lower CD4+ T cell counts. Associated with immune senescence and the inflammation associated with aging. Positively correlated with sCD14(El-Sadr et al., 2006).
Interleukin-8 (IL-8)	Secreted by some epithelial and white blood cells in response to HIV-1 exposure (Nazli et al., 2010).
Interleukin-10 (IL-10)	Anti-inflammatory immune modulation inhibiting IFN-γ and IL-2 production. Associated with IBD. Produced by programmed death (PD-1) triggered monocytes (Said et al., 2010).
Interleukin-17 (IL-17)	Promotes a pro-inflammatory effect and recruitment of neutrophils. Produced by a subset of Th17 cells (Brenchley et al., 2008).
Interleukin-21 (IL-21)	Cytokine with crucial role of B cell differentiation; decreased in HIV infection. (Ruffin et al., 2012)
Interleukin-22 (IL-22)	Secreted by Th17 cells to promote epithelial healing and proliferation caused by inflammation. Can also act synergistically, amplifying other pro-inflammatory cytokines such as IL-17 and cause hyperplastic tissue remodeling as seen in acanthosis (Kitchens & Thompson, 2005; Zheng et al., 2007).
Interleukin-23 (IL-23)	Stimulates production of IL-22 and/or IL-17 by Th17 cells in the GALT. Plays a key role in IBD (Weaver et al., 2013).
TNFα	Response of enterocytes in response to HIV gp120. Induces inflammation and contributes to the breakdown of tight gap junctions disrupting barrier function (Nazli et al., 2010).
C-reactive protein (CRP)	An inflammatory biomarker elevated in HIV disease. Associated with increased risk of CVD, all-cause mortality, AIDS and non-AIDS events, and with detectable HIV viral loads (El-Sadr et al., 2006).
Coagulation	
D-dimer	Coagulation biomarker also associated with higher levels of sCD14 (El-Sadr et al., 2006).

Note. cART = combination antiretroviral therapy; IBD = irritable bowel disease; CVD = cardiovascular disease.

Table 2: Inflammatory Symptoms and Their Association with IL-6 and sCD14 Increase

Symptoms frequently reported by PLWH	Early ART	sCD14	IL-6
Abdominal pain			
Anxiety			X
Changes in body weight/fat	X	X	X
Cognitive decline	X	X	X
Diarrhea	X		X
Fatigue	X		X
Fever or night sweats	X		X
Headaches	X		
Insomnia	X		X
Joint pain/Stiffness			X
Loss of strength		X	X
Muscle pain			X
Nausea/Vomiting	X		
Peripheral neuropathy	X		X
Reduction in appetite			
Sadness	X		X
Sexual problems			
Shortness of breath/Cough	X		X
Skin problems	X		

Note. The symptoms listed are common symptoms currently reported in HIV disease as referenced in the section of this article: *Symptoms in HIV Disease*. The column labeled *Early ART* indicates symptoms, which were often attributed to side effects of early era ART, including highly toxic antiretroviral drugs (e.g., zidovudine monotherapy, stavudine, didanosine, indinavir, nelfinavir). The column labeled *sCD14* indicates symptoms with established associations with sCD14. The column labeled *IL-6* indicates symptoms with established associations with IL-6. Blank cells indicate frequently reported HIV-associated symptoms without reported associations with early ART or IL-6 and sCD14.

PLWH = people living with HIV infection; sCD14 = soluble CD14; IL-6 = interleukin-6; ART = antiretroviral therapy; IL = interleukin;

IDENTIFYING SYMPTOM PATTERNS IN PEOPLE LIVING WITH HIV DISEASE

by

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Format adapted for thesis or dissertation

Abstract

Symptoms guide disease management, and patients frequently report HIV-related symptoms, but HIV symptom patterns reported by patients have not been described in the era of improved antiretroviral treatment. The objectives of our study were to investigate the prevalence and burden of symptoms in people living with HIV and attending an outpatient clinic. The prevalence, burden, and bothersomeness of symptoms reported by patients in routine clinic visits during 2011 were assessed using the 20-item HIV Symptom Index. Principal component analysis was used to identify symptom clusters and relationships between groups using appropriate statistic techniques. Two main clusters were identified. The most prevalent and bothersome symptoms were muscle aches/joint pain, fatigue, and poor sleep. A third of patients had 7 or more symptoms including the most burdensome symptoms. Even with improved antiretroviral drug side effect profiles, symptom prevalence and burden, independent of HIV viral load and CD4+ T cell count, are high.

Key words: fatigue, HIV, pain, symptom burden, symptom prevalence, symptoms

Identifying Symptom Patterns in People Living with HIV Disease

HIV disease has always been associated with a high symptom burden, yet clusters of symptoms have not been defined in the current literature encompassing the contemporary era of improved combination antiretroviral therapy (cART). Available evidence has indicated poor agreement in provider and patient assessments of the symptom experience, and patient reports of symptoms are often overlooked or under-recognized by health care providers (Justice et al., 2001; Justice, Rabeneck, Hays, Wu, & Bozzette, 1999). Patient-reported symptoms may drive treatment interruption or discontinuation leading to poor health outcomes and decreased quality of life and/or function (Deeks, 2011; Erlandson et al., 2013; Kempf et al., 2009). Therefore, acknowledging symptoms and incorporating symptom management into clinical visits is an important strategy to improve patient-provider relations and health outcomes.

Many factors are associated with the development of symptoms in the context of HIV disease, including co-morbidities, treatment side effects, and inflammatory processes (Dodd et al., 2001; Lenz, Pugh, Milligan, Gift, & Suppe, 1997). Within the symptom experience, symptoms interact with each other by moderating and mediating one another (Lenz et al., 1997). For example, pain symptoms may lead to sleep dysfunction and fatigue, which in turn may increase the experience of pain. Depressive symptoms are also associated with increased experience of pain (Merlin et al., 2012) and the combined effect of sleep and unresolved pain may further influence depression or sadness. This dynamic relationship between symptoms makes research targeting the symptom experience challenging (Cheung, Le, & Zimmermann, 2009; Lenz et al., 1997). Situational factors arising from the social and physical environment can also influence

the development, experience, and interpretation of symptoms (Dodd et al., 2001; Lenz et al., 1997).

Other disease models use symptom patterns to guide management and diagnosis. For instance, polyphagia, polydipsia, polyuria, and weight loss represent a symptom cluster associated with hyperglycemia and a diagnosis of diabetes (American Diabetes Association, 2014). The symptom cluster of bloating, abdominal pain, chronic diarrhea, and/or constipation may indicate exacerbation of irritable bowel syndrome or inflammatory bowel disease (Tontini, Vecchi, Pastorelli, Neurath, & Neumann, 2015). The objectives of our study were to investigate the prevalence and burden of self-reported symptoms in people living with HIV disease (PLWH) attending routine care at an outpatient clinic in the Southern United States. This knowledge could help clinicians understand symptom patterns and inform them of underlying disease processes as seen in other diseases, such as diabetes or inflammatory bowel disease.

Methods

Study Design and Subjects

The University of Alabama at Birmingham (UAB) 1917 Clinic is a Ryan White funded ambulatory HIV clinic providing comprehensive medical care and social services including primary and specialty HIV care, mental health, and dental care; serving 3,000 adults living with HIV. Patients are able to access the site's Liver Clinic for hepatitis treatment and clinical trials. The UAB 1917 Clinic is the largest HIV health care facility in the state of Alabama. Providers are infectious disease board-certified physicians and HIV specialty nurse practitioners. Practitioners provide specialty care clinics in dermatology, endocrinology, neurology, palliative care, psychiatry, and women's health.

Prior to scheduled clinic appointments, all patients privately report symptoms at an electronic kiosk using the HIV Symptom Index, a 20-item survey routinely used for clinical care and research with PLWH to capture prevalence and magnitude of HIV-related symptoms. The index was developed to identify and describe symptoms for the purpose of developing targeted interventions. It is a useful tool to consider patterns of symptoms and the impact on patient quality of life (Justice et al., 2001). Patients identify symptoms experienced and then rate each reported symptom as to the level of bothersomeness on a 5-point Likert-type scale ranging from *symptom not present* (0) to *bothers me a lot* (4). The HIV Symptom Index has demonstrated construct validity with high test-retest reliability (intra-class correlation coefficient = 0.92), and internal consistency ($\alpha = 0.79$; Justice et al., 2001; Whalen, Antani, Carey, & Landefeld, 1994).

Data collected from patients using the HIV Symptom Index are automatically entered into the Center for AIDS Research Network of Integrated Clinical Systems (CNICS) database as part of an ongoing longitudinal study following HIV disease outcomes (Kitahata et al., 2008). We chose a 12-month timeframe to conduct a retrospective analysis of patients seen in 2011 to capture all seasons and investigate the patterns of symptoms reported by patients with HIV disease. The CNICS database includes patient demographic and clinical information, and was used to identify all eligible subjects between 19 and 79 years of age seen in the 1917 Clinic for routine office visits between January 1, 2011 and December 31, 2011. For the purpose of this article, patients were considered to have a diagnosis of HIV regardless of being symptomatic or asymptomatic. The UAB Institutional Review Board approved the study.

Data Analysis

Descriptive statistics were used to summarize demographics, symptoms, and disease characteristics. For the purpose of calculating symptom prevalence, we selected the first visit of 2011 for each patient (Justice et al., 2001). A symptom was present and counted if the score was greater than 0 (*I do not have the symptom*) and reported as 1 to 4 on the HIV Symptom Index, with 1 = *I have the symptom, does not bother me*, 2 = *bothers me a little*, 3 = *bothers me some*, 4 = *bothers me a lot*. Symptom distress was defined by a score of 2–4 on the HIV symptom. Symptom burden was defined as the count of symptoms reported.

To examine if there were differences in symptom prevalence in earlier versus later linkage to HIV infection care, we reported symptoms as bothersome for patients diagnosed less than 12 months (n = 147) and patients diagnosed greater than or equal to 12 months (n = 1,738). Only the first clinic visit of the year per patient was used for this comparison using chi-square analysis. Note that the recorded date of diagnosis was not representative of the date of infection nor did it allow for calculating the true time since infection, but rather provided an estimate. Kendall's tau was used to estimate and test the association between HIV-1 viral load, CD4+ T cell count, and symptom burden count.

A principal component analysis with oblimin rotation (Abdi & Williams, 2010) was conducted on within-subject mean item scores of all 20 items on the HIV Symptom Index across visits and stratified by HIV-1 viremia to determine clusters of symptoms that co-varied or clustered together independent of other subsets of symptoms at varying levels of viremia.

Missing data were defined as data that were not entered by the patient, where the

patient left the question blank, and the data were deleted list-wise for analysis. Only 804 patients responded to all 20 symptoms on the HIV Symptom Index for the first visit of the year. Statistical analyses were performed using IBM SPSS Version 22 (Armonk, NY: IBM Corp.).

Results

Symptom Description

In total, 5,738 clinic visits were documented for the 1,945 patients seen for routine HIV care, with 1,885 patients reporting at least one symptom during the year. The mean clinic visit count during the 12-month study period was three encounters per patient. Mean age of study subjects was 44 years, with 96% of patients on HIV antiretroviral therapy, 76% of patients having a viral load of < 500 copies/mL, and 71% having a CD4+ T cell count of > 500 cells/mm^3 (Table 1). Almost a third of patients (31%) had a high symptom burden, reporting 7 or more symptoms with 8% of all patients having 10 or more symptoms including those ranking as most bothersome. There was no statistically significant correlation between symptom burden and viral load or CD4+ T cell count. Two main clusters were identified encompassing both physical and psychological symptoms in the clinic population studied. No statistically significant difference between presence of symptoms based on HIV-1 RNA viremia > 500 copies/mL and viral load suppression (< 500 copies/mL of HIV-1 RNA) was found. The most prevalent symptoms reported were poor sleep, muscle aches/joint pain, fatigue, nervous/anxious, and sadness (Table 2; Figure 1). Twelve of the 20 symptoms assessed were reported as bothersome in more than 30% of the sample (Table 2). Of all symptoms reported, the symptoms rated as *most bothersome* were muscle aches/joint pain, fatigue,

bloating/abdominal pain, numbness/pain in feet, poor sleep, nervous/anxious, and sex problems. Of symptoms reported as bothersome, those *bothering patients a lot* (scoring 4 on the scale) were muscle aches/joint pain, numbness/pain in feet, sex problems, poor sleep, fatigue, and bloating/abdominal pain. Muscle aches/joint pain, fatigue, and poor sleep were present in the top five most prevalent and most bothersome symptoms reported (Table 2; Figure 1).

We examined differences in symptoms based on race and gender using chi-square analysis. No statistically significant differences in reported symptoms based on gender were found. However, for race, specifically between African American, White, and Hispanic groups, there were differences in fatigue, sadness, poor sleep, nervous/anxious, and memory loss with White patients reporting a higher prevalence for these symptoms than non-White groups ($p < .01$). Hispanics reported higher prevalence of sex problems than Whites and African Americans ($p < .01$). Whites and Hispanics reported higher muscle aches/joint pain and diarrhea than African Americans ($p < .01$). African Americans and Hispanics reported a higher prevalence of headaches than Whites ($p = .03$). There were no statistically significant differences in bloating/abdominal pain, numbness/pain in feet, cough/shortness of breath, poor appetite, nausea/vomiting, fever/chills/sweats, or feeling dizzy.

When examining differences in whether persons reported symptoms as bothersome, Whites reported that symptoms of fatigue, memory, sadness, anxiety, poor sleep, and sex ($p < .01$), muscle aches/joint pain, and nausea ($p = .02$) bothered them more than the rates of bothersomeness reported by African Americans for these symptoms. Diarrhea ($p < .01$) and hair loss ($p = .03$) bothered Whites, but African

Americans were more likely to report these symptoms as *bothers a lot* than Whites. African Americans reported rash as being more bothersome than Whites ($p < .01$).

Symptom Clusters

Across all patient routine clinic visits, four symptom clusters were identified. The two main clusters identified attributed 42% and 7% of total variability in the 20 symptoms reported, having Eigen values greater than 1.0 (Table 3). Eleven of the 20 symptoms loaded on Factor 1 and explained 41% of the total variation in symptoms reported. The symptom cluster represented in Factor 2 accounted for 7% of the total variation. The remaining two clusters accounted for 5% of the variability each and had Eigen values of 1.0. The loadings (correlations between symptom and factor) of symptoms on each factor or cluster are displayed in Table 3, and Figure 2 displays a Venn diagram illustrating the breakdown of symptoms of the major clusters, including their overlap. In this sample, the 20-item index had an internal consistency of $\alpha = .92$.

Symptom Cluster 1. Of the symptoms reported in cluster 1, the most prevalent symptoms occurring in at least a third of the sample population were muscle aches/joint pain (48%), fatigue (47%), numbness/pain in feet (36%), and headache (36%). Cough/shortness of breath (28%), fever/chills/sweats (28%), and bloating/abdominal pain (25%) were prevalent in at least a quarter of those reporting symptoms. Muscle aches/joint pain (85%), fatigue (84%), bloating/abdominal pain (83%), and numbness/pain in feet (82%) were reported as the most bothersome with at least 40% of those reporting muscle aches/joint pain (42%) and numbness/pain in feet (40%) being reported as *bothers a lot* on the HIV Symptom Index.

Symptom Cluster 2. Of the symptoms reported in cluster 2, the most prevalent,

occurring in at least a third of the sample population, were poor sleep (51%), muscle aches/joint pain (48%), fatigue (47%), nervous/anxious (43%), sadness (40%), and memory loss (35%). Sex problems occurred in 28% of patients reporting symptoms. Furthermore, of those reporting symptoms, muscle aches/joint pain (85%), fatigue (84%), poor sleep (82%), and nervous/anxious (81%) were reported as the most bothersome. Muscle aches/joint pain (42%) and numbness/pain in feet (40%) were reported as *bothers a lot* in at least 40% of those reporting symptoms on the HIV Symptom Index. Nearly a third reported poor sleep (35%) and sex problems (38%) as *bothers a lot*.

Associations between Symptom Clusters

There was a strong correlation between Symptom Cluster 1 and Symptom Cluster 2 ($r = .53$, $p < .01$). Fatigue and muscle pain/joint aches loaded on both factors and were reported among the most prevalent and most bothersome symptoms. The high prevalence of fatigue and muscle pain/joint aches persisted in subjects with both clusters with HIV-1 viremia ($\geq$ 500 copies/mL) or aviremia (< 500 copies/mL). There were no statistical differences in clusters based on gender or race.

In symptom clusters in patients with HIV-1 viral loads > 500 copies/mL, symptom clusters did not have any overlap. The first cluster accounted for 42% of the variability in reported symptoms, with rash added to the symptoms listed in comparison to the full population model. The second cluster loaded symptoms with an inverse correlation between weight variables and appetite, and accounted for 8% of the variability of symptoms reported (Table 4).

In patients with HIV-1 viral loads < 500 copies/mL, muscle aches/joint pain dropped from the first cluster model and fatigue did not load on the second cluster. The

first cluster accounted for 40% of the variability of symptoms reported (Table 4). Loss of appetite loaded on both clusters. The second cluster, accounting for 7% of the variability, did not differ from the full population model.

Symptom Burden

Symptoms reported by each patient at the first visit of the year ranged from 0 to 16 (median 3 symptoms). Of all patients, 29% reported no symptoms, while 28% reported 1-3 symptoms, 14% reported 4-6 symptoms, 10% reported 7-9 symptoms, 14% reported 10-15 symptoms, and 5% reported 16 symptoms in the symptom clusters. In patients who reported symptoms, the association between symptom burden and viral load and between symptom burden and CD4+ T cell count was evaluated for a subset of patients without missing data for viral load (n = 804) and for a subset of patients without missing data for CD4+ T cell count (n = 655), using Kendall's tau. No association emerged between viral load and symptom burden (τ = .032, p = .20) or CD4+ T cell count and symptom burden (τ = -.015, p = .622). There was no statistically significant correlation between PHQ-9 scores and symptom burden. However, there was a weak association with PHQ-9A scores (measuring anxiety) and symptom burden ($r[752]$ = .07, p = .035).

Symptom Distress and Recent HIV Diagnosis

We compared the proportion of symptoms reported as bothersome by patients diagnosed for less than 12 months versus more than 12 months. For patients diagnosed more than 12 months, a higher proportion of patients reported fatigue (p = .002), numbness/pain in feet (p = .041), memory loss, and fat deposit/weight gain (p = .007) as bothersome; this same group experienced a lower proportion of patients who reported

sadness (p = .006), rash (p = .002), and loss of appetite (p = .010) as bothersome. These results are displayed in Table 5. No other symptom in the symptom index had statistically significant differences between time of recorded diagnosis and distress of symptom report.

Discussion

High symptom prevalence, distress, and burden were identified in this sample of patients with HIV infection despite high levels of HIV-1 suppression and immunologic stability. Physical and mental health symptoms were both common. The most prevalent symptoms were fatigue, muscle pain/joint pain, sadness, numbness/pain in feet, and poor sleep, which have been reported in inflammatory-related conditions. This was consistent with recent work conducted by McGowan et al. (2014) where fatigue, poor sleep, and muscle ache/joint pain were among the most prevalent and distressing symptoms. The majority of the most prevalent symptoms were represented in Symptom Cluster 1 regardless of HIV-1 viremia. Poor sleep and muscle aches/joint pain were among the most prevalent symptoms.

Of people reporting any symptom, more than 80% reported their symptoms as bothersome, with a significant proportion of patients reporting some symptoms as bothering them a lot. Symptoms perceived as bothersome can affect quality of life and even health outcomes. Symptoms such as pain, poor sleep, and sexual issues should be proactively addressed in clinical visits. Pain has been associated with mental health disorders and has frequently been reported with other symptoms (Merlin et al., 2012). However, the report of perceived adverse effects or symptoms indicate possible underlying inflammation, which has been implicated as a key predictor in HIV disease

progression, early aging, and non-HIV related morbidity and mortality (El-Sadr et al., 2006; Sandler et al., 2011). Inflammatory markers have not been investigated in the context of symptom development in HIV disease but should be given consideration to further understand the underlying physiologic processes of symptom development in HIV disease.

Few publications have reported symptom prevalence using the HIV Symptom Index. However, in validating the HIV Symptom Index, Justice et al. (2001) reported symptom prevalence in 115 PLWH in 1998-1999. Overall symptom prevalence has decreased from a median of 15 symptoms reported to a median of 3 as reported in our study. The most common symptoms in 1999 versus 2011 were fatigue (81% vs. 47%), diarrhea (77% vs. 24%), anxiety (77% vs. 43%), sadness (76% vs. 40%), and difficulty sleeping (76% vs. 51%). In our study, the most common symptoms were poor sleep (difficulty sleeping; 51%), muscle aches/joint pain (48%), fatigue (47%), nervous/anxious (anxiety; 43%), and sadness (40%). Although there has been an overall decrease in reported symptom prevalence, fatigue, sleeping difficulties, anxiety, and sadness have remained as the most common symptoms reported. Diarrhea has improved decreasing from 77% to 24%, which is to be expected given improvements in protease inhibitors. However, muscle aches/joint pain has become one of the more prevalent symptoms, although decreasing in overall frequency from 72% to 48%. Symptom burden has changed and decreased with improvements in cART but persists despite viral suppression. Earlier HIV regimens had high side effect profiles and, unbeknownst at the time, were the cause of high levels of inflammation. Now that side effect profiles have improved, patients may be dealing with the effects of underlying pathophysiological

processes of inflammation manifesting symptoms and the symptomatic effects from developed or developing co-morbidities such as cardiovascular, bone, and liver disease.

The *most bothersome* symptoms in 1999 versus 2011 were fatigue (77% vs. 84%), sadness (67% vs. 77%), anxiety (66% vs. 81%), sleep difficulties (65% vs. 82%), and diarrhea (61% vs. 71%). The *most bothersome* symptoms reported also changed with the highest reported symptoms being muscle aches/joint pain (85%), fatigue (84%), bloating/abdominal pain (83%), and numbness/pain in feet and poor sleep (82%). While fatigue remained as a bothersome and prevalent symptom, pain (muscle, joint, abdominal, and neuropathic) became more prevalent and psychological symptoms (e.g., sadness and anxiety) became less bothersome. Of note, the 2001 study (Justice et al., 2001) reflected different geographic and ethnographic populations and a smaller sample than in our study, but were used to compare reported symptoms using the same instrument. While symptoms have diminished overall, symptoms still persist and, in those experiencing symptoms, they contribute to a greater patient burden. Racial differences in symptom experiences reported are crucial because they indicate potential barriers to adherence in already vulnerable populations. If side effects are likely to develop for particular medications, discussing these symptoms with patients in advance may address perceived threats, and provide steps and interventions that may support a higher likelihood of continuing therapy or seeking immediate health care in the event of symptom development (Janz & Becker, 1984).

Symptom burden impacts adherence to cART and health outcomes along the HIV disease care continuum (Baran et al., 2014; Gay et al., 2011; Kempf et al., 2009; Mugavero, Amico, Horn, & Thompson, 2013). During clinic visits and while addressing

adherence to cART, providers should be particularly sensitive to identifying and managing symptoms that are causing patient distress, regardless of the source of the symptoms. While individual symptoms of the identified clusters are a part of the larger complete review of systems in a clinical assessment, asking patients specifically if they have symptoms of muscle or joint pain, poor sleep, numbness/pain in feet, fatigue, bloating/abdominal pain, and sex problems will help clinicians manage strategies targeted to patient concerns. Given the apparent symptom clusters observed in our study, the review of systems may need to be reorganized to recognize symptom cluster patterns in clinics focused on HIV disease management, proactively focusing on symptom clusters identified in persons with suppressed viral load versus viremia. With a better understanding of possible underlying physiological factors, clinicians may develop targeted strategies to address and resolve symptom clusters experienced by patients. Furthermore, clinicians may want to focus on managing symptoms most distressing to their patient populations.

Patients who had been diagnosed with HIV for 12 or more months were bothered by fatigue, neuropathy, fat deposit/weight gain, and memory loss. These symptoms were likely related to long-term cART; however, newer therapies have lower side effect profiles. Other explanations included long-term effects of inflammation (Wilson et al., 2014). Fatigue (Klimas, Broderick, & Fletcher, 2012), neuropathy (Harezlak et al., 2011; Zheng et al., 2011), obesity (Koethe et al., 2013), and cognitive decline including memory loss (Ancuta et al., 2008; Deeks, 2011; Kamat et al., 2012) have been associated with inflammation. Understanding the etiology of symptoms, especially those identified in chronic HIV disease, has been an ongoing consideration in clinical HIV research. Are

symptoms a result of side effects or the ongoing effects of inflammation and residual viral replication? Further studies validating these symptoms and the variables contributing to their presence are warranted.

Other symptoms, including poor appetite, sadness, and rash, tended to become less bothersome after 12 months in clinical care. It is possible that a decrease in reported sadness was related to adjustment to diagnosis after the first 12 months; this emphasizes the importance of supporting patients through the first year of HIV diagnosis. According to the HIV treatment cascade in the United States, roughly 40% of persons diagnosed with HIV infection and linked into care are actually retained in care (Mugavero et al., 2013). Therefore, identifying symptoms that are particularly distressing to patients should be anticipated and addressed to improve retention in care, especially during the first 12 months of diagnosis and linkage to care.

Limitations and Future Research

While our study had adequate statistical power to identify relevant symptom clusters and associations, there were a number of limitations, including not categorizing symptom clusters based on immune status or function. The patient sample was recruited at a single clinic at an academic institution with a proportionally well-controlled population in the U.S. Southeast, so the results may not be generalizable to other areas. But the findings of symptom prevalence results were consistent with studies of other HIV-infected populations (Edelman, Gordon, & Justice, 2011; Swan et al., 2014).

Our study did identify symptom clusters and confirmed that symptoms were still common in patients with well-controlled HIV disease. Many of these symptoms could be attributed to cART, but given the newer therapies and lower side effect profiles, this is

unlikely to be the sole explanation. While symptom burden has decreased in comparison to earlier reports in which symptoms were attributed to high viremia and medication side effects, the findings from our study and newer evidence suggests other explanations, such as inflammatory effects due to immune activation, for the consistent prevalence of symptoms. Research in other geographical clinic populations is needed to validate these results with longitudinal investigation of various factors. Predicting symptoms will help clinical scientists identify management strategies to reduce or alleviate symptoms when warranted. Even though this study was meant to describe the prevalence and burden of symptoms experienced and reported by PLWH, a significant limitation of our study was that we did not have a comparison cohort of persons without HIV infection. Comparisons between infected and uninfected populations would support interpretation of the context of the HIV symptom experience. Information on co-morbidities, outside of those reported, was not collected. We did not have access to substance abuse information. This limited our study to control for substance use, which can influence symptoms. However, previous studies in the same clinic population have shown substance use reported by 3% of the clinic population (Merlin et al., 2012).

We did not control for or consider medication side effects in our analysis; however, most of the symptoms (i.e., diarrhea, rash, and nausea/vomiting) associated with medication side effects were not ranked among the most prevalent symptoms reported. Variability in literacy, health numeracy, and health literacy of the study sample could have impacted the accuracy of self-reported data and may have impacted the study findings (Gakumo, Vance, Moneyham, Deupree, & Estrada, 2013). As the medical community strives to achieve improved patient-centered health outcomes, additional

steps to explore aspects of symptom burden are warranted. The results of our study demonstrated a need to develop and test symptom management strategies that predictably reduce symptom burden and improve clinical management and patient quality of life. Now that people are living longer with HIV and developing co-morbidities from inflammation, further research is warranted to examine differences in co-morbidities and the effect of inflammation on symptoms. Investigating symptoms in the context of co-morbidities and chronic inflammation in HIV populations is also warranted.

Conclusion

Even with improvements to cART side effect profiles, symptom prevalence and symptom burden continue to be highly independent of HIV viral load and CD4+ T cell count in PLWH. Clinicians should target inquiries to patients regarding highly prevalent symptoms and identify bothersome symptoms for management.

Key Considerations

- Patients living with HIV disease continue to have high symptom burdens despite viral suppression and decreased side effect profiles.
- Symptoms experienced in HIV disease may have underlying pathophysiology due to inflammation even in the context of viral suppression.
- Nurses should proactively ask patients about symptoms that contribute to distress.
- Nurses should help patients discuss symptoms with their clinicians.

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Table 1: *Demographic and Clinical Characteristics of Patients with HIV Included in the Study*
*(*N = *1,945)*

Age (years)	
Mean (range)	44 (19-78)
Gender	
Male	1,511 (78%)
Female	434 (22%)
Race/ethnicity	
African American	1,024 (53%)
Caucasian	883 (45%)
Hispanic	22 (1%)
Other	16 (1%)
Plasma HIV RNA (copies/mL)	
< 50 – 200	1,403 (73%)
201 – 499	64 (3%)
500 – 1,000	40 (2%)
1,001 – 10,000	125 (7%)
≥ 10,001	294 (15%)
Transmission Risk Factors	
Heterosexual	694 (26%)
MSM	1,029 (53%)
IVDU	162 (8%)
Other	60 (3%)
Insurance	
Private	609 (31%)
Public	717 (37%)
Uninsured	619 (32%)
CD4 Count in cells/mm^3	
< 50	62 (4%)
51 – 200	142 (9%)
201 – 350	255 (16%)
351 – 500	317 (20%)
> 500	828 (51%)
Other Characteristics	
Currently on cART	1,873 (96%)
Depression Diagnosis	276 (11%)
Anxiety Diagnosis	249 (10%)
Current Smokers	889 (35%)
Hepatitis C co-infection	221 (11%)

Abbreviations: IVDU = intravenous drug use; MSM = men who have sex with men; cART = combination antiretroviral therapy.

Table 2

Symptoms Ranked by Prevalence and Distress for Symptoms Identified in Main Symptom Clusters

Most Prevalent Symptoms	**Total *N* Reporting on HIV Symptom Index***	**A. Prevalence of persons reporting symptom as present on HIV Symptom Index**		**B. Reporting Symptom Bothersome on HIV Symptom Index**		**C. Reporting Symptom as Bothers A Lot* from those reporting symptom as Bothersome**	
	N	***n***	**%**	***n***	**%**	***n***	**%**
Poor sleep	1,784	908	51	747	82	264	35
Muscle aches/Joint pain	1,815	865	48	732	85	310	42
Fatigue	1,774	834	47	676	84	208	31
Nervous/Anxious	1,782	763	43	618	81	198	32
Sadness	1,784	714	40	547	77	135	25
Numbness/Pain in Feet	1,808	658	36	540	82	214	40
Headache	1,796	645	36	485	75	134	28
Memory Loss	1,797	626	35	456	73	93	20
Sex problems	1,797	503	28	397	80	152	38
Cough/Shortness of breath	1,792	508	28	374	74	102	27
Fever/Chills/Sweats	1,797	489	27	387	79	93	24
Dizzy	1,760	444	25	336	76	63	19
Bloating/Abdominal Pain	1,785	442	25	366	83	105	29
Poor Appetite	1,785	428	24	316	74	97	31
Diarrhea	1,784	423	24	300	71	76	25
Nausea/Vomiting	1,792	376	21	270	72	62	28

*Note: *N* size is equal to those reporting the symptom on the HIV Symptom Index. Total *N* differed by symptom due to missing data for particular symptom. Prevalence is the number *(n*) of people reporting the symptom as 1-4: 0 = *I do not have the symptom*, 1 = *have, but does not bother*, 2 = *bothers a little*, 3 = *bothers some*, 4 = *bothers a lot*. Column A reports the % of persons reporting *having the symptom*. Column B reports the % bothersome for the proportion of people reporting the symptom between 2 and 4; this excludes persons reporting that they do not have the symptoms and those that reported the symptom did not bother them. Column C reports the number of persons who reported the symptom as *bothers a lot* of those reporting symptom as bothersome in Column B.

Table 3

Principal Components Analysis Determining Patterns of Symptoms Reported

N = 1,885				
HIV Symptom Index (*N* = 20)	Factor 1	Factor 2	Factor 3	Factor 4
Fatigue	**.45**	**.46**	-.02	-.14
Fever / Chills / Sweats	**.76**	-.02	.05	.03
Dizziness	**.61**	.22	.01	-.06
Numbness / Pain in feet	**.34**	.26	.18	-.29
Memory Loss	.21	**.58**	.05	-.07
Nausea / Vomiting	**.73**	.04	-.04	.17
Diarrhea	**.56**	.13	-.03	.15
Sadness	.18	**.77**	-.11	.05
Nervous / Anxious	.22	**.75**	-.11	.04
Poor sleep	.25	**.63**	-.05	.02
Rash	.18	-.16	.66	.06
Cough/Shortness of breath	**.64**	-.11	.19	-.03
Headache	**.74**	.04	-.01	-.04
Loss of appetite	**.37**	.27	.18	.55
Bloating / Abdominal pain	**.54**	.10	.22	-.22
Muscle aches / Joint pain	**.36**	**.37**	.15	-.20
Sex problems	-.25	**.72**	.20	.01
Fat deposit / Weight gain	.05	.30	.22	-.60
Weight loss / Wasting	.07	.30	.40	.62
Hair loss changes	-.05	.10	.73	-.04
Eigen Values	8.14	1.41	1.04	1.02
Proportion	0.41	.07	.05	.05

Note. Factor 1 (Symptom Cluster 1) contributed to 41% of the variance in symptoms while Factor 2 (Symptom Cluster 2) contributed 7%. Factors 1 and 2 had a strong inter-factor correlation of .53 (p value < .001). Factors 3 and 4 were excluded from further analysis due to low variability.

Table 4

Principal Components Analysis Determining Patterns of Symptoms Reported Stratified by HIV-1 Viremia < 500 copies/mL and ≥ 500 copies/mL

N=1,884				
HIV Symptom Index ($N = 20$)	Aviremia < 500 copies/mL (n = 1,437)		Viremia ≥ 500 copies/mL (n = 447)	
	Factor 1	Factor 2	Factor 1	Factor 2
Fatigue	**.44**	-.15	**.28**	.18
Fever / Chills / Sweats	**.75**	.02	**.73**	.04
Dizziness	**.61**	-.08	**.58**	.09
Numbness / Pain in feet	.26	-.30	.30	.30
Memory Loss	.22	-.08	**.32**	.21
Nausea / Vomiting	**.76**	.14	**.71**	-.19
Diarrhea	**.61**	.13	**.54**	-.12
Sadness	.21	.05	-.06	-.05
Nervous / Anxious	.25	.03	-.01	-.15
Poor sleep	.29	.04	-.01	.03
Rash	.14	.06	**.52**	.17
Cough/Shortness of breath	**.59**	-.01	**.64**	.16
Headache	**.77**	-.06	**.43**	.00
Loss of appetite	**.41**	**.56**	**.65**	**-.39**
Bloating / Abdominal pain	**.54**	-.25	**.49**	.28
Muscle aches / Joint pain	.28	-.19	.26	.27
Sex problems	-.27	.02	.03	.20
Fat deposit / Weight gain	.04	**-.59**	-.08	**.75**
Weight loss / Wasting	.06	**.63**	**.73**	-.30
Hair loss changes	-.07	-.02	**.43**	**.44**
Eigen Values	8.1	1.4	8.4	1.5
Proportion	.40	.07	.42	.07

Table 5

Proportion of Symptoms Reported as Bothersome at < 12 Months of Diagnosis Versus ≥ 12 Months of Diagnosis of HIV Infection

Symptom Reported as Bothersome	< 12 months *n* = 147 %	≥ 12 months *n* = 1,738 %	*P* value*
Fatigue	**32**	**39**	**.002**
Fever/Chills/Sweats	21	22	.905
Dizzy	19	19	.635
Numbness/Pain in feet	**21**	**31**	**.041**
Memory loss	**19**	**26**	**.007**
Nausea/Vomiting	18	15	.089
Diarrhea	18	17	.744
Sad	**41**	**30**	**.006**
Anxious/Nervous	40	34	.288
Poor Sleep	39	42	.430
Rash	**22**	**12**	**.002**
Cough/Shortness of breath	21	21	.739
Headache	30	27	.588
Loss of Appetite	**26**	**17**	**.010**
Bloating/Abdominal pain	20	21	.208
Muscle aches/Joint pain	35	41	.201
Sex Problems	17	22	.130
Fat deposit/Weight gain	**11**	**20**	**.008**
Wasting/Weight Loss	17	14	.314
Hair loss	9	11	.351

Note. *Pearson Chi-Square used to determine difference between groups.

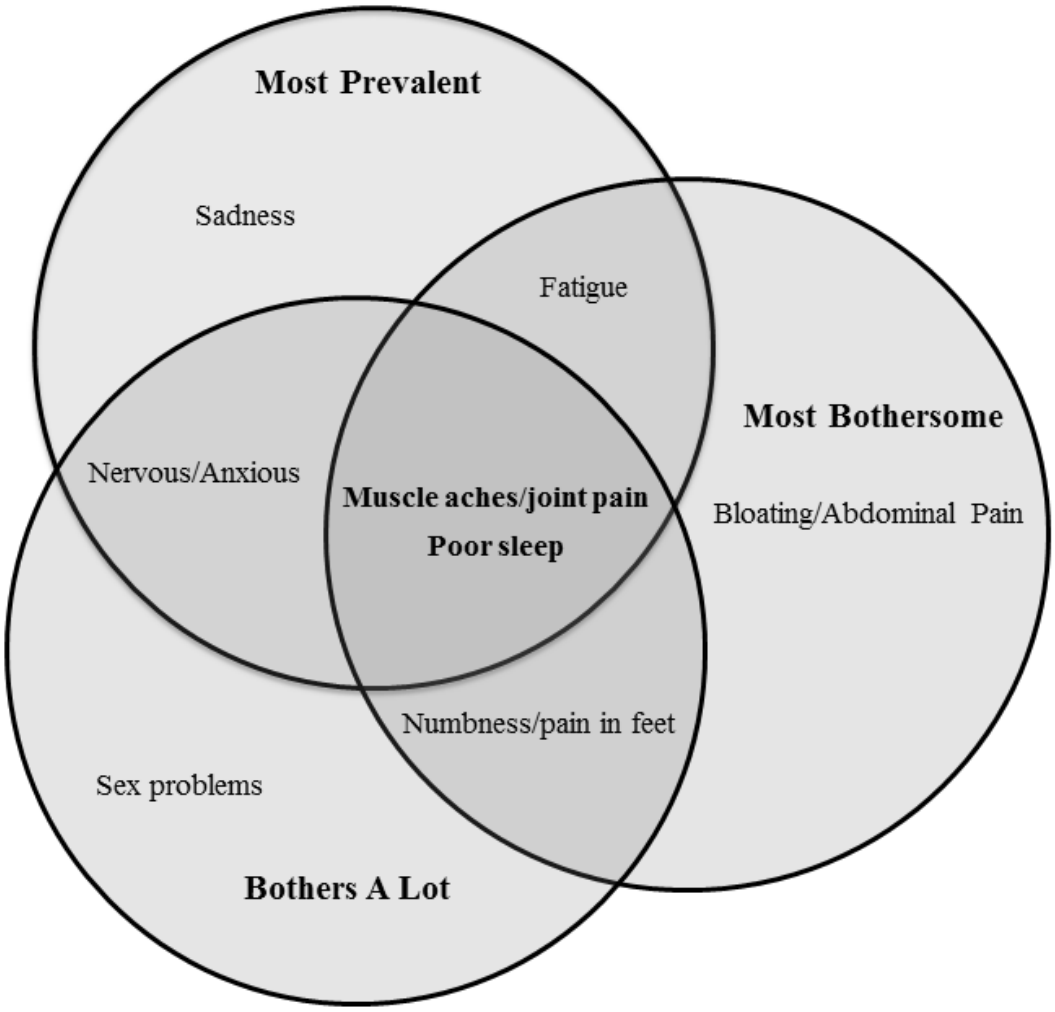

Figure 1. Venn diagram of the top 5 ranked symptoms with *prevalence, most bothersome,* and *bothers a lot* reported on the HIV Symptom Index.

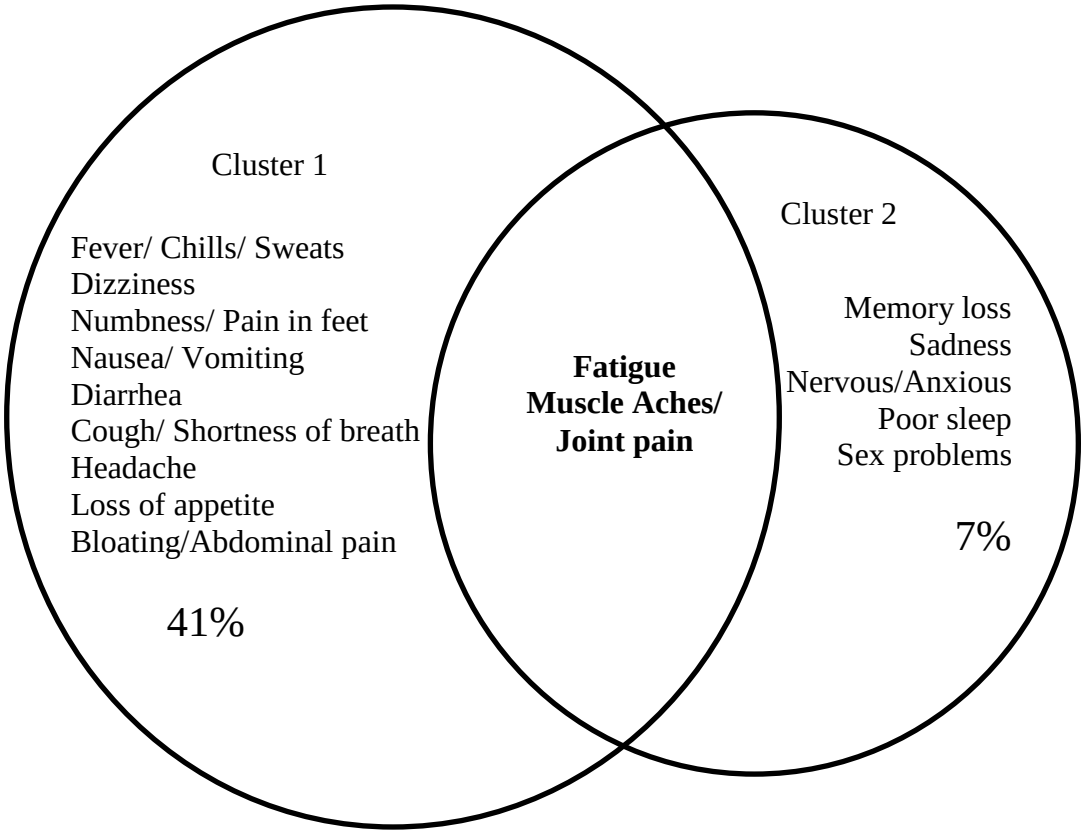

Figure 2. Venn diagram of the two main symptom clusters.

A RETROSPECTIVE ANALYSIS OF HIV-ASSOCIATED GASTROINTESTINAL SYMPTOM BURDEN AND INFLAMMATION IN THE VETERANS AGING COHORT STUDY (VACS)

by

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A Retrospective Analysis of HIV-Associated Gastrointestinal Symptom Burden and Inflammation in the Veterans Aging Cohort Study (VACS)

Abstract

Background: HIV-associated gastrointestinal (GI) barrier dysfunction leads to microbial translocation, which along with Interleukin-6 (IL-6) and D-dimer, predict HIV disease progression and mortality. We hypothesized that common GI symptoms in HIV disease, in the context of HIV-1 viral suppression, might be associated with the inflammatory biomarkers predictive of poor outcomes in HIV.

Methods: Among 1,418 HIV+ participants from the Veterans Aging Cohort Study Biomarker Cohort, we investigated whether the association between reported GI symptoms (bloating/abdominal pain, nausea/vomiting, diarrhea, and poor appetite), measured by the 20-item HIV Symptom Index, might predict microbial translocation secondary to epithelial barrier dysfunction. We used logistic regression with minimization of the Akaike Information Criteria to determine the best model fit to predict high levels of inflammation (sCD14, IL-6, D-dimer) and confounding variables to predict GI symptoms. We adjusted final models for intravenous (IV) drug use and compared this to the full model without IV drug use.

Results: The odds of being in the highest quartile of sCD14 was associated with poor appetite (OR 95% CI: 1.96 [1.29-2.97] p =.01). Nausea/vomiting (OR 95% CI: 2.06 [1.48-2.87] p = .00), bloating/abdominal pain (OR 95% CI: 1.54 [1.15-2.06] p = .00), and poor appetite (OR 95% CI: 1.58 [1.22-2.05] p = .00), were associated with the highest quartile of IL-6 (2.4-3.9 pg/mL). Poor appetite (OR 95% CI: 1.66 [1.20-2.31] p =.01) and nausea/vomiting (OR 95% CI: 1.76 [1.22-2.53] p =.01) were associated with highest

levels of D-dimer. HIV-1 RNA was associated with diarrhea, nausea/vomiting, and poor appetite, in crude but not adjusted models with the exception of poor appetite.

Conclusion: HIV-associated GI symptoms are independently associated with higher levels of inflammatory biomarkers. However, inflammatory biomarkers are not associated with symptoms in fully adjusted models. HIV-1 RNA was not associated with GI symptoms in multivariate models except with poor appetite. Poor appetite was strongly associated with viral load, sCD14, IL-6, and D-dimer, and may be a clinical indicator of poor outcomes in HIV disease progression.

A Retrospective Analysis of HIV-Associated Gastrointestinal Symptom Burden and Inflammation in the Veterans Aging Cohort Study (VACS)

Chronic inflammation from immune activation has been identified as a driver of HIV disease progression and early aging in persons with HIV infection (Deeks, 2011; Hsu & Sereti, 2016; Hunt, 2012). Monocytic activation as expressed by soluble CD14 (sCD14) has been identified as a key predictor of mortality and early aging in HIV disease (Sandler et al., 2011; So-Armah et al., 2016). Microbial products are able to escape the gut lumen due to the inflammatory effects of HIV infection on the permeability of the GI epithelial barrier after changes to the gut- associated lymph tissue (GALT) (Brenchley et al., 2006; Klatt et al., 2010; Somsouk et al., 2015). Elevated levels of sCD14, in response to circulating bacteria in circulation from the gastrointestinal (GI) tract have been associated with a 6-fold increased risk of mortality as compared to those with lower levels of sCD14 (Sandler et al., 2011). Microbial translocation has a strong role as a driver of immune activation and therefore, HIV disease progression (Zevin, McKinnon, Burgener, & Klatt, 2016).

Dysfunction of the GI epithelial barrier leads to increased permeability of the GI tract, allowing microbial products to translocate from the inner lumen into circulation (Estes et al., 2010). As in similar conditions of GI epithelial barrier dysfunction and microbial translocation such as Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS), GI symptoms occur and are commonly reported in persons living with HIV disease (Koboziev, Karlsson, & Grisham, 2010).

Almost a quarter of patients with suppressed HIV-1 experience some GI symptom such as bloating/abdominal pain, poor appetite, diarrhea, and/or nausea/vomiting, with

over 70% of those reporting GI symptoms considering them bothersome (Wilson et al., 2015). Furthermore, over 30% of HIV-infected patients discontinue or alter their cART regimen due to GI symptoms within 12 months of initiation, with the majority of them attributing this discontinuation/alteration to medication side effects (Gay et al., 2011; Kempf et al., 2009). However, little is known of the link between inflammation and functional HIV-associated GI symptoms. There is limited evidence in the HIV population of whether this hallmark epithelial barrier inflammation and dysfunction can be clinically recognized by the presence of self-reported GI symptoms (Wilson et al., 2014).

In addition to sCD14, the non-specific inflammatory markers, interleukin-6 (IL-6) and D-dimer, have a strong association with HIV disease progression, early aging, non-AIDS-related morbidity, and increased mortality among people living with HIV disease (Deeks, 2011; Sandler et al., 2011). Systemic inflammation and intestinal dysfunction have been linked to the following conditions: Depression (Krishnadas & Cavanagh, 2012); alcohol dependence (Leclercq, De Saeger, Delzenne, de Timary, & Starkel, 2014; Leclercq, Matamoros, et al., 2014); peripheral neuropathy (Zheng et al., 2011); fat redistribution (Johnson et al., 2004); and fibromyalgia (Pimentel et al., 2004); and cognitive dysfunction and memory loss (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011); and early aging (Deeks, 2009, 2011; Kohman, 2012). The process known as microbial translocation is associated with systemic immune activation and subsequent chronic inflammation and coagulation (Estes et al., 2010). Microbial translocation induced immune activation is a strong predictor of disease progression and a target for intervention research (Ancuta et al., 2008; Brenchley & Douek, 2008; Brenchley et al., 2006; Estes et al., 2010; Kamat et al., 2012). Persons with higher levels

of sCD14, D-dimer, IL-6, and C-reactive protein were more likely to progress to AIDS and non-AIDS events in HIV disease than those with lower levels and the HIV-uninfected (Duprez et al., 2012; El-Sadr et al., 2006; Nixon & Landay, 2010; Rodger et al., 2009; Sandler et al., 2011). It is unclear if these specified inflammatory biomarkers are associated with GI symptom prevalence related to epithelial barrier dysfunction and inflammation (Brenchley et al., 2006; Estes et al., 2010).

GI symptoms commonly reported by HIV-infected patients resemble symptoms experienced with IBD/IBS and share similar physiologic processes (Kilbourne et al., 2002). Loss of Th17 type CD4 cells in the GALT, gut inflammation, GI symptoms, and microbial translocation are similar events experienced in both HIV-uninfected IBD/IBS and in those with HIV disease; GI symptoms may be an indication of possible underlying HIV immune-mediated inflammation (Kamat, Ancuta, Blumberg, & Gabuzda, 2010; Wilson et al., 2014).

The main purpose of this investigation was to explore if the occurrence of GI symptoms is associated with epithelial barrier dysfunction and if symptoms would therefore predict elevations in sCD14 among a cohort of people living with HIV classified as United States Veterans. As a secondary outcome, we sought to understand what HIV-associated variables were related with the outcome of GI symptoms.

Methods

Study Participants. The Veterans Aging Cohort Study (VACS) is an ongoing prospective observational cohort and represents over 120,000 Veterans with and without HIV infection in care at 9 Veterans Administration (VA) medical centers across the

United States. The broad objective of the VACS is to examine the relationship between HIV disease and aging, comparing HIV disease progression, socioeconomic indicators, patient health behaviors, patient outcomes, and the patient-provider relationship between racially diverse, aging HIV-infected and non-infected adult Veterans. Furthermore, the purpose of the VACS is to understand the role of comorbid medical and psychiatric disease in determining clinical outcomes in HIV infection. The VA has a national, fully electronic medical-record system that includes all routine clinical, administrative, and comprehensive longitudinal data. HIV-infected participants recruited from VA infectious disease clinics are matched by age, race/ethnicity, and sex with patients seen at the general medicine clinic at each site (Justice et al., 2006). Participants self-complete the HIV Symptom Index, a validated 20-item questionnaire (Justice et al., 2001). Surveys are managed using Teleform^{TM} v7.0 ICR Technology, a software package for automated form entry that designs, scans, verifies, and enters data. Blood samples were analyzed for inflammatory markers (e.g., IL-6, D-dimer, and sCD14) collected using serum separator and ethylenediaminetetraacetic acid blood collection vials and shipped to the central repository at the Massachusetts Veterans Epidemiology Research and Information Center in Boston, Massachusetts. Demographic and medical information were also collected (Justice et al., 2006). Data were extracted on study participants from the biomarker cohort of 1525 HIV-infected Veterans enrolled between the years 2005 and 2008.

For this study, we used the following inclusion criteria of Veterans enrolled in the VACS Biomarker Cohort: 1) completed the HIV Symptom Index and 2) given a blood sample with sCD14, IL-6, and D-dimer results.

Symptoms Measurement: The HIV Symptom Index

GI symptoms (bloating/abdominal pain, nausea/vomiting, diarrhea, and poor appetite) in the VACS were self-reported by participants using the HIV Symptom Index, a validated 20-item scale to measure patient reported symptoms associated with HIV-disease (Justice et al., 2001). Symptoms were reported based on frequency and bothersomeness with a recall period of four weeks (Bozzette, Hays, Berry, & Kanouse, 1994; Cleary et al., 1993; Justice et al., 2001; Lenderking, Testa, Katzenstein, & Hammer, 1997; Whalen, Antani, Carey, & Landefeld, 1994) . The HIV Symptom Index is a standard instrument available for use in clinical trials and clinical care for the identification of bothersome symptoms common in HIV; it is comprehensive and comprehensible to patients to complete (Justice et al., 2001) .

Symptom Prevalence. Symptoms were considered *present* if the participant responds in the past 4 weeks, "I have this symptom and it doesn't bother me."(1), "bothers me a little" (2), "it bothers me" (3), and "it bothers me a lot" (4). Symptoms were considered *absent* if it was reported as "I don't have this symptom" (0). Participants who reported their symptoms "bothers me a little" (2), "it bothers me" (3), or "it bothers me a lot" (4) were recorded as having symptom distress. Although we considered a symptom present, we did not consider responses of "I have this symptom and it doesn't bother me" (1), as contributing to symptom distress.

Biological Markers

Biological markers were analyzed as continuous and ordinal independent variables. Categorical cut-offs for lab results were consistent with previous VACS cutoffs (Justice et al., 2012).

Soluble CD14. Microbial translocation was measured by levels of sCD14 with a detectable range of 40-3,200 ng/mL (Brenchley et al., 2006). Increased sCD14 indicates the presence of gram-negative bacteria in the blood. sCD14 has been shown to determine the degree of severity in epithelial barrier dysfunction and is a surrogate marker for detection of microbial translocation (Estes et al., 2010). Quantikine sCD14 immunoassay was used to measure sCD14 (R & D Systems, Inc., Minneapolis, MN).

IL-6 is a classic marker of inflammation. Circulating bacterial products stimulate the release of IL-6 (Stehle et al., 2012). IL-6 serum levels measurements were at the last known at time of symptom self-report using the HIV Symptom Index and had a detectable range of 0.4-10,000 pg/mL. IL-6 was measured as a continuous variable and stratified based on previous standardized quartile levels consistent with previous VACS studies.

D-dimer is a fibrin degradation product and marker of coagulation. D-dimer reflects thrombotic activity measured by the STAR automated coagulation analyzer (Diagnositca Stago, Parsippany, New Jersey) using an immune-turbidometric assay (Liatest D-DI). The detectable range was 0.01-20 µg/mL.

Covariates

We examined differences in GI symptoms for age, gender, race/ethnicity, reported annual income, years of education, body mass index, and ART information. Cocaine, Intravenous (IV) drug use, alcohol use, and smoking use were determined by self-report screening. IV drug use was evaluated separately due to the large amount of missing data greater than 5%.

We calculated odds ratios for GI symptoms using the lowest or the most normative and clinically significant group as the reference category. For instance, for CD4 count variable, we used the normative group as in CD4 count > 500 cells/mm^3 and for HIV-1 viral load, <500 copies was used as the reference category; for other variables we used the group with the smallest effect size (i.e. education). Adherence data was not available and therefore, HIV-1 viral load was used as a proxy for adherence with HIV-1 viral load <500 copies/mL as the reference assuming that adherence to cART would achieve an HIV-1 viral load of less than 500 copies/mL.

Other measures. Alcohol consumption was measured using the Alcohol Use Disorders Identification Test (AUDIT-C) is a brief 3-item alcohol screening tool scored on a 0-12 scale; hazardous alcohol use and active alcohol use disorders were determined using the scoring criteria of the AUDIT-C of >4 for men and >3 for women (Dawson, Grant, Stinson, & Zhou, 2005). Fibrosis was measured using the FIB-4 scoring system using a formula of platelet count, age, and liver function tests (AST and ALT) to predict severity of liver disease (Sterling et al., 2006). The FIB-4 screening identifies mild fibrosis with a FIB-4 score <1.39 and significant fibrosis with a FIB-4 score of > 2.05 (Trang, Petersen, & Snyder, 2008). CD4 lymphocyte counts, HIV-1 log10 RNA, and hepatitis C (HCV) antibody status were extracted from laboratory files. IV drug use was self-reported as yes/no but there was no data on what drug was injected. Other illicit drugs (i.e., marijuana, cocaine, methamphetamine) were self-reported as yes/no. Separated cART components were recorded as current medication regimens. However, adherence to components and historical information was not available. All variables were extracted from the VACS database, which draws from the VA electronic health record.

Detailed information is published in the *Veterans Aging Cohort Study: Overview and description* (Justice et al., 2006).

Statistical Analysis

We compared the probability of having GI symptoms as odds ratios (OR). We assessed the statistical significance of the effect of each of the symptoms using Type 3 analysis of effect and chi-squared tests for categorical variables, Fisher's exact test for dichotomous variables, and t-tests for continuous variables. All tests with the exception of comparisons with viral load categories were non-parametric. All tests were two-tailed with a selection restricting the alpha level as models were built. We standardized continuous predictors using the standard deviation. Biomarkers were analyzed as a continuous variable and stratified based on previous standardized quartile levels consistent with previous VACS studies. Clinically significant covariates of GI symptoms were fit in a full logistic regression model. Using a backward elimination process manually, final reduced models were selected with minimization of the Akaike Information Criteria (AIC) and are presented along with univariate association. We used logistic regression to determine the best fit model of predicting GI symptoms using minimization of the AIC (Hosmer et al., 2013). Type 3 tests were used to determine categorical elimination with the alpha set at 0.157 (Hosmer, Lemeshow, & Sturdivant, 2013; Weisberg, 2013). We did not correct for multiple comparisons because there was frequent covariation between biomarkers. Missing data were examined for systematic causes and deleted listwise; otherwise, we imputed the missing value for symptom report only (Altman & Bland, 2007). Power calculations were based on 2-sided testing at the 95% confidence level, using nQuery Advisor version 7.0 software. We required a sample

size of n = 937 to detect a small effect size of d = .2 in a continuous outcome (t-test), assuming 30% (n = 280) had a symptoms versus 70% that did not have the symptom. With a sample size of n = 937, the detectable OR was 1.50, assuming the proportion of events of a binary outcome was 0.5 among those without the symptom.

Results

Patient Study Cohort

Our final sample included a total of 1,418 HIV+ patients 25 to 75 years of age, with a median age of 51.8 years (IQR 25, 75: 46.6, 57.2). In the sample, 1,378 (97%) were male and 40 (3%) were female. The median CD4 T-cell count was 390 cells/mm^3 (IQR: 244, 582), CD8 T-cell count was 874 cells/μL (IQR: 591, 1,228), and HIV-RNA was 75 copies/mL (IQR: 50, 1,870). Among the sample, 978 (69%) participants were African American, 275 (19%) were Caucasian, and 118 (8%) were Hispanic. Eighty-three percent of participants were on a protease inhibitor-based ART regimen for a mean of 1,811 days. Less than 1% used integrase inhibitors.

Proportions of patients experiencing bloating/abdominal pain, nausea/vomiting, diarrhea, and loss of appetite are presented in Figure 1. Of those participants experiencing symptoms, 43% had 1 symptom, 26% had 2 symptoms, 18% had 3 symptoms, and 13% had all 4 GI symptoms. Because of the low number of females (*n* = 70, 3%) in the study, we did not examine differences in biological sex assignment among our variables; however, we included them in the analysis because there were no statistical differences between symptoms with males and females based on presence of symptoms. Demographic information for the study population is presented in Table 1.

Table 1. Patient demographic and clinical characteristics of HIV patients included the study (N = 1,418)

Age (years)	
Mean (range)	52 (25-77)
Gender	**N (%)**
Male	1,378 (97%)
Female	40 (3%)
Race/ethnicity	
African American	978 (69%)
Caucasian	275 (19%)
Hispanic	118 (8%)
Other	47 (4%)
Body Mass Index kg/m^2	
<19 underweight	57 (4%)
19-29 normal/overweight	1,133 (80%)
>30 obese	221 (16%)
Plasma HIV RNA in copies/mL	
<500	978 (69%)
≥500	440(31%)
CD4 Count in cells/mm^3	
<50	62 (4%)
50-199	219 (16%)
200-499	652 (46%)
≥500	482 (34%)
Substance Use*	
Hazardous Alcohol	1,093 (78%)
IV Drug Use (past 12 months)	51 (8%)
Current Smoker	700 (49%)
Past Smoker	373 (26%)
Cocaine use	289 (20%)
GI Symptoms	
Bloating/Abdominal Pain	595 (42%)
Nausea/Vomiting	338 (24%)
Diarrhea	654 (46%)
Poor Appetite	522 (37%)
Mean Burden (IQR)	2 (1, 3)
sCD14 ng/mL	
<2010	997 (70%)

2010 -< 2310	179 (13%)
2310 - < 2710	131 (9%)
≥ 2710	111 (8%)
IL-6 pg/mL	
<1.6	478 (34%)
1.6 - <2.4	346 (24%)
2.4 - <3.9	306 (22%)
≥3.9	288 (20%)
D-dimer µg/mL	
≤0.18	405 (29%)
0.18 - <0.34	443 (31%)
0.34 - <0.64	332 (23%)
≥0.64	238 (17%)
Other Characteristics	
Hepatitis C co-infection	658 (46%)
Irritable Bowel Syndrome Diagnosis	2 (.1%)
Inflammatory Bowel Disease Diagnosis	85 (6%)
Peptic Ulcer Disease Diagnosis	25 (2%)

Percentile cutoffs for sCD14, IL-6, and D-dimer, are consistent with VACS

*based on reporting numbers

sCD14 = soluble CD14, IL-6 = Interleukin-6, VACS = Veterans Aging Cohort Study, IV = Intravenous, IQR = Interquartile Range

Symptom Prevalence and Distress

The most prevalent GI symptom was diarrhea (46%), followed by bloating/abdominal pain (42%), poor appetite (37%), and nausea/vomiting (24%). Sixty-nine percent of persons experiencing bloating/abdominal pain, 67% experiencing diarrhea, 65% of those experiencing poor appetite, and 58% of people with nausea/vomiting reported their GI symptom as bothersome.

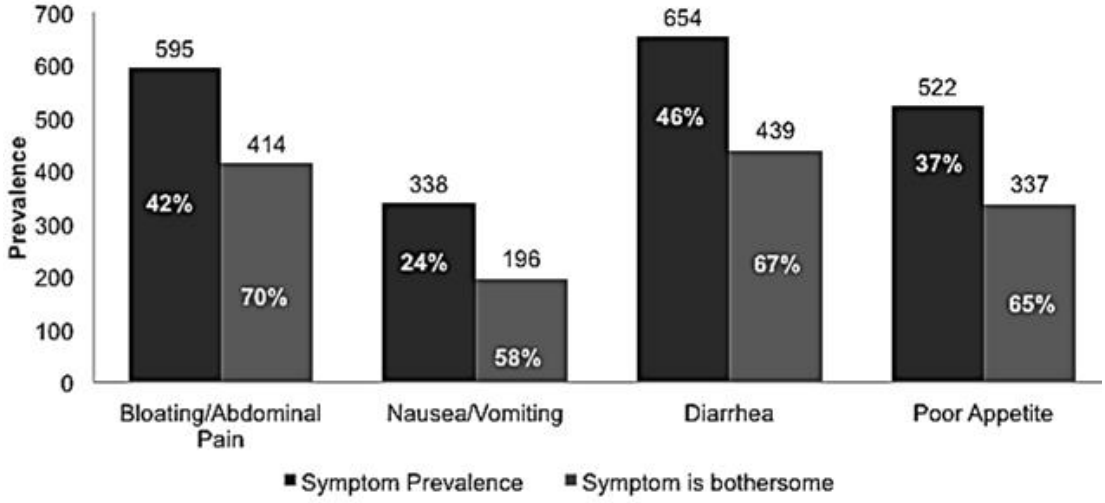

Figure 1: Prevalence of symptoms reported as bothersome among those reporting GI symptoms.

Covariates of GI Symptoms

Crude and adjusted model results are presented in Tables 2-5. Because GI symptoms were so prevalent, we modeled each GI symptom as an outcome variable to examine which independent variables contributed to the development of that symptom. IV drug use seemed to be a strong confounder of GI symptoms based on univariate analysis so we fit the model without IV drug use and then adjusted the model for this variable. There were 629 responses to *IV Drug Use* which was a missing response rate of 56%. Therefore, we removed IV drug use from the full reduced multivariate models and then adjusted for IV drug use, which limited the sample used to n = 605. Of the 51 participants reporting IV drug use 61% reported bloating/abdominal pain, 39% nausea, 55% diarrhea, and 63% poor appetite.

Bloating/abdominal pain was best predicted by being Caucasian, engaging in hazardous alcohol use, HCV, a CD4 count less than 500 cells/mm^3, and levels of IL-6 between 2.4-3.9 pg/mL. When adjusting for IV drug use, bloating/abdominal pain was

predicted by binge drinking (OR 95% CI: 1.39 [.98-1.96]), HCV/HIV co-infection (OR 95% CI: 1.35 [.94-1.94]), and a detectable HIV-1 viral load above 500 copies/mL (OR 95% CI: 1.31 [.91-1.83]). Persons identifying as using IV drugs were 2 times more likely to report bloating/abdominal pain (OR 95% CI: 2.10 [1.15-3.81]). The effect size was small but there was a significant difference between the full model r^2 = .026, and the reduced model (r^2 = .017) and between the reduced model and the IV drug use model (r^2 = .028) as seen in Table 2.

As presented in Table 3, poor appetite was best predicted by older age, hazardous alcohol use, having HCV, and a CD4 count of less than 500 cells/mm^3, a HIV-1 viral load > 500 copies/mL, and levels of IL-6 between 2.4-3.9 pg/mL. Being African-American or Hispanic, and smoking were associated with a decrease in report of poor appetite. Using IV drugs was associated with 77% greater odds of reporting poor appetite than those not reporting a poor appetite. IV drug use (OR 95% CI: 1.77 [.92-3.41]), HCV (OR 95% CI: 1.39 [.99-1.95]), hazardous alcohol use (OR 95% CI: 1.37 [.95-1.98]), and levels of IL-6 between 2.4 and <3.9 pg/mL (OR 95% CI: 1.99[1.26-3.14]) were associated with poor appetite. Viral load > 500 copies/mL was associated with poor appetite (OR 95% CI: 1.50 [1.05-2.14]). The effect size was small but there was a significant difference between the full model (r^2 = .061), and the reduced model (r^2 = .052) and between the reduced model and the IV drug use model (r^2 = .039.).

As seen in Table 4, nausea/vomiting was best predicted by weight measured by a higher body mass index, those with higher IL-6 levels and D-dimer, and CD4 counts below 500 cells/mm^3. The odds of having nausea/vomiting was 1.6-fold higher in those that were obese than those underweight. IL-6 levels in the 2.4-3.9 pg/mL were 2 times

more likely than those with low levels to have nausea/vomiting. Being older and non-Caucasian was associated with a decrease in nausea/vomiting. When adjusted for IV drug use, there was no change in age as a predictor; however, race, BMI, D-dimer, and CD4 count dropped from the model. HCV (OR 95% CI: 1.48 [.99-2.22]), fibrosis score (OR 95% CI: 1.29 [1.00-1.65]), and engaging in hazardous alcohol use (OR 95% CI: 1.48 [.99-2.22]) were predictive of nausea/vomiting, and IL-6 increased the likelihood of nausea/vomiting up to 2-fold.

Diarrhea was best predicted by being Caucasian, engaging in hazardous alcohol use, and CD4 counts less than 500 cells/ mm^3. Those with CD4 count <50 cells/ mm^3 were 2-fold more likely to report diarrhea than those with a CD4 count >500 cells/mm^3. Smokers were 25% less likely to report diarrhea. When the model for diarrhea was adjusted for IV drug use, persons with obesity were 42% less likely to have diarrhea than those not obese. Diarrhea was also associated with binge drinking (OR 95% CI: 1.35 [.96-1.89]) and increased IL-6 levels >1.6 pg/mL. The effect size was small but there was a significant difference between the full model r^2 = .034, and the reduced model (r^2 = .022) and between the reduced model and the IV drug use model (r^2 = .019).

Discussion

The aim of this study was to explore GI symptomatic predictors of inflammatory biomarkers, specifically sCD14, IL-6, and D-dimer among a cohort of PLWH classified as United States Veterans. We sought to explore whether the dysfunction of the epithelial barrier could be detected by symptom and/or immune response (sCD14) to circulating translocated microbial products from the gut or the additional correlated biomarkers of poor outcomes in HIV disease (IL-6, D-dimer). The results did not support a clinically

significant association of inflammatory biomarkers sCD14, IL-6, and D-dimer with GI symptoms as an outcome in adjusted final models.

Higher levels of sCD14 and IL-6 predicted a greater likelihood of loss of appetite than those with normal levels of sCD14 and IL-6. The GI symptom most predictive of epithelial barrier dysfunction measured by sCD14 was a loss of appetite. Persons experiencing loss of appetite in association with elevated sCD14 may have microbial translocation and chronic inflammation from immune activation. This finding is consistent with previous research that demonstrated loss of appetite may be related to the secretion of a pro-inflammatory cytokine, high mobility group box protein-1 (HMGB1) from apoptotic HIV-1 infected cells. This protein in combination with microbial products has a role in promoting immune activation in HIV infection including the induction of IL-6 (Agnello, Wang, Yang, Tracey, & Ghezzi, 2002; Troseid, Sonnerborg, & Nowak, 2011). There is some evidence to suggest that microbial products provoke pro-inflammatory mechanisms which lead to loss of appetite (Yue et al., 2015). Further research is needed to examine the relationship among these variables. Symptoms should warrant further diagnostic investigation in clinical practice. Therefore, symptoms are more likely to be used to initiate further diagnostic investigation into systemic inflammation and immune activation. Prior to having effective evidence-based strategies to reducing inflammation and immune activation, lifestyle changes can be utilized. Strategies such as exercise and improving sleep have demonstrated effectiveness in decreasing C-reactive protein, a non-specific marker of inflammation, and IL-6 in PLWH (Cutrono et al., 2015; Wirth et al., 2015)

Poor appetite had a non-linear association with sCD14. HCV increased report of poor appetite by 64%. This finding is consistent with studies demonstrating elevated evidence of microbial translocation HIV/HCV co-infection due to the increased intestinal permeability resulting from liver disease. Fibrosis stage may be dependent on microbial translocation induced immune activation (Sacchi et al., 2015). The findings of other studies report that fibrosis is actually protected by elevated levels of sCD14 by down-regulation of inflammation and that it is TNF-α that actually contributes to the progression of liver disease in the course of HCV (Marchetti et al., 2014). Targeting HCV treatment in people living with HIV/HCV co-infection may decrease levels of microbial translocation and immune activation with sustained virological response to chronic HCV antiviral treatment in ART treated individuals (Nystrom, Stenkvist, Haggblom, Weiland, & Nowak, 2015).

Persons using IV drugs were twice as likely to have a poor appetite. While information was not available on the specific drugs used IV from the data provided in this study, opiate-based substances such as heroin, narcotics, OxyContin, pain relievers, and cocaine are commonly used drugs for injection. Prescription opioids were associated as an antecedent to almost 80% of those people who initiate using heroin (Muhuri, Gfroerer, Davies, & Substance Abuse and Mental Health Services Administration, 2013).

This finding does coincide and bring attention to the effect of opioid compounds on the dysfunction of the epithelial barrier, alteration of the gut microbiome, and subsequent microbial translocation and chronic inflammation (Banerjee et al., 2016). This represents an important area of research because the chronic use of opioids, whether

illicit IV or prescribed, may lead to progression of HIV disease (Meng, Sindberg, & Roy, 2015). Yet, it is noted clinicians must address pain and substance abuse as an illness.

Limitations of this study were that we did not have data concerning adherence to medications outside of viral load suppression. We did not have information on depression or surrogates for depression. Blood samples were collected between 2005 and 2007 limiting the participants to the context of the ART prescribed during those time periods. There have certainly been significantly improved agents released on the market. The nature of this study was a retrospective exploration supported by a hypothesized framework presented in Wilson et al. (2014). Based on this current study, there are highlighted variables associated with key predictors of HIV disease progression sCD14 and IL-6. Furthermore, poor appetite is a symptom worthy of further investigation as many other contributions could be associated with this symptom.

Conclusion

A major finding was that, loss of appetite was an independent predictor of high levels of plasma sCD14; however, other GI symptoms did not predict epithelial barrier dysfunction measured by sCD14. This result could mean that identifying those with poor appetite may help identify those patients at risk for worse health outcomes or progression of HIV disease. Viral load was not associated with GI symptoms with statistical significance. Loss of appetite was also more likely to occur among Veterans with HCV, fibrosis, and IV drug use. Such findings are not surprising and support the importance of treating HCV early and addressing substance abuse in this population. Since higher levels of IL-6 were associated with diarrhea, nausea/vomiting, and poor appetite, this supports the combination of treatment strategies to reduce inflammation, and heal and repair the

GI epithelial lining in addition to anti-viral treatment for HIV and HCV. Research is needed to develop and test safe and effective interventions that manage such symptoms in this population. Clinicians can also use GI symptoms to trigger an assessment for liver disease and hazardous drug use. Furthermore, poor appetite may be an indication of microbial translocation and alert clinicians to take steps to protect the GI epithelial barrier with dietary changes and supplementation with probiotics. GI symptoms as well as inflammation of the epithelial barrier may be restored with the use of probiotics. Probiotics have demonstrated extensive benefits including reduction of microbial translocation by promoting healing of the GI epithelial barrier and stimulation of the regulatory effects of the immune system (d'Ettorre et al., 2015; Wilson, Moneyham, & Alexandrov, 2013).

This study also brings attention to substance abuse. There has been growing concern about use of opioid compounds in the community at risk for and infected with HIV, illegally or by prescription. While there is concern to discontinue clinical use of opioids for use in pain management, there is greater concern that this could move patients toward unmonitored use through IV drugs. Therefore, research into pain management in the context of symptom management and inflammation should also be investigated.

There are implications for inflammation in the early aging, cardiovascular events, and cognitive decline seen in HIV disease being associated with shortening of telomeres, which are caps protecting the ends of chromosomes (Fitzpatrick et al., 2007; Jurk et al., 2014; Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013; Valdes et al., 2010). Systemic chronic inflammation is associated with both the normal process of aging and that associated with disease by inducing reactive oxygen species (ROS), or oxidative

stress, causing shortening and lymphocyte immunosenescence (Jurk et al., 2014). Senescence-associated ROS and IL-6 through the NF-KB pathway reinforce the feedback loop that contributes to senescence (Jurk et al., 2014). Overall, telomere dysfunction and cellular senescence is intensified by chronic inflammation (Jurk et al., 2014). Furthermore, NF-κβ mediated inflammation (IL-6) has the ability to stimulate cellular proliferation including tumor cells, is involved in signaling apoptosis, and impairs tissue regeneration (Jurk et al., 2014). In otherwise healthy populations, leukocyte telomere length has been associated as well in cognitive decline, which normally begins around mid-life and advances with age, and is associated with cognitive performance (Valdes et al., 2010). Oxidative stress and chronic inflammation lead to lymphocyte turnover and increases erosion of telomere caps (Valdes et al., 2010). Further research would be required to test these assumptions; however, identifying inflammation early and developing strategies would hold promise to support improvements in health outcomes in PLWH. There is some evidence that physical activity and improvement in sleep protects telomere damage (Lee et al., 2014; Soares-Miranda et al., 2015). Additionally, in the context of our results in this study, active HCV is associated with shortening of telomeres. HCV was associated with poor appetite (OR, 95% CI: 1.69, 1.34-2.13, $p < .00$) and bloating/abdominal pain (OR, 95% CI: 1.40, 1.2-1.76, $p < .00$) in multivariate models. This further supports early intervention with HCV treatment in the HIV/HCV co-infected population.

The main purpose of this investigation was to explore if the occurrence of GI symptoms is associated with epithelial barrier dysfunction and if symptoms would therefore predict elevations in sCD14 among a cohort of PLWH classified as US

Veterans. We found that epithelial barrier dysfunction was associated with poor appetite, and a trend toward bloating/abdominal pain. As a secondary outcome, we sought to understand what HIV-associated variables were related with the outcome of GI symptoms and identified several clinical factors such a inflammation, specifically IL-6, liver disease, CD4+ count, and IV drug use that contributed to the presence of GI symptoms independent of viral load with the exception of poor appetite. Poor appetite appeared to be a clinical indicator of factors associated with key predictors of poor outcomes in HIV disease (e.g., inflammation and detectable HIV-1 viral load). Rethinking symptom management to include considerations to underlying pathology will enhance the clinical encounter by addressing the symptom burden and the disease process.

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Table 2: Frequency of bloating/abdominal pain with association and effect of confounding measures

	Total N Reporting	**BLOATING/ABDOMINAL PAIN (*n* = 595)**						
Patient Characteristics	1,418	Frequency (%) for Symptom Present or Mean (±SD)	Unadjusted OR [95% CI]	*P* value	Reduced Model (*n*=1376) OR [95% CI]	*P* value	Adjusted Model With IV Drug Use (*n* = 605)	*P* value
Age in years	1,418	51.7 (±7.9)	1.00* [.98-1.01]	*.72*				
Gender	1,418			*79*				
Female		16 (40%)	1.00					
Male		579 (42%)	1.08 [.57-2.06]					
Body Mass Index (kg/m^2)	1,411			*.55*				
<19, underweight		20 (35%)	1.00					
19-29, normal/overweight		476 (42%)	1.34 [.77-2.34]					
>30, obese		95 (43%)	1.40 [.76-2.56]					
Race/Ethnicity	1,418			.06		.02		
Caucasian		126 (45%)	1.00		1.00			
African American		388 (40%)	.77 [.59-1.02]		.69 [.52-.91]			
Hispanic		57(48%)	1.11 [.72-1.70]		.98 [.63-1.52]			
Other		24 (51%)	1.23 [.66-2.29]		1.13 [.601-2.11]			
Education	1,407			.96				
Less than 8th grade		8 (50%)	1.52 [.52-4.43]					
Some high school		33(40%)	1.00					
HS graduate(GED)		195 (42%)	1.07 [.66-1.73]					
Some college		272 (43%)	1.14					

			[.72-1.83]					
College graduate		53 (40%)	1.03 [.59-1.80]					
Post graduate		43 (43%)	1.13 [.60-2.12]					
Intravenous Drug Use	629			*.00*				
Yes		31 (61%)	2.31 [1.28-4.15]				2.10 [1.15-3.81]	.02
No		232 (40%)	1.00					
Alcohol Use Indicators								
Hazardous Alcohol Use (AUDIT-C)	1,408			*.03*		.06		
Yes		150 (48%)	1.33 [1.04-1.72]		1.29 [.99-1.67]			
No		443 (41%)	1.00		1.00			
Binge Drinking	1,400			*.17*			1.39 [.98-1.96]	
Yes		356 (44%)	1.16 [.94-1.44]					
No		234 (40%)	1.00					
No drinking past 12 months	1,405			*.16*				
Yes		234 (40%)	.86 [.69-1.06]					
No		359 (44%)	1.00					
Tobacco Use	1,418							
Current	700			*.41*				
Yes		305 (44%)	1.12 [.86-1.45]					
No (non-smoker)		141 (41%)	1.00					
Past	373			*.80*				
Yes		149 (40%)	.96 [.71-1.29]					
No (non-smoker)		446 (41%)	1.00					
Hepatitis C diagnosis	1,418			*.00*		.00		.11
Yes		306 (47%)	1.42 [1.15-1.75]		1.40 [1.2-1.76]		1.35 [.94-1.94]	

No		289 (38%)	1.00		1.00			
FIB-4 Score	1,408	2.0 (±2.2)	1.17 * [1.04-1.33]	*.01*			1.43 [1.09-1.86]	.01
Biomarkers:								
CD4 count (cells/mm^3)	1,415			*.01*		.05		
≤50		27 (44%)	1.39 [.81-2.37]		1.67 [.95-2.94]			
50-199		102 (47%)	1.57 [1.13-2.17]		1.45 [1.03-2.05]			
200-499		292(45%)	1.46 [1.15-1.86]]		1.36 [1.05-1.75]			
≥500		172 (36%)	1.00		1.00			
HIV-1 Viral Load (copies/mL)	1,415			*.17*				.12
<500		398 (41%)	1.00				1.00	
≥500		197(45%)	1.17 [.94-1.47]				1.31 [.91-1.83]	
Soluble CD14 (ng/mL)	1,418			*.19*				
<2010		410(41%)	1.00					
2010 – < 2310		79 (44%)	1.13 [.82-1.55]					
2310 – < 2710		50 (38%)	.88 [.61-1.29]					
≥ 2710		56 (50%)	1.46 [.98-2.16]					
IL-6 (pg/mL)	1,414			*.01*		.07		
<1.6		165 (32%)	1.00		1.00			
1.6 - <2.4		184 (23%)	.93 [.69-1.23]		.84 [.63-1.13]			
2.4 - <3.9		143 (25%)	1.54 [1.15-2.06]		1.28 [.95-1.73]			
≥3.9		103 (21%)	1.17 [.87-1.57]		.95 [.69-1.30]			
D-dimer (µg/mL)	1,416			*.89*				

≤0.18	165 (27%)	1.00
0.18 - <0.34	184 (31%)	1.03 [.79-1.35]
0.34 - <0.64	143 (24%)	1.10 [.82-1.48]
≥0.64	103 (18%)	1.11 [.80-1.53]

Notes:
*OR for a standard deviation increase.

Value presented in the 2nd column is for bloating presence within each row category. Therefore, any percentages are of those reporting the presence of bloating/abdominal pain within the last 4 weeks.

First reduced model fitted without IV drug use due to low numbers of people using IV drugs.

P values for Categorical Variables are reflective of the result for the Type 3 or Wald test.

Reduced model built using backward selection and minimizing the Akaike Information criterion.

Pseudo R^2 (Full Model) = .026
Pseudo R^2 (Reduced Model)= .017
Pseudo R^2 (IVD Model) = .028

Table 3: Frequency of poor appetite with association and effect of confounding measures

	Total N Reporting	POOR APPETITE (***n*** **= 690)**						
Patient Characteristics	1,418	Frequency (%) for Symptom Present or Mean (±SD)	Unadjusted OR [95% CI]	*P* value	Reduced Model (*n*=1376) OR [95% CI]	*P* value	Adjusted Model With IV Drug Use (*n* = 605)	*P* value
Age in years	1,418	51.6 (± 7.9)	.93 [.84-1.04]	.20	.90 [.80-1.01]	.08		
Gender	1,418			.76				
Female		21 (53%)	1.00					
Male		669 (50%)	.91 [.59-2.06]					
Body Mass Index (kg/m^2)	1,411			.13				
<19, underweight		35 (64%)	1.00					
19-29, normal/overweight		549 (50%)	.56 [.32-.98]					
>30, obese		106 (50%)	.57 [.31-1.04]					
Race/Ethnicity	1,418			.36		.01		
Caucasian		146 (54%)	1.00		1.00			
African American		460 (49%)	.82 [.62-1.07]		.61 [.45-.82]			
Hispanic		57 (48%)	.81 [.53-1.26]		.65 [.41-1.03]			
Other		27 (57%)	1.16 [.62-2.16]		1.00 [.53-1.92]			
Education	1,418			.14				
Less than 8th grade		9 (60%)	1.23 [.40-3.78]					
Some high school		45 (55%)	1.00					
HS graduate(GED)		244 (54%)	.95 [.59-1.52]					
Some college		306 (49%)	.80					

			[.50-1.27]					
College graduate		55 (43%)	.62					
			[.35-1.08]					
Post graduate		31 (42%)	.59					
			[.31-1.12]					
Intravenous Drug Use	629			.02				.09
Yes		35 (70%)	2.06				1.77	
			[1.10-3.85]				[.92-3.41]	
No		295 (53%)	1.00					
Alcohol Use Indicators	1,408							
Hazardous Alcohol Use (AUDIT-C)				.00				.09
Yes		178 (58%)	1.49		1.34		1.37	
			[1.15-1.92]		[1.03-1.76]		[.95-1.98]	
No		512 (48%)			1.00			
Binge Drinking				.08				
Yes		418 (52%)	1.21					
			[.98-1.50]					
No		272 (47%)	1.00					
No drinking past 12 months				.08				
Yes		272 (47%)	.83					
			[.67-1.03]					
No		418 (52%)	1.00					
Tobacco Use	1,418			.01		.08		
Current								
Yes		364 (54%)	1.20		.93			
			[.92-1.55]		[.70-1.24]			
No (non-smoker)		164 (49%)	1.00		1.00			
Past								
Yes		162 (44%)	.81		.71			
			[.60-1.08]		[.52-.98]			
No (non-smoker)		164 (49%)	1.00		1.00			
Hepatitis C diagnosis	1,418			.00		.00		.06
Yes		363 (57%)	1.63		1.69		1.39	
			[1.32-2.02]		[1.34-2.13]		[.99-1.95]	
No		327 (44%)	1.00		1.00			

FIB-4 Score	1,408	1.94 (±1.76)	1.28 [1.11-1.49]	.00	1.11 [.96-1.30]	.17		
Biomarkers:								
CD4 count (cells/mm^3)	1,418			.00		.01		
≤50		38 (66%)	2.84 [1.61-5.04]		1.98 [1.05-3.71]			
50-199		117 (55%)	1.86 [1.34-2.59]		1.35 [.94-1.94]			
200-499		348 (54%)	1.78 [1.40-2.27]		1.50 [1.17-1.94]			
≥500		187 (40%)	1.00		1.00			
HIV-1 Viral Load (copies/mL)	1,418			.00		.04		.02
<500		444 (47%)	1.00		1.00		1.00	
≥500		246 (58%)	1.61 [1.27-2.02]		1.31 [1.01-1.71]		1.50 [1.05-2.14]	
Soluble CD14 (ng/mL)	1,418			.01				
<2010		459 (47%)	1.00					
2010 – < 2310		93 (53%)	1.26 [.91-1.74]					
2310 – < 2710		71 (55%)	1.38 [.95-2.00]					
≥ 2710		67 (64%)	1.96 [1.29-2.97]					
IL-6 (pg/mL)	1,414			.00		.00		.02
<1.6		195 (42%)	1.00		1.00		1.00	
1.6 - <2.4		153 (45%)	1.13 [.85-1.50]		1.04 [.77-1.39]		1.23 [.79-1.93]	
2.4 - <3.9		181 (60%)	2.05 [1.53-2.76]		1.73 [1.27-2.37]		1.99 [1.26-3.14]	
≥3.9		161 (58%)	1.87 [1.38-2.53]		1.48 [1.06-2.06]		1.67 [1.05-2.64]	
D-dimer (µg/mL)	1,416			.01				
≤0.18		172 (43%)	1.00					

0.18 - <0.34	221 (51%)	1.37 [1.04-1.80]
0.34 - <0.64	168 (53%)	1.44 [1.08-1.95]
≥0.64	129 (56%)	1.66 [1.20-2.31]

Notes:
*OR for a standard deviation increase.

Value presented in the 2nd column is for poor appetite presence within each row category. Therefore, any percentages are of those reporting the presence of poor appetite within the last 4 weeks.

First reduced model fitted without IV drug use due to low numbers of people using IV drugs.

P values for Categorical Variables are reflective of the result for the Type 3 or Wald test.

Reduced model built using backward selection and minimizing the Akaike Information criterion.

Pseudo R^2 (Full Model) =.061
Pseudo R^2 (Reduced Model) =.052
Pseudo R^2 (IVD Model) = .039

Table 4: Frequency of nausea/vomiting with association and effect of confounding measures

	Total N Reporting	NAUSEA/VOMITING (*n* =338)						
Patient Characteristics	1,418	Frequency (%) for Symptom Present or Mean (±SD)	Unadjusted OR [95% CI]	*P* value	Reduced Model (*n*=1376) OR [95% CI]	*P* value	Adjusted Model With IV Drug Use (*n* = 605)	*P* value
Age in years	1,418	50.7 (±7.34)	.83 [.74-94]	.00	.76 [.66-.87]		.75 [.61-.93]	.01
Gender	1,418			.86				
Female		10 (25%)	1.00					
Male		328 (24%)	.94 [.45-1.94]					
Body Mass Index (kg/m^2)	1,411	26.2 (±4.9)		.20		.06		
<19, underweight		14 (25%)	1.00		1.00			
19-29, normal/overweight		259 (23%)	.91 [.49-1.69]		1.06 [.55-2.06]			
>30, obese		63 (29%)	1.22 [.63-2.39]		1.61 [.79-3.31]			
Race/Ethnicity	1,418			.63		.01		
Caucasian		73 (27%)	1.00		1.00			
African American		224 (23%)	.82 [.61-1.11]		.55 [.39-.77]			
Hispanic		30 (25%)	.94 [.58-1.54]		.66 [.39-1.12]			
Other		11 (23%)	.85 [.41-1.75]		.62 [.29-1.32]			
Education	1,418			.17				
Less than 8th grade		7 (44%)	3.53 [1.13-10.97]					
Some high school		15 (18%)	1.00					
HS graduate(GED)		116 (25%)	1.52 [.85-2.74]					
Some college		159 (25%)	1.52					

			[.72-2.07]			
College graduate		24 (18%)	1.02 [.50-1.80]			
Post graduate		17 (23%)	1.33 [.61-2.89]			
Intravenous Drug Use	629			.05		.24
Yes		20 (39%)	1.86 [1.03 -3.36]		1.47 [.78-2.77]	
No		149 (25%)	1.00		1.00	
Alcohol Use Indicators	1,408					
Hazardous Alcohol Use (AUDIT-C)				.00		
Yes		99 (31%)	1.65 [1.25-2.17]		1.40 [.95-2.08]	.09
No		238 (22%)	1.00		1.00	
Binge Drinking				.28		
Yes		203 (25%)	1.15 [.89-1.48]			
No		131 (22%)	1.00			
No drinking past 12 months				.37		
Yes		139 (23%)	.89 [.70-1.14]			
No		199 (25%)	1.00			
Tobacco Use	1,418			.01		
Current						
Yes		189 (27%)	1.29 [.95-1.74]			
No (non-smoker)		77 (22%)	1.00			
Past						
Yes		72 (19%)	.83			
No (non-smoker)		77 (22%)	1.00			
Hepatitis C diagnosis	1,418			.00		.06
Yes		196 (30%)	1.85 [1.44-2.36]		1.48 [.99-2.22]	
No		142 (19%)	1.00			
FIB-4 Score	1,408	2.15 (±2.68)	1.24 [1.09-1.40]	.00	1.29 [1.00-1.65]	.05

Biomarkers:								
CD4 count (cells/mm^3)	1,418			.00		.01		
≤50		19(31%)	2.16 [1.19-3.89]		2.03 [1.06-3.87]			
50-199		71 (32%)	2.34 [1.62-3.39]		1.96 [1.31-2.92]			
200-499		165 (25%)	1.65 [1.23-2.22]		1.41 [1.03-1.93]			
≥500		82 (17%)	1.00		1.00			
HIV-1 Viral Load (copies/mL)	1,418			.01				
<500		214 (22%)	1.00					
≥500		123 (29%)	1.40 [1.08-1.81]					
Soluble CD14 (ng/mL)	1,418			.42				
<2010		235 (24%)	1.00					
2010 – < 2310		38 (21%)	.87 [.59-1.29]					
2310 – < 2710		32 (24%)	1.04 [.69-1.60]					
≥ 2710		33 (30%)	1.37 [.89-2.11]					
IL-6 (pg/mL)	1,414			.00		.03		.04
<1.6		89 (19%)	1.00		1.00		1.00	
1.6 - <2.4		75 (22%)	1.21 [.86-1.71]		1.03 [.71-1.49]		1.68 [.99-2.85]	
2.4 - <3.9		98 (32%)	2.06 [1.48-2.87]		1.60 [1.11-2.30]		2.01 [1.19-3.40]	
≥3.9		76 (26%)	1.57 [1.11-2.22]		1.03 [.69-1.55]		1.98 [1.15-3.40]	
D-dimer (µg/mL)	1,416			.01		.09		
≤0.18		84 (21%)	1.00		1.00			
0.18 - <0.34		97 (22%)	1.07 [.77-1.49]		1.10 [.77-1.56]			
0.34 - <0.64		82 (25%)	1.25		1.12			

		[.89-1.77]	[.760-1.64]
≥0.64	75 (32%)	1.76	1.67
		[1.22 -2.53]	[1.09-2.55]

Notes:
*OR for a standard deviation increase.

Value presented in the 2nd column is for nausea/vomiting presence within each row category. Therefore, any percentages are of those reporting the presence of nausea/vomiting within the last 4 weeks.

First reduced model fitted without IV drug use due to low numbers of people using IV drugs.

P values for Categorical Variables are reflective of the result for the Type 3 or Wald test.

Reduced model built using backward selection and minimizing the Akaike Information criterion.

Pseudo R^2 (Full Model) =.070
Pseudo R^2 (Reduced Model) = .062
Pseudo R^2 (IVD Model) = .057

Table 5: Frequency of diarrhea with association and effect of confounding measures

	Total N Reporting	DIARRHEA (*n* = 654)						
Patient Characteristics	1,418	Frequency (%) for Symptom Present or Mean (±SD)	Unadjusted OR [95% CI]	*P* value	Reduced Model (*n*=1376) OR [95% CI]	*P* value	Adjusted Model With IV Drug Use (*n* = 605)	*P* value
Age in years	1,418	51.7 (± 8.1)	.96 [.86-1.06]					
Gender	1,418			.41				
Female		21 (53%)	.77 [.41-1.44]					
Male		633 (46%)						
Body Mass Index (kg/m^2)	1,415			.25				.14
<19, underweight		24 (42%)	1.00				1.00	
19-29, normal/overweight		515 (45%)	1.45 [.67-1.96]				1.05 [.46-2.39]	
>30, obese		113 (51%)	1.44 [.80-2.59]				.58 [.23-1.50]	
Race/Ethnicity	1,418			.00		.00		
Caucasian		153 (56%)	1.00		1.00			
African American		428 (44%)	.62 [.47-.81]		.57 [.43-.76]			
Hispanic		48 (41%)	.55 [.35-.85]		.49 [.32-.77]			
Other		25 (53%)	.91 [.49-1.69]		.80 [.42-1.50]			
Education	1,407			.22				
Less than 8th grade		11 (69%)	3.33 [1.06-10.47]					
Some high school		33 (40%)	1.00					
HS graduate(GED)		205 (44%)	1.17 [.73-1.89]					
Some college		306 (48%)	1.42 [.89-2.27]					

College graduate		62 (47%)	1.36 [.78-2.38]					
Post graduate		34 (45%)	1.26 [.67-2.36]					
Intravenous Drug Use	629			.25				.24
Yes		28 (55%)	1.40 [.79-2.49]				1.43 [.79-2.60]	
No		269 (47%)	1.00					
Alcohol Use Indicators	1,408							
Hazardous Alcohol Use (AUDIT-C)				.01				
Yes		166 (53%)	1.40 [1.09-1.80]		1.38 [1.06-1.79]			
No		484 (44%)	1.00		1.00			
Binge Drinking				.05				
Yes		394 (48%)	1.24 [1.00-1.53]				1.35 [.96-1.89]	.08
No		252 (43%)	1.00					
No drinking past 12 months								
Yes		265 (43%)	.81 [.66-1.00]					
No		389 (48%)	1.00					
Tobacco Use	1,418			*.39*		.11		
Current								
Yes		314 (45%)	.84 [.65-1.08]		.75 [.57-.99]			
No (non-smoker)		170 (49%)	1.00		1.00			
Past								
Yes		170 (46%)	.86 [.64-1.16]		.77 [.57-1.05]			
No (non-smoker)		170 (49%)	1.00		1.00			
Hepatitis C diagnosis	1,418			.22				
Yes		315 (48%)	1.14 [.93-1.41]					
No		339 (45%)	1.00					
FIB-4 Score	1,408	1.9 (±2.1)	1.12 [1.00-1.26]	.06				

Biomarkers:							
CD4 count (cells/mm^3)	1,418			.00		.00	
≤50		34 (55%)	1.87 [1.10-3.18]		2.13 [1.22-3.74]		
50-199		113 (52%)	1.64 [1.89-2.26]		1.60 [1.15-2.24]		
200-499		316 (49%)	1.45 [1.14-1.83]		1.43 [1.12-1.83]		
≥500		190 (39%)	1.00				
HIV-1 Viral Load (copies/mL)	1,418						
<500		441 (45%)	1.00				
≥500		213 (48%)	1.15 [.92-1.44]				
Soluble CD14 (ng/mL)	1,418			.34			
<2010		457 (46%)	1.00				
2010 – < 2310		78 (44%)	.91 [.66-1.26]				
2310 – < 2710		59 (45%)	.97 [.67-1.40]				
≥ 2710		60 (54%)	1.39 [.94-2.06]				
IL-6 (pg/mL)	1,414			.04			
<1.6		196 (41%)				1.00	
1.6 - <2.4		165 (48%)	1.31 [.99-1.73]			1.54 [.99-2.41]	
2.4 - <3.9		155 (51%)	1.48 [1.11-1.97]			1.69 [1.09-2.64]	
≥3.9		138 (48%)	1.32 [.99-1.78]			1.38 [.88-2.17]	
D-dimer (µg/mL)	1,416			.90			
≤0.18		186 (46%)					
0.18 - <0.34		202 (46%)	.99 [.75-1.29]				
0.34 - <0.64		151 (46%)	.98				

		[.73-1.31]
≥0.64	115 (48%)	1.10
		[.80-1.52]

Notes:
*OR for a standard deviation increase.

Value presented in the 2nd column is for diarrhea presence within each row category. Therefore, any percentages are of those reporting the presence of diarrhea within the last 4 weeks.

First reduced model fitted without IV drug use due to low numbers of people using IV drugs.

P values for Categorical Variables are reflective of the result for the Type 3 or Wald test.

Reduced model built using backward selection and minimizing the Akaike Information criterion.

Pseudo R2 (Full Model) = .034
Pseudo R^2 (Reduced Model) = .022
Pseudo R^2 (IVD Model) = 0.019

CHAPTER 6: SUMMARIZING DISCUSSION

Symptoms assist providers in detecting underlying abnormalities. Chronic inflammation and immune activation from microbial translocation are key predictors in HIV disease progression. Detecting possible immune activation and inflammation from patient reported symptoms may help clinical providers to make treatment decisions and recommendations that will decrease inflammation, improve symptoms and HIV disease outcomes. Prior to developing strategies that will improve microbial translocation, we must fully understand which patients are likely to experience this problem. Similar gut dysfunction is found in IBD/IBS, diseases which have GI symptoms similar to those associated with HIV; however, the only symptom to be found associated with microbial translocation in our studies was loss of appetite, a symptom not generally associated with IBD/IBS (Berkes, Viswanathan, Savkovic, & Hecht, 2003; McGuckin, Eri, Simms, Florin, & Radford-Smith, 2009).

In the first article presented in Chapter 2 of this thesis, *A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection* (Wilson, Moneyham, & Alexandrov, 2013), we provided a comprehensive review presenting the case for investigating probiotics in PLWH. Since we began this investigation probiotics have been successfully studied in both humans and non-human primates with successful outcomes demonstrating a reduction in inflammatory biomarkers and healing of the epithelial barrier (d'Ettorre et al., 2015; Stiksrud et al., 2015).

We then used the conceptual framework presented in Chapter 3, *Connecting the Dots: Could Microbial Translocation Explain Commonly Reported Symptoms in HIV Disease?* (Wilson et al., 2014), to design studies presented as reprinted and preprinted

manuscripts in Chapter 4, *Identifying Symptoms in HIV Disease*, and Chapters 5, *A Retrospective Analysis of HIV-associated Symptoms and Microbial Translocation in the Veterans Aging Cohort Study*. We sought to investigate if the patterns of symptoms reported in outpatient settings by PLWH were inflammatory related. In our study, two different clusters or patterns of symptoms emerged, including mental and constitutional complaints; all were associated with inflammation in uninfected populations. This work provided a conceptual framework to follow in building the subsequent research trajectory. We first aimed to identify symptoms reported by patients seeking ambulatory HIV care and whether the reported symptoms formed a pattern associated with inflammation. These results were published in *Identifying Symptoms in HIV Disease* (Wilson et al., 2015), and reported an up-to-date prevalence and burden of symptoms, and symptom clusters in PLWH. A total of 15 symptoms were reported within two symptom clusters. Two symptoms (aggregated muscle aches/joint pain and fatigue) presented in both clusters. Eleven symptoms contributed to 41% of the variance in symptoms reported and 10 of the 11 symptoms have been associated with inflammation (Wilson et al., 2014). GI symptoms, bloating, nausea/vomiting, diarrhea, and loss of appetite were included in this cluster.

With these GI symptoms in mind, we sought to investigate the hypothesis that GI symptoms were associated with GI epithelial barrier dysfunction [sCD14]. We used sCD14, a pro-inflammatory cytokine released from monocytes in response to microbial translocation as a marker of epithelial barrier dysfunction; microbial translocation is an end result and indicator of GI barrier dysfunction (Estes et al., 2010a). This final article, *A Retrospective Analysis of HIV-associated Symptoms and Microbial Translocation in*

the Veterans Aging Cohort Study, addressed the association between elevated levels of circulating microbial products. Findings from this study suggested that loss of appetite may be an indication of underlying epithelial barrier dysfunction [sCD14], (OR, 95% CI: 1.70 [1.15 - 2.51, p =.01).

Similarly to investigating monocytic activation to microbial translocation, we also sought to investigate whether elevated levels of IL-6 and D-dimer, evidence of non-specific immune activation, were associated with the presence of symptoms. We found that there is an association with elevations in inflammatory biomarkers sCD14, IL-6, and D-dimer and loss of appetite. Loss of appetite is also associated with high elevations in the upper quartile of IL-6 (OR, 95% CI: 1.58, 1.22 - 2.05, p <.00) and D-dimer (OR, 95% CI: 1.53, 1.15 - 2.02 p < .00). Nausea/vomiting were associated with elevated levels of IL-6 and D-dimer. Bloating was associated with elevated levels of IL-6. LPS induces IL-6 (Agnello, Wang, Yang, Tracey, & Ghezzi, 2002; Lee, Cho, & Kim, 2015).

Since we did not have access to adherence data, we used viral load as a surrogate marker for adherence to cART. PLWH that were not HIV-1 suppressed were 40% more likely than those who were HIV-1 suppressed (<500 copies/mL) to experience nausea/vomiting and 61% more likely than those HIV-1 suppressed (<500 copies/mL) to experience a loss of appetite. Loss of appetite was a clinically significant GI symptom having a moderate association with inflammatory biomarkers and unsuppressed HIV-1 viral load. Loss of appetite may be a likely predictor of poor outcomes in HIV disease as there is an association with elevated inflammatory biomarkers predictive of unsuppressed viral load, morbidity, and non-AIDS related mortality. Changes to sCD14 are closely

associated with IL-6 and D-dimer which are associated with non-AIDS morbidity and mortality in cART treated patients (Nixon & Landay, 2010; Tenorio et al., 2014).

These findings are consistent with studies that have identified high mobility group protein-1 (HMGB-1) and its association with gram-negative bacteria and loss of appetite (Agnello et al., 2002; Troseid, Sonnerborg, & Nowak, 2011; Yang, Wang, Czura, & Tracey, 2005). HMGB-1 is produced by monocytes, the same white blood cell types that release sCD14 in response to LPS (Agnello et al., 2002). Reduction of microbial translocation is a goal to improve and decrease levels of sCD14, D-dimer, and IL-6 to reduce risk of a non-AIDS morbid event (Nixon & Landay, 2010; Tenorio et al., 2014). We discussed in Chapter 5 possible strategies tested for anti-HMGB-1 administration, but this has not been tested in the HIV population for this symptom but may be an effective strategy to reduce microbial translocation effect on monocytes. However, symptoms are alarms to underlying pathology and blocking the trigger is only suppressing the alert and not reducing the effect or outcome. Therefore, appetite may return due to a reduction of monocytic activation, IL-6, but the microbial translocation remains.

Possible Treatment Strategies for Microbial Translocation

Microbial translocation and inflammation is an emerging field gaining global attention. This field of research certainly has gained momentum in the past few years since being described in HIV-infected humans in 2006 (Brenchley et al., 2006). Strategies to resolve microbial translocation are being borrowed from other disease models and tested in the HIV population. As strategies are being tested, researchers are still trying to gain a more comprehensive understanding of the mechanisms of immune activation at the molecular level. Yet, there are a few strategies that have evolved since

the publication of the published article presented in Chapter 2, *A Systematic Review of Probiotics as a Potential Intervention to Restore Gut Health in HIV Infection* (Wilson et al., 2013).

Rifaximin

In an effort to decrease levels of microbial translocation induced immune activation, researchers attempted to lower immune activation by reducing gut microbial translocation with an antibiotic used in patients with cirrhosis shown to improve symptoms and reduce LPS levels in the blood (Vlachogiannakos et al., 2009). Rifaximin had a minimal effect on monocyte immune activation to microbial products (i.e., sCD14) and generalized immune activation but not on LPS. Furthermore, there were minimal differences in IL-6 after 8 weeks but not on D-dimer. While Rifaximin demonstrated safety in having no deaths, there were significant GI side effects, including nausea, constipation, flatulence, anorexia, and abdominal pain (Tenorio et al., 2014). Overall, the effect of Rifaximin on microbial translocation in patients treated with cART, demonstrated very small and transient changes between study arms and there were unexplained fluctuations in immune biomarkers unrelated to the treatment. Additionally, the investigators disclosed that the study time points, duration, and limited precision of the selection criteria imposed significant limitations on the ability to determine if there was a clinically meaningful impact on microbial translocation and immune activation.

Probiotics

Probiotics have demonstrated effectiveness in decreasing these levels of pro-inflammatory cytokines. Stiksrud and Colleagues (2015) published data showing a reduction in coagulation and inflammatory biomarkers with the use of probiotics

containing *Lactobacilli* and *Bifidobacteria*. The median change in D-dimer decreased by 106 ng/mL (p = .03). The levels of IL-6 decreased from a median of 1.29 pg/mL to 1.06 pg/mL (p = .06). The non-probiotic comparison group had stable levels of IL-6 and D-dimer. In this study, levels of sCD14 did not change, nor did other levels of epithelial barrier function, tryptophan, kynurenine, nor kynurenine/tryptophan ratio; however, there were only 14 subjects in the study not giving it significant power to achieve the expected results (Stiksrud et al., 2015).

A similar pilot clinical trial was conducted by d'Ettorre, Giancarlo Ceccarelli and Colleagues (2015) in Europe using a more comprehensive combination of probiotic strains: *Streptococcus salivarius* ssp. *Termophilus, Bifidobacteria, Lactobacilli,* ssp. *Bulgarius,* and *Streptococus faecium* for 48 weeks and twice a day. They noted that cART did not normalize the levels of systemic immune activation. After taking these probiotics, there was a reduction in immune activation markers (CD38+ and HLA-DR on CD4+ T cells). IL-6 reduced from 5.97 ± 6.48 pg/mL pre-probiotic and 1.81 ± 2.0 pg/mL post-probiotic (p = .037). There was a positive correlation between sCD14 and IL-6 with a Spearman rank coefficient of 0.621 and p = .003. Additionally, there was improvement in digestion, reduction in intestinal bloating, diarrhea, constipation, and a small subset reported a reduction in allergic episodes and improvement in fatigue (d'Ettorre et al., 2015). Both studies lacked the sample size to conduct multivariate analysis controlling for confounders.

Another study conducted by Hensley-McBain, Klatt, and Colleagues (2016) offers fecal transplantation, an alternative to probiotics, to alter the intestinal microbiome in non-human primates. This study demonstrated there was significant increase in

peripheral CD4+ Th17 cells and Th22 cells, and a reduction in CD4+ T cell activation in GI tissues after fecal transplantation with stool from healthy (Simian Immunodeficiency Virus; SIV-) rhesus macaque donors. The microbiome reverted back to the pre-transplant milieu 2 weeks after transplant in 4 of the 6 animals. There were no adverse effects from this procedure (Hensley-McBain et al., 2016). Albeit, the results warrant further studies in human models with HIV disease and the alteration of the microbiome holds promise to improve disease management. Integrating the microbiome in studies and examining the correlation between the microbiome and the etiology of disease will support maintaining optimal health (Zhang, Lun, & Tsui, 2015).

Probiotic use is an effective nursing intervention used safely in other disease models with beneficial effects on the gut, immune function, and inflammation. Wilson, Moneyham, and Alexandrov (2013) provided evidence that probiotics could support improving aspects of the gut physiology, barrier integrity, and immune function. This has been demonstrated in studies showing improvements in microbial translocation by reducing the inflammatory sequelae at the gut level and improving HIV disease outcomes (d'Ettorre et al., 2015; Klatt et al., 2013). It is the hope that not only will probiotics serve as a promising adjunct therapy to reduce symptoms but also improve quality of life in PLWH.

We presented a theoretical framework which introduced the conceptual pathway for GI symptoms and systemic symptoms in the context of inflammation and microbial translocation (Wilson et al., 2014). With the inflammation and dysfunction of the gut, it was hypothesized that this would be indicative of symptoms at the GI level. Because of the dysfunction of the gut, we would expect to see the effect of microbial translocation

through monocytic response with sCD14 and subsequent effects of rises in IL-6 and D-dimer (Armah et al., 2012; Brenchley et al., 2006; Estes et al., 2010a; Somsouk et al., 2015). Part of this framework described the inflammation that would be the result of circulating microbial products possibly being associated with the development of systemic symptoms such as pain, fatigue, and cognitive decline, elements seen also in aging (Deeks, 2011; Vance, McDougall, Wilson, Debiasi, & Cody, 2014; Wilson et al., 2014).

In a retrospective study examining symptom patterns among PLWH on cART, we found that commonly reported symptoms were also associated with biomarkers of inflammation in the uninfected population (Wilson et al., 2015). However, we had limited evidence showing that this was the case in HIV disease. So we investigated the association of symptoms and HIV-associated inflammatory biomarkers (sCD14, IL-6, and D-dimer) predictive of mortality and HIV disease progression in the Veteran population. We confirmed an association with inflammatory biomarkers and certain GI symptoms. This was a cross-sectional study, therefore, we were not able to determine causality.

In this sample of the United States Veteran population, we were able to determine variables associated with GI symptom outcomes. Substance use and Hepatitis C were also associated with GI symptoms. Substance abuse and immune function were significant confounding variables contributing to the presence of GI symptoms. IV drug use had significant changes to the systemic inflammatory and symptom response. The majority of substances used for injection IV are opiate-based (e.g. heroin, opioid-based narcotics, cocaine) (Azar et al., 2015; Conrad et al., 2015). One of the concerns with use

of opiate-based pharmacological derivatives and analgesic is the effect on the gut. Opiates have demonstrated to alter the microbiome and create gut barrier disruption in animal models (Banerjee et al., 2016; Meng et al., 2015; Meng et al., 2013). In human models, opiates have a well-known effect on the functional properties of the gut (Kurz & Sessler, 2003) but could also accelerate and modulated HIV disease progression (Meng et al., 2015).

Opiates, Pain Management, and the Gut

Pain management strategies often include opioid prescriptions which carry risks associated with decreased functional status, hyperalgesia, tolerance, bowel dysfunction and microbial translocation (Edelman et al., 2013; Grunkemeier, Cassara, Dalton, & Drossman, 2007; Kurz & Sessler, 2003; Meng et al., 2015). The increase in microbial translocation, demonstrated in animal models, could lead to hyper-immune activation and chronic inflammation, augmenting HIV disease progression (Brenchley & Douek, 2008; Sandler et al., 2011). The most prevalent symptom and factor independently associated with functional impairment of mobility, self-care, and usual activities in PLWH is pain (Merlin et al., 2013; Wilson et al., 2015). With functional impairment, there is a link to higher levels of immune activation and IL-6 levels in PLWH; this finding persists even in persons with a suppressed viral loads less than 50 copies/mL (Erlandson et al., 2013). There were no significant differences in markers of microbial translocation between PLWH with low functional status and PLWH with high functional status (Erlandson et al., 2013). Chronic pain symptoms commonly reported include abdominal pain; muscle ache, joint pain, and neuropathic pain are associated with inflammation (Merlin et al., 2012; Wilson et al., 2014). Among individuals with HIV disease, the prevalence of long-

term opioid prescription use for pain relief ranges from 39-85% (Gaither et al., 2014; Merlin et al., 2012; Merlin et al., 2013).

Non-opioid pain treatment strategies both pharmaceutical and non-pharmacologic can safely manage pain and reduce adverse effects (Tauben, 2015). Some patients perceive non-opioid management plans to be less effective than opioid plans and often demand stronger medications that will alleviate the chronic pain experienced. Once on opioid-based regimens, patients demand to be left on opioids and even escalate the dose of opioids as the current analgesic effect becomes ineffective (Brush, 2012). This may be due to the highly motivational properties of opioid action in the brain (Cui et al., 2014; Ting et al., 2013).

Long-term exposure to opioids stimulates the release of pro-inflammatory cytokines, specifically IL-1β, IL-6, and TNF-α, promoting inflammation and causing hyperalgesia or increased pain; these same cytokines predict disease progression in HIV (Busch-Dienstfertig & Stein, 2010; Kulmatycki & Jamali, 2007; Sommer & Kress, 2004; Stein & Lang, 2009). Opioids may accelerate the progression of HIV disease as a result of increased inflammation, and decreased functional status. It is unclear in Chapter 5 which specific drugs were used but this does highlight the need for further research because the resulting inflammation plays a key role in the HIV disease progression (Brenchley et al., 2008; d'Ettorre, Paiardini, Ceccarelli, Silvestri, & Vullo, 2011; Estes et al., 2010b; Gordon et al., 2010; Sommer & Kress, 2004). The inflammatory effects of opioid chronic pain management on patient outcomes may accelerate HIV disease progression. Furthermore, alteration to the gut occurs with the use of opioids due to opioid-receptor modulation in the GI tract.

Opioid [Narcotic] bowel syndrome (NBS) is characterized by chronic abdominal pain, reduced GI motility, nausea and vomiting worsening with continued and escalated doses of opioids (Grunkemeier et al., 2007). It is unknown if the chronic use of opioids leads to alterations of the GI epithelial barrier as a characteristic of NBS. It is plausible that additional local stimulation of receptors to release pro-inflammatory cytokines will promote further inflammation and permeability of the gut tight gap junctions and subsequent microbial translocation.

Meng and colleagues provided evidence of pro-inflammatory cytokine (IL-17) from Th-17 cells altering the gastrointestinal barrier leading to microbial translocation in the murine model. In addition, IL-6 and IL-1β were mediators of IL-17, which are significantly increased in HIV and in opioid use (Meng et al., 2015). Higher levels of microbial translocation would lead to increases in immune activation and chronic inflammation augmenting HIV disease progression (Estes et al., 2010a; Klatt et al., 2010).

Future research is required into examining symptoms and opiate use, also associated with increased HIV progression and transmission risk (Conrad et al., 2015). It is necessary to conduct a study examining the association of systemic symptoms with microbial translocation. In addition, cataloging differences in the gut microbiome and the effect of alpha diversity on levels of microbial translocation in the context of symptoms is warranted.

Addressing Co-Morbidities to Reduce Symptom Burden

Hepatitis C, fibrosis, and hazardous alcohol use were identified as potential confounders of GI symptoms: bloating/abdominal pain, nausea/vomiting, and loss of

appetite. Diarrhea only had a univariate association with hazardous drinking. Microbial translocation and immune activation may modulate liver fibrosis stage in HIV-infected patients co-infected with hepatitis C (Marchetti et al., 2014; Marquez, Fernandez Gutierrez Del Alamo, & Giron-Gonzalez, 2016; Sacchi et al., 2015). Research is needed to investigate whether new treatments for hepatitis C and addressing alcohol use in patients would support a reduction of symptom burden, improve liver function, and reduce microbial translocation (Wang et al., 2014). Also, screening for hepatitis C and hazardous alcohol use are critical to improving patient outcomes, reducing symptom burden, and monocytic immune activation (Carrico et al., 2015; Wang et al., 2014).

Diarrhea was the most commonly reported GI symptom (46%) and considered bothersome by 67% of those experiencing the symptom. In the reduced model, CD4+ T cell counts less than 500 cells/mm^3, hazardous alcohol use, and white race was most predictive of diarrhea. CD4+ T cell count <50 cells/mm^3 were twice as likely to report diarrhea, than those persons with CD4+ T cell counts >500 cells/mm^3. This highlights the importance of achieving optimal therapeutic goals of cART of a CD4+ T cell count of >500 cells/mm^3 to address this symptom.

Bloating/abdominal pain was the second most prevalent GI symptom (42%) and considered bothersome by 69% of those experiencing the symptom. Taking into consideration other clinical factors that may influence bloating/abdominal pain, hazardous alcohol use, hepatitis C, having a low CD4+ T cell count of less than 500 cells/mm^3, and having non-specific immune activation with elevated levels of IL-6 were most predictive. While there was a small effect size of r = .13, the factors contributing to

bloating/abdominal pain can be addressed through substance abuse, HIV-1, and hepatitis C treatment.

Nausea/vomiting was best predicted by higher body mass index and by those with higher levels of IL-6 and D-dimer, and CD4+ T cell counts below 500 cells/mm^3. This symptom, nausea/vomiting, may be an indication higher risk for non-AIDS morbidity. Similar to bloating/abdominal pain, diarrhea, and loss of appetite in adjusted models, CD4+ T cell counts below 500 cells/mm^3 were critical confounders for poor outcomes in HIV disease.

Methodological Strengths and Limitations

One of the major limitations of this research thesis was that all data was retrospective and subject to the design of the original study purpose. Therefore, there were limitations to data received especially in determining adherence to medications. We did not have specifics of drug of choice for IV drug use which would have strengthened our understanding of the results and assumptions. Conversely, the retrospective database offered us a large sample size to achieve sufficient power to determine effect sizes. Because the area of study was exploratory, having retrospective data supported the analysis and overcame the obstacle of time it would have taken to explore the questions in a prospective sample. The effect sizes and results will support determining future sample sizes for prospective studies as we continue to explore the relationship between symptoms and inflammation. Examining inflammation and epithelial barrier dysfunction in the VACS would limit the generalizability to Veterans. However, the VACS is a national study consisting of sites in different regions.

Implications for Early Aging in HIV

The impetus of this research is the threat of inflammation on the health of PLWH. Inflammation has critical effects on DNA telomeres, damaging the cellular structure and creating cellular senescence (Fitzpatrick et al., 2011; Zanet et al., 2014). Telomere length has been associated with cardiovascular events leading to mortality (Fitzpatrick et al., 2007; Fitzpatrick et al., 2011). The link between shortened telomeres and biomarkers of inflammation in HIV disease are certainly contributors to the effects of HIV and microbial translocation have on aging, cognitive decline, end-organ damage, cancers and non-AIDS related co-morbidities and mortality (Deeks, 2011; Jurk et al., 2014; Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013; Valdes et al., 2010). The urgency of developing strategies and interventions to prolong or protect the telomeres is of significant value in the promotion of health in PLWH. In Chapter 4, we presented an article in which poor sleep was the number one symptom presenting in 51% of the sample of PLWH with 82% of those experiencing the symptom reporting that having poor sleep was bothersome. Sleep duration has seen associated with preserving the length of telomeres in PLWH and may be a clinical strategy to promote health (Lee et al., 2014; Wirth et al., 2015).

Interestingly, there are three major differences in gut microbiota seen between adults and children. Children have a significantly higher prevalence of *Bifidobacteria* and *Enterobacteria* than seen in the elderly with similar levels of *Bacteroides-Porphyromonas-Prevotella* between the elderly and children (Hopkins, Sharp, & Macfarlane, 2001). More specifically decreasing *Bifidobacteria* and increased levels of *Bacteroides* species were associated with advancing age (Hopkins, Sharp, & Macfarlane,

2002). PLWH have a significant shift in their gut microbiota with lowers the diversity of the species which has similar diversity to the elderly in the gut which has been associated with chronic gut inflammation (Mutlu et al., 2014). Increased levels of *Bacteroides* in the context of deficient of *Bifidobacteria* and *Fermicutes* may be the key to chronic inflammation and the subsequent early aging seen in PLWH (Nowak et al., 2015).

Nursing Implications

This research has identified important variables contributing to the symptom burden experienced by PLWH. The results discussed will forward the field of nursing research and clinical practice by reframing symptom assessment and management. While the results of these studies were meant to provide contribution to the field of symptom management research, it is only a part of the puzzle; not meant to convey that symptoms have been cured or that lives could be saved by resolving microbial translocation. Conversely, the GI system is a profound and important organ to maintaining homeostasis of the human host. Efforts to reduce inflammation and promote healing of the GI epithelial barrier are needed to reduce the effects of microbial translocation in PLWH are important in the field of nursing research. Clinically, nurses should begin to consider and address the underlying pathophysiology leading to the symptom development in addition to alleviating the symptom mechanism alone; this is the science of healing. The development of symptom management strategies that will not only improve gut function but improve the effects of subsequent inflammation are needed in the HIV clinical field. Plans to identify probiotic strains which work in combination to repair and improve the epithelial barrier function as well as decrease microbial translocation will be a study in the near future and have already begun in the AIDS Clinical Trial Group [A5350].

Nursing science should also investigate possible educational techniques to improve dietary intake that reduces or does not contribute to inflammation of the GI epithelial barrier. This would include multidisciplinary investigation with nutrition science to examine the effects of different foods such as, sugar, gluten, dairy, and processed substances genetically modified for human consumption, on the gut. The strategies presented are meant to be in conjunction with and as adjunct therapy to treatments targeted toward infectious pathogens (e.g. cART and HCV therapy). The purpose of this journey is to achieve healing of the gut and microbiome to support the achievement of homeostasis in PLWH.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVALS FROM UNIVERSITY OF ALABAMA AT BIRMINGHAM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator: WILSON, NATALIE

Co-Investigator(s):

Protocol Number: **X131220001**

Protocol Title: *Microbial Translocation & Symptoms on Adherence in the Veterans Aging Cohort Study: Secondary Data Analysis*

The IRB reviewed and approved the above named project on 1-3-14. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 1-3-14

Date IRB Approval Issued: 1-3-14

IRB Approval No Longer Valid On: 1-3-15

HIPAA Waiver Approved?: N/A

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
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Principal Investigator:	WILSON, NATALIE
Co-Investigator(s):	
Protocol Number:	**X131220001**
Protocol Title:	*Microbial Translocation & Symptoms on Adherence in the Veterans Aging Cohort Study: Secondary Data Analysis*

The IRB reviewed and approved the above named project on 1-20-15. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 1-20-15

Date IRB Approval Issued: 1-20-15

IRB Approval No Longer Valid On: 1-20-16

HIPAA Waiver Approved?: N/A

Member - Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

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Principal Investigator:	WILSON, NATALIE
Co-Investigator(s):	
Protocol Number:	**X131220001**
Protocol Title:	*Microbial Translocation & Symptoms on Adherence in the Veterans Aging Cohort Study: Secondary Data Analysis*

The IRB reviewed and approved the above named project on 1-5-16. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 1-5-16

Date IRB Approval Issued: 1-5-16

IRB Approval No Longer Valid On: 1-5-17

HIPAA Waiver Approved?: N/A

Marian Dass

Expedited Reviewer
Member - Institutional Review Board
for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

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APPENDIX B

INSTITUTIONAL REVIEW BOARD FOR THE VETERANS ADMINISTRATION BIRMINGHAM MEDICAL CENTER AND VACS INFORMED CONSENT FOR HUMAN SUBJECTS

Birmingham VA Institutional Review Board (IRB)
Birmingham Veterans Affairs Medical Center
Research Service
VA Research Service (151) • 700 South 19th Street • Birmingham, AL 35233 • 205-933-8101 • Fax: 205-933-4471

EXEMPTION

Date: November 19, 2013
From: Kevin W. Harris, MD, PhD, Chairperson Kevin W. Harris 11/26/13
Investigator: Natalie L. Wilson, DNP, ANP-BC, FNP, MPH, MSN, AAH
Protocol: Microbial Translocation, Gastrointestinal Symptoms on Adherence in the Veterans Aging Cohort Study: A Secondary Data Analysis
ID: 01533 Prom#: N/A Protocol#: N/A

The following items were reviewed and determined to be exempt from IRB review:

- Miscellaneous - HIPPA Waiver (11/06/2013)
- Miscellaneous - Informed Consent Waiver (11/06/2013)
- Initial Review Application (11/06/2013)
- Checklist for Reviewing Privacy, Confidentiality & (11/06/2013)

Exemption from IRB review was granted on 11/19/2013. This exemption from IRB review will be reported to the fully convened IRB on 11/27/2013.

This study will need to be reviewed and approved by the R&D Committee and the Information Security Officer prior to the initiation of research.

The study must be reviewed annually by the R&D Committee.

The following other committee reviews are scheduled:
Associate Chief of Staff/Research and Development [12/04/2013]
Research and Development Committee [12/04/2013]

Approval by each of the following is required prior to study initiation (unless Exempt):
Birmingham VA Institutional Review Board (IRB)
Research and Development Committee

Approval for study initiation is contingent upon your compliance with the requirements of the Research Service for the conduct of studies involving human subjects.

The Birmingham VAMC IRB is not connected with, has no authority over, and is not responsible for human research conducted at any other institution, except where a Memorandum of Understanding specifies otherwise. Separate consent forms, initial reviews, continuing reviews, amendments, and reporting of serious adverse events are required if the same study is conducted at multiple institutions.

Birmingham VA Institutional Review Board (IRB)
Birmingham Veterans Affairs Medical Center
Research Service
VA Research Service (151) • 700 South 19th Street • Birmingham, AL 35233 • 205-933-8101 • Fax: 205-933-4471

IRB APPROVAL - Continuing Review

Date: December 1, 2014
From: Kevin W. Harris, M.D., Ph.D., Chairperson
Investigator: Natalie L. Wilson, DNP, ANP-BC, FNP, MPH, MSN, AAH
Protocol: Microbial Translocation, Gastrointestinal Symptoms on Adherence in the Veterans Aging Cohort Study: A Secondary Data Analysis
ID: 01533 Prom#: N/A Protocol#: N/A

The following items were reviewed and approved through Expedited Review:

- Continuing Review (11/13/2014)
- Miscellaneous - Preview of data tables (12/01/2014)
- Miscellaneous - Add & Remove staff (11/13/2014)

Add: Corilyn Ott, Josh Richman, Miriam Kempf, Juan Vargas.
Remove: Mark Litaker

Expedited Approval [Expedited under Federal Regulation: 45 CFR 46.110(b)(1)(5) / VA Regulation: 38 CFR 16.110(b)(1)(5)] was granted on 12/01/2014 for a period of 12 months and will expire on 12/08/2015. Your Continuing Review is scheduled for 11/11/2015. This Expedited review will be reported to the fully convened Birmingham VA Institutional Review Board (IRB) on 12/10/2014.

In reviewing the submission, the Chair had no issues of concern and determined this study met the criteria for expedited review as set forth in 45 CFR 46.110(b)(1)(5) / VA Regulations: 38 CFR 16.110(b)(1)(5). This determination is based on 1) some or all of the research appearing on the category list and found by the reviewer(s) to involve no more than minimal risk & 2) Research involving materials (data, documents, records, or specimens) that have been collected or will be collected solely for non-research purposes (such as medical treatment or diagnosis). (NOTE: some research in this category may be exempt from the HHS regulations for the protection of human subjects.

Approval by each of the following is required prior to study continuation (unless Exempt):
Birmingham VA Institutional Review Board (IRB)

Approval for study continuation is contingent upon your compliance with the requirements of the Research Service for the conduct of studies involving human subjects.

The Birmingham VAMC IRB is not connected with, has no authority over, and is not responsible for human research conducted at any other institution, except where a Memorandum of Understanding specifies otherwise. Separate consent forms, initial reviews, continuing reviews, amendments, and reporting of serious adverse events are required if the same study is conducted at multiple institutions.

Birmingham VA Institutional Review Board (IRB)
Birmingham Veterans Affairs Medical Center
Research Service
VA Research Service (151) • 700 South 19th Street • Birmingham, AL 35233 • 205-933-8101 • Fax: 205-933-4471

IRB APPROVAL - Continuing Review

Date: November 3, 2015
From: Kevin W. Harris, M.D., Ph.D., Chairperson Kevin W. Harris 10/3/15
Investigator: Natalie L. Wilson, DNP, ANP-BC, FNP, MPH, MSN, AAH
Protocol: Microbial Translocation, Gastrointestinal Symptoms on Adherence in the Veterans Aging Cohort Study: A Secondary Data Analysis
ID: 01533 Prom#: N/A Protocol#: N/A

The following items were reviewed and approved through Expedited Review:

- Abstract - 10-20-15 (10/20/2015)
- Continuing Review - Progress Report 10-20-15 (10/20/2015)
- Miscellaneous - Personnel List 11-3-15 (11/03/2015)
- Miscellaneous - PI Continuing Review Memo 11-3-15 (11/03/2015)
- Miscellaneous - MISC IRB Submission 10-20-15 -Remove Mike Conner and Juan Vargas (03/05/2009)
- Checklist for Reviewing Privacy, Confidentiality & - 11-3-15 (No Changes) (11/03/2015)

Expedited Approval was granted on 11/03/2015 for a period of 12 months and will expire on 11/02/2016. Your Continuing Review is scheduled for 10/12/2016. This Expedited review will be reported to the fully convened Birmingham VA Institutional Review Board (IRB) on 11/18/2015.

The following other committee reviews are scheduled:
Associate Chief of Staff/Research and Development [11/05/2015]

Approval by each of the following is required prior to study continuation (unless Exempt):
Birmingham VA Institutional Review Board (IRB)

Approval for study continuation is contingent upon your compliance with the requirements of the Research Service for the conduct of studies involving human subjects.

The Birmingham VAMC IRB is not connected with, has no authority over, and is not responsible for human research conducted at any other institution, except where a Memorandum of Understanding specifies otherwise. Separate consent forms, initial reviews, continuing reviews, amendments, and reporting of serious adverse events are required if the same study is conducted at multiple institutions.

VA Department of Veterans Affairs

VA RESEARCH CONSENT FORM

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** VAMC: Pittsburgh (646)

LAY TITLE: This is a smaller substudy within the larger Veterans Aging Cohort Study (VACS) to examine the extent of influence that personal genetic heredity may have on veterans' healthcare outcomes.

STUDY CONTACT INFORMATION:
If you have a general question about this research study you may call **Carol Rogina at 412-688-6000 ext. 81-5291** or any of the investigators listed below.

If you experience a medical problem that you feel may be related to this study, please call **Dr. Adeel Butt at 412-688-6000 ext. 81-6179 during the day or page him at 412-698-8748. You may also call the toll free VHA after-hours call center at 1-866-482-7488.** In the case of a medical emergency contact your local emergency medical service or go to your local emergency room.

INVESTIGATOR CONTACT INFORMATION:

Adeel Butt, MD
(Site Primary Investigator)
Assistant Professor of Medicine
VA Pittsburgh Healthcare System
University of Pittsburgh
3601 Fifth Avenue, Suite 3A
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Pittsburgh, PA 15213
E-mail: butta@dom.pitt.edu
Tel: 412-688-6000 ext. 81-6179
Pager: 412-698-8748

Joseph Conigliaro, MD, MPH
(Overall Primary Investigator)
Associate Professor of Medicine
VA Pittsburgh Healthcare System
University of Pittsburgh
University Dr. C
Pittsburgh, PA 15240
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Tel: 412-688-6000 ext. 81-6477

Erika Hoffman, MD
(Co-Investigator)
Assistant Professor of Medicine
VA Pittsburgh Healthcare System
Staff Physician
University of Pittsburgh

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IRB Designee _____

VA RESEARCH CONSENT FORM
(Page 2 of 13)

Subject Name: _____ Last 4 SSN:_____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

University Dr. C, 11E-107 (130U)
Pittsburgh, PA 15240
Email: erika.hoffman@med.va.gov
Tel: 412-688-6000 ext. 81-4072

STUDY SPONSOR:
This study is sponsored by the National Institute of Health **(NIH)**, and the National Institute of Alcoholism and Alcohol Abuse **(NIAAA)**.

PURPOSE OF THE RESEARCH STUDY / BACKGROUND:
The purpose of this research study is to study alcohol, aging and their relationships to the kinds of health conditions a person has, the health care the person gets, the things a person does to improve their health, the things a person does that may harm their health and important results such as whether or not a person comes to appointments, takes their medicines, has complications, experiences side effects from their medications, has a good quality of life and lives longer. The study will compare these conditions among HIV positive and negative veterans in care at 8 VAMC's around the nation. You have already agreed to participate as one of approximately 6000 patients by completing an initial questionnaire when you first enrolled, and others at yearly intervals for the duration of this study, as long as the study is funded and you continue to be willing to participate.

Patients already enrolled in this main observational study with general medical conditions cared for in the primary care clinics as well as patients receiving care for chronic HIV infection will be asked to participate in donating a small sample of blood for initial testing and banking for future research studies on conditions related to alcohol, aging, comorbidity, and side effects of treatment.

DESCRIPTION OF THE RESEARCH STUDY:
A sample (approximately 4 tablespoons) of your blood will be obtained by needle puncture of a vein. If you do not come in for an appointment when it is time for a blood draw, a research staff member designated by the Primary Investigator will give you a phone call or send you a letter as a reminder to come in. Initial routine tests will be done including tests to determine evidence of liver and cardiac injury, and bone marrow function. Other tests will be done locally for a complete blood count, including a platelet count. This data will be entered into your medical report. Additional studies related to alcohol,

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Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

aging, comorbidity and drug toxicity will be conducted on your specimens in the future based upon a formal review process. Samples will be shipped to and stored for the Veterans Aging Cohort Study at the Massachusetts VA Epidemiology Research and Information Center (MAVERIC) in Boston, MA.

VACS Samples: In addition to the tests that will be run immediately on your blood samples, VACS wants to store away samples of your blood at MAVERIC so that we can better study the alcohol, aging and their relationships to the kinds of health conditions veterans develop. As we learn more about these conditions, we will be better able to identify important blood tests to perform on your blood samples to identify factors associated with these conditions. If a researcher proposes to use your VACS blood samples for conditions not listed, a meeting of the VACS Community Advisory Board, or CAB, will be convened to discuss the proposal. The proposal may or may not be approved by this advisory committee. VACS will retain complete control of blood samples stored at MAVERIC. Because of special concerns around DNA testing, VACS and MAVERIC will work with the VA DNA Bank to ensure that samples banked for DNA testing have an extra layer of protection of your rights to privacy.

The DNA Bank: A DNA Bank is a place where DNA and plasma is stored for scientists to use in future studies. In some ways, a DNA Bank is a lot like a safety deposit box at your local bank. A person's DNA and plasma is placed in a secure location and only scientists who meet strict requirements are allowed access to the DNA. The purpose of this DNA Bank is to store DNA and plasma from individuals, such as you, who may or may not be HIV positive or may have other medical conditions (such as diabetes.) Scientists will then be able to use your DNA to identify inherited traits of various diseases. We plan to look at genes that may be passed on to you that might be responsible for your medical condition. Some medical conditions that we are interested in are substance use and addiction, vascular disease, diabetes, liver injury, mental illness and infectious diseases. If a researcher would like to use your DNA for conditions not in this list, a meeting of the Veterans Advisory Group, or VAG, will be convened to discuss the proposal. The proposal may or may not be approved by this advisory committee.

Consent: We will ask your permission to collect a blood sample from you. If you agree to donate blood to the Veterans Aging Cohort Study and DNA to the DNA Bank, you will need to sign this consent form. After you sign this form we will take a 57.5 ml. blood sample (less than 4 tablespoons) from your vein

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Subject Name: _____ Last 4 SSN:_____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

and store it in test tubes. As mentioned above, samples will be shipped and stored for VACS at the VA in Boston, where the DNA and plasma will be collected from the blood cells.

By signing this consent form, you are agreeing to give the Veterans Aging Cohort Study and the DNA Bank access and control of your blood samples and your clinical information. This allows both VACS and the DNA Bank to retain, preserve, or destroy these samples and allows us to use them in future research studies for an unlimited amount of time.

In addition, the blood sample that you are giving to the Veterans Aging Cohort Study and the DNA Bank may result in new products, tests or discoveries. In some instances, these may have potential commercial value and may be developed and owned by the investigators and/or others. However, participants in the VACS DNA Bank do not retain any property rights to the materials. Therefore, you would not share in any financial benefits from these products, tests or discoveries.

Use of your VACS Samples: Your blood samples will be used only for research related to the Veterans Aging Cohort Study. All studies and the scientists who do them, must first be reviewed by the VACS Steering Committee to oversee the ethics, safety and scientific activity of the studies.

Use of your DNA: Your DNA will be used only for research on the way that genes relate substance use and addiction, vascular disease, diabetes, liver injury, mental illness and infectious diseases. It will be used by investigators interested in the cause of such illnesses and developing potential treatments for HIV. All DNA research must first be reviewed by two VA advisory committees that work with the DNA Bank to oversee the ethics, safety and scientific activity of the studies. If a researcher would like to use your DNA for conditions not in the above list, a meeting of the Veterans Advisory Group (VAG) will be convened to discuss the research. The research may or may not be approved by this advisory committee.

RISKS AND BENEFITS:
The risk of drawing blood is very small, but includes minor pain, skin bruising, bleeding from where the needle goes in, or anxiety about needles. There is also the possibility of fainting and infection at the site of the blood draw. These risks are the same as for a standard blood test. Individuals trained and experienced in obtaining blood samples so as to minimize these risks will perform this procedure.

Subject's Initials_____

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IRB Designee _____

[redacted]

VA RESEARCH CONSENT FORM
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Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

Your blood sample may be inadvertently lost as a result of equipment failure or other unforeseen events.

There is the possibility of unplanned release of information from your medical records. The chance that this information will be given to an unauthorized person without your permission is very small. Possible problems with the unplanned release of information include discrimination when applying for insurance and employment. Similar problems may occur if you disclose information yourself or agree to have your medical records released.

Genetic information about you will not be revealed to others, including your relatives, without your permission. We will not release any information about you or your family to any insurance company or employer unless you sign a document allowing release of information. In general, genetic testing may tell researchers something about how health or illness is passed on to you by your parents or from you to your children. You should consider the possible effects on your emotional well being. How might you feel about yourself and your life if you learned that you and your children might be at increased risk of some disease, especially if there were no treatment? This could cause stress, anxiety or depression. Additional genetic counseling and advice are available from the National Institutes of Health to help you understand the nature and implications of findings about you and your family.

Also, relationships with other family members may be affected by finding out risks they have but did not want to know. An example would be if your children, brothers or sisters find out that they have risks for health problems because of information found out about you. Genetic testing can also be used to determine if people are directly related. These tests sometimes show that people were adopted or that their biological parent is someone other than their legal parent. If these facts were not known previously they could be troubling. It is our policy to not discuss such information unless it has direct medical or reproductive implications for you or your family. By agreeing to participate in this study, you do not waive any rights that you may have regarding access to and disclosure of your records. For further information on those rights, you can contact the principle investigator of this study.

The ability to look at human DNA is a new development, and there is a very small risk that disclosure of genetic information could affect you and your family by its psychological impact, and disclosure could make it harder for you and your family to obtain employment, adopt children, or get medical and life insurance.

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IRB Designee KP

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

Although this study does not provide you with any direct health benefit or treatment, you will contribute to a greater understanding of the way that genes affect various medical conditions. In addition, the results of future research with your DNA may assist patients with these health conditions, their relatives and the general public.

ALTERNATIVES TO PARTICIPATION:
Your participation in this research and donation of blood for storage of both blood and DNA is voluntary and the alternative is not to participate. If you choose not to participate, this decision will not affect your medical care, or your participation in the VACS study in any way.

INVESTIGATOR INITIATED WITHDRAWAL:
The investigator(s) may stop your participation in this study without your consent for reasons such as: it will be in your best interest; you do not follow the study plan; or you experience a study-related injury.

VOLUNTARY PARTICIPATION/RIGHT TO WITHDRAW:
You understand that you do not have to take part in this study, and your refusal to participate will involve no penalty or loss of rights to which you are entitled. You may withdraw from this study at any time without penalty or loss of VA or other benefits to which you are entitled. If you withdraw, you may be asked to return for a final study visit in order to assure your safety. You must withdraw in writing in order to withdraw your permission for us to continue to use the protected health information we have already collected about you. Even if you withdraw your permission for us to use the information about you, we are required by regulatory agencies to record any information that relates to the safety of any study-related intervention.

You have the right to request that we destroy the blood samples you donated for VACS use. If you choose to withdraw your remaining samples will be destroyed. Should you decide you want your samples destroyed, please contact Beth Dombrowski (VACS Project Coordinator) at (203) 932-5711 extension 5371.

Similarly, you also have the right to withdraw your DNA from the DNA Bank at any time. If you choose to withdraw, your DNA sample will be destroyed and will not be used for more testing. Any information obtained before you withdraw will be stored at the DNA Bank Coordinating Center in Palo Alto,

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IRB Designee 16

Department of Veterans Affairs

VA RESEARCH CONSENT FORM
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Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

California. In order to withdraw your DNA from the DNA Bank, you will need to contact Beth Dombrowski at (203) 932-5711 extension 5371.

PATIENT STATEMENT:
You have been told of the risks or discomforts and possible benefits of the DNA Bank. You understand that you do not have to donate your blood or DNA to VACS or the DNA Bank, and your refusal to participate will involve no penalty or loss of rights to which you are entitled. You may withdraw your samples from the either VACS or the DNA Bank at any time without penalty or loss of benefits to which you are entitled. The results of research which uses your blood or DNA may be published, but your records will not be revealed unless required by law.

Please read each sentence below and think about your choice. After reading each sentence, please mark your choice.

PLEASE CHECK ONLY ONE BOX BELOW

[ ] --I agree to both blood testing and DNA testing. My blood samples will be stored at MAVERIC and my DNA and plasma will be stored at the DNA bank.
[ ] --I agree to blood testing ONLY. My blood samples will be stored at MAVERIC.
[ ] --I agree to DNA testing ONLY. My DNA and plasma will be stored at the DNA bank.
[ ] --I DO NOT AGREE to either blood testing or DNA testing.

NOTE: if this last option is checked, you will not be enrolled in the blood sampling study.

Sometimes health information that was not already collected (such as smoking history or current health status) may be important to know for future research. If additional health information is needed, I am willing to be re-contacted by the study team to provide this information.

Yes [ ] No [ ]

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IRB Designee IG

Department of Veterans Affairs

VA RESEARCH CONSENT FORM
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Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

FEEDBACK OF RESEARCH FINDINGS:

VACS Samples:
VACS will probably not reveal the results of blood testing to you or your physician. An exception would be made if the test results could help you:

1. Do something for your own health
2. Do something for the health of family members
3. Provide information about future risks to your health or your family's health

We do not think it is very likely that you will need to be told about your test results. If this does turn out to be necessary, the VACS Steering Committee will find the best way to give you and your physician the most helpful information. If you would prefer that we do not give this information to anyone, including yourself, please tell us by checking the box below and signing your name and date.

☐ **I do not wish VACS to give me or anyone else, including my family and my physician, the results of my blood testing.**

_____ Patient's signature _____ Date

DNA Samples:
The DNA Bank will probably not reveal the results of genetic testing to you or your physician. An exception would be made if the test results could help you:

1. Do something for your own health
2. Do something for the health of family members
3. Provide information about future risks to your health or your family's health

We do not think it is very likely that you will need to be told about your test results. If this does turn out to be necessary, the DNA bank oversight committees will find the best way to give you and your

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IRB Designee _____

Department of Veterans Affairs

VA RESEARCH CONSENT FORM
(Page 9 of 13)

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

physician the most helpful information. If you would prefer that we do not give this information to anyone, including yourself, please tell us by checking the box below and signing your name and date.

☐ **I do not wish the DNA Bank to give me or anyone else, including my family and my physician, the results of my genetic testing.**

_____ Patient's signature _____ Date

MEDICAL TREATMENT:
In the event that you sustain injury or illness as a result of your participation in this VA approved research study, conducted under the supervision of one or more VA employees, all medical treatment (emergent as well as medical treatment beyond necessary emergent care) will be provided by the VA.

However, if such injury or illness occurred as a result of your failure to follow the instructions for this study, you may not be eligible for free care unless you have independent eligibility for such care under Federal Law.

FINANCIAL COMPENSATION:
If you sustain an injury or illness as a result of participating in this research study, you may be eligible to receive monetary compensation for your damages pursuant to applicable federal law.

COST AND PAYMENTS:
There will be no cost to you for your participation in this study, however if you are receiving medical care and services from the VA that are not part of this study, and you are a veteran described in federal regulations as a "category 7" veteran, you may be required to make co-payments for the care and services that are not required as part of this research study.

You will be paid $20.00 for your blood sample.

VA FORM 10-1086 JUNE 1990 (revised 02/2004) **Subject's Initials**_____

VAPHS IRB No. 1 approved this version 6-15-05 of the consent form on 6-16-05 Extension beyond 10-24-05 requires re-approval by the IRB.
IRB Designee _____

VA Department of Veterans Affairs

VA RESEARCH CONSENT FORM
(Page 10 of 13)

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

PRIVACY AND CONFIDENTIALITY:

- Information that will be used: Your privacy is protected by a Certificate of Confidentiality issued by the Public Health Service, Department of Health and Human Services. If you agree to donate a sample to the DNA Bank, sensitive genetic information will be generated during this study and this Certificate will help researchers avoid involuntary disclosures which could expose subjects, and their families, to adverse economic, legal, psychological, and social consequences. If you have an adverse experience during the course of the study, your entire medical record may be used and disclosed as clinically necessary as well as pursuant to federal and state laws and regulations.

  To protect your privacy, your blood sample will have only a code number so that it cannot be identified by your name. In addition, your DNA sample will be identified by a code number that will be different from the code number used in the VACS study and the code number used for your blood sample. Only the researchers at the DNA Bank Coordinating Center in Palo Alto will be able to connect them to each other. At no time will your name, address or any other identifying information be released for research purposes. We are doing everything possible to prevent anyone outside the study from learning your private genetic information, and the risk to your privacy is extremely small.

- The People/Organizations Who May Use or Disclose the Information: Your information will be used only as specified above and under the direction of Dr. Adeel Butt and his/her research team. Your private information may also be used by employees of the VA Pittsburgh Healthcare System Research and Development Office, as necessary, to perform their duties regarding research.

- The People/Organizations Who Will Receive the Information: Your blood samples will be shipped to and stored at the Massachusetts VA Epidemiology Research and Information Center (MAVERIC) in Boston, MA. Scientists may use your DNA and plasma for use in future studies. You understand that every effort will be made to make sure that the information about you obtained from this study will be kept strictly confidential. Information on research participants may be provided to federal and regulatory agencies within the scope of these studies. There is a possibility that your records, including identifying information such as your name and address, may be reviewed by the Department of Veterans Affairs or other Federal government agencies. The Committee for the Protection of Human Subjects that approved this study may have access to this informed consent form to ensure that guidelines designed to protect participants in research studies are followed correctly.

VAPHS IRB No. 1 approved this version 6/15/05 of the consent form on 6-16-05. Extension beyond 10-24-05 requires re-approval by the IRB.
IRB Designee _____

[redacted] Department of Veterans Affairs

VA RESEARCH CONSENT FORM
(Page 11 of 13)

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

Once your private information is released to outside entities as specified above, further disclosure will be limited by federal and state privacy laws and regulations. Your information may also be disclosed to the Education and Compliance Officer of the VA Pittsburgh Healthcare System in order to perform audit and compliance duties. You understand that your private health information may also be reviewed by the institutional review board, which is a group at this hospital that oversees all research. You do understand that research records, just like hospital medical records, may be released or disclosed pursuant to applicable federal and state law as well as to federal and state agencies that are responsible for oversight of medical research. You also understand that medical information may be shared with your healthcare provider(s) with your consent, and possibly without your consent if permissible under federal laws and regulations. Finally, you consent to the publication of the study results so long as the information about you is anonymous and/or disguised so that your identity will not be disclosed.

Future publications by authors using data from VACS blood samples and DNA data will be listed at the website: www.vacohort.org.

- Expiration Date or Event: Your blood samples and DNA will be stored until the studies are over, and then they will be destroyed. The decision to end the studies will be made by the scientific investigators and a special committee that will watch over these studies.

RESEARCH SUBJECTS' RIGHTS:
You have read or have had read to you all of the above. Dr. Adeel Butt or his/her authorized representative has explained the study to you and answered all of your questions. You have been fully informed of the risks, discomforts, and possible benefits of the DNA bank and this research study. You understand that you do not have to donate your blood to VACS or your DNA to the DNA bank, and your refusal to participate will involve no penalty or loss of rights. You may withdraw your samples at any time. The results of research which uses your blood or DNA may be published, but your records will not be revealed unless required by law. You have been fully informed of other treatment choices available to you.

[redacted] approved this consent on 6-15-05 of the consent form on 6-16-05. Extension beyond 10-24-05 requires re-approval by the IRB.
Designee [signature]

VA Department of Veterans Affairs	VA RESEARCH CONSENT FORM (Page 12 of 13)

Subject Name: _____ Last 4 SSN:_____ Date: _____

Title of Study. **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

In case there are medical problems or questions, you have been told you can call Dr. Adeel Butt at 412-688-6000 ext. 81-6179, page him at 412-698-8747 during the day, or you may call the toll free VHA after-hours call center at 1-866-48-7488.

You understand your rights as a research subject, and you voluntarily consent to participate in this research study. You understand what the study is about and how and why it is being done. You will receive a copy of this signed consent form.

If you have any questions about the research or your rights as a participant in this study, you can call Dr. Steven H. Graham, Associate Chief Of Staff/R&D, VA Subcommittee on Human Studies (SHS) at 412-688-6104.

As long as the study is renewed as required by the SHS, your signature on this document is valid for the duration of the entire research study and you understand that you will be notified of any changes in the study that will affect you.

6/15/05 of the consent
6-16-05 Extension
10-24-05 requires
re-approval by the IRB.
IRB Designee

VA Department of Veterans Affairs

VA RESEARCH CONSENT FORM

Subject Name: _____ Last 4 SSN: _____ Date: _____

Title of Study: **Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study (VACS-DNA Bank Substudy)**

Principal Investigator: **Dr. Adeel Butt, M.D.** AMC: Pittsburgh (646)

By signing this form, you agree to participate in this research study.

Subject's Signature	Date	
Signature of Subject's Representative*	Subject's Representative (Print)	Date
Signature of Witness	Witness (Print)	Date
Investigator/Person Obtaining Consent**	Researcher (Print)	Date

***Only allowed if subject is not competent. (Such subjects cannot be enrolled without specific IRB approval to enroll incompetent subjects.)**

**If person other than the Investigator is obtaining consent, he/she must be listed on the IRB-approved "List of Authorized Representatives to Administer Informed Consent."

Version 6/2005

I approved this
6-15-05 the consent
6-16-05 Extension
10-24-05 requires
approval by the IRB.
Designee

APPENDIX C

GENETIC TISSUE AND BLOOD BANKING FOR VACS PROTOCOL

Genetic Tissue and Blood Banking for the Veterans Aging Cohort Study

Amy C. Justice, MD, PhD – Principal Investigator for the Veterans Aging Cohort Study (VACS)

Philip Lavori, PhD – Principal Investigator for CSP #478 Genetic Tissue Banking Initiative

Version 2.4
June 14, 2005

I. Executive Summary

This proposal encompasses two related banking substudies (a DNA Bank and a Blood Bank) to be incorporated into the ongoing Veterans Aging Cohort Study (VACS). These substudies are to be a cooperative venture between VACS, National Institute of Alcoholism and Alcohol Abuse (NIAAA--our funder), and VA Cooperative Study Program (CSP). As we have with all other components of our study, VACS will provide centralized coordination of the 8 sites in our ongoing study including all IRB communications, the contact and consenting of patients (all of whom are already participating in the main VACS study), and all budgetary issues. NIAAA is providing the funding for these substudies as well as for the main VACS study. NIAAA is funding our project as a cooperative agreement and, as such, also has a scientific advisor (Dr. Kendall Bryant) who is a member of the VACS Steering Committee and will have ongoing input on these substudies. Through discussions with Dr. Phil Lavori, Dr. Louis Fiore, and Dr. Michael Gaziano, CSP has initially agreed to participate in this project in two ways. First, the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC) will be the banking processing and storage center for both the VACS DNA Banking and the VACS Blood Banking substudies. Second, all DNA samples will be controlled by the CSP DNA Banking Coordinating Center (DNACC). Specifically, the DNACC will: review and approve all IRB protocols and patient consent documents related to DNA banking, hold the only crosswalk between the DNA sample identification numbers and VACS study ID numbers, and (through the DNACC Scientific Advisory committee, the Ethics Oversight Committee, and the Veterans Advisory Group) have final decision making power regarding all proposals for analyses of VACS DNA samples and all decisions regarding patient safety with respect to VACS DNA sample test results.

We are describing these studies together because we are proposing a single blood draw, a single consent form, and a single IRB submission to cover both substudies. The consent form (see appendix E) makes it clear that the patient can choose to participate in one or both substudies or may choose not to participate in either substudy and still continue participation in VACS. We are convinced that this is the preferred approach. To try to conduct these two studies completely separated from each other would confuse patients, double the paperwork burden for the coordinators and the IRBs at all sites and centers involved. Further, by describing the protocol and the protection of privacy for these two substudies in parallel we emphasis what is alike and different about DNA banking and blood banking in general.

II. Summary of VACS

VACS is sponsored primarily by the National Institute of Alcoholism and Alcohol Abuse (NIAAA) at the National Institutes for Health (NIH) and is supported by Department of Veterans Affairs. VACS is a prospectively consented, 5-year longitudinal, observational study of 3000 HIV+ and 3000 HIV- age-race-site group matched controls receiving care at 8 VA Medical Centers across the country: Atlanta, Baltimore, Bronx, Houston, Los Angles, New York, Pittsburgh and Washington, DC. The West Haven VAMC serves as the VACS Coordinating Center for the 8 sites. The broad objective of VACS is to examine the relationship between HIV disease, HIV treatment, alcohol use, and comorbid disease and the effect of these conditions on patient outcomes in order to design effective interventions. We are in the third year of a 5-year funding period and have enrolled 98% of our target sample.

We propose routine banking of DNA specimens and blood on all consenting HIV positive subjects and a 50% age-race-site stratified sample of the controls. It is also our intent to make DNA and blood specimens available for the future research on HIV, HIV treatment toxicity,

alcohol, and comorbid illness and link the results to the clinical data collected as part of VACS. In addition, our blood banking protocol also encompasses prospective assays not routinely performed (but of scientific merit). This project is one in a series of tissue banking substudies proposed for CSP studies. Like other studies, the storage and management of the DNA samples is under the control of the central CSP DNA Bank. The CSP DNA Bank is responsible for all the ethical, legal, and social issues that arise in such activities, as well as for the physical security of the samples, management of sample-splitting for assay, and maintenance of all resulting data.

The storage and management of other blood banking samples is under the control of MAVERIC with supervision from the VACS coordinating center and PI. The VACS Coordinating Center and MAVERIC are responsible for all the ethical, legal, and social issues that arise concerning blood and blood product management activities, as well as for the physical security of the samples, management of sample-splitting for assay, and maintenance of all resulting data.

The DNA Bank and the Blood Bank are a collaboration among VACS, the National Institute of Alcoholism and Alcohol Abuse, the Department of Veterans Affairs CSP Coordinating Center – Palo Alto, the Human Genetics Center at Stanford University and the Massachusetts Epidemiological Research and Information Center (MAVERIC). The procedures and controls of the DNA Bank and the Blood Bank ensure the protection of individual rights while facilitating the pursuit of responsible medical research and treatment goals. It is designed to be able to serve a wide range of studies and to provide methods and examples to the fields of clinical genetics and pathophysiologic research more generally. The separate procedures and controls of the DNA and Blood Banks ensure the protection of individual rights while facilitating the pursuit of responsible medical research and treatment goals.

The overall purpose and aim of the VACS DNA and Blood Banks are to 1) build a DNA bank for the storage of DNA and plasma from samples taken from VACS participants, 2) build a blood and blood product bank for the storage of blood products taken from VACS participants and 3) run some important clinical assays on blood samples that are not routinely collected on veterans in care. The DNA will be used for future studies of genetic factors associated with HIV, its treatment, alcohol, and comorbid conditions.. The Blood bank will be used for current and future studies of general pathophysiologic factors associated with HIV, its treatment, alcohol, and comorbid conditions. The DNA and Blood banks will continue so long as research continues using VACS data.

III. Introduction and Background

DNA Bank

To fully realize the potential of the new tools being perfected by genomic science, it is necessary to link the genetic information with accurate and detailed clinical information, including onset, course, and outcome of disease. Banking of genetic tissue is the first step in allowing researchers to link the clinical information with disease outcome and is beginning to be adopted as a regular part of clinical trials and observational studies. Eventually genotyping and phenotyping methods may become routine assessments. Well-designed prospective observational studies with genetic tissue banking will provide unique and valuable opportunities to gain insights into the genetic basis of onset and course of diseases and the variation in response to treatments.

HIV/AIDS is a high priority condition for the VA and the VA is uniquely positioned to study the long-term effects of HIV treatment and common comorbid conditions such as alcohol use and abuse. The VA is the single largest provider of healthcare to HIV infected individuals in the United States. Last year the VA cared for more than 19000 veterans with HIV. The VA has a

QUERI and a Technical Advisory Group focused exclusively on the care of those with HIV infection. Finally, care for HIV infection requires treatment with multiple medications from multiple classes for the remainder of the patient's life. Long-term treatment will likely be associated with a host of cumulative drug toxicities that may "masquerade" as age associated comorbidities such as diabetes, heart disease, liver disease etc. Alcohol use and abuse will likely have major ramifications on adherence, drug toxicity, immune response, and risky sexual behavior leading to increased rates of HIV transmission.

The creation of this bank will have significance both for the VA and for the larger U.S. society in understanding the natural history of HIV and associated conditions. The bank assists the VA in achieving its mission to provide for the health of veterans in two primary ways. First VACS provides the VA with crucial epidemiological data on the population of veterans in long-term treatment for HIV infection. Second, VACS will provide an important mechanism for recruiting veterans with HIV into clinical drug trials and other studies that may yield improved outcomes and further understand of this disease. VACS DNA and Blood Banks will provide an unparalleled opportunity for researchers examining the optimal management of HIV.

Blood Bank

While conducting the VACS study, we have noted that many tests of importance to our study aims are not routinely ordered in the course of VA clinical practice. Additionally, many other blood tests are still in development. Many useful tests are expected to be standardized and available in the near future.

We propose routine banking of blood specimens. Our blood sampling protocol would encompass both prospective assays not routinely performed (but of scientific merit) and future tests currently in development.

Additionally, establishing a baseline on VACS patients will provide a sample by which to validate the results of other studies which may lack the diverse patient population or the ability to comprehensively link laboratory data to detailed information about the patient and the clinic in which they receive care.

More specifically, VACS justifies blood banking for the following reasons:

1. To better understand the effect of medical and psychiatric comorbidity on HIV disease we need assays still in development including: standardized tests of HIV-1 fitness (rather than viral load), panels of specific immune function (rather than CD-4 count), assays of viral resistance.

2. To better understand the effect of HIV on medical and psychiatric comorbidity (for example anemia or alcohol abuse) we need the ability to test for causes of anemia such as mean corpuscular volume, reticulocyte count, RDW, B12, folate, iron levels, and erythropoietin levels which are not routinely ordered in the clinics. Additionally, better assays of alcohol exposure are currently in development.

3. We will need banked specimens available for future work in exploring the etiology of observed associations in our cohort. Also, we expect that independent investigators will need specimens from a well-characterized population in order to explore hypothesized pathophysiologic relationships.

IV. The VACS DNA Bank

The VACS DNA Bank will extract and store DNA and plasma from blood samples collected from consenting VACS participants. The primary aim of the VACS DNA Bank is to bank these specimens for future use by investigators interested in the cause of and potential treatments for HIV, alcohol use and abuse, and associated comorbid conditions.

V. Background and Significance

A. VACS

The Veterans Aging Cohort Study (VACS), funded by the NIAAA and now in its 3rd year of 5 years of funding, provides a unique and invaluable infrastructure on which to build DNA and Blood banking and testing. The primary study will include 3,000 HIV positive veterans and 3,000 HIV negative age-race-site group matched veteran controls who have provided written consent for full electronic access to all electronic medical record data (administrative, clinical progress note, laboratory data, all pharmacy data, all pathology data, etc). In addition, all subjects complete baseline and follow-up self completed surveys that include comorbid disease, SF-12 quality of life data, symptom data, substance use data (including a careful characterization of all alcohol, cigarette and illicit drug use), utilization data, and patient satisfaction with care data). Subjects also give permission for their primary provider to complete a survey. The provider survey covers the provider's impressions of patient health behaviors and symptom burden, patient severity of illness, and the providers comfort with managing a list of common comorbid conditions. Half the subjects have also participated in an in-depth telephone interview regarding alcohol use and treatment adherence.

Our long range goal is to design and implement interventions to improve long-term patient relevant outcomes among patients aging with HIV infection complicated by major, common, and often overlapping comorbid conditions. Because alcohol use, abuse, and dependence is common among our patients we will first focus on the role of alcohol in determining outcomes. Future interventions are likely to include computerized risk assessments coupled with reminders for clinicians and nurse-delivered brief motivational interventions.

These interventions require detailed information concerning specific health risks tailored to the individual patient. Thus, our immediate goal is to develop sustained funding for a long-term cohort study of veterans with and without HIV infection to inform intervention design.

Our primary study **specific aims** among HIV positive and negative veterans in care are:

1. Test the unadjusted and adjusted association of alcohol use/abuse (alcohol) with process measures.
2. Test the unadjusted and adjusted association of alcohol use/abuse (alcohol) with outcomes.
3. Assess patient and provider attitudes concerning alcohol use/abuse.

By adding DNA to our protocol, we will be able to determine the degree to which human genetic variation (in susceptibility to HIV, HIV treatment, and comorbid conditions including alcohol abuse) influences important outcomes in our study. By adding Blood Banking and some initial testing to our protocol, we will be able to participate in the development of important markers of viral fitness, alcohol toxicity, mitochondrial injury and other clinical conditions in need of better screening and diagnostic tests. Further, we will also be able to participate in other important research regarding the pathophysiology of HIV, HIV treatment, alcohol, and related comorbid conditions.

B. Genetics, HIV, HIV Treatment, and Comorbid Illness

Human genetics is of increasing importance in the understanding of HIV disease and its management. It has important ramifications with respect to 1) HIV infection and disease progression; 2) HIV treatment effectiveness; and 3) HIV treatment toxicity and comorbid disease.

The course of HIV infection varies widely among individuals(1;2). Long-term nonprogressors may remain asymptomatic with normal CD4 cell counts despite more than a decade of untreated HIV infection. In contrast, rapid progressers develop AIDS within 5 years. In addition, some persons remain uninfected despite repeated exposure to HIV. Human allelic variation has been shown to have important implications for individual susceptibility to HIV infection and disease progression. These polymorphisms can be classified into three general categories (1-3): a) those that control viral entry into susceptible cells (chemokine and chemokine receptor polymorphisms), b) genes involved in immune regulation (e.g., interleukin-10, interleukin-4, tumor necrosis factor-alpha, and mannose-binding lectin), and c) genes involved in the adaptive immune recognition by T cells (e.g., human leukocyte antigen). Further elucidating the functional role of these factors via biotechnological assays is expected to further enhance our understanding of the pathogenesis of HIV-1 infection, and, eventually, to enrich our therapeutic arsenal with novel antiviral agents or strategic treatment approaches.

Human genetics also play an important role in determining the effectiveness of antiretroviral treatment (4). For example, the P-glycoprotein drug efflux transporter is a determinant of oral bioavailability and central nervous system penetration of protease inhibitors and may affect drug penetration to other tissue compartments that can serve as sanctuaries for HIV infection. Polymorphisms in the MDR1 gene regulating P-glycoprotein expression are associated with differences in drug disposition, with some data indicating that different genotypes are associated with differences in plasma PI levels and magnitudes of CD4 cell count recovery under therapy.

Human genetics also play an important role in predisposing individuals to adverse effects of drug treatment, whether these effects are exceptional and dramatic (e.g., abacavir hypersensitivity conferred by HLA-B*5701 and a haplotypic Hsdp70-Hom variant) (5;6) or commonplace and difficult to differentiate from the effects of "true" comorbid disease (i.e., diseases that the patient would have experienced even if they did not have HIV or received antiretroviral treatment). Examples of the latter category include diabetes, heart disease, and hypertension(7-10). Just as genetic susceptibility cause some individuals to be predisposed to these conditions in general, it is likely that genetic susceptibility influences the probability that a patient will develop these conditions when exposed to the added risk of long-term antiretroviral therapy. It is also plausible that there may be important interactions between genetic predisposition and the likelihood of developing the condition (or experiencing an exacerbation) while on antiretroviral therapy.

Of note, human genetics is known to play an important role in determining the likelihood of alcohol abuse and addiction(11-15). As this comorbid condition is a primary focus of our study, we are eager to gain an understanding of the role of genetic predisposition in the development and course of alcohol addiction in our population.

Finally, it is important to note that we have only described some of what is already known about the role of human genetic variation in determining outcomes with HIV and long term HIV treatment. It is likely that new insights may be gained in the future with the use of the VACS DNA Bank.

C. Rationale for Banking DNA in Veterans with HIV and their Controls

While we have justified DNA and Blood Banking in HIV in general, there are also important reasons to do this banking among veterans in care. First, veterans in care with and without HIV are more likely to be African American or Hispanic and from lower socioeconomic status than those participating in other observational studies of HIV infection. There are also at least 10 years older than the national average (median age among veterans in care with HIV infection is 49 years). As such, they provide an ideal group in which to study the combined effects of HIV, long-term HIV treatment, and concomitant comorbid disease. Finally, the VA is the single largest provider of HIV care in the United States. This national system of care will provide an important infrastructure supporting ongoing research on these subjects and in disseminating the findings of our research that have important clinical implications.

D. Overview of VACS

Participants: VACS has nearly completed the baseline enrollment of 3,000 HIV positive veterans and 3,000 HIV negative (age-race-site group matched veteran controls). As of June 11th, 2004, the enrollment has reached 2942 HIV+ and 2929 HIV- or 98% (5871/6000) of our target. Patients are enrolled from the infectious disease clinic (HIV+) and the general medical clinic (HIV-) at 8 separate VA facilities (Atlanta, GA; Baltimore, MD; Bronx, NY; Washington DC; Houston, TX; Los Angeles, CA; New York, NY; and Pittsburgh, PA).

Procedures: Veterans participants are enrolled in their respective clinics after having signed a written consent which includes consent for full access to all electronic medical records, consent to complete a written and telephone survey, consent to contact their physician and consent to be recontacted in the future.

Once enrolled, medical record data is collected at regular intervals using electronic means, the patient and their primary provider complete annual surveys, and a subset of patients complete annual telephone surveys. The patient completed survey includes demographic data (race, sex, income, education), comorbid diseases and HIV associated discases, health behaviors (risky sexual practices, exercise, smoking, weight and height, alcohol and drug use), quality of life (SF-12 and a symptom index), utilization (VA and nonVA), satisfaction with care, and functional status. The provider survey includes information about the provider, their relationship with the patient, their comfort with managing comorbid diseases, their assessment of how sick the patient is and their awareness of the patients health behaviors and comorbid conditions.

VI. *Previous DNA Bank Work*

To define a standard methodology for managing genetic information from clinical trials, the VA CSP has established a central DNA Bank for its studies. The VACS is one in a series of clinical trials to make use of the DNA Bank. The VACS-DNA Bank represents a collaboration among the Palo Alto Cooperative Studies Program Coordinating Center (CSPCC), the Massachusetts Veterans Epidemiologic Research and Information Center (MAVERIC), the Human Genetics Center at Stanford University, and the VACS Coordinating Center.

A. Palo Alto Cooperative Studies Program Coordinating Center (CSPCC)

This current project builds on the experience of the Palo Alto CSPCC DNA Bank Coordinating Center (DNACC) in establishing and overseeing DNA banks for 1) CSP#395: Beta-Blocker Evaluation of Survival Trial (BEST), co-sponsored by the DVA CSP and the National Heart, Lung and Blood Institute, 2) CSP #410: Iron (Fe) in Atheriosclerosis Study (FeAST), 3)

CSP#453: The Homocysteine Study (HOST) and 4) CSP#481: The Home INR Study (THINRS), all sponsored by the DVA CSP. These banks were established to provide a resource for investigators examining possible genetic links in heart failure, atherosclerosis, kidney disease, and thrombotic disease and sensitivity to blood thinners, respectively, and to help scientists identify at-risk populations who may benefit from early intervention.

DNA sample collection for the BEST DNA Bank began in 1996 and continued through 1999 when the trial terminated. Over 1,000 blood samples were collected from BEST study participants with Class III or IV heart failure. The DNACC designed, implemented, and oversaw the BEST DNA Bank sample collection. Currently the DNACC oversees the use of the BEST DNA in collaboration with the BEST DNA Bank Oversight Committee. The Oversight Committee is an independent panel comprised of cardiologists, medical ethicists, epidemiologists, biostatisticians, attorneys specializing in medical ethics, a patient advocate, and sponsor representatives. The Committee is charged with evaluating the scientific and ethical merit of all proposals for use of the BEST DNA. In addition, the DNACC works with outside investigators to carry out statistical analysis and prepare manuscripts based on DNA analyses. The first manuscripts to come out of the BEST DNA Bank work are expected in late 2005.

The DNACC's experience with the BEST DNA Bank was the foundation for the CSP #478 Genetic Tissue Banking effort. In 1999 the DNACC began a collaboration with MAVERIC to begin banking samples from CSP studies. In 2000 we began DNA collection for our demonstration project, the FeAST study. DNA Bank sample collection for the HOST study began in 2002. We continue to bank samples from both the FeAST and HOST studies. We do not expect to utilize the DNA banked as part of these studies until the trials terminate. . Currently the DNACC is gearing up to begin DNA Sample collection for the THINRS trial. THINRS DNA Bank enrollment is expected to begin in September 2003.

The DNACC manages the FeAST-DNA, HOST-DNA, and THINRS-DNA banks with the help of three Oversight Committees, the Scientific Advisory Committee, the Ethics Oversight Committee and the Veterans Advisory Group. The Committees aid in guiding the DNA Bank policy and collection methods. In addition, the DNACC maintains the secured DNA Bank database and crosswalk coding systems. The database and crosswalk protect the confidentiality of the DNA specimens.

B. Massachusetts Veterans Epidemiology Research and Information Center

The Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC) was established in 1997 as one of three centers within the DVA to foster epidemiologic research in veterans and disseminate relevant findings to the VA community. A Core Laboratory was created within MAVERIC at the VA Boston Health Care System (Jamaica Plain Division) to provide VA researchers with a convenient, high-quality and low-cost mechanism to include biological specimen handling, storage and analysis in clinical studies. The laboratory is a fully-equipped, state-of-the-art biological specimen collection and processing center.

The laboratory quickly became nationally recognized through its participation in the DNA Bank project (CSP #478) and soon established itself as a prototype for tissue banking facilities. A recently completed expansion project established a liquid nitrogen freezer farm that doubled the storage capacity of the laboratory (to store approximately 1,000,000 aliquots each of 1.5 ml).

The laboratory has the ability to supply customized blood kits and to coordinate the collection, processing and storage of serum, plasma, buffy coats and other biological specimens. The

laboratory has incorporated real-time DNA extraction into the standard operating procedures to improve the quantity and quality of DNA obtained from whole blood. In addition to providing technical support regarding specimen collection and shipping, MAVERIC Core Laboratory staff assists researchers in understanding the vagaries of specimen analysis, quality control and storage.

Currently the MAVERIC Core Lab houses approximately 200,000 samples from over 15,000 veterans. The following studies have banked specimens at the laboratory:

Two Completed Collections:
CSP#387 Combination Hemotherapy and Mortality Prevention: CHAMP
CSP# 458 National Survey of Gulf War Veterans

Ten Ongoing Collections:
CSP# 453 Homocysteine-Lowering Survival Trial - HOST
CSP#465 Glycemic Control and Complications in Diabetes Mellitus Type 2
CSP#465 Glycemic Control and Complications in Diabetes Mellitus Substudies
CSP#478 Genetic Tissue Bank; Iron in Atherosclerosis Study - FeAST
CSP# 478 Genetic Tissue Bank; Homocysteine-Lowering Survival Trial - HOST
CSP#500 The Occurrence of ALS Among Gulf War Veterans
CSP#512 Options In Management with Anti-Retroviral - OPTIMA
CSP#710B Normative Aging Study
CSP#714B Prevalence and Determinants of Osteoporosis - VALOR
CSP#719B Early Stage Prostate Cancer Study
CSP#499a Aging Cohort to Improve Veterans' Health - Active

Three Transferred Archived Collections:
Normative Aging Study (archived specimens)
CSP#363 HDL Intervention Trial -HIT
CSP#366 A Genetic Linkage Study of Schizophrenia

VII. VACS DNA and Blood Banks Organization

This proposal encompasses two related banking substudies (a DNA Bank and a Blood Bank) to be incorporated into the ongoing Veterans Aging Cohort Study (VACS). We are describing these studies together because we are proposing a single blood draw, a single consent form, and a single IRB submission to cover both substudies. The consent form (Appendix E) makes it clear that the patient can choose to participate in one or both substudies or may choose not to participate in either substudy and still continue participation in VACS. We are convinced that this is the preferred approach. To try to conduct these two studies completely separated from each other would confuse patients, double the paperwork burden for the coordinators and the IRBs at all sites and centers involved. Further, by describing the protocol and the protection of privacy for these two substudies in parallel we emphasis what is alike and different about DNA banking and blood banking in general.

Within these two separate substudies, there are some separate and some shared components. These components, described below, provide the VACS DNA and Blood Banks with adequate resources to enroll patients and collect both DNA and blood specimens, to conduct analyses, to store the specimens for future use, to manage and maximize the scientific use of both DNA and blood banks, and to address the ethical, legal, and social implications of this material.

Shared Components

VACS Coordinating Center: The VACS Coordinating Center is located at the West Haven, CT VA and coordinates all activities of the 8 sites participating in our study. VACS personnel will be responsible for recruiting patients, obtaining informed consent, and coordinating the draws for both DNA Bank and Blood Bank samples. As they are for all other aspects of our study, VACS personnel (the VACS study coordinator, supervised by the VACS PI, Dr. Amy Justice) will be responsible for updating consent information and handling IRB queries related to collection of DNA and Blood specimens and protecting the subject confidentiality. When needed, the VACS coordinator will forward questions concerning DNA banking issues to the DNA coordinating center and then, in turn, circulate the final response to VACS sites. The VACS Coordinating Center will also insure that all required documentation is forwarded to MAVERIC and to the DNA Bank Coordinating Center. This organization will help insure that each site coordinator has "one stop shopping" for all VACS related questions. TheVACS study coordinator in West Haven will in turn log all questions and ensure that they are appropriately referred. The VACS study coordinator will also ensure that a timely response is received at the sites. This will help maintain the strong working relationships we have carefully cultivated at all our participating sites during the course of our study.

The VACS Coordinating Center will serve as to first contact for all proposals regarding testing of DNA or Blood Banked specimens. Proposals will be submitted on standard forms (Blood Bank proposals on the existing VACS subanalysis proposal (Appendix f) found on our website: www.vacohort.org; The Statement of Research Intent for DNA Analyses Form [Appendix G], once approved, will also be made available on our website). The VACS PI, Steering Committee, Site PIs/CoPIs, and a representative from the Community Advisory Board will initially vet all proposals for feasibility and scientific merit. All proposals for DNA banking specimen analyses will then be forwarded with comment to the DNA Coordinating Center for external review. Proposals for Blood banked specimen analyses will be directly approved, rejected, or revisions requested by the VACS Coordinating Center after undergoing our internal review. This includes review by the Tissue Committee for issues of scientific merit and feasibility and then full review by the Steering Committee (includes a Community Advisory Board member) and the Site PI/CoPI committee.

Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC): A collaboration between VA Cooperative Studies Program, the VA Boston Healthcare System and the Boston and Harvard University Schools of Medicine and Public Health, MAVERIC is a national VA resource to foster epidemiologic research with a core laboratory for blood specimen processing and storage. MAVERIC is a fully approved VA Tissue Bank and will serve as the processing and storage center for both the DNA (Genetic Tissue Core Laboratory) and Blood (Tissue Core Laboratory) Banks described in this study (for details of each, see below). MAVERIC will provide supplies for the collection of specimens for DNA and blood banks, receive, process, and separate DNA and Blood specimens for separate storage and handling.

Separate Components

Tissue Core Laboratory (TCL): Housed at the MAVERIC laboratory, the TCL serves as a central repository for blood specimens from study populations studied in DVA CSP clinical trials. The TCL has the capacity to track, process and store blood, cells, serum, and plasma specimens. Under the direction of the VACS Coordinating Center, the Core Laboratory ships blood specimens to investigators with approved requests for use of the Blood bank.

Genetic Tissue Core Laboratory (GTCL): Housed at the MAVERIC laboratory, the GTCL serves as a central repository for DNA specimens from study populations studied in DVA CSP

clinical trials. The GTCL has the capacity to a)extract and store DNA, b) immortalize cell lines where indicated, and c) perform certain DNA analyses. Under the direction of the DNA Coordinating Center, the Core Laboratory ships DNA specimens to investigators with approved requests for use of the DNA bank.

DNA Bank Coordinating Center (DNACC): A subunit of the Palo Alto CSP Coordinating Center, the DNACC administers the DNA banks for all CSP studies in its portfolio, and provides statistical expertise within the CSP for genetic analyses. The DNACC provides guidelines for the collection of DNA specimens, including a template informed consent document for use in studies. Regulatory documents for the DNA bank will also be maintained at the DNACC. Research staff at the DNACC will provide or obtain the expertise needed to deal with the ethical, legal, and social considerations associated with banking and using genetic tissue.

DNA Scientific Advisory Committee (SAC): A group of individuals with expertise in genetics, epidemiology, molecular biology and specific disease areas has been assembled to help guide policy for the use of the bank and to provide technical and scientific advice to the DNA Coordinating Center. The Scientific Advisory Committee reviews new CSP studies during the planning phase, and determines which of those studies should be considered for genetic tissue collection. It makes recommendations concerning the specimens that should be collected (e.g., buccal or blood cells) and advises on issues such as the proper quantity of specimens for banking, storage requirements, and additional clinical information that should be collected to ensure the utility of the DNA specimens for later analysis. The Scientific Advisory Committee meets periodically to review and recommend approval of proposals for use of the bank. A list of the Scientific Advisory Committee members is provided in Appendix B. The Study Chair (or designated investigators with relevant expertise) of each client study participates in the SAC discussions and decisions regarding the use of samples from that parent study.

DNA Ethics Oversight Committee (EOC): This committee meets regularly to review provisions for the protection of human research volunteers, and to provide a review of the activities of the DNA bank (including utilization of samples). It reports to the VA Chief Research and Development Officer through the Palo Alto Coordinating Center, much as the Data Monitoring Boards and Human Rights Committees of other CSP studies. It is composed of experts in the legal and ethical implications of genetics research in humans, as well as experts in the relevant scientific disciplines. The Ethics Oversight Committee membership list is provided in Appendix C.

DNA Veterans Advisory Group (VAG): A group of veterans who provide their perspectives on the ethical and social implications of DNA banking in veteran subjects of CSP studies. The advice of this group helps to shape future operations of the DNA bank, which is designed to adapt to meet the changing social consensus on the use of such information. Some examples of agenda items include the "disclosure" provisions (whether, when, and how to inform subjects about genetic information that may be important to them or their families), subgroup sensitivities to certain research topics (minority group differences in genetic vulnerabilities and the genetics of stigmatized disorders, for example), and commercial use of DNA Bank information. The Veterans Advisory Group will meet routinely to evaluate new research proposals for the DNA specimens that seek to study conditions not already specified in both the informed consent and protocol. As members of this group are themselves veterans receiving care, this step will help insure that any new research will serve to improve veterans' health care.

Community Advisory Board (CAB): The Community Advisory Board consists of a group of veterans already enrolled in the Veterans Aging Cohort Study. The group comprises veteran

representatives from all 8 VACS study sites and meets quarterly by conference call. Members of this advisory board have considerable input into survey design, procedures to protect confidentiality, and the future direction of VACS projects. This board will be consulted before beginning blood banking or DNA banking, and members will be fully informed as to procedures to be implemented. They will also evaluate new research proposals that will utilize data from the blood bank specimens and that seek to study conditions not already specified in the consent form and the study protocol. As members of this group are themselves veterans receiving care, this step will help to insure that any new research will serve to improve veterans' health care.

VIII. Study Design and Methods

A. Subject Selection

All subjects previously enrolled in the main VACS observational study are eligible to participate in either the DNA or blood banking protocols. We will collect samples on all consenting HIV positive subjects and 50% of HIV negative subjects over an 18-24 month period. Assuming an 80% participation rate, this will result in 2400 HIV+ and 1200 HIV- subject samples or 3600 subject samples overall.

B. Informed Consent (Appendix E)

The DNA and Blood Bank informed consent process is independent of the VACS informed consent process. After enrollment in VACS is completed, we will provide veterans with information about DNA and blood banking and obtain informed consent from veterans who are willing to participate in this component of the study. Veterans may choose to participate in VACS but decline to take part in the DNA and/or the Blood banking component. Consents will be handled using the established mechanisms in VACS.

During the consent process, study personnel will discuss the potential risks with every interested participant to ensure as full an understanding as possible. It will also be explained that a certificate of confidentiality has been obtained from the Department of Health and Human Services to protect against involuntary disclosure that could expose subjects and their families to potentially adverse economic, legal, psychological, and social consequences.

C. Collection, Handling, Shipping, and Storage of Specimens

All samples will be collected in VA Medical Centers currently participating in VACS. Once a veteran consents to participate in either the DNA Bank or the Blood Bank or both, study personnel will use the Vacutainer System (standard in VAMC clinics, butterfly needles will be used only on patients in whom Vacutainers was infeasible) to collect the specimens.

If the patient consents only to DNA banking 18 mls (2 9 ml plastic tubes) of blood will be collected in EDTA. If the patient consents only to Blood banking 9.5 ml (1 tube) will be collected in SST and a total of 30 mls or 4 tubes=[(5 tubes, 9 ml each)+ (1 tube X 3 ml)] will be collected in EDTA. Thus, if a patient participates in both studies, a total of 7 tubes and 57.5 mls or less than 4 Tablespoons of blood will be drawn.

Before shipping the samples to MAVERIC, the 3 ml. EDTA tube of blood from the Blood banking collection will be sent to the local site laboratory for a complete blood count with platelets, differential, MCV, RDW and reticulocyte count and this data will be entered into the

clinical laboratory report as well as forwarded to the VACS coordinating center. The remaining specimens will be shipped overnight to the Massachusetts VA Epidemiology Research and Information Center (MAVERIC) at the Boston VAMC, for processing and banking.

The site coordinator will place preprinted coded numerical label (specimen code) on each DNA and blood sample set to ensure that no identifying subject information is sent to MAVERIC. For all samples, the site coordinator will place a duplicate preprinted coded label on the Participation Form and fax it to the DNACC. At the DNACC, the information from the case report form will be entered into a protected crosswalk database that links clinical and DNA information. The site coordinator will send a copy of the Participation Form to the VACS coordinating center to be filed with the patient consent forms. At the VACS Coordinating Center, the information from the case report form will be entered into a protected crosswalk database that links clinical and blood banking information.

Once a specimen arrives at the GTCL, it will be assigned a GTCL barcode number. The GTCL will prepare the following aliquots for the DNA Bank:

4 vials of EDTA plasma (2 per 9 ml EDTA)
2 vials of buffy coat (1 per 9 ml EDTA)

The plasma will be transferred to labeled cryovials. DNA will be stored at –80C; plasma will be stored at -80C or in liquid nitrogen tanks. All samples will be stored in two separate freezers in the unlikely event that a freezer undergoes an unplanned thaw cycle.

Once a specimen arrives at the TCL, it will be assigned a TCL barcode number. The TCL will prepare the following aliquots for blood banking:

2 vials of serum (2 per 9.5 ml SST)
6 vials of EDTA plasma (2 per 9 ml EDTA)
4-6 vials of PBMC (5 x 10^6 per ml)
2 vials of RBC

Serum and plasma will be separated from cells and transferred to labeled cryovials. Serum, plasma, and RBC specimens will be stored at -80C or in liquid nitrogen tanks. PBMC will be stored in freezing medium at -150C in vapor phase liquid nitrogen tanks. All samples will be stored in two separate freezers. DNA and Blood samples will be maintained as long as ongoing data analyses continue in the VACS.

Management of Identifying Information—DNA Bank

At regular intervals, the GTCL personnel will provide the DNACC with a log that contains the specimen code numbers (from the preprinted labels used at the site at collection time) that correspond to the GTCL barcode numbers they have assigned at processing time. The GTCL then destroys the log ensuring that the only link to the specimen code and the barcode resides at the DNACC (VACS can connect the patient study ID to the specimen code, and the GTCL can connect the specimen code to the barcode, but without the log, neither can connect the study ID to the barcode). The DNACC will implement additional coding systems if determined necessary to protect the confidentiality of participants. In total, the DNACC will maintain three separate datasets: the clinical study data, the DNA assay results, and the crosswalk between the two sets of numbers indexing subjects in these databases. This will assure confidentiality while providing a mechanism to ultimately link the genetic information to the clinical data. The GTCL will receive only numerically coded specimens and information on the specimen type date and time obtained and conditions of collection. No identifying information that could potentially link the specimen to the subject will be maintained at the GTCL database. Transfer of genetic material to other laboratories for analysis will be controlled by the DNACC.

Management of Identifying Information—Blood Bank
At regular intervals, the TCL personnel will provide the VACS Coordinating Center with a log that contains the specimen code numbers (from the preprinted labels used at the site at collection time) that correspond to the TCL barcode numbers they have assigned at processing time. The TCL then destroys the log ensuring that the only link to the specimen code and the barcode resides at the VACS Coordinating Center (the TCL can connect the specimen code to the barcode, but without the log, neither can connect the study ID to the barcode. The TCL will receive only numerically coded specimens and information on the specimen type date and time obtained and conditions of collection. No identifying information that could potentially link the specimen to the subject will be maintained at the TCL database. Transfer of blood and blood product material to other laboratories for non human genetic analysis will be controlled by the VACS Coordinating Center.

IX. Application Procedures and Bank Utilization for Future Studies

The VACS DNA bank is open to VA and non-VA scientists in academia and industry who submit written proposals describing the intended use of the DNA for a specified disease population and outlining genetic analyses. Proposals will be initially screened for feasibility and priority by the VACS study team and they will make nonbinding recommendations to the DNA bank. Proposals have to meet the scientific and ethical standards determined by the bank advisory committees, the DNACC and DVA CSP. The application procedure is a multi-step process depicted in Figure 2 and described below:

1. The investigator submits a Statement of Research Intent (SORI) to the VACS study team. The SORI is a brief description of the research (App. E). They review the SORI and make nonbinding recommendations to the DNA Scientific Advisory Committee.

2. The Scientific Advisory Committee will review the SORI and decide if the research is feasible and meritorious or reject the proposed research.

3. If the SAC accepts the SORI, the investigator will be requested to submit a detailed proposal within a specified time period.

4. The SAC will review the detailed proposal and, if accepted, will recommend review by the Ethics Oversight Committee.

5. The Ethics Oversight Committee will review the proposal and consider the ethical implications of the research. If the EOC accepts the proposal, then the investigator will be notified that DNA Bank access will be granted.

6. If the SAC or the EOC has concerns about the proposal they may request that the CSPCC HRC review the proposal to ensure the research is ethical and appropriate.

7. In order to keep veterans involved in the process, any one of the Committees may request the input from the Veterans Advisory Group by submitting specific issues for review. In addition, the Veteran's Advisory Group will be consulted if a proposal seeks to study a condition other than those listed in the informed consent: substance use and addiction, vascular disease, diabetes, liver injury, mental illness, and infectious diseases.

Once access to the Bank has been granted to the investigator and the recipient institution, the official who can make legal commitments on behalf of the applicant institution will be required to sign a Material Transfer Agreement. The Material Transfer Agreement summarizes the terms and

conditions of the agreement to access the DNA Bank, to include transfer of DNA specimens. This statement addresses the assumption of all risks and responsibility in connection with the receipt, handling, storage and use of DNA samples by the recipient institution. It further commits the client to indemnify and hold harmless the VA CSP and the Genetic Tissue Core Laboratory from any claims, costs, damage, or expenses resulting from the use of tissues provided by the DNA bank.

A. Statistical Analysis and Requests for Clinical Data

The DNACC serves as the gatekeeper to all requests for analyses involving linked genetic and clinical data. The VACS Coordinating Center serves as the gatekeeper for all clinical data requests. The VACS Coordinating Center, SAC, and the EOC will review all requests. VACS Coordinating Center, using their established protocol, will review all clinical data variables requested. SAC and EOC will review genetic assays proposed. The DNACC will obtain other approvals, as it considers necessary. In order to preserve the confidentiality of the linked genetic and clinical data to the greatest extent possible, and to ensure the appropriateness of the statistical analyses, the VACS Coordinating Center and the DNACC will collaborate with the requesting investigator to perform the analyses required for the research proposal. The investigator will provide the results of genetic analyses in a mutually agreed upon format to the DNACC and these results will become part of the DNA Bank database.

In the event the DNACC is unable to conduct the analyses within a reasonable time frame, the DNACC may agree to prepare an anonymized data set for the investigator, linked with the genetic data by the GTCL barcode number. The data set and data dictionary will be provided based on the investigator's needs, with the review and approval of the SAC, EOC and concurrence of the DNACC, subject to final approval by the CRDO.