BREAST CANCER RISK IN HIV: SOCIODEMOGRAPHIC FACTORS AND VIRAL ETIOLOGY

by

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Abstract

Background: Epidemiologic studies suggest that the risk of breast cancer is lower comparing women with versus without HIV; however, it remains unclear why. Evidence indicates that sociodemographic differences between women with and without HIV do not completely explain this phenomenon. The overarching goal of this dissertation was to assess breast cancer risk factors and incidence among women with HIV to explore potential mechanisms driving this association. Specifically, this dissertation sought to: (aim 1) characterize breast cancer trends over time accounting for the competing risk of death, (aim 2) quantify differences in estrogen comparing women with versus without HIV, and (aim 3) assess the association between HIV viremia and breast cancer risk.

Methods: For aims 1 and 3 of this dissertation, the large size and representativeness of the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) was leveraged to conduct two prospective, longitudinal cohort studies. Both aims were assessed from 1996-2016 among women with HIV who initiated antiretroviral therapy (ART) and had no history of any cancer. For aim 2, differences in estrogen comparing women with versus without HIV were examined using a cross-sectional analysis within a nested substudy of the Women's Interagency HIV Study, a longitudinal prospective cohort of women with and without HIV across the United States.

Results: Breast cancer risk did not change over time after accounting for the competing risk of death, though there were significant declines in all-cause mortality. There were no clinically impactful differences in total or free estradiol comparing women with versus without HIV. There was also no association between HIV viremia (measured using cumulative viral load on ART) and breast cancer risk among women with HIV who

initiated ART. When the exposure (HIV viremia) was lagged, increasing viremia signaled an inverse association with breast cancer, which got stronger with longer exposure lag.

Conclusions: Declining mortality in women with HIV did not influence trends in breast cancer risk over time and may not contribute to the reduced risk of breast cancer observed among women with HIV. There were no differences in total or free estradiol among premenopausal women with and without HIV; however, this should continue to be investigated in postmenopausal women, where breast cancer risk is highest. Finally, although no significant association was observed between HIV viremia and breast cancer, estimates trending in a protective direction should be further explored. As women with HIV continue to age, becoming increasingly at risk for breast cancer, understanding potential differences in etiology is needed and continuing appropriate breast cancer screening remains an important aspect of clinical care.

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Chapter 1: Introduction

HIV in the United States

State of the US Epidemic: Declining Mortality and an Aging Population

As of 2018, there were approximately 1.1 million individuals living with HIV in the United States (US).[1] People with HIV (PWH) in the US represent several distinct sociodemographic groups (key populations) that all differ considerably from the general US population. Since the first diagnosis of HIV in the US, there have been substantial changes in mortality. In the 1980s and early 1990s, mortality among PWH was incredibly high. Among individuals diagnosed with AIDS between 1981-1986, 50% died within that same time period.[2] The establishment of effective antiretroviral treatment (ART) in 1996 led to immediate and dramatic declines in mortality.[3–6] One study found that, among people diagnosed with AIDS, there was a 32.5% annual percent decline in HIV-attributed mortality from 1995-1998.[6] Large decreases in mortality continued to be observed in the 2000s, with the most notable declines in HIV-attributed deaths.[4,6–9] In the last decade, though mortality is still declining, the downward trend has stabilized.[10] Now, latest national estimates (2017) indicate that among PWH, 66% of deaths were attributed to non-HIV related causes.[10]

With these remarkable declines in mortality among PWH, life expectancy is now approaching that of the general population.[11–14] As of 2013, a 20-year-old with HIV who is treated with ART is expected to live into their early 70s, based on estimates from a nationally representative sample of PWH in the US and Canada.[12] In a more contemporary analysis of this sample comparing life expectancy in 2004-2007, 2008-2011, and 2012-2015, there were increases in life expectancy overall, though disparities in improvements were evident by race among men who have sex with men (MSM) and women.[14] Black MSM and Black women both had relatively lower improvements in life expectancy compared to white MSM and white women across each time period.[14]

Effective ART regimens consist of three or more different HIV medications. There are several classes of available HIV medications that each act on different mechanisms to prevent viral replication: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, CCR5 antagonists, post-attachment inhibitors and integrase inhibitors.[15] Integrase inhibitors were first made available in 2007, are now the most commonly prescribed class, and are the recommended first-line regimen for treatment.[16] Over time, treatment guidelines were also revised to treat PWH at higher thresholds of CD4 count and earlier in infection, and now advocate for treating all individuals with HIV as soon as possible following diagnosis, irrespective of immune status.[16]

These advances in treatment and clinical guidelines have led to a shift in the age distribution of PWH in the United States—now a population mostly 50 years and older,[1] and increasingly at risk for several age-related morbidities. Such conditions include cardiovascular disease,[17–20] bone density loss,[21,22] kidney[23,24] and liver disease,[25–27] diabetes,[28–30] and non-AIDS-defining cancers (NADCs).[31,32] The risk of age-related, non-AIDS defining comorbidities in PWH is exacerbated not only by increases in life expectancy, but also by ART treatment side effects, chronic inflammation, and the high prevalence of smoking.

Protease inhibitors and nucleoside/nucleotide reverse transcriptase inhibitors have been associated with lipodystrophy.[33–37] More contemporary regimens, namely integrase inhibitors, have also been associated with weight gain, especially among women with HIV.[38] Other metabolic complications have been observed in treated HIV including dyslipidemia and insulin resistance.[39–43] PWH on treatment also experience chronic inflammation and immune activation at younger ages than in the general population, putting them at increased risk for age-related health outcomes at younger

ages.[44] The prevalence of smoking is also high among PWH in the US, with estimates ranging from 40-70% versus 16% in the general US population.[45–51] Taken in sum, as the age distribution of this population shifts to older ages, their older age combined with these additional risk factors associated with HIV infection leaves PWH at increased incidence and risk for these comorbidities relative to the US general population.

HIV in Women in the United States

Women with HIV represent 25% of PWH in the United States.[1] Though men have a higher incidence in the US compared to women, women appear to experience more severe disease. One study found that though women have 40% less circulating HIV virus, they have a 1.6 times higher risk of AIDS when matched on HIV viral load.[52] Women with HIV in the US represent a diverse subpopulation with substantial clinical and demographic differences by race. Women with HIV are predominantly Black (58%), followed by Hispanic (20%), and white (16%).[1] Black women with HIV are more likely to have a higher body mass index (BMI) compared to white women with HIV.[53–55] Though mortality among women with HIV overall has substantially declined, Black women with HIV have higher mortality compared to white women with HIV: in 2018, the mortality rate among Black women with HIV was 12.2 per 100,000 compared to 0.2 per 100,000 in white women.[1] In 2015, Black women with HIV were also less likely to achieve viral suppression compared to white women with HIV.[56]

Women with HIV in the US face unique challenges in the context of this aging population. With the majority of PWH over age 50, most women with HIV are going to be entering menopause (median age at menopause in the US general population is 51 years).[57–59] Though some studies report earlier onset of menopause comparing women with versus without HIV, the research is equivocal.[60–64] Clarifying the onset of

menopause is complicated by the high prevalence of women with HIV reporting amenorrhea, with estimates ranging from 5%-48% compared women without HIV.[65–67] A meta-analysis (5 studies in the US and one in Nigeria) found a 70% increased odds of amenorrhea comparing women with versus without HIV.[68] Importantly, in one of the US studies, women with HIV were three times more likely to report amenorrhea compared to women without, and most had follicle stimulating hormone (FSH) levels below 25 mIU/mL, which is consistent with premenopausal concentrations.[61] Other studies in the United States have also found that Mullerian Inhibiting Substance (a marker of ovarian reserve) was comparable by HIV status.[60,62,63]

The association with amenorrhea but not biomarkers of ovarian reserve indicates that HIV status may be not be associated with earlier menopause (primary ovarian failure); but rather, is associated with secondary ovarian failure.[60] Though gonadal dysfunction has been well documented in men with HIV, little is known about women with HIV. Such gonadal dysfunction in women could be the result of several factors including stress, chronic inflammation, and higher prevalence of drug use and smoking relative to the general US population.

Cancer Burden in People with HIV

The burden of cancer among PWH can be broadly characterized by the incidence of NADCs as opposed to AIDS-defining cancers (ADCs), which are Kaposi Sarcoma, cervical cancer, and Non-Hodgkin Lymphoma. This distinction between NADCs and ADCs has implications for the etiology and burden of these diagnoses in PWH. Specifically, ADCs are attributed to severe immune suppression and uncontrolled viremia in PWH.[69] By contrast, NADCs are associated with not only HIV-related factors (e.g. chronic inflammation), but more broadly are associated with demographic and

lifestyle factors such as race/ethnicity, smoking, and obesity. Certain NADCs may also have viral etiology related to coinfection with HPV, cytomegalovirus, or hepatitis B and/or C.[31]

Prior to the advent of effective ART, ADCs were the predominant cancer types among PWH, with Kaposi Sarcoma being the most common, followed by Non-Hodgkin Lymphoma, and then cervical cancer.[70] In the era of effective treatment, the burden of cancer diagnoses in PWH is shifting such that the incidence of ADCs continues to decrease while NADCs are rising.[69–71] In the HIV/AIDS Cancer Match Study, NADCs comprised only 8% of all cancers among people with AIDS in 1991-1995, and increased to 48% of all cancers in 2001-2005.[32] Currently, NADCs are the leading non-AIDS cause of death among PWH.[72] Lung cancer, anal cancer, liver cancer, and Hodgkin's Lymphoma are among the most common NADCs in PWH.[73,74] In fact, compared to the US general population, the risks of lung, liver, and anal cancer are higher in PWH.[73–76]

Though the etiology underlying this excess cancer risk in PWH is currently under investigation, it is possible that chronic inflammation associated with HIV and a higher prevalence of viral coinfections among PWH play a role.[31,77] Though the risk of NADCs are generally higher or comparable to that of the general US population, studies have also demonstrated that the risk of breast and prostate cancer are lower in PWH.[78–83] The hormone-dependent nature of both breast and prostate cancer motivates investigation into hormonal pathways that could confer a reduction in risk.

Breast Cancer in Women with and without HIV

Breast Cancer Epidemiology in the General US Population

Female breast cancer (henceforth referred to as breast cancer) is the most common cancer diagnosis among women in the US (30% of all cancers in women), with

an estimated 281,000 diagnoses for 2021, and is the second leading cause of cancer death in women.[84] The lifetime risk for breast cancer in the general population is 13%.[85] Breast cancer is a diagnosis associated with older age, with the mean age at diagnosis of 62 years.[85,86] With increases in breast cancer screening, approximately 60% of breast cancer diagnoses are classified as local cancers.[85]

Though there has historically been a disparity in breast cancer incidence by race, with a lower risk among Black women compared to white women, this disparity has narrowed over time, with the risk in both groups now almost equivalent.[87] This narrowing has been attributed to declines in breast cancer incidence among white women, and modestly increasing rates in Black women.[87] The decrease in breast cancer incidence is thought to be related to declines menopausal hormone therapy use following observations of increased adverse effects.[88–90] Reasons why Black women have not experienced similar declines in incidence remain unclear, but could be related to the lower use of menopausal hormone therapy comparing non-Hispanic Black to non-Hispanic white women at that time.[91] Anderson and Barrington have hypothesized that this could also be related to changes in body mass index (BMI), or differences in clinical care for Black compared to white women that lead to differential rates of hysterectomy and reporting of vasomotor symptoms,[88] but further research is needed to confirm these hypotheses.

Recent evidence indicates that there are racial differences by age group: in women who are younger than 60, breast cancer risk is higher in Black compared to white women, while for women over 60, the risk is higher for white compared to Black women.[87] There is also a persistent disparity in breast cancer mortality: the mortality rate is 40% higher in Black compared to white women.[87] There are further racial disparities by stage at diagnosis, with 56% of breast cancers classified as local stage in Black

women compared to 66% among white women.[85] These disparities reflect the underlying social construct of race- socioeconomic and cultural differences in the context of systemic structural inequities- rather than a biological association. Several other risk factors contribute to breast cancer occurrence including genetic predisposition and family history of breast cancer, obesity, and high estrogen levels.

Breast Cancer Epidemiology in Women with HIV

Breast cancer has not been well characterized among women with HIV, in part due to the lack of sample size for sufficient cases to arise. In the US, women with HIV who are diagnosed with breast cancer are predominantly Black, with estimates ranging from 49-64%.[80,92] In one study, 40% of women with HIV diagnosed with breast cancer were between the ages of 40-49, followed by 33% in the 50-59 age range.[80] In the same analysis, 37% of women had a prior AIDS diagnosis;[80] though this has not necessarily been associated with an increased risk of breast cancer among women with HIV.[93] As observed in the general population, most breast cancer diagnoses among women with HIV are local stage; however, women with HIV are more likely to be diagnosed at advanced stages of disease compared to women without HIV in the US.[79,80,92,94]

Comparison of Breast Cancer Incidence and Mortality in Women with versus without HIV

Few large studies have assessed the association between HIV status and breast cancer risk. The largest analyses were conducted in the HIV/AIDS Cancer Match Study (HACM, a linkage of HIV and cancer registries in the US). The first analysis found a lower risk of breast cancer comparing women with AIDS to women in the general population (standardized incidence ratio [SIR] of 0.69 (95% confidence interval [CI] 0.62–0.77)) in 1980-2002.[79] Within a subset of HACM, a similar analysis was completed among women with only HIV and assessed breast cancer diagnoses in the 5-years

following HIV registration. Here, a non-statistically significant decreased risk was observed (SIR=0.80, 95% CI 0.5, 1.10).[95] The most contemporary analysis included women with HIV/AIDS in the era of effective treatment (1996-2012) and found a lower risk of breast cancer compared to uninfected women (SIR= 0.63, 95% CI 0.58 to 0.68).[80]

Beyond HACM, another study found a reduced risk of breast cancer in the early treatment era (standardized rate ratio [SRR]=0.70, 95% CI 0.30, 1.90), and a null effect in the effective treatment era (SRR=1.10, 95% CI 0.70, 1.80).[78] A small study conducted within the Women's Interagency HIV Study (7 breast cancer diagnoses) found a 30% reduced risk comparing women with versus without HIV.[96] Another small analysis (3 breast cancer diagnoses) at the Johns Hopkins University AIDS Service clinic found a non-significant 40% reduced breast cancer risk (SIR=0.60, 95% CI 0.10, 1.80).[97] Given the limited number of cases, these findings should be interpreted cautiously (**Table 1-1**). Studies have also focused on breast cancer diagnoses occurring within specific timeframes around AIDS diagnosis. These studies demonstrate similar inverse associations.[98,99] Lastly, a meta-analysis combining estimates from several difference studies/countries also found a protective effect of HIV status on breast cancer incidence, with a 26% reduced risk of breast cancer comparing women with versus without HIV.[76]

Despite the lower risk of breast cancer among women with HIV, they are at a higher risk for all-cause and breast cancer-specific mortality. Two distinct studies from the Surveillance, Epidemiology and End Results (SEER)-Medicare linked database were conducted. One, among adults with nonadvanced cancer, found that the risk of all-cause mortality was significantly higher among women with compared to without HIV (hazard ratio [HR]=1.50, 95% CI 1.01, 2.24), though breast cancer-specific mortality was not significantly different by HIV status.[100] The second found a three-fold increased risk of all-cause mortality and 2.8-fold increased risk of breast cancer-specific mortality

accounting for the competing risk of death.[92] These findings were consistent when assessing stage-adjusted mortality in an analysis of the National Cancer Database (2004-2014) (**Table 1-1**).[94]

Potential Mechanisms of Reduced Breast Cancer Risk

Sociodemographic Risk Factors Among Women with versus without HIV

It is possible that sociodemographic differences between women with HIV versus women in the US general population could drive this association. On average, women with HIV are younger,[101] predominantly Black,[101,102] and may be less likely to be appropriately screened for breast cancer due to barriers in accessing healthcare.[103] Further, prior to advancements in ART, women with HIV experienced high mortality at young ages. As such, these women may not have been living long enough until recently to reach comparable rates of breast cancer, resulting in a low occurrence of breast cancer.[104]

Research suggests that these explanations for the reduced risk of breast cancer on their own are insufficient. Studies have demonstrated that for breast cancer, age at diagnosis does not differ by HIV status,[105] and analyses reporting a reduced risk of breast cancer were all age-standardized to the general US population (**Table 1-1**). As previously mentioned, though the risk of breast cancer in the general population is modestly lower comparing Black to white women in the US, this gap in incidence is narrowing. Prior works assessing differences in breast cancer risk by HIV status also accounted for race through standardization and still found a protective effect (**Table 1-1**). Studies characterizing differences in mammography are equivocal: one study found that mammography was more common in women with versus without HIV,[106] suggesting women with HIV have more engagement with healthcare systems. Other studies have

found similar or lower rates of mammography compared to women without HIV.[107–109] Though studies have not directly addressed the aforementioned bias created by differences in life expectancy, contemporary analyses of breast cancer risk, when mortality among women with HIV is more comparable to that of the general population, still find a reduced risk of breast cancer.[80]

Plausible HIV-related Mechanisms in Breast Cancer Etiology

In the absence of strong evidence in support of sociodemographic explanations for this decreased risk, virologic, HIV-specific mechanisms should be considered. Emerging evidence suggests that HIV infection may induce estrogen suppression.[110,111] This is important because, especially for postmenopausal breast cancer, estradiol (the most potent downstream metabolite of estrogen) has a well-established role in the risk and progression of breast cancer.[112–114] There is some evidence indicating an interaction between viral load and estrogen. Among women with HIV, one study found that within a menstrual cycle, as estradiol levels increase, viral load simultaneously decreases.[115] Evidence on whether estradiol is lower comparing women with versus without HIV is unclear, and studies have not adequately accounted for demographic and clinical differences between women with and without HIV in large studies.[62,111,116–119] The aforementioned high prevalence of amenorrhea also suggests that there could be hormonal dysfunction among women with HIV.

There is preliminary data that also supports a direct biological relationship between HIV (the virus itself) and breast cancer. HIV binds to two sites to gain entry and infect CD4 cells: a CD4 cell receptor, and either, 1) the CCR5 immune receptor, 2) the CXCR-4 immune receptor, or 3) both immune receptors.[120] CXCR-4 tropic HIV binds exclusively to a CD4 receptor and the CXCR-4 receptor. This same immune receptor is expressed on the surface of breast cancer cells, in addition to ductal carcinoma *in situ*

and atypical ductal hyperplasia (a pre-cancer), but not in normal breast epithelium.[93,111,121–123] Research has shown that CXCR-4 tropic HIV can induce apoptosis in neoplastic ductal cells, likely through this hypothesized mechanism.[124] One study further demonstrated that among women with HIV, lower breast cancer risk was strongly associated with CXCR-4 tropism.[93] Certain HIV treatments including protease inhibitors and nucleoside antagonists, have also shown tumoricidal effects and slow progression of AIDS-defining cancers.[125–128]

Persisting Knowledge Gaps & Challenges

Though substantial strides have been made towards understanding cancer etiology among PWH, there are several areas that require further investigation, especially in the context of breast cancer. Effective ART has been available for almost two decades leading to remarkable declines in mortality, and life expectancy among women with HIV continues to increase over time. We do not know how these changes in mortality and life expectancy may have influenced trends in breast cancer among women with HIV. Estrogen is a well-known risk factor for breast cancer observed in the general US population; however, whether estrogen differs between women with versus without HIV is unknown. Studies assessing estrogen are often small in sample size, and do not account for differences between women with and without HIV that could inform estrogen concentrations. Previous studies assessing breast cancer risk in the context of HIV indicate that sociodemographic and clinical factors do not fully explain the reduced risk observed in women with HIV. Yet, there is limited research among women with HIV exploring the direct association between HIV viral load and breast cancer.

To date, research addressing these questions have been hindered by insufficient source populations from which breast cancer cases could arise. One of the largest studies assessing breast cancer in women with HIV contained approximately 600 cases.

These large sources of data for breast cancer among women with HIV are often limited in the ability to address potential confounding factors such as traditional risk factors for breast cancer and/or clinical factors related to HIV infection (e.g. HIV viral load, and immune status). Studies assessing differences in risk factors for breast cancer, like estrogen, are difficult to conduct because there are few large, longitudinal cohorts of comparable women with versus without HIV that also have information available on potential confounding factors.

Dissertation Aims and Implications of the Research

To address these gaps in the current knowledge on breast cancer and HIV, this dissertation sought to complete the following aims:

1. **To characterize breast cancer risk over calendar time from 1996-2016 in the context of changes in mortality among women with HIV who have initiated ART.**

To address this research question, I estimated trends in the hazard and cumulative incidence of breast cancer accounting for the competing risk of death in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD).[129] The NA-ACCORD is a consortium of interval and clinical cohorts of PWH in care across the United States and Canada (**Figure 1-1**). PWH are eligible for inclusion if they successfully link into HIV care, defined as attending at least two HIV care visits in 12 months. Every cohorts utilizes standardized data collection methods, and submits data on participant demographic characteristics, laboratory measurements, diagnoses, prescriptions, and vital status. The NA-ACCORD currently comprises roughly 180,000 participants 14% of which are cis-gender women. The NA-ACCORD participants are representative of PWH in the US more broadly with respect to age, sex, and race/ethnicity. (**Table 1-2**).

It is widely known that mortality among women with HIV has declined since the establishment of effective ART treatment in 1996. I hypothesized that as mortality declines over time, there would be an initial increase in breast cancer risk that would stabilize and remain constant over time. This would indicate mortality may have influenced breast cancer risk early in the modern treatment era, but that in later years, as risk stabilized, changes in mortality and life expectancy did not impact breast cancer risk. If this hypothesis was confirmed, the reduced risk observed comparing women with versus without HIV may be due to factors beyond life expectancy and mortality, providing the motivation to continue exploring additional factors that could drive the association between HIV and breast cancer.

2. **To quantify differences in total and free estradiol as well as sex hormone binding globulin (SHBG) between women with and without HIV, and among women with HIV by viral suppression status.**

To complete this aim, I estimated differences in these biomarkers using quantile regression to account for differences in sociodemographic and clinical characteristics by HIV status, and among women with HIV, by viral suppression status. The data source for this analysis was the Women's Interagency HIV Study (WIHS), (now the MACS/WIHS Combined Cohort Study).[130] The WIHS was established in 1993 and includes six consortia with multiple sites across the country. Women are recruited to the WIHS if they have HIV or are considered at high-risk for HIV. Thus far, there have been four recruitment waves. The method of enrollment into the study is designed to ensure that women are comparable with respect demographic and clinical factors (see **Table 1-3**). At all sites, study visits are conducted every 6 months, during which structured interviews are conducted to ascertain medical history, ART, and lifestyle factors.

Physical and gynecologic exams are also completed in addition to HIV status, HIV viral load and biological specimen collection.

I hypothesized that total estradiol would not differ by HIV status, or viral suppression status among women with HIV, but free estradiol would be lower and SHBG would be higher comparing women with versus without HIV. Among women with HIV, free estradiol would be lower and SHBG would be higher comparing women who did not achieve viral suppression compared to those achieving viral suppression. Little is known regarding estradiol levels among women with compared to without HIV; irrespective of whether my hypotheses were confirmed, these findings would shed light on the hormonal profile of estrogen and SHBG among women with HIV. If my hypotheses were confirmed, this would suggest that estrogen suppression is a potential mechanism driving the reduced risk of breast cancer observed in women with HIV.

3. **To assess the association between cumulative HIV viral load since ART initiation and breast cancer risk.**

To address this aim, I used joint longitudinal survival models to estimate the association between cumulative viral load on ART, a metric capturing the cumulative burden of HIV viremia over the course of treatment, and breast cancer risk. I additionally assessed cumulative viral load at 1-5 years prior to diagnosis/censoring. For this aim, I leveraged the large sample size, availability of longitudinal viral load measurements, and confounding factors from the NA-ACCORD. I hypothesized that cumulative viral load on effective ART would be inversely associated with the risk of breast cancer. Further, that the association between cumulative viral load and breast cancer would strengthen when assessed in the years prior to diagnosis. The findings from this aim would provide novel insight into the association between HIV viremia and breast cancer. If the hypotheses were confirmed, these findings would demonstrate that HIV itself may be a mechanism

leading to a reduced risk of breast cancer that requires further investigation. Additionally, if there was an association between HIV and breast cancer, these findings could help clarify the etiologically relevant time period for when HIV would act on tumor development.

Taken in sum, the completion of these dissertation aims will help fill current gaps in the literature regarding HIV and breast cancer risk by assessing potential hypotheses that could explain the reduced risk seen in women with HIV. Findings from this work have the potential to inform our broader understanding of breast cancer etiology, the burden of breast cancer in the context of HIV as well as other chronic infectious diseases, and the course of clinical care for women with HIV. As the population of women with HIV continues living longer at older ages, understanding breast cancer occurrence and etiology is critical for cancer prevention and surveillance.

Chapter 1 Tables

Table 1-1. Summary of the literature on breast cancer incidence and mortality comparing women with versus without HIV in the US

Breast cancer incidence studies					
Study Population	**Era**	**Factors accounted for**	**Estimate of Association**	**% difference in women with HIV**	**Sample Size**
HIV/AIDS Cancer Match Study[79] (AIDS only)	1980-2002	Age at first live birth Parity Body Mass Index Smoking Oral contraceptive use Age African ancestry	Standardized incidence ratio	**31% reduced risk**	313 diagnoses 85,268 women
HIV/AIDS Cancer Match Study[95] (HIV only, 5-years following diagnosis)	1979-2002	Age Race/ethnicity Calendar year Registry	Standardized incidence ratio	20% reduced risk	34 diagnoses 19,785 women
HIV/AIDS Cancer Match Study[80] (HIV/AIDS)	1996-2012	Age Race/ethnicity Calendar year Registry	Standardized incidence ratio	**37% reduced risk**	688 diagnoses 893,506 women
HIV Outpatient Study/ Adult Spectrum of Disease Study[78]	1992-1995	Age Race	Standardized rate ratio	**30% reduced risk**	29 diagnoses 38,298 person-years
	1996-1999			20% reduced risk	
	2000-2003			10% increased risk	
Women's Interagency HIV Study[96]	1993-2001	Age Race	Rate ratio	30% reduced risk	7 diagnoses 1,950 women
Johns Hopkins University AIDS Service[97]	1996-2005	Age Race Calendar year	Standardized incidence ratio	40% reduced risk	3 diagnoses 832 women
New York Cancer/AIDS Registries[98]	1981-1994	Age Race Region Differential survival	Standardized incidence ratio	20% reduced risk	47 diagnoses 1,288 women
HIV/AIDS & Cancer registries from six states[99]	1980-1989	Age Race Calendar year Registry	Standardized incidence ratio	No cases	0 diagnoses 8,886 women
	1990-1995			**60% reduced risk**	14 diagnoses 35,396 women
	1996-2002			20% reduced risk	28 diagnoses

					27,282 women
All-cause and breast cancer specific mortality among women with breast cancer					
Study Population	**Era**	**Factors accounted for**	**Estimate of Association**	**X-fold risk in women with HIV**	**Sample Size**
Surveillance, Epidemiology, and End Results–Medicare[100]	1996-2012	Age Race/ethnicity Median income Year Stage Treatment type Time between diagnosis and treatment	All-cause mortality (hazard ratio)	**1.5 fold increased risk**	24 deaths among 50 women with HIV out of 139,270 women
			Breast cancer specific mortality following initial treatment (hazard ratio)	1.9 fold increased risk	Breast cancer specific deaths not reported due to small case count
Surveillance, Epidemiology and End Results (SEER)-Medicare (2000-2013)[92]	2001-2011	Age and year diagnosis Race/ethnicity Marital status Comorbidity score Medicare eligibility reason Poverty indicator Summary stage Treatment type	All-cause mortality (hazard ratio)	**3-fold increased risk**	84 deaths in 176 women with HIV among 164,080 women total
			Breast-cancer specific mortality (subdistribution hazard ratio)	**2.8 fold increased risk**	46 deaths in 176 women with HIV among 164,080 women total
National Cancer Database (2004-2014)[94]	2004-2014	Age Race Calendar year Household income Stage Treatment Health insurance type Treatment facility	All-cause mortality (hazard ratio)	**1.9 fold increased risk**	399 deaths in 957 women with HIV among 1,100,058 women total

Listed in the order they are presented in text.

Bold indicates statistically significant estimate.

Green text indicates statistically significant decrease in risk, red text indicates statistically significant increase in risk.

Table 1-2. Characteristics of NA-ACCORD participants compared to people with HIV in the US using CDC data as of 2018

	NA-ACCORD[a]		PWH in US[b]	
Characteristics	N	%	N	%
Age				
18-19	90	0.2	--	--
20-24	1,010	1.8	26,914	2.6
25-29	3,474	6.2	70,839	7.0
30-34	4,276	7.6	87,843	8.6
35-39	4,546	8.1	93,817	9.2
40-44	4,404	7.8	96,965	9.5
45-49	6,259	11.1	123,900	12.2
50-54	8,411	14.9	158,278	15.5
55-59	8,343	14.8	153,473	15.0
60-64	6,860	12.1	104,620	10.3
65+	8,824	15.6	102,197	10.0
Sex				
Male	48,372	85.6	774,422	75.5
Female	7,786	13.8	240,787	23.5
Transgender	339	0.6	10,362	1.0
Race/ethnicity				
Non-Hispanic white	22,953	40.6	304,131	29.7
Non-Hispanic Black	22,190	39.3	422,994	41.3
Hispanic	7,110	12.6	231,317	22.6
Other/unknown	4,244	7.5	66,538	6.5
HIV transmission risk				
Male-to-male sexual contact	20,244	35.8	576,787	55.4
Injection drug use[c]	8,026	14.2	178,629	17.2
Heterosexual contact	8,953	15.9	269,596	25.9
Other/unknown	19,274	34.1	15,339	1.5

Note: Table sourced from: https://naaccord.org/vital-statistics

[a] Participants with at least one CD4 count or viral load measurement between 1/1/2006-12/31/2018

[b] Data from the HIV Surveillance Report, http:/www.cdc.gov/hiv/library/reports/hiv-surveillance.html.Published_May_2020). Total population includes children, adolescents and adults; sex and HIV transmission risk include adults and adolescents only. Weighted percentages presented.

[c] Includes those reporting both male-to-male sexual contact and injection drug use

Table 1-3. Selected demographic and clinical characteristics among WIHS participants by recruitment wave at the last WIHS visit

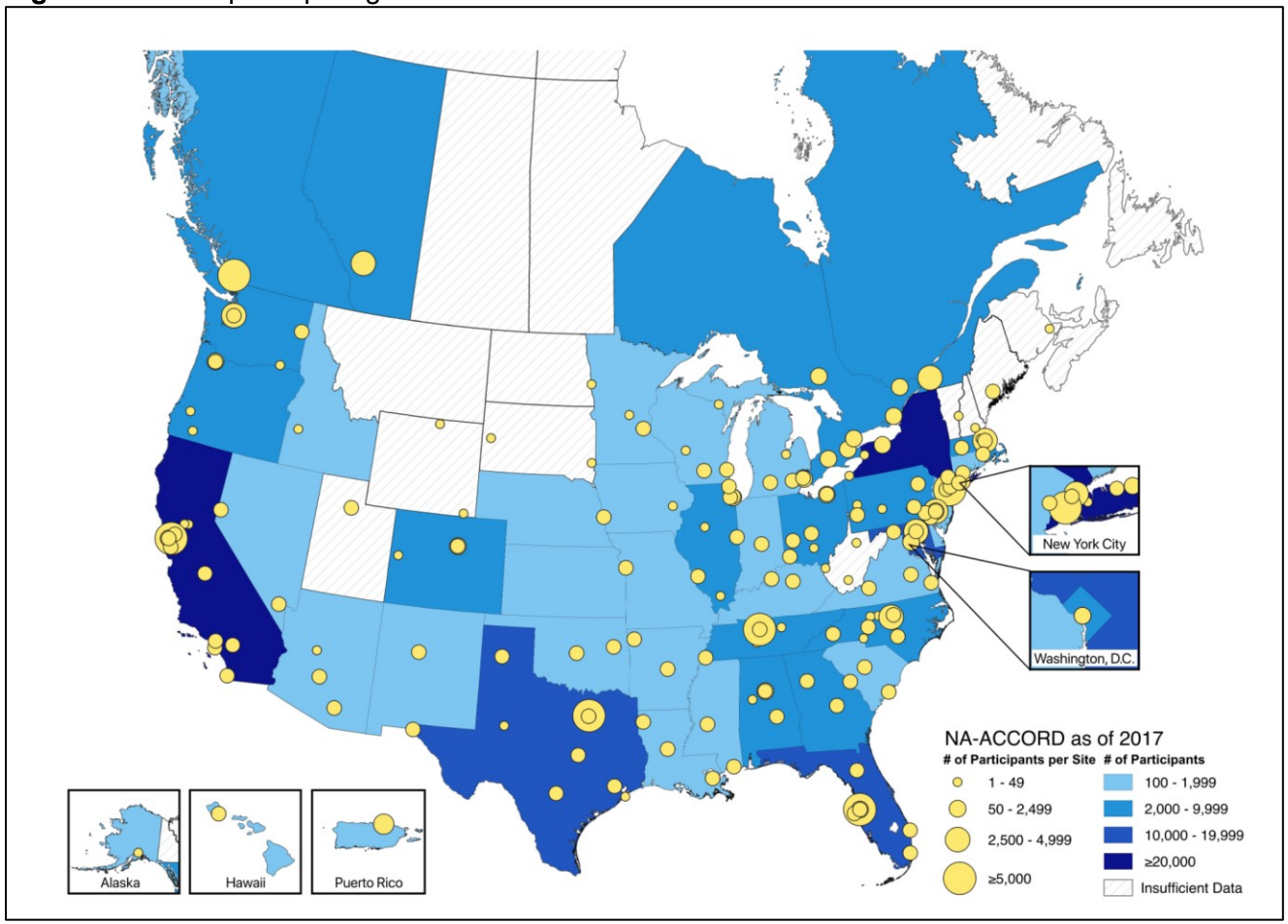
	Wave 1 (1994-95)		Wave 2 (2001-02)		Wave 3 (2011-12)		Wave 4 (2013-15)		Total	
	HIV -	HIV+	HIV -	HIV+	HIV -	HIV+	HIV -	HIV+	HIV -	HIV+
	N=196	N=519	N=215	N=356	N=77	N=210	N=214	N=576	N=702	N=1,661
Median Age (IQR)	56 (49, 61)	56 (52, 61)	43 (37, 50)	48 (42, 52)	53 (47, 58)	49 (42, 53)	46 (37, 53)	47 (39, 53)	49 (41, 55)	51 (44, 56)
Race/ethnicity										
NH Black	128 (65)	308 (59)	135 (63)	245 (69)	65 (84)	163 (78)	178 (83)	477 (83)	506 (72)	1,193 (72)
NH white	19 (10)	91 (18)	13 (6)	21 (6)	2 (3)	18 (9)	19 (9)	52 (9)	53 (8)	182 (11)
Other	49 (25)	120 (23)	67 (31)	90 (25)	10 (13)	29 (13)	17 (8)	47 (8)	143 (20)	286 (17)
Current smoker	74 (38)	160 (31)	87 (40)	113 (32)	44 (57)	99 (47)	102 (48)	253 (44)	307 (44)	625 (38)
Alcohol use, past 6 months (drinks/week)										
None	92 (47)	310 (60)	76 (35)	211 (59)	39 (51)	101 (48)	84 (39)	318 (55)	291 (41)	940 (57)
0-7	50 (26)	137 (26)	91 (42)	23 (26)	22 (29)	71 (34)	82 (38)	199 (35)	245 (35)	500 (30)
>7-12	15 (8)	15 (3)	15 (7)	15 (4)	5 (6)	8 (4)	11 (5)	17 (3)	46 (7)	55 (3)
>12	22 (11)	26 (5)	17 (8)	29 (8)	10 (13)	20 (10)	35 (16)	39 (7)	84 (12)	114 (7)
Menopausal status (last visit)										
Premenopausal	26 (13)	47 (9)	108 (50)	122 (34)	18 (23)	72 (34)	97 (45)	214 (37)	249 (35)	455 (27)
Perimenopausal	23 (12)	50 (10)	36 (17)	74 (18)	12 (15)	34 (16)	29 (13)	82 (14)	100 (14)	230 (14)
Postmenopausal	129 (66)	392 (76)	56 (26)	160 (45)	46 (60)	94 (45)	84 (39)	277 (48)	315 (45)	923 (56)
History of cancer	16 (8)	47 (9)	6 (3)	21 (6)	2 (3)	5 (2)	2(1)	10 (2)	26 (4)	83 (5)
Undetected VL	N/A	351 (68)	N/A	238 (67)	N/A	143 (68)	N/A	420 (73)	N/A	1,152 (69)
Evert ART use	N/A	510 (98)	N/A	347 (97)	N/A	198 (94)	N/A	555 (96)	N/A	1,610 (97)

Abbreviation: IQR, interquartile range; NH, non-Hispanic; ART, antiretroviral therapy.

Note: Adapted from Adimora et al. Cohort Profile: The Women's Interagency HIV Study (WIHS). Int J Epidemiol. 2018 Apr 1;47(2):393-394i. doi: 10.1093/ije/dyy021.

Chapter 1 Figures

Figure 1-1. Sites participating in the NA-ACCORD as of 2017



Note: Figure sourced from: https://naaccord.org/

Chapter 2: Aim 1

Trends in breast cancer hazard and cumulative incidence among women with HIV

Abstract

Background: Studies suggest a lower risk of breast cancer in women with versus without HIV. These estimates may be biased by the lower life expectancy and younger age distribution of women with HIV. This aim evaluated this bias and characterized secular trends in breast cancer among women with HIV initiating antiretroviral therapy (ART). I hypothesized breast cancer risk would initially increase over time as mortality decreased and then stabilize.

Methods: This aim was conducted among women with HIV prescribed ART in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) from 1997-2016. Breast cancer hazard (cause-specific hazard ratios [csHR]) and cumulative incidence accounting for the competing risk of death (subdistribution hazard ratios [sdHR]) were estimated to assess changes in breast cancer risk over time. This was assessed overall (1997-2016) and within/across calendar periods. Analyses were adjusted for race/ethnicity and were inverse probability weighted for cohort. Cumulative incidence was graphically assessed by calendar period and race/ethnicity.

Results: There were 11,587 women observed from 1997-2016 in the analytic study population, contributing 63 incident breast cancer diagnoses and 1,353 deaths (73,445 person-years [median follow-up=4.5 years]). Breast cancer cumulative incidence was 3.2% for 1997-2016. There were no secular trends in breast cancer hazard or cumulative incidence. There were annual declines in the hazard and cumulative incidence of death (csHR and sdHR: 0.89, 95% CI 0.87, 0.91) which remained within and across calendar periods. There were no significant differences in breast cancer incidence by race/ethnicity or calendar period.

Conclusions: These findings contradict the hypothesis of increasing breast cancer risk with declining mortality over time among women with HIV, suggesting a limited impact of changing mortality on breast cancer risk. Additional inquiry is merited as survival improves among women with HIV, and this population continues aging.

Publication information:

Coburn SB, Shiels MS, Silverberg MJ, Horberg MA, Gill MJ, Brown TT, Visvanathan K, Connor AE, Napravnik S, Marcus JL, Moore RD, Mathews WC, Mayor AM, Sterling TR, Li J, Rabkin CS, D'Souza G, Lau B, Althoff KN; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of the International Epidemiology Databases to Evaluate AIDS. Secular Trends in Breast Cancer Risk Among Women With HIV Initiating ART in North America. J Acquir Immune Defic Syndr. 2021 May 1;87(1):663-670. doi: 10.1097/QAI.0000000000002627. PMID: 33492023; PMCID: PMC8026587.

Introduction

People with HIV (PWH) in the United States have made substantial gains in life expectancy, although disparities within this population by sex, race/ethnicity, and HIV acquisition risk group endure.[11,13,14] As this population ages, with the majority now >50 years of age,[1] PWH are increasingly at risk for common chronic comorbidities seen in the general population, including non-AIDS defining cancers (NADCs).[31,131] Over time the cancer burden has shifted from predominantly AIDS-defining cancers to NADCs.[69,71,72,132] The mechanisms driving this shift are likely multifactorial, and differential by cancer site/type. Potential factors include population-level aging, risk factor prevalence (e.g. smoking), human papillomavirus/other coinfections, and immune suppression.[73–76]

Some studies suggest the risk of breast cancer in women with HIV is lower compared to women in the general population,[78–80] despite the high prevalence of obesity and alcohol abuse in women with HIV,[133,134] as well as chronic inflammation associated with long term infection, which are relevant risk factors for breast cancer. In the HIV/AIDS Cancer Match study (HACM), two analyses found protective effects of HIV infection on breast cancer: standardized incidence ratio (SIR) 0.69 (95% CI 0.63, 0.7) in 1980-2002,[79] and SIR 0.63 (95% CI 0.58, 0.68) in 1996-2012.[80] A third study found a reduced risk of breast cancer from 1992-1995 (standardized rate ratio [SRR]=0.70, 95% CI 0.30, 1.90), and a null effect from 2000-2003 (SRR=1.10, 95% CI 0.70, 1.80).[78]

Observational studies cast doubt on racial and age differences between women with versus without HIV as drivers of these associations. Though women with HIV have a younger age distribution, and are predominantly Black (among whom breast cancer risk is modestly lower),[101] prior estimates account for these factors.[78–80] Also, the racial disparity in breast cancer risk in the general population by race is narrowing.[135] Differences in screening has also been postulated as explaining this disparity.[103,105,136]

Though research is sparse, estimates vary on whether mammography is more common, less common or comparable in women with versus without HIV.[106–109]

The changing mortality rate among women with HIV from early treatment eras to the modern antiretroviral therapy (ART) era could also lead to artificially lower rates of disease. Specifically, it is unclear whether the previously documented low risk of breast cancer might be attributed to the younger age distribution of women with HIV relative to the general population, where breast cancer risk is typically not elevated, and/or that women with HIV had a lower life expectancy compared to women in the general US population.[13] Though prior studies have used age-standardization to mitigate this bias, analyses addressing breast cancer risk in older women with HIV have not addressed the competing risk of death, which would allow for a more complete understanding of trends in breast cancer among women with HIV.[73]

Thus, the objectives of this aim were to: 1) characterize breast cancer risk over calendar time in the context of changes in mortality; and 2) evaluate the role of the potential bias produced by changing mortality (shifts in the age distribution and increased life expectancy) in breast cancer risk among women with HIV who have initiated ART.

Methods

Study Population

The study population included women with HIV participating in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD).[129] Briefly, the NA-ACCORD is a consortium of single- and multisite cohorts of adults with HIV in the U.S. and Canada. Individuals are eligible for inclusion if they attended two or more HIV care visits within twelve months (i.e. they successfully linked into HIV care). Each cohort employs standardized data collection, submitting data on enrolled participant

characteristics, diagnoses, laboratory measures, prescribed medications, and vital status to the Data Management Core (University of Washington, Seattle, WA). Data are harmonized across cohorts and evaluated for quality control prior to being transmitted to the Epidemiology/Biostatistics Core (Johns Hopkins University, Baltimore, Maryland).

Cis-gender women were included in this nested study if they were ≥18 years old, prescribed ART, under observation for at least six months from 1996 through 2016, had no history of any cancer (including non-melanoma skin cancer), and had data available on race/ethnicity (**Figure 2-1)**. This excluded 224 first-time AIDS-defining cancer diagnoses, 1 woman was subsequently diagnosed with breast cancer, and 93 died. Twenty NA-ACCORD cohorts provided data on women meeting these criteria.

Outcome: Breast Cancer

First-time, incident breast cancer diagnosis was ascertained and validated using a standardized abstraction protocol as described elsewhere.[73] Abstraction included manual review of medical charts and pathology reports for cancer site and histopathology, and/or linkages with cancer registries.

Death

Deaths in the NA-ACCORD are ascertained via linkages with: 1) provincial, state, or national death registries (includes the US National Death Index Plus), 2) medical record abstraction following notification of death to the attending physician, 3) outreach to family and/or friends, and 4) monitoring of obituaries. Though cohorts participating in the NA-ACCORD have differing methods of ascertainment, mortality rates are comparable between cohorts with and without registry linkages.[12] Lags in death index matching were accounted for by administratively censoring women at December 31st 2016. Death was defined as all-cause mortality.

Covariates

Age was estimated from year of birth. Race/ethnicity was categorized as: Non-Hispanic Black, Non-Hispanic white, Hispanic, or other race/ethnicity. This was selected to reflect racial/ethnic differences in breast cancer risk observed in the general population,[135] as well as racial/ethnic differences in mortality among women with HIV.[1] This also ensured large enough sample size for stable estimates, and is consistent with the literature on breast cancer risk in women with HIV.[80] ART exposure was defined using the first occurrence of a prescription for effective antiretroviral therapy for at least 30 days; effective ART was defined using treatment guidelines.[16]

Statistical Analyses

Women contributed person-time and events beginning 6 months after the latest of the following dates: January 1st, 1996, enrollment into the NA-ACCORD, age 25, ART initiation date, or cohort-specific cancer validation start date. The six-month period following the start date was added to mitigate including prevalent breast cancer diagnoses and to exclude immortal person-time.[137,138] Women were censored from the analysis on the earliest of the following dates: incident breast cancer diagnosis, death, administrative censoring on December 31st, 2016, cohort-specific cancer validation end date, or loss-to-follow-up. Loss-to-follow-up was defined as the last HIV laboratory measurement (either CD4 count or HIV viral load measurements) prior to a two-year gap. Although women could enter as early as 1996, person-time and events prior to 1997 were excluded due to limited observation during this year (**Figure 2-1)**. The timescale for this analysis was age, allowing women of the same age to be compared by calendar period with respect to risk of breast cancer or death, effectively controlling for the influence of age on breast cancer risk.

To characterize secular changes in the risk of breast cancer, I estimated cause-specific hazard ratios (csHR) and sub-distribution hazard ratios (sdHR) for the association between time-updated calendar time and time to breast cancer. I also estimated the csHR and the sdHR for the association between calendar time and time to death. Comparisons of the csHRs and sdHRs for both breast cancer and death can elucidate the contribution of death to the hazard of breast cancer over time.[73] As described by Silverberg et al., in the era of effective ART, women with HIV are experiencing increased life expectancy over time. Assuming the competing risk of death is the only reason breast cancer risk appears lower in women with compared to without HIV, the hazard rate (the instantaneous risk of breast cancer: csHR), may not vary over time, but the cumulative incidence (the risk of breast cancer given surviving to a certain time: sdHR) would be expected to increase as a result of decreased mortality.[73]

I also graphically assessed breast cancer and all-cause mortality rates over calendar time by plotting lowess smoothed incidence rates (the ratio of lowess smoothed annual breast cancer/death counts over lowess smoothed person-time [days] for each calendar year).

Proportionality of hazards was assessed using interaction terms between calendar year and age. Calendar year was modelled continuously (centered at 2006) to assess annual trends overall (1997-2016) and using linear splines to assess annual trends within time-updated calendar periods (1997-2001, 2002-2006, 2007-2011, and 2012-2016). Calendar periods approximated changes in ART treatment guidelines. I additionally assessed changes in breast cancer risk across calendar periods, comparing 1997-2001 to all later periods. All models were adjusted for race/ethnicity, and regression analyses were repeated additionally adjusting for having an AIDS-defining illness within a year prior to ART initiation (yes or no). Models were weighted as the inverse probability of being in each cohort (or subsite for multisite cohorts) to account for

differences across cohorts. The cumulative incidence of breast cancer over age was graphically examined using non-parametric estimators accounting for the competing risk of death stratified by calendar period and race/ethnicity.

Results

Characteristics of the Study Population

There were 11,587 women (**Figure 2-1**) contributing 73,445 person-years, 63 incident breast cancer diagnoses, and 1,353 deaths from 1997-2016, yielding a breast cancer incidence rate of 8.6 cases (95% CI 6.7, 11.0) per 10,000 person-years and mortality rate of 184.2 deaths (95% CI 174.7, 194.3) per 10,000 person-years (**Table 2-1**). The median age at analysis entry was 40.0 years (IQR: 33.5, 47.0 years), and median age at exit was 47.0 years (IQR: 39.1, 54.1 years). The median duration of follow-up for women was 4.5 years (IQR: 2.3, 9.5 years). Women were predominantly Non-Hispanic Black (59.4%) and did not have an AIDS-defining illness in the year prior to ART initiation (91%) (**Table 2-1**). There was variability in the distribution of race/ethnicity over calendar year (average annual percent change ≤15% per category) and AIDS-defining illness in the year prior to ART (average annual percent change <10% per category).

Secular Changes in Cause-Specific Hazard of Breast Cancer and Death

The cause-specific hazard assessed overall (1997-2016) for breast cancer was stagnant, and for death declined over time (**Table 2-2**). There was no change in the cause-specific hazard of breast cancer (csHR: 0.98, 95% CI 0.92, 1.05) per one-year increase in calendar year. There was a 11% decrease in the cause-specific hazard of death per one-year increase in calendar year (csHR 0.89, 95% CI 0.87, 0.91).

The cause-specific hazard was also assessed within calendar periods (**Table 2-2)**. The cause-specific hazard of breast cancer within calendar periods did not significantly change over time and was characterized by modest fluctuation. This was also observed when comparing breast cancer risk across calendar periods, where there was no significant difference in breast cancer hazard comparing later periods to 1997-2001. When visually assessing the rate of breast cancer over time using lowess smoothed incidence rates over calendar year, there was an initial small increase in breast cancer which peaked from 1997 to 2008, but little change was observed after this (**Figure 2-2A**).

There was a consistent decline in the hazard of death within calendar periods (**Table 2-2)**. In 1997-2001, there was a 11% decline per year in the hazard of death (csHR: 0.89, 95% CI 0.79, 1.00). In 2002-2006 there was an 8% annual decrease in the hazard of death (csHR: 0.92, 95% CI 0.85, 0.99), followed by a 16% decrease in the annual hazard of death in 2007-2011 (csHR: 0.84, 95% CI 0.77, 0.92). This stabilized in 2012-2016, with a non-significant 10% annual decline in the hazard of death (csHR: 0.90, 95% CI 0.77, 1.06). When evaluated across calendar periods, compared to 1997-2001, the risk of death significantly increasingly declined for 2002-2006 (csHR: 0.62, 95% CI 0.44, 0.87), 2007-2011 (csHR: 0.37, 95% CI 0.26, 0.51), and 2012-2016 (csHR: 0.16, 95% CI 0.11, 0.24). This was consistent with the lowess smoothed all-cause mortality rates by calendar year, where there was a steady decline in all-cause mortality with increasing calendar year (**Figure 2-2B**).

Secular Change in Cumulative Incidence of Breast Cancer and All-Cause Death

The cumulative incidence of breast cancer (estimated via the breast cancer sdHRs) followed similar trends to the breast cancer csHRs (**Table 2-2**). There was no significant annual change in the cumulative incidence of breast cancer assessed from

1997-2016, within calendar period, or across calendar periods. The cumulative incidence of death followed a markedly similar pattern to the cause-specific hazard of death, characterized by an 11% annual decrease in the cumulative incidence of death (sdHR: 0.89, 95% CI 0.87, 0.91) and declines in the annual cumulative incidence of death in 1997-2001, 2002-2006, 2007-2011, and 2012-2016 of 11%, 8%, 16% and 10% (**Table 2-2**). Compared to 1997-2001, cumulative incidence of death was significantly lower with each increasing calendar period.

All regression analyses were repeated additionally adjusting for AIDS-defining illness in the year prior to ART, which did not substantively change estimates (**Appendix 2-1**).

Cumulative Incidence of Breast Cancer by Calendar Period

The incidence of breast cancer fluctuated with calendar period. The overall cumulative incidence of breast cancer from 1997-2016 was 3.2% (95% CI 2.1%, 4.7%). The cumulative incidence of breast cancer was 2.3% (95% CI 0.1%, 6.2%) in 1997-2001, 2.3% (95% CI 1.2%, 3.9%) in 2002-2006, 4.5% (95% CI 2.1%, 8.4%) in 2007-2011, and 3.8% (95% CI 1.7%, 7.2%) in 2012-2016. Assessed graphically, there were no discernable patterns in breast cancer cumulative incidence with age as the timescale with respect to calendar periods (**Figure 2-3A**).

Cumulative incidence was highest among Hispanic women at 4.7% (95% CI 1.5%, 11.1%), followed by Non-Hispanic white women at 4.2% (95% CI, 1.7%, 8.4%), Non-Hispanic Black women at 2.6% (95% CI, 1.5%, 4.3%), and women of other race/ethnicity at 0.1% (95% CI, 0.2%, 3.3%). There were no differences by race/ethnicity, until the end of follow-up, where the data are sparse (**Figure 2-3B**). There was limited follow-up beyond age 65, so estimates should be interpreted cautiously (**Appendix 2-2**).

Discussion

In a large sample of women with HIV prescribed ART in North America with no history of any cancer, there were no trends in either the hazard or cumulative incidence of breast cancer over calendar time accounting for age (timescale), race/ethnicity, and cohort. The hazard and cumulative incidence of death demonstrated consistent declines over time when assessed from 1997-2016 and within/across calendar periods. Cumulative incidence of breast cancer was 3.2% over 16 years of follow-up in women with HIV initiating ART with no history of any cancer (median follow-up 4.5 years) for the years 1997-2016. There were no significant differences in breast cancer risk by calendar period or race/ethnicity.

These findings suggest a limited impact of changing mortality on breast cancer risk among women with HIV initiating ART. Despite declines in mortality (as both the csHRs and sdHRs for death indicated), there was no change in breast cancer risk over time. If mortality substantively influenced breast cancer incidence among women with HIV, we would expect to see a pattern of increasing breast cancer cumulative incidence with increasing calendar year; however, we observed no difference in breast cancer cumulative incidence when stratified by calendar period.

These results align with an analysis in HACM, which found that the annual percent change in breast cancer incidence rates did not change over time from 1996-2010.[71] Another study conducted in HACM projecting breast cancer incidence through 2030 found no change in the rate of breast cancer from 2006-2012.[70] To compare these findings to this aim, one must assume the incidence rate is a reasonable estimate of the average hazard in these calendar periods; however, the incidence rate is not directly comparable to the hazard, cumulative incidence, or lowess incidence rates examined in this analysis. In the era of effective ART, my observation of declining mortality over calendar time has been well-documented among PWH.[8,72,139] This has been observed in

women with HIV,[140] though in the US, relative to men, the magnitude of this decline in mortality is smaller for women.[141]

Breast cancer incidence in this analysis is markedly lower than the commonly cited 12% lifetime risk among women in the general population.[142] There are substantial differences in the underlying populations that generated our estimate (women with HIV initiating ART with no history of cancer, and under observation in the NA-ACCORD), and those in the general population of women. Moreover, given the limited follow-up among women beyond age 65, and the increased risk of breast cancer in women 65 and older observed in the general population, this estimated risk likely does not reflect lifetime breast cancer risk in women with HIV. No direct comparisons should be made, though this notable disparity merits additional investigation. To my knowledge, breast cancer lifetime cumulative incidence has not been estimated in women with HIV initiating ART.

These observations could be an artifact of the increasing number of women enrolling in the NA-ACCORD, entering the analysis (**Appendix 2-2**) and the integration of cancer diagnosis observation into each cohort in later years. Cohorts/subsites enter and exit this analysis at varying timepoints according to when cohorts began participation in the NA-ACCORD and began capturing cancer diagnoses. It is possible that fluctuation in cohort participation over time could lead to differential cancer ascertainment over time, though models accounted for cohort/subsite. At the individual level, women enter and leave the study at different timepoints, and I assumed that women who are lost-to-follow-up are represented by the women who remain.

I did not adjust for secular trends in factors related to breast cancer incidence such as changes in screening practices, guidelines, insurance coverage, risk factors for breast cancer (e.g. reproductive factors, alcohol abuse, obesity), or breast cancer risk in the general population due to limited data availability and sample size constraints. Therefore, this aim cannot isolate the effect of mortality alone on breast cancer risk over

time. Another assumption was that the age-specific risk of cancer was constant by birth cohort (e.g. a 35-year-old woman with treated HIV in 1996 is comparable to a 35-year-old woman with treated HIV in 2007). I attempted to mitigate this bias by limiting our data to women who initiated ART and were under observation using a conservative definition of loss-to-follow-up. The small number of breast cancer cases (n=63) limited the precision of these estimates. There was little follow-up observed among women older than 65, restricting the ability to assess lifetime breast cancer risk among women with HIV and may have led to under-ascertainment of cases. Lastly, by restricting to women without a history of cancer, a healthier subset of women with HIV who have initiated ART was selected, especially since AIDS-defining cancer were excluded. Therefore, findings may not be applicable to all women with HIV, where associations with calendar time may differ.

These findings add to the existing limited literature on breast cancer among women with HIV using a large cohort of women with HIV accessing care and initiating ART in North America with moderate duration of follow-up and validated cancer diagnoses. This offers additional evidence that changing mortality is not a mechanism driving lower risk of breast cancer in women with compared to without HIV. This is a novel evaluation of breast cancer over time among women with HIV which assesses the competing risk of death. Further, by limiting the analyses to women initiating ART who are engaged in care (as per our loss-to-follow-up criteria), potential under ascertainment of outcomes was minimized.

The number of women living with HIV over age 50 will continue rising as life expectancy increases and mortality decreases. A rise in breast cancer incidence is plausible given that declining HIV-associated mortality has resulted in an older and aging population of women with HIV who are at the most risk for breast cancer. This was not supported by these findings where there was no substantial change in breast cancer risk

or cumulative incidence over time despite declines in mortality; however, given limited follow-up beyond age 65, it is possible that the threshold for breast cancer risk has not been reached in this population. Additionally, even with no change in risk/hazard, there could be an absolute increase in the number of cases among these women over time.

Breast cancer should continue to be tracked in large cohorts of women with HIV to determine if there are trends over a longer time period at older ages. Further investigation will be required to clarify the role of not only mortality, but also secular trends in the underlying population on breast cancer risk in women with HIV. Comparisons to an appropriate population of women without HIV should be considered to determine if there are disparities by HIV status in breast cancer risk over time.

Chapter 2 Tables

Table 2-1. Characteristics of women with HIV (N=11,587), NA-ACCORD, 1997-2016

	Median	IQR
Age at entry	40.0	33.5, 47.0
Age at exit	47.0	39.1, 54.1
Calendar year entry	2004	2000, 2010
Calendar year exit	2014	2007, 2016
Follow-up (years)	4.5	2.3, 9.5
	N	%
Race/ethnicity		
Non-Hispanic Black	6,887	59.4
Non-Hispanic white	2,313	20.0
Hispanic	1,504	13.0
Other	883	7.6
ADI in the year prior to ART initiation		
No	10,511	90.7
Yes	1,076	9.3
	N	Rate per 10,000 Person-Years
Breast cancer	63	8.6
All-cause death	1,353	184.2

Abbreviations: IQR. Interquartile range; ADI, AIDS-defining illness; ART, antiretroviral therapy.

Table 2-2. Secular change in breast cancer and death by calendar time, 1997-2016

	Cause-specific hazard		Subdistribution hazard	
	csHR[a]	95% CI	sdHR[a]	95% CI
Breast cancer				
Annual	0.98	0.92, 1.05	1.01	0.94, 1.07
Within periods[b]				
1997-2001	1.02	0.66, 1.59	1.07	0.68, 1.68
2002-2006	1.00	0.76, 1.33	1.02	0.77, 1.35
2007-2011	0.95	0.77, 1.18	0.97	0.79, 1.20
2012-2016	1.01	0.74, 1.37	1.02	0.75, 1.38
Across periods[c]				
1997-2001	ref	--	ref	--
2002-2006	0.76	0.18, 3.32	0.88	0.20, 3.82
2007-2011	0.79	0.22, 2.80	1.01	0.28, 3.61
2012-2016	0.77	0.22, 2.74	1.07	0.30, 3.75
Death				
Annual	**0.89**	**0.87, 0.91**	**0.89**	**0.87, 0.91**
Within periods[b]				
1997-2001	0.89	0.79, 1.00	0.89	0.79, 1.00
2002-2006	**0.92**	**0.85, 0.99**	**0.92**	**0.85, 0.99**
2007-2011	**0.84**	**0.77, 0.92**	**0.84**	**0.77, 0.92**
2012-2016	0.90	0.77, 1.06	0.90	0.77, 1.06
Across periods[c]				
1997-2001	ref	--	ref	--
2002-2006	**0.62**	**0.44, 0.87**	**0.62**	**0.44, 0.87**
2007-2011	**0.37**	**0.26, 0.51**	**0.37**	**0.26, 0.52**
2012-2016	**0.16**	**0.11, 0.24**	**0.16**	**0.11, 0.24**

Abbreviations: cause-specific hazard ratio, csHR; subdistribution hazard ratio (sdHR); CI, confidence interval.

[a] Adjusted for race/ethnicity and inverse probability weighted for cohort/subsite

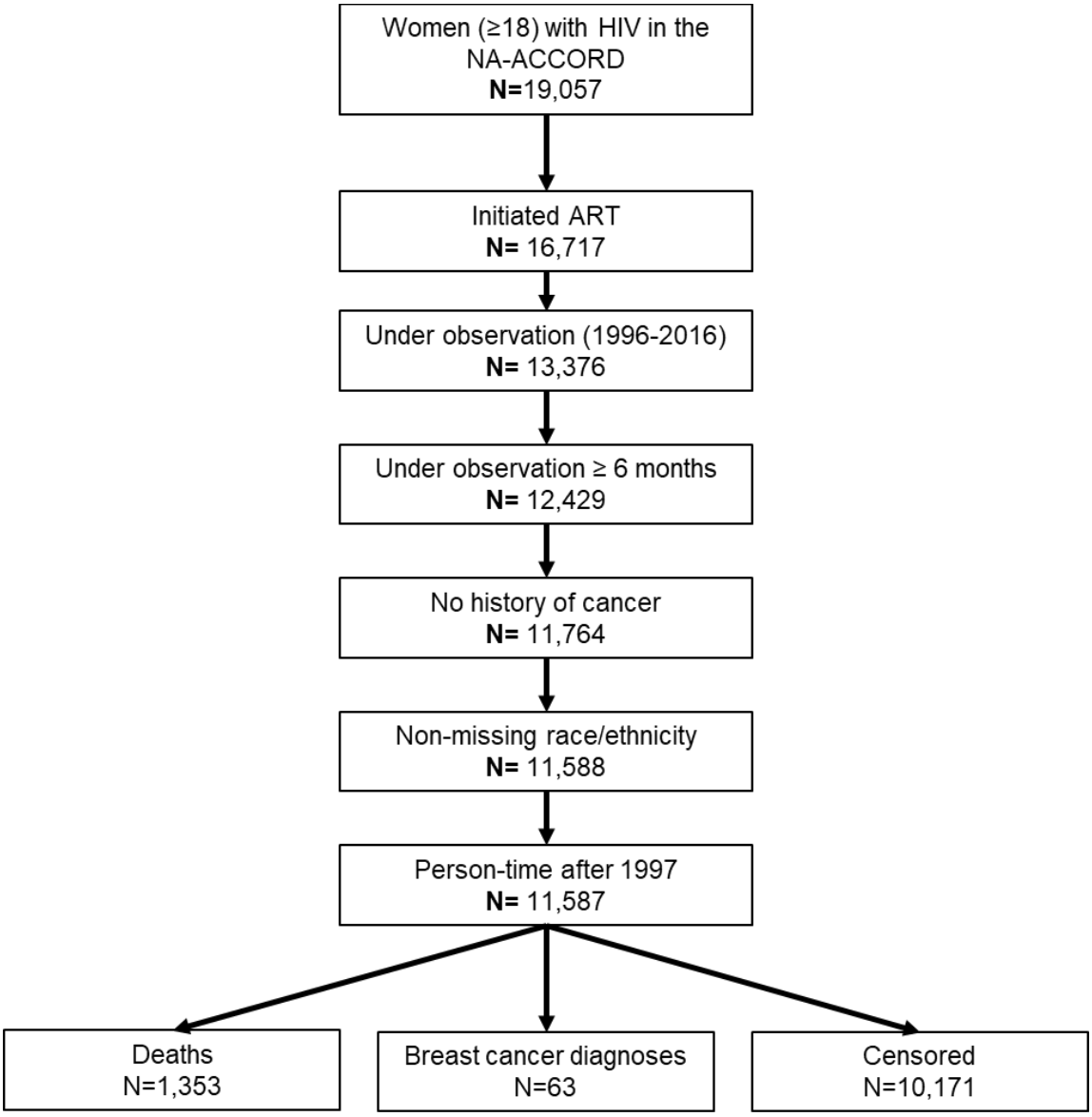
[b] Interpreted as: annual change in breast cancer hazard within the stratum

[c] Interpreted as: change in breast cancer hazard over each calendar period compared to 1997-2001

Statistically significant estimates are bolded.

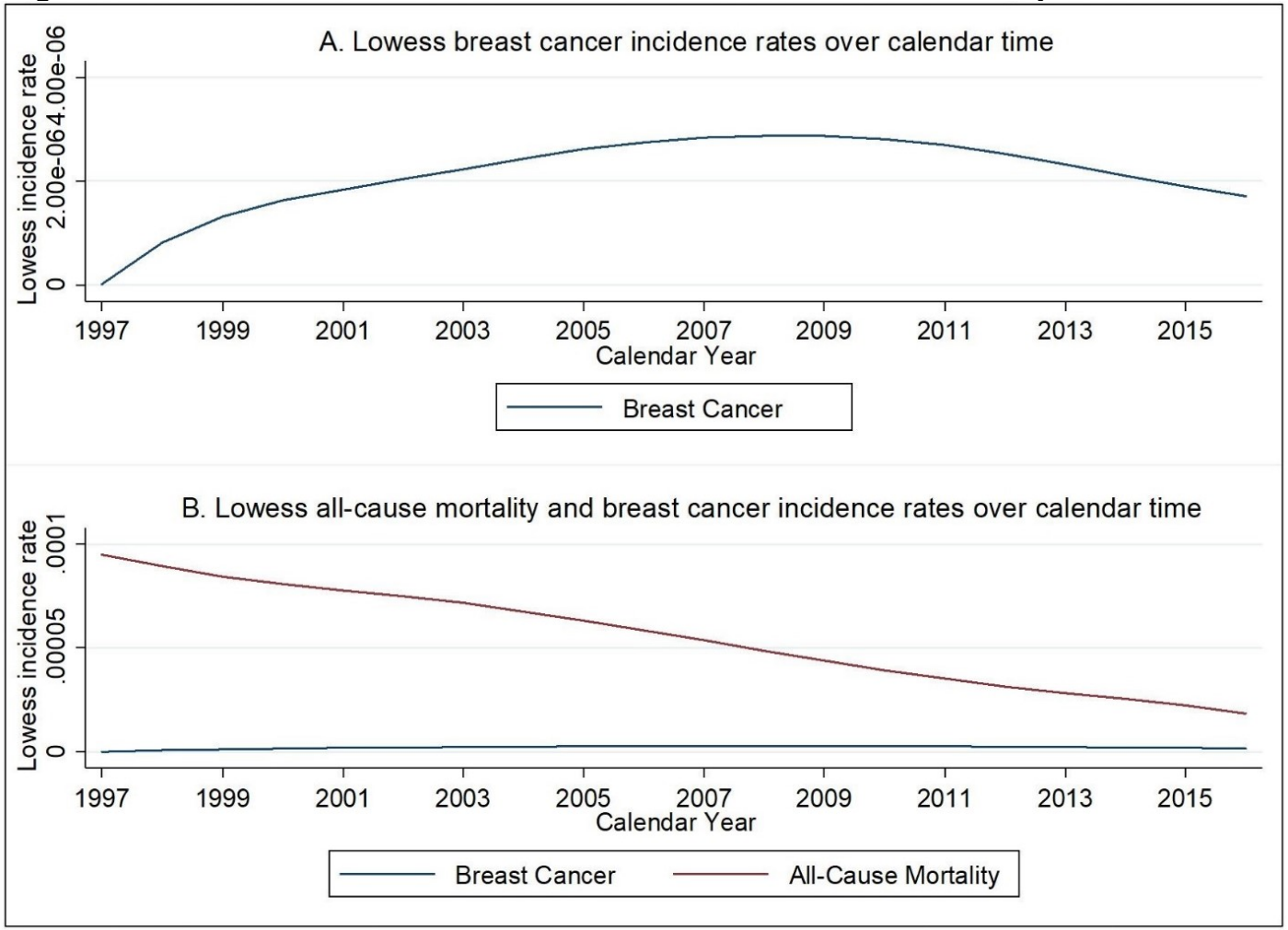
Chapter 2 Figures

Figure 2-1. Flow diagram for inclusion into the analytic study population, NA-ACCORD, 1997-2016



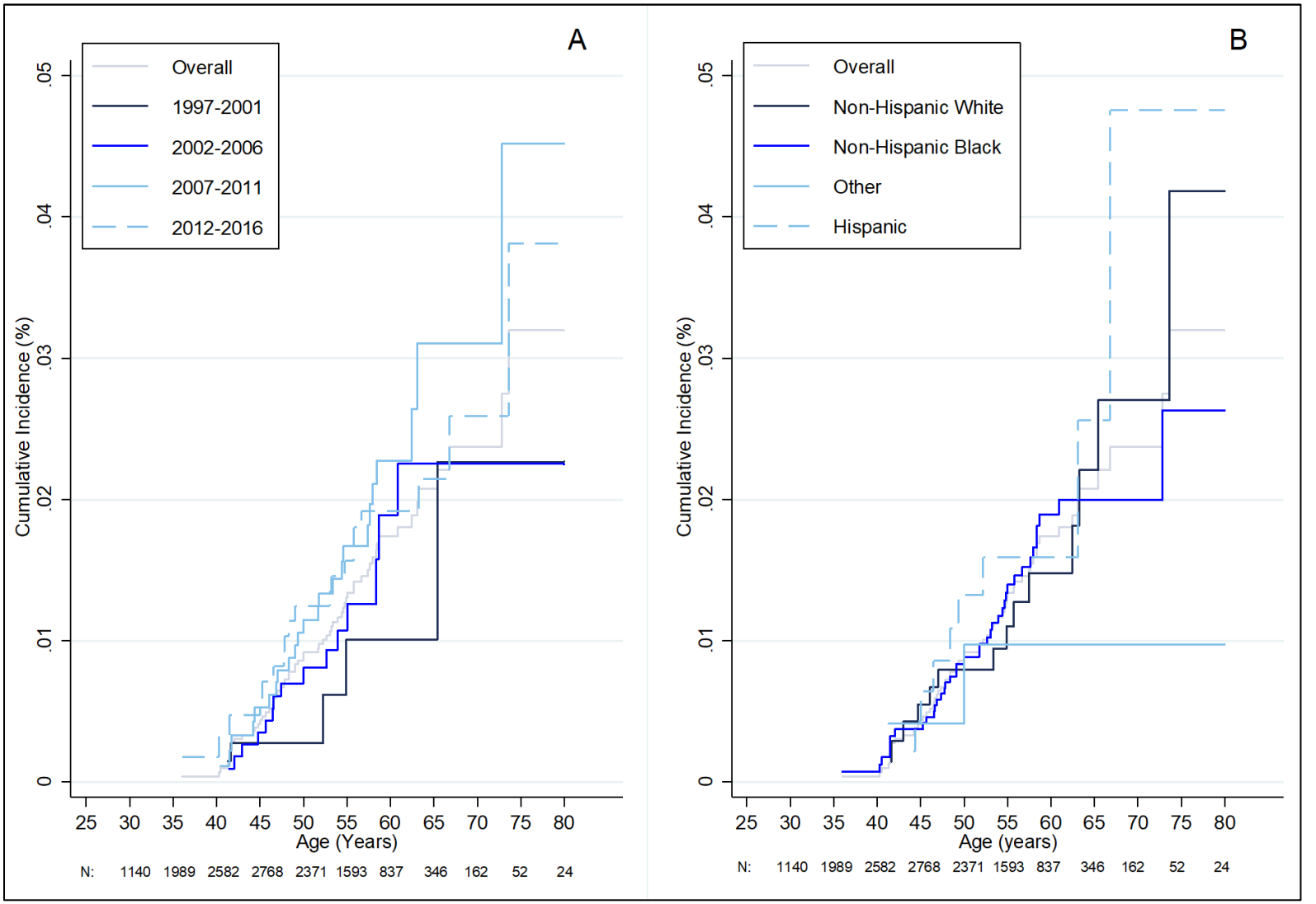
Abbreviations: antiretroviral therapy, ART.

Figure 2-2. Lowess smoothed incidence rates for breast cancer and all-cause mortality over calendar time



Note: Figure 2-2B plots both breast cancer and mortality lowess incidence rates on the same axis, while Figure 2-2A plots breast cancer rates long on a magnified axis. That y-axes are on difference scales.

Figure 2-3. Cumulative incidence of breast cancer over age by calendar period and race/ethnicity (N=11,587), NA-ACCORD, 1997-2016



Panel A: Cumulative incidence of breast cancer stratified by calendar periods; Panel B: Cumulative incidence of breast cancer stratified by race/ethnicity

Note: Truncated at age 80 due to limited person-years (<100 person-years)

Chapter 2 Appendices

Appendix 2-1 Supplemental Tables

Supplemental Table 2-1. Secular change in breast cancer and death by calendar time adjusting for AIDS-defining illness

	Cause-specific hazard		Subdistribution hazard	
	csHR[a]	95% CI	sdHR[a]	95% CI
Breast cancer				
Annual	0.98	0.92, 1.05	1.01	0.95, 1.07
Within periods[b]				
1997-2001	1.03	0.67, 1.59	1.09	0.70, 1.68
2002-2006	1.00	0.75, 1.32	1.02	0.76, 1.35
2007-2011	0.95	0.77, 1.18	0.98	0.79, 1.21
2012-2016	1.01	0.74, 1.37	1.02	0.75, 1.38
Across periods[c]				
1997-2001	ref	--	ref	--
2002-2006	0.77	0.18, 3.33	0.89	0.21, 3.82
2007-2011	0.79	0.22, 2.81	1.01	0.28, 3.62
2012-2016	0.78	0.23, 2.70	1.09	0.32, 3.68
Death				
Annual	**0.89**	**0.87, 0.91**	**0.89**	**0.87, 0.91**
Within periods[b]				
1997-2001	0.89	0.80, 1.00	0.89	0.80, 1.00
2002-2006	**0.92**	**0.85, 0.99**	**0.92**	**0.85, 0.99**
2007-2011	**0.84**	**0.77, 0.92**	**0.84**	**0.77, 0.92**
2012-2016	0.90	0.77, 1.06	0.90	0.77, 1.06
Across periods[c]				
1997-2001	ref	--	ref	--
2002-2006	**0.62**	**0.44, 0.87**	**0.62**	**0.44, 0.87**
2007-2011	**0.37**	**0.26, 0.51**	**0.37**	**0.26, 0.52**
2012-2016	**0.16**	**0.11, 0.24**	**0.16**	**0.11, 0.24**

Abbreviations: cause-specific hazard ratio, csHR; subdistribution hazard ratio (sdHR); CI, confidence interval.

[a] Adjusted for race/ethnicity and inverse probability weighted for cohort/subsite

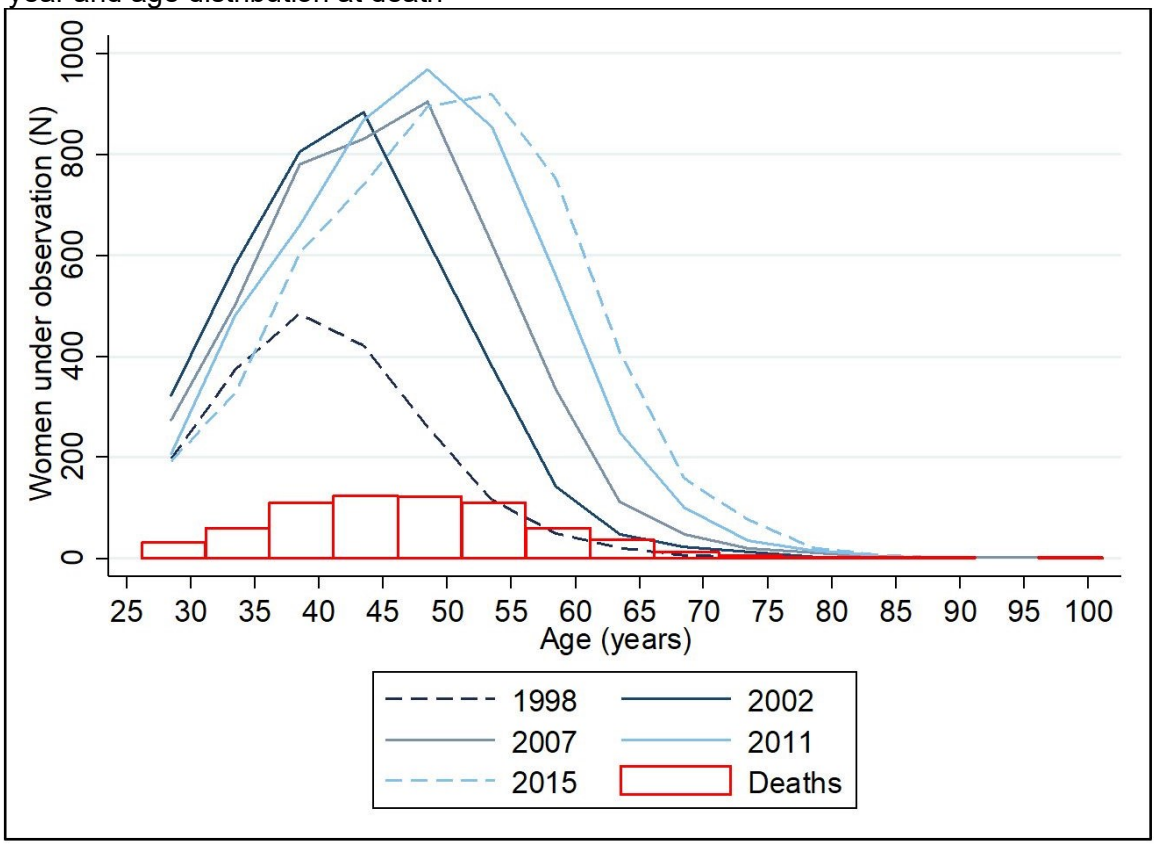
[b] Interpreted as: annual change in breast cancer hazard within the stratum

[c] Interpreted as: change in breast cancer hazard over each calendar period compared to 1997-2001

Statistically significant estimates are bolded.

Appendix 2-2 Supplemental Figures

Supplemental Figure 2-1. Age distributon of women under observation by calendar year and age distribution at death



Chapter 3: Aim 2

Differences in estradiol by HIV status, and among women with HIV, by viral suppression status

Abstract

Background: Characterizing estradiol among women with HIV has implications for breast cancer risk but has not been well-explored. Studies suggesting serum estradiol concentrations are lower in women with versus without HIV are limited in sample size and have not accounted for confounding or sex hormone binding globulin (SHBG). This aim sought to quantify differences in circulating estradiol and SHBG among women with and without HIV.

Methods: I used data from the sex steroid substudy conducted within the Women's Interagency HIV Study (WIHS) from 2003-2006. The WIHS is a multisite cohort of women with and without HIV in the US. Premenopausal women were included if they reported having a period the past six months, were not pregnant, had no history of oophorectomy, no breastfeeding in the last six months, and no exogenous hormone use in the past twelve months. All samples were collected on days 2-4 at the start of the menstrual cycle. I calculated geometric means of total/free estradiol (pg/mL) and SHBG by HIV status. I used weighted (by sociodemographic and clinical factors) quantile regression to quantify estradiol/SHBG differences at the 25th, 50th, and 75th percentile by HIV status, and among women with HIV, by viral suppression status.

Results: Among 643 women, the median age was 37 years (IQR 32, 42), 63% were Black, and 51% reported being current smokers. Total estradiol was significantly lower at all percentiles in women with suppressed viral load vs. women without HIV (ranging 4-10 pg/mL lower). Free estradiol at the 75th percentile was 0.29 pg/mL lower comparing women with suppressed viral load to women without HIV (95% CI -0.57, -0.02), and among women with HIV, free estradiol was 0.44 pg/mL lower in women with suppressed versus not suppressed viral load (95% CI -0.74, -0.13). SHBG was significantly higher in

women with unsuppressed viral load compared to women without HIV at the 25th and 50th percentile (β=10, 95% CI 3.50, 16.50; β=12, 95% CI 3.41, 20.58).

Conclusions: Total and free estradiol were slightly lower in women with versus without HIV, though differences were not clinically meaningful. SHBG was significantly higher comparing women with unsuppressed viral load to women without HIV, which should be investigated in future studies. Overall, these findings merit further exploration in a larger, contemporary sample of women with and without HIV, who are pre and postmenopausal, especially given the population-level aging observed in women with HIV.

Introduction

There is evidence that people living with HIV experience gonadal and hormonal dysfunction.[60,143] Though this is well documented in men with HIV,[144] there is less known in women with HIV. Understanding the impact of HIV infection on endogenous sex steroid hormones in women, particularly estrogen, is critical because these hormones modulate the immune system[145] and are associated with chronic comorbidities, including breast cancer (both pre and post-menopausal)[113,114,146–149] and cardiovascular disease.[150,151] Few studies have demonstrated that breast cancer risk is significantly lower comparing women with versus without HIV, and estrogen suppression has been hypothesized as a potential mechanism;[78–80] though this has not been explored. Further, as women with HIV have life expectancies approaching those of the general population, and the age distribution of women with HIV shifts toward post-menopause,[1,13,14] these comorbidities are increasingly relevant and such information could inform reproductive health care.

Existing epidemiologic research addressing sex hormones in women with HIV is scarce, and whether there is hormonal dysregulation is unclear. Some studies indicate a high prevalence of amenorrhea in women with HIV (estimates varying from 5%-48%) compared to uninfected women. [65,66,115] One meta-analysis found that the odds of amenorrhea was 70% higher in women with versus without HIV.[68] By contrast, others show no association with HIV status.[61,152–154] Prior studies have also suggested earlier onset of menopause; though these findings are not definitive, and defining menopausal status is difficult due to how commonly amenorrhea is observed in women with HIV.[60–64] Plausible mechanisms that may explain hormonal dysfunction in women with HIV include high prevalence of opioid use and smoking, stress, weight loss, and the proinflammatory state associated with HIV, but further research is needed.

Characterizing hormonal dysregulation in women with HIV is complicated by natural fluctuations in hormones within the menstrual cycle in premenopausal women. Additionally, the association between HIV, specifically HIV viral load, and estrogen may vary within the menstrual cycle: one study found that plasma viral load varied within the menstrual cycle, with viral load falling a median of 0.16 $\log_{10}$ copies/mL from the early follicular phase to the mid-luteal phase.[115] Women with HIV may also take mediations/narcotics that can suppress ovulation more frequently than women in the general population. Therefore, in women with HIV, it is difficult to determine whether hormonal dysfunction is due to menopause, hypothalamic dysregulation (due to stress/illness), lifestyle factors, or anovulation.

Biomarker measurements offer a direct method of assessing hormonal dysfunction in women with HIV. Estradiol is an important biomarker of hormonal dysfunction because it is the most biologically active form of estrogen and most abundant prior to menopause.[155] The research thus far is equivocal. One study suggested that total estradiol was lower in premenopausal women with versus without HIV and that other hormones, such as testosterone, may also differ by HIV status in women.[111] Other studies have found no difference in total estradiol by HIV status (though estradiol was not the primary outcome) [62,116,117], or that estradiol was higher in women with versus without HIV.[118,119]

These studies were limited in sample size and did not account for relevant covariates contributing to potential differences in estrogen levels such as body mass index (BMI), smoking, and among women with HIV, treatment, or immune status. Importantly, these works also did not consider the role of sex hormone binding globulin (SHBG) or assess free estradiol. When estradiol circulates in the blood, it is usually bound to albumin, or more commonly SHBG. While estradiol is bound to SHBG, it cannot enter cells, activate receptors, or carry out its function. The remaining estradiol

(unbound) is free estradiol, and can interact with cells and their receptors, carrying out its function. Thus, free estradiol, as opposed to total estradiol, is most relevant to adverse health outcomes. Assessment of both SHBG and free estradiol is critical in fully characterizing the association between estradiol and HIV status. Previous studies have also not considered the association between HIV viremia and estradiol concentrations among women with HIV.

The objective of this aim was to quantify differences in total and free estradiol as well as SHBG between women with and without HIV, and among women with HIV by viral suppression status using a large sample of US women with and without HIV who are socio-demographically comparable.

Methods

Study Population

Data for this study were previously collected by the Women's Interagency HIV Study (WIHS), now the MACS/WIHS Combined Cohort Study. The WIHS is a multicenter prospective, interval cohort study of women with and at high risk for HIV in the US.[130] Established in 1993, there are six consortia comprised of multiple sites across the country. Study visits are conducted every six months, including a structured interview (assessing medical history, antiretroviral therapy [ART], lifestyle factors, and healthcare utilization), physical and gynecologic exams, HIV status and HIV viral load, and biological specimen collection. There have been four recruitment waves since the inception of the WIHS. As of 2019, the WIHS joined with the Multicenter AIDS Cohort Study of gay and bisexual men to form the MACS/WIHS Combined Cohort Study. The data are maintained by the Department of Epidemiology at the Johns Hopkins Bloomberg School of Public Health.

Women participating in the sex steroid substudy nested within the WIHS were eligible for our study.[62,111] The sex steroid substudy took place from visits 18-25 (2003 to 2006), during which serum samples were collected to assess cycle-specific sex steroid hormones (estradiol, inhibin-B, SHBG, and follicle-stimulating hormone [FSH]) to assess the effect of HIV on the age of onset for diminishing ovarian reserve. Samples were collected among women at one time point on one day on days 2-4 of the start of her menstrual cycle. Women were eligible if they reported having a period the past six months, were not pregnant, had no history of unilateral or bilateral oophorectomy, reported no breastfeeding in the last six months, and reported no exogenous hormone use (including hormonal contraceptives and hormone replacement therapy) in the prior twelve months.

Women were included in this study if they met these inclusion criteria, had complete data regarding inclusion criteria, and had an estradiol measurement (n=643). Women were additionally excluded if they: 1) self-reported being menopausal (n=6), 2) did not have HIV viral load measured at the biomarker visit (n=2); 3) had a high level of total estradiol (>350 pg/mL, [n=1]) given the inclusion criteria and when during the menstrual cycle estradiol was supposed to be measured; and 4) were missing covariates included in regression analyses (n=9) (**Figure 3-1**).

Biomarker Measurements

Total estradiol was assessed continuously and measured in picograms per mL (pg/mL). Estradiol was measured using serum samples on the Coat-A-Count solid-phase radioimmunoassay (Siemens Medical Solutions, Malvern, Pennsylvania) with six-dilution calibration standards and a zero control (lower limit of quantification=20 pg/mL).[62,111] SHBG was tested using a chemiluminescent assay run on the Siemens DPC Immulite (Siemens DPC, Washington DC) using their reagents, and was assessed continuously

and measured in nanomoles per liter (nmol/L). Estradiol and SHBG were both performed by Quest Diagnostic Laboratories (Baltimore, Maryland). Of the 643 women included, 431 also had SHBG measured at the same visit and laboratory. Free estradiol was calculated using total estradiol, SHBG and a constant for albumin.[156]

Covariates

Race/ethnicity was defined categorically: non-Hispanic white, non-Hispanic Black, Hispanic, and other race/ethnicity. These categories were selected to ensure sufficient sample size to conduct regression analyses and maximize precision. Covariates assessed at the time of biomarker measurements included: body mass index (BMI) (analyzed continuously as kg/m^2), smoking status (current, former, never), alcohol use (abstain, 1-7 drinks/week, 8-12 drinks/week, 12+ drinks/week), age at menarche (years of age), parity (nulliparous, 1, 2, 3, 4+ births), age (years), and Hepatitis C status (measured using HCV RNA among those seropositive at entry into the WIHS). Among women with HIV, current ART status was collected (no therapy versus on therapy), as was cumulative duration of protease inhibitor use, and CD4 count (assessed continuously, cells/mm^3). Missing values for BMI and CD4 count were carried forward from the visit prior (6-12 months) as needed (n=13 and n=1, respectively).

Exposure Assessment

HIV status was stratified into three categories: women without HIV, women with HIV and suppressed viral load defined as HIV RNA <200 copies/mL, or women with HIV and unsuppressed viral load (HIV RNA ≥ 200 copies/mL). This cut off for viral load was used because it is a clinically relevant marker with respect to HIV transmission, has been associated with adverse health outcomes, and is the current threshold used to define virologic failure used by the Department of Health and Human Services.[157,158]

Comparisons were made between women without HIV to women with HIV with suppressed, or unsuppressed viral load (two pairwise comparisons). Among women with HIV, women were compared by suppression status at the biomarker visit. To explore the temporality of the association between viral suppression and estradiol/SHBG, among women with HIV, we also assessed the viral suppression status at the last visit prior (within 6-12 months) to the biomarker measurement.

Statistical Analyses

First, differences in demographic, clinical and reproductive factors by HIV status were evaluated using non-parametric 2-sample tests for continuous covariates and chi-square tests for categorical variables. Second, I calculated geometric means for total estradiol, free estradiol, and SHBG by HIV status, and among women with HIV, by viral suppression status. T-tests on the log transformed values of these biomarkers was utilized to assess statistically significant differences by HIV status and viral suppression status.[159] Geometric means were selected as opposed to arithmetic means to reduce sensitivity to skewed values.

Third, I utilized weighted quantile regression to estimate differences in total estradiol, free estradiol, and SHBG by HIV status. Pairwise comparisons were made with women without HIV as the reference group to: a) women with HIV and suppressed viral load, and b) women with HIV and unsuppressed viral load. Among women with HIV, women with suppressed versus unsuppressed viral load at the biomarker visit and 6-12 months prior to the visit were also compared.

Quantile regression assesses whether there is a shift in the distribution of the outcome at a particular quantile.[160,161] For instance, at the 50th percentile (i.e., median), quantile regression assesses if there is a difference in the median estradiol levels between women with HIV and suppressed viral load as compared to women without

HIV. This can be done for any percentile (1^{st}, 2^{nd}, … 99^{th}) and allows for assessment of shift in the entire distribution, not only at the central tendency (i.e., mean or expected value that generalized linear models typically are modeling). Therefore, this approach can provide a comprehensive examination of the entire distribution of the outcome. This methodology is also advantageous when the outcome is not normally distributed,[161] which was evident in this analytic population. These biomarkers were assessed at three separate quantiles: 25^{th} percentile, 50^{th} percentile, and 75^{th} percentile to capture associations with HIV status across the distribution.

Stabilized inverse probability weights were utilized to address confounding by several measured covariates.[162] Time-fixed weights were estimated using multinomial and logistic models where HIV status was the outcome, with following variables as predictors: race/ethnicity, log-transformed BMI, smoking status, current Hepatitis C status, alcohol use, age at menarche, parity, and current age. Models among women with HIV only were additionally weighted for ART status and CD4 count. Robust variance estimation was used to calculated standard errors. Estimates from the weighted quantile regression for each percentile (1^{st}-99^{th}) were then graphically assessed to describe the distribution of estradiol stratified by HIV status.

Subgroup analyses were conducted excluding women with total estradiol levels >150 pg/mL (n=13). These were considered likely to be inaccurate due to measurement error given that estradiol measurements were supposed to be collected on days 2-4 of the menstrual cycle. I also conducted subgroup analyses among women with both FSH and inhibin-B data available who also had FSH below 20 pg/mL and detectable inhibin-B (n=289) to mitigate the inclusion of women who may be menopausal.

Results

Characteristics of the Study Population

There were 643 women who participated in the sex steroid substudy meeting our inclusion criteria (**Table 3-1**). Overall, women were predominantly non-Hispanic Black (63%), had a median age of 37 (IQR 32, 42) years, and were Hepatitis C negative (86%). Fifty-one percent of women reported currently smoking, and 9% reported problematic drinking (>7 drinks/week). Comparing women with versus without HIV, BMI was statistically significantly lower (p=0.01), reported current smoking was lower (p=0.001), and reported drinking was lower (p<0.01). There were also statistically significant differences in parity comparing women with versus without HIV (p=0.03). Age at menarche was similar in women with and without HIV, with an overall median of 12 years.

Sixty-eight percent of participants were women with HIV. Among women with HIV, 60% were not virally suppressed at the biomarker visit (n=263), and 40% (n=177) were virally suppressed. Women with unsuppressed viral load were more commonly non-Hispanic Black relative to those achieving suppression. Women with suppressed viral load were more likely to currently be on ART (87%) compared to women without viral suppression (53%), (p <0.01). Women with suppressed viral load had higher CD4 counts compared to unsuppressed women (524 vs. 367 cells/μL), p=0.03) and were more likely to also be suppressed at the visit 6-12 months prior (76% vs. 18%, p<0. 01). The duration of protease-inhibitor (PI) use was comparable by suppression status (p=0.26). Among women with HIV, women with unsuppressed viral load were more likely to be smokers (55% vs. 33%). Current self-reported problematic drinking was lower in women with suppressed viral load.

Geometric Means of Total Estradiol, Free Estradiol and SHBG

Geometric means of total estradiol were statistically significantly lower in women with compared to without HIV (**Table 3-2**): (36.9 pg/mL vs. 41.6 pg/mL, p=0.006). Among women with HIV, total estradiol was lower in women who achieved suppression: 38.2 pg/mL among unsuppressed women vs. 35.1 pg/mL among suppressed women, but was not statistically significant. Total estradiol was significantly lower in women with suppressed versus unsuppressed viral load when assessed at the visit prior (p=0.03). Free estradiol did not differ comparing women with versus without HIV (p=0.21). Among women with HIV, free estradiol was significantly higher in women who did not achieve suppression (1.4 vs. 1.2 pg/mL, p=0.01), which was similar when suppression status was assessed at the visit prior (p=0.02). SHBG was higher in women with versus without HIV (58.8 nmol/L vs. 47.0 nmol/L, p<0.01). Among women with HIV, SHBG was not significantly different by viral suppression status assessed at the biomarker measurement or at the visit prior (p=0.10, and p=0.51, respectively).

Regression Estimated Differences in Total Estradiol, Free Estradiol, and SHBG

Quantile regression estimates revealed potential differences in total estradiol by HIV status (**Table 3-3**). In weighted analyses, women with suppressed viral load compared to women without HIV had lower total estradiol at the 25th, 50th and 75th percentile. Median total estradiol was 5 pg/mL lower among women with suppressed viral load compared to women without HIV (β= -5, 95% CI -9.99, -0.01). Therefore, while women without HIV had an expected median of 40 pg/mL, women with suppressed viral load had an expected median of 35 pg/mL. The 25th percentile of total estradiol was also 4 pg/mL lower comparing women with unsuppressed viral load to women without HIV (β= -4, 95% CI -7.77, -0.23). The 75th percentile of total estradiol was 10 pg/mL lower in women with suppressed viral load compared to women without HIV (β= -10, 95% CI -

16.25, -3.75). Among women with HIV, there were no statistically significant differences in total estradiol when viral suppression was assessed at the same time as the total estradiol measurement; though women with suppressed viral load consistently had lower total estradiol than women with unsuppressed viral load. When suppression was assessed at the visit prior, the 25th percentile was 6 pg/mL lower in women with suppressed viral load compared to women with unsuppressed viral load (β= -6, 95% CI -9.54, -2.46).

With respect to free estradiol, unweighted analyses indicated that the 75th percentile was lower in women with suppressed viral load compared to women without HIV (β= -0.29, 95% CI -0.57, -0.02) (**Table 3-4**). Among women with HIV, the 75th percentile was -0.44 lower in suppressed compared to unsuppressed women (95% CI -0.74, -0.13). When viral suppression was assessed at the visit prior, this association remained, and median free estradiol was additionally lower in suppressed compared to unsuppressed women (β= -0.18, 95% CI -0.32, -0.04). However, upon accounting for confounding, these associations were no longer statistically significant.

The largest differences by HIV status were observed for SHBG (**Table 3-5**). In weighted analyses, the 25th and 50th percentile were both statistically significantly higher comparing women with unsuppressed viral load to women without HIV: the 25th percentile was 10 nmol/L higher (95% CI 3.50, 16.50), and the 50th percentile was 12 nmol/L higher (95% CI 3.41, 20.58). The 75th percentile was also estimated to be 10 nmol/L higher, though this was not statistically significant. The remaining comparisons between women without HIV to women with suppressed viral load were not significant, ranging from 2-3 nmol/L. There were also no significant differences in SHBG by viral suppression status among women with HIV; though the greatest differences were observed at the 75th percentile of SHBG.

The graphical depiction of each percentile for total estradiol, free estradiol and SHBG by HIV status, and among women with HIV, viral suppression status at the time of the biomarker measurement are shown in **Figure 3-2**. While the shape of the distribution is similar across groups (right skewed), the distribution of total estradiol among women with HIV is shifted towards slightly lower values. The shape and position of the distribution of free estradiol is very similar comparing women with and without HIV. The shape of the distribution for SHBG is also similar across groups; however, the distribution among women without HIV is shifted towards lower values, especially for women with HIV and were not virally suppressed.

In subgroup analyses among women with total estradiol levels below 150 pg/mL, findings were similar (**Appendix 3-1**). In subgroup analyses among women who had FSH and inhibin-B data, with FSH <20 pg/mL and detectable inhibin-B, quantile regression estimates were also comparable to the main analysis (**Appendix 3-1**). Of note, and slightly different from the main analysis, the 75th percentile of free estradiol was significantly lower comparing women with suppressed viral load to women without HIV in weighted analyses. Among women with HIV, the 50th and 75th percentile of free estradiol was also lower comparing women with suppressed to unsuppressed viral load.

Discussion

In a sample of comparable premenopausal women with and without HIV in the US, though there were statistically significant differences in total and free estradiol, these differences were not likely to be clinically meaningful. I observed comparable levels of total and free estradiol in women with versus without HIV, with the modest differences observed when comparing women without HIV to women with suppressed viral load. There were noteworthy higher levels of SHBG comparing women with versus without HIV, especially for women with unsuppressed viral load. There were no significant

differences in SHBG by suppression status in women with HIV. The estimated shape of the distribution of total and free estradiol for each group were visually comparable, while the distribution of SHBG was shifted towards lower values for women without HIV compared to women with HIV.

High SHBG could impact the amount of free estradiol in circulation, leading to lower free estradiol concentrations. This was not consistently observed in this analysis, where significant, though modest differences in free estradiol were only observed at the 75th percentile of the estradiol distribution comparing women without HIV to women with women with suppressed viral load, and among women with HIV, by suppression status.

Our assessment of total estradiol was comparable to prior works (including an additional analysis of the WIHS sex steroid substudy), where total estradiol ranged from 32-44 pg/mL for women without HIV, and 33-37 pg/mL for women with HIV.[111,116,118] There are few studies in large groups of women that are socio-demographically similar to the WIHS; however, these biomarkers are commonly assessed in the context of breast cancer risk in the general population. In two case-control studies assessing breast cancer odds in the Nurses' Health Study, the geometric means for total estradiol were 45 and 51 pg/mL, free estradiol was 0.6 pg/mL in both analyses and SHBG was 59 and 62 nmol/L (all measured during early follicular phase, among controls).[113,163,164] Differences between these estimates and the women without HIV in our sample are likely due to substantial differences in the source populations.

Beyond the breast cancer literature, an analysis from the Harvard Moods and Cycles study found mean early follicular (days 1-5 of the menstrual cycle) estradiol was 30 pg/mL at the baseline assessment.[165] One study from the Study of Women's Health Across the Nation (SWAN) study, a community-based multiethnic prospective cohort study, also found mean total estradiol of 76 pg/mL, mean free estradiol was 0.8 pg/mL, and mean SHBG of 45 nmol/L among pre and perimenopausal women (assessed

predominantly during early follicular phase).[166] The relatively higher estradiol and lower SHBG compared to our sample could be explained by sociodemographic differences between the source populations, and/or the inclusion of serum measurements beyond the early follicular phase (19% of the sample had a random fasting measurement).

There are not comparable studies using regression techniques to characterize the association between these biomarkers and HIV. My analysis indicating a modest to null association between total estradiol and HIV status has been seen in univariate analyses of early follicular total estradiol, including within the WIHS.[62,111,116] By contrast, another study found that maximum follicular estradiol was higher in women with HIV (148 pg/mL) compared to women without HIV (111 pg/mL), all of whom reported regular menses.[119] The discrepancy between these findings and my research may be attributed to the timing and assessment of maximum total estradiol over the entire follicular phase, or differences in the source populations. There were no comparable studies examining free estradiol in women with compared to without HIV. Higher SHBG levels comparing women with versus without HIV has been reported previously in the WIHS sex steroid substudy (58.5 nmol/L in women with HIV versus 47.0 nmol/L in women without HIV); however, comparable studies assessing SHBG in women with versus without HIV were not found.[111]

The observation that, among women with HIV, total and free estradiol were modestly lower in women with suppressed versus unsuppressed viral load contradicts one other study which found that luteal estradiol was higher among women with a lower viral load (<3.05 logbDNA vs. > 3.05 logbDNA) among women with no exposure to ART.[167] That said, the differences I observed, though statistically significant, are not likely physiologically impactful. Women achieving viral suppression may have a longer duration of infection relative to women not achieving suppression. It is possible that longer cumulative exposure to HIV may impact estradiol differently than viral

suppression captured at one time point. Though data on HIV infection date were not available, I assessed differences in time from ART initiation as a proxy for duration of infection and found no differences by suppression status. This limited difference in total or free estradiol by suppression status I observed may be related to the fact that these women are participating in an interval cohort study and could be generally healthier than the broader population of women with HIV, where there may be larger or qualitative differences in viral suppression as it relates to estradiol. Alternatively, selecting only women reporting a period in the six months prior may have made the comparison groups more homogenous than what would be expected in the real world, obscuring potential differences.

Although this is one of the largest studies assessing differences in estradiol and SHBG by HIV status, sample size was limited for inferences at the extreme ends of the distribution and should be interpreted cautiously. This is a cross-sectional analysis so inferences cannot be made regarding the temporality of the association between viral suppression and estradiol. Specifically, this study cannot address the association between duration of exposure to HIV and estradiol, which may differ from cross-sectional associations. Exposure and outcome measurement were only assessed at one time point, which makes them prone to measurement error. Measurement error of estradiol is compounded by the use of radioimmunoassay (RIA), as opposed to a liquid chromatography with tandem mass spectrometry (LC MS/MS) assay to measure estradiol, the gold standard. Free estradiol was calculated using standard equations that assume linear binding affinity of estradiol to SHBG, which may not represent the true relationship between SHBG and estradiol. Lastly, estradiol was measured in 2003-2006 and findings may not be generalizable to women living with HIV today.

This aim represents a novel assessment of total and free estradiol as well as SHBG by HIV status accounting for confounding by important covariates. This work

demonstrates the importance of accounting for SHBG when assessing estradiol among women with versus without HIV and motivates further investigation in a larger, more contemporary sample. Given the increased risk of hormonally-related comorbidities with increasing age, and the shift in the age distribution of women with HIV towards older ages, it will also be especially important to understand the changing distributions of estradiol among peri and post-menopausal women with HIV as it compares to the general population of women.

Chapter 3 Tables

Table 3-1. Demographic and clinical factors by HIV status among women in the sex steroid substudy (WIHS)

	Women with HIV (unsuppressed viral load)		Women with HIV (suppressed viral load)		Women without HIV		
	N=263		N=177		N=203		
	Median/N	IQR/%	Median/N	IQR/%	Median/N	IQR/%	p-value[a]
Age (years)	38	33,42	37	32,42	37	29, 44	0.58
Race							0.82
Non-Hispanic white	12	4.6	16	9.0	11	5.4	
Non-Hispanic Black	179	68.1	98	55.4	129	63.5	
Hispanic	67	25.5	56	31.6	55	27.1	
Other	5	1.9	7	4.0	8	3.9	
BMI	27.8	23.7, 32.8	28	24.3, 34.2	29.5	24.8, 36.0	**0.01**
Current smoking status							**<0.01**
Current	145	55.1	58	32.8	125	61.6	
Former	47	17.9	43	24.3	36	17.7	
Never	71	27.0	76	42.9	42	20.7	
Current alcohol use							**<0.01**
Abstain	125	47.5	102	57.6	69	34.0	
1-7 drinks/week	113	43.0	66	37.3	110	54.2	
8-12 drinks/week	11	4.2	3	1.7	9	4.4	
>12 drinks/week	14	5.3	6	3.4	15	7.4	
Hepatitis C status							0.79
Negative	225	85.6	153	86.4	176	86.7	
Positive	38	14.4	24	13.6	27	13.3	
Age at menarche (years)							0.27
11 or less	75	28.5	47	26.6	68	33.5	
12-13	109	41.4	88	49.7	88	43.3	
>13	79	30.0	42	23.7	47	23.2	
Parity							**0.03**
0	42	16.0	35	19.8	49	24.1	
1	39	14.8	32	18.1	41	20.2	

2	58	22.1	45	25.4	29	14.3	
3	50	19.0	23	13.0	36	17.7	
4+	74	28.1	42	23.7	48	23.6	
Current ART							**<0.01**
No therapy	123	46.8	23	13.0			
On therapy	140	53.0	154	87.0			
Duration of any PI use							0.26
None	138	52.5	80	45.2			
<1 year	35	13.3	22	12.4			
1-<3 years	45	17.1	43	24.3			
3+ years	45	17.1	32	18.1			
HIV suppression at prior visit							**<0.01**
Suppressed	47	17.9	135	76.3			
Unsuppressed	216	82.1	42	23.7			
Current HIV RNA (copies/mL)	7600	1600, 32000	80	80, 80			**<0.01**
Current CD4 count (cells/mm^3)	367	245, 519	524	394, 722			**0.03**

Abbreviations: interquartile range, IQR; body mass index, BMI; antiretroviral therapy, ART; protease inhibitor, PI.

[a] Non-parametric 2-sample test for continuous variables and chi-square test for categorical variables comparing all women with HIV versus without HIV. For variables relevant to HIV, statistical tests were conducted comparing suppressed versus unsuppressed

Bolded values demonstrate statistically significant difference by HIV status

Table 3-2. Geometric means of estradiol and SHBG by HIV status and viral suppression status

	Women with HIV						Women without HIV					
	Estradiol (pg/mL)		Free estradiol (pg/mL)		SHBG (nmol/L)		Estradiol (pg/mL)		Free estradiol (pg/mL)		SHBG (nmol/L)	
	N=440		N=308		N=308		N=203		N=123		N=123	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Overall	36.9	35.2, 38.8	1.4	1.3, 1.4	58.8	44.5, 62.2	41.6	38.9, 44.6	1.4	1.3, 1.6	47.0	42.5, 52.0
Suppressed							X	X	X	X	X	X
Yes	35.1	32.7, 37.8	1.2	1.1, 1.3	55.1	50.2, 60.5	X	X	X	X	X	X
No	38.2	35.8, 40.8	1.4	1.3, 1.5	61.1	56.8, 65.7	X	X	X	X	X	X
Suppressed at visit prior							X	X	X	X	X	X
Yes	34.7	32.3, 37.3	1.2	1.1, 1.3	57.1	52.2, 62.4	X	X	X	X	X	X
No	38.6	36.1, 41.3	1.4	1.3, 1.5	59.9	55.6, 64.6	X	X	X	X	X	X

Abbreviations: sex hormone binding globulin, SHBG; 95% confidence interval, 95% CI; suppressed, suppression of HIV RNA.
"X" indicates not applicable.

Table 3-3. Weighted quantile regression estimates for the association between HIV status and viral suppression status with total estradiol at the 25th, 50th, and 75th percentile, N=643

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	**-4**	**-7.57, -0.43**	**-4**	**-7.47, -0.53**
p50	**-5**	**-9.48, -0.52**	-4	-9.75, 0.75
p75	-2	-9.07, 5.07	-2	-9.44, 5.44
HIV suppressed vs. HIV negative[b]				
p25	-3	-6.93, 0.93	**-4**	**-7.77, -0.23**
p50	**-6**	**-10.27, -1.73**	**-5**	**-9.99, -0.01**
p75	**-10**	**-15.89, -4.11**	**-10**	**-16.25, -3.75**
Suppressed vs. unsuppressed[c]				
p25	1	-2.47, 4.47	-3	-8.22, 2.22
p50	-1	-4.86, 2.86	-4	-11.15, 3.15
p75	**-8**	**-14.42., -1.58**	-9	-18.68, 0.68
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-1	-4.67, 2.67	**-6**	**-9.54, -2.46**
p50	-3	-6.68, 0.68	-7	-14.77, 0.77
p75	**-7**	**-13.88, -0.12**	-11	-22.29, 0.29

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.
[a] At the stated percentile
[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use
[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count
Statistically significant estimates are bolded.

Table 3-4. Weighted quantile regression estimates for the association between HIV status and viral suppression status with free estradiol at the 25th, 50th, and 75th percentile, N=431

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	-0.07	-0.22, 0.08	-0.04	-0.20, 0.12
p50	-0.04	-0.23, 0.15	-0.01	-0.21, 0.21
p75	0.15	-0.18, 0.47	0.15	-0.18, 0.47
HIV suppressed vs. HIV negative[b]				
p25	-0.07	-0.23, 0.08	-0.04	-0.23, 0.16
p50	-0.15	-0.33, 0.03	-0.11	-0.33, 0.11
p75	**-0.29**	**-0.57, -0.02**	-0.18	-0.48, 0.11
Suppressed vs. unsuppressed[c]				
p25	-0.01	-0.17, 0.17	-0.01	-0.24, 0.24
p50	-0.11	-0.26, 0.04	-0.07	-0.32, 0.18
p75	**-0.44**	**-0.74, -0.13**	-0.40	-0.88, 0.08
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-0.07	-0.24, 0.10	0.01	-0.24, 0.25
p50	**-0.18**	**-0.32, -0.04**	-0.04	-0.28, 0.20
p75	**-0.40**	**-0.73, -0.07**	-0.15	-0.60, 0.31

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.
[a] At the stated percentile
[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use
[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count
Statistically significant estimates are bolded.

Table 3-5. Weighted quantile regression estimates for the association between HIV status and viral suppression status with sex hormone binding globulin at the 25th, 50th, and 75th percentile, N=431

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	**12**	**5.28, 18.72**	**10**	**3.50, 16.50**
p50	**13**	**5.23, 20.77**	**12**	**3.41, 20.58**
p75	**17**	**3.54, 30.46**	10	-7.18, 27.18
HIV suppressed vs. HIV negative[b]				
p25	6	-0.20, 12.20	3	-2.99, 8.99
p50	6	-2.19, 14.19	3	-7.32, 13.32
p75	6	-5.80, 17.80	2	-14.87, 18.87
Suppressed vs. unsuppressed[c]				
p25	-6	-12.10, 0.10	2	-5.17, 9.17
p50	-7	-15.17, 1.17	-3	-16.28, 10.28
p75	-11	-23.18, 1.18	-6	-22.98, 10.98
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-5	-11.59, 1.59	4	-6.73, 14.73
p50	-3	-10.73, 4.73	7	-5.72, 19.72
p75	-8	-21.99, 5.99	11	-24.94, 46.94

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.
[a] At the stated percentile
[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use
[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count
Statistically significant estimates are bolded.

Chapter 3 Figures

Figure 3-1. Flow diagram for inclusion into the analytic population

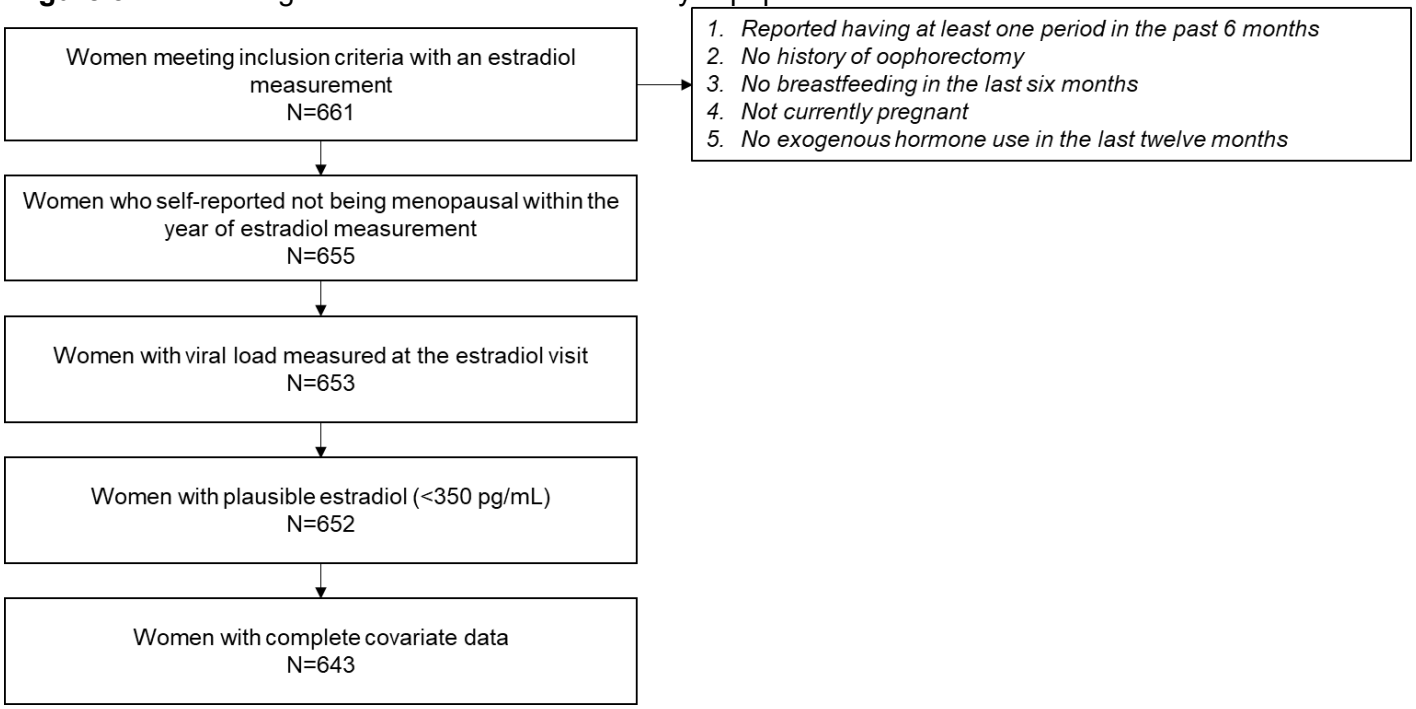
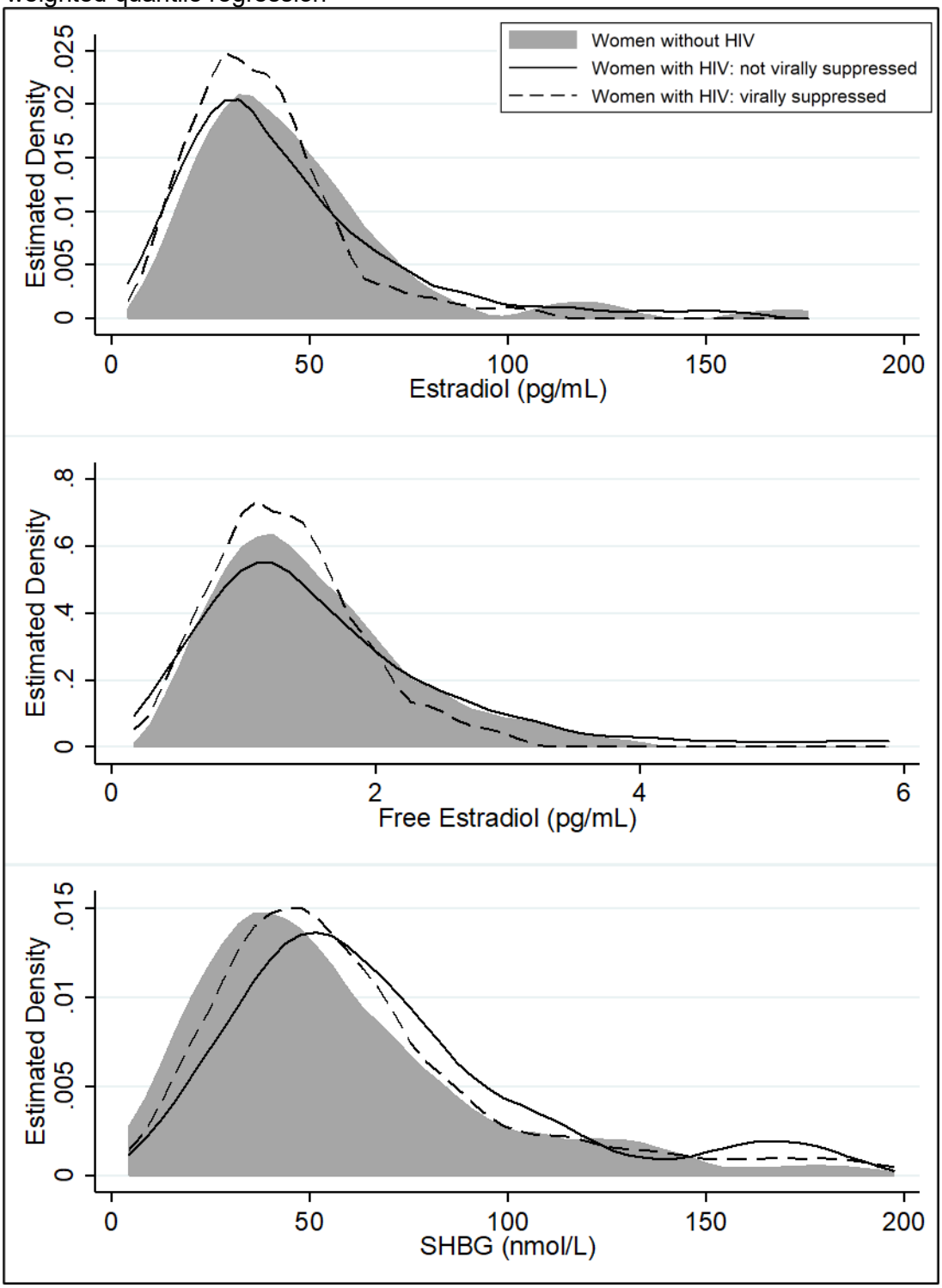


Figure 3-2. Estimated distribution of estradiol and SHBG by HIV status using weighted quantile regression



Abbreviations: sex hormone binding globulin, SHBG.

Chapter 3 Appendix

Appendix 3-1 Supplemental Tables

Supplemental Table 3-1. Weighted quantile regression estimates for the association between HIV status and viral suppression status with total estradiol at the 25th, 50th, and 75th percentile among women with total estradiol < 150 pg/mL, N=630

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	**-4**	**-7.37, -0.63**	**-4**	**-7.58, -0.42**
p50	**-5**	**-9.50, -0.50**	-3	-8.03, 2.03
p75	-2	-8.09, 4.09	-2	-8.43, 4.43
HIV suppressed vs. HIV negative[b]				
p25	-3	-6.94, 0.94	-4	-8.09, 0.09
p50	**-6**	**-10.29, -1.71**	-4	-9.03, 1.03
p75	**-9**	**-14.90, -3.10**	**-9**	**-14.96, -3.04**
Suppressed vs. unsuppressed[c]				
p25	1	-2.48, 4.48	-3	-8.22, 2.22
p50	-1	-4.87, 2.87	-3	-10.16, 4.16
p75	**-7**	**-13.47, -0.53**	-9	-18.72, 0.72
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-1	-4.47, 2.47	**-6**	**-9.74, -2.26**
p50	-3	-6.89, 0.89	-7	-14.73, 0.73
p75	-7	-12.22, 0.22	-10	-20.00, 0.01

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.
[a] At the stated percentile
[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use
[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count
Statistically significant estimates are bolded.

Supplemental Table 3-2. Weighted quantile regression estimates for the association between HIV status and viral suppression status with free estradiol at the 25th, 50th, and 75th percentile among women with total estradiol < 150 pg/mL, N=424

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	-0.07	-0.22, 0.08	-0.04	-0.20, 0.12
p50	-0.07	-0.27, 0.12	-0.04	-0.26, 0.18
p75	0.15	-0.17, 0.46	0.18	-0.13, 0.50
HIV suppressed vs. HIV negative[b]				
p25	-0.07	-0.23, 0.08	-0.04	-0.23, 0.16
p50	-0.11	-0.29, 0.07	-0.11	-0.33, 0.11
p75	**-0.29**	**-0.58, -0.01**	-0.11	-0.39, 0.17
Suppressed vs. unsuppressed[c]				
p25	-0.01	-0.16, 0.16	-0.01	-0.24, 0.24
p50	-0.04	-0.18, 0.10	-0.04	-0.29, 0.22
p75	**-0.44**	**-0.72, -0.16**	-0.33	-0.77, 0.11
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-0.04	-0.20, 0.13	0.04	-0.22, 0.29
p50	-0.15	-0.30, 0.01	-0.07	-0.34, 0.19
p75	**-0.40**	**-0.67, -0.13**	-0.18	-0.61, 0.24

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.

[a] At the stated percentile

[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use

[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count

Statistically significant estimates are bolded.

Supplemental Table 3-3. Weighted quantile regression estimates for the association between HIV status and viral suppression status with sex hormone binding globulin at the 25th, 50th, and 75th percentile among women with total estradiol < 150 pg/mL, N=424

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	**12**	**5.25, 18.75**	**10**	**3.19, 16.81**
p50	**14**	**6.04, 21.96**	**13**	**4.39, 21.61**
p75	**19**	**5.50, 32.50**	12	-2.45, 26.45
HIV suppressed vs. HIV negative[b]				
p25	6	-0.20, 12.20	3	-3.32, 9.32
p50	7	-1.19, 15.19	4	-6.06, 14.06
p75	7	-4.19, 18.19	3	-11.31, 17.31
Suppressed vs. unsuppressed[c]				
p25	-6	-12.35, 0.35	2	-5.20, 9.20
p50	-7	-14.92, 0.92	-2	-15.59, 11.59
p75	**-12**	**-23.74, -0.26**	-6	-23.34, 11.34
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-4	-10.61, 2.61	5	-5.86, 15.86
p50	-4	-11.98, 3.98	7	-5.42, 19.42
p75	-8	-21.48, 5.48	9	-26.91, 44.91

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.

[a] At the stated percentile

[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use

[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count

Statistically significant estimates are bolded.

Supplemental Table 3-4. Weighted quantile regression estimates for the association between HIV status and viral suppression status with total estradiol at the 25th, 50th, and 75th percentile among women with detectable inhibin-B and FSH <20 pg/mL, N=354

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	-3	-6.43, 0.43	-2	-5.71, 1.71
p50	-4	-8.93, 0.93	-5	-10.38, 0.38
p75	-4	-11.36, 3.36	-4	-11.53, 3.53
HIV suppressed vs. HIV negative[b]				
p25	-3	-6.85, 0.85	-3	-7.79, 1.79
p50	**-7**	**-11.56, -2.44**	**-7**	**-12.16, -1.84**
p75	**-11**	**-17.20, -4.80**	**-11**	**-18.38, -3.62**
Suppressed vs. unsuppressed[c]				
p25	0	-4.09, 4.09	-3	-8.74, 2.74
p50	-3	-6.81, 0.81	-5	-10.43, 0.43
p75	**-7**	**-13.43, -0.57**	-9	-15.09, 0.09
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-2	-6.29, 2.29	-6	-12.60, 0.60
p50	-3	-6.82, 0.82	**-7**	**-13.79, -0.21**
p75	-4	-11.19, 3.19	-8	-20.20, 4.20

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.

[a] At the stated percentile

[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use

[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count

Statistically significant estimates are bolded.

Supplemental Table 3-5. Weighted quantile regression estimates for the association between HIV status and viral suppression status with free estradiol at the 25th, 50th, and 75th percentile among women with detectable inhibin-B and FSH <20 pg/mL, N=352

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	-0.07	-0.24, 0.10	-0.04	-0.19, 0.12
p50	-0.07	-0.28, 0.13	-0.01	-0.22, 0.22
p75	-0.11	-0.46, 0.24	-0.15	-0.55, 0.25
HIV suppressed vs. HIV negative[b]				
p25	-0.07	-0.25, 0.10	-0.04	-0.23, 0.15
p50	**-0.22**	**-0.42, -0.02**	-0.15	-0.36, 0.07
p75	**-0.55**	**-0.89, -0.21**	**-0.44**	**-0.80, -0.08**
Suppressed vs. unsuppressed[c]				
p25	0.01	-0.16, 0.16	-0.04	-0.25, 0.18
p50	-0.15	-0.29, 0.01	**-0.18**	**-0.36, -0.01**
p75	**-0.44**	**-0.75, -0.14**	**-0.48**	**-0.84, -0.12**
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-0.04	-0.21, 0.14	-0.01	-0.30, 0.30
p50	**-0.18**	**-0.34, -0.02**	-0.15	-0.37, 0.08
p75	**-0.37**	**-0.67, -0.06**	-0.11	-0.56, 0.34

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.

[a] At the stated percentile

[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use

[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count

Statistically significant estimates are bolded.

Supplemental Table 3-6. Weighted quantile regression estimates for the association between HIV status and viral suppression status with sex hormone binding globulin at the 25th, 50th, and 75th percentile among women with detectable inhibin-B and FSH <20 pg/mL, N=352

	Unweighted coefficients		Weighted coefficients	
	Expected Difference[a]	95% CI	Expected Difference[a]	95% CI
HIV unsuppressed vs. HIV negative[b]				
p25	**12**	**5.26, 18.74**	**10**	**1.91, 18.09**
p50	**16**	**7.56, 24.44**	**12**	**2.95, 21.05**
p75	**17**	**3.21, 30.79**	12	-3.13, 27.13
HIV suppressed vs. HIV negative[b]				
p25	**6**	**0.52, 11.48**	3	-3.94, 9.94
p50	9	-0.77, 18.77	5	-5.52, 15.52
p75	9	-3.30, 21.30	6	-10.13, 22.13
Suppressed vs. unsuppressed[c]				
p25	-6	-12.79, 0.79	-4	-11.64, 3.64
p50	-7	-15.26, 1.26	-5	-15.89, 5.89
p75	-8	-21.34, 5.34	-6	-20.87, 8.87
Suppressed vs. unsuppressed (at visit prior)[c]				
p25	-2	-9.04, 5.04	7	-2.87, 16.87
p50	-2	-10.03, 6.03	4	-8.35, 16.35
p75	-5	-19.13, 9.13	3	-23.03, 29.03

Abbreviations: 95% confidence interval, 95% CI; p25, p50, p75, 25th, 50th and 75th percentiles.
[a] At the stated percentile
[b] IPWe for: age, race/ethnicity, smoking status, Hepatitis C status, age at menarche, parity, log BMI, and alcohol use
[c] IPWe for: age, race/ethnicity, smoking status, age at menarche, Hepatitis C status, parity, log BMI, alcohol use, therapy status, CD4 count
Statistically significant estimates are bolded.

Chapter 4: Aim 3

Association between cumulative viral load on antiretroviral therapy and breast cancer risk

Abstract

Background: It has been suggested that the lower risk of breast cancer reported in women living with HIV may be related to HIV viremia. Breast cancer among women with HIV has not been explored in the context of long-term infection or suppressive antiretroviral therapy (ART). This aim sought to quantify the association between cumulative viral load (cVL) and breast cancer risk in women with HIV initiating ART.

Methods: Women with HIV in the North American AIDS Cohort Collaboration on Research and Design ≥18 years of age with no known prior cancer diagnosis (any cancer), ≥2 viral load measurements, and ≥6 months of follow-up from 1996-2016 were included. Women were followed from ART initiation (first prescription) to the earliest of incident breast cancer diagnosis, death, loss to follow-up (2 consecutive years with no viral load measurement), December 31, 2016, or date through which cohorts validated cancer diagnoses. Log_{10} cVL (viral copies/mL per year over the duration of follow-up) was calculated from ART initiation to the end of follow-up. Joint longitudinal survival models were used to estimate the longitudinal process of cVL as a linear predictor of breast cancer risk. Hazard ratios were adjusted (aHR) for age and calendar year at ART initiation, as well as AIDS diagnosis prior to ART. cVL on ART was lagged 1-5 years to account for breast cancer latency.

Results: There were 30 breast cancer diagnoses in 5,839 women with HIV contributing 36,829 person-years. Median follow-up was 5.0 (IQR 2.4, 9.2) years, median baseline age was 41 (IQR 34, 49) years, and median baseline calendar year was 2007 (IQR: 2002, 2011). Women were predominantly Black (61%), and 45% had an AIDS diagnosis prior to ART. Median cVL at the end of follow-up was 18,374 (IQR: 1,611, 103,966) copies*years/mL. There was no association between log_{10} increase in cVL and breast

cancer (aHR: 0.90, 95% CI 0.62, 1.30). Estimates that were annually lagged trended increasingly protective, with a 22% reduced risk in breast cancer with a 5-year lag in cVL (aHR: 0.78, 95% CI 0.56, 1.10) but remained non-statistically significant.

Conclusions: There was no association between cVL on ART and breast cancer; though when the exposure was lagged, estimates were increasingly protective, remaining non-statistically significant. These findings merit further exploration in populations of women with HIV with long duration of follow-up and larger numbers of validated breast cancer diagnoses. This would allow for additional adjustment for factors related to breast cancer risk and account for the competing risk of death. Given these results, future research on breast cancer in HIV should consider the cumulative burden of viremia and that the etiologically relevant time period may be years before diagnosis.

Introduction

In recent years, with marked advancements in antiretroviral therapy (ART), people with HIV (PWH) who are on treatment are living longer and experiencing life expectancy comparable to the general population in the United States.[12,13,72,168] This trend of improved longevity and survival corresponds with an increase in common chronic comorbidities, including certain non-AIDS defining cancers (NADCs).[71,73,74,131,169] While breast cancer is the most common cancer among women in the United States, very little is known about the risk of breast cancer in women with HIV. A few studies have suggested the risk of breast cancer may be lower in women with versus without HIV, though the research is sparse, studies are often limited in sample size, and findings are inconsistent across calendar periods.[74,76,78–80,96,170]

Prior studies have directly accounted for differences in age and race/ethnicity, and still observed a lower risk of breast cancer comparing women with and without HIV. Reduced breast cancer risk in the early years of the HIV epidemic may have been due to lower life expectancy among women with HIV at the time; however, more contemporary analyses (when survival has improved) still find a lower risk of breast cancer.[80] Among women with HIV, studies assessing breast cancer risk stratified by AIDS diagnosis and CD4 count found significantly lower than expected rates of breast cancer irrespective of stratification, indicating that immune status also may not completely explain breast cancer differences by HIV status.[78–80]

It is possible that differences in screening practices could also explain this reduced risk. Studies in large cohorts of comparable women with and without HIV are limited, and findings are equivocal. One study found mammography was more common in women with versus without HIV, possibly due to more opportunities to interact with healthcare systems,[106] while others have observed similar to lower rates of mammography.[107–109] Using stage at diagnosis as a proxy for screening, one study found

breast cancer stage was comparable by HIV status,[136] though others have indicated later stage at diagnosis in women with versus without HIV.[94] In one of the previously mentioned studies comparing breast cancer incidence, the risk of large tumors (typically not detected via mammography) was also lower in women with HIV, suggesting a lack of a screening effect.[80]

This raises the possibility of a direct influence of HIV viremia on breast cancer. Indeed, there is preliminary evidence that supports a potential biological mechanism. One possible explanation is that the CXCR-4 immune receptor, which is the binding site for CXRC-4 tropic HIV, is expressed on breast cancer cells as well as in ductal carcinoma *in situ* and atypical ductal hyperplasia (a breast pre-cancer) but not in normal breast epithelium.[93,111,121–123] Indeed, CXCR-4 tropic HIV is able to induce apoptosis in cancerous ductal cells.[124] Further, one case-control study among women with HIV found that breast cancer risk was strongly inversely associated with CXCR-4 tropic HIV.[93]

Studies analyzing breast cancer in women with HIV have focused predominantly on relative comparisons to the general population. Few have assessed the association between viral load and cancer risk, a widely accessible clinical measurement.[171] Among women with HIV, the association between viral load and breast cancer has not fully explored: only one other study assessed this association using HIV viral load measured at one time point.[93] Such a measurement does not reflect the variability, duration of exposure, or the cumulative burden of viral load over time.

Therefore, the objective of this aim was to characterize the association between cumulative HIV viral load since ART initiation and breast cancer risk in a large sample of women with HIV with long duration of follow-up.

Methods

Study Population

The study population for this aim included women with HIV participating in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD).[129] Briefly, the NA-ACCORD is a consortium of single-site/multisite cohorts among adults with HIV in the U.S. and Canada. Individuals who successfully link into care, defined as attending two or more HIV care visits in twelve months, are enrolled in the NA-ACCORD. Each cohort employs standardized data collection, submitting data on enrolled participant characteristics, diagnoses, laboratory measures, prescribed medications, and vital status to the Data Management Core (University of Washington, Seattle, WA). Data are then harmonized across cohorts and evaluated for quality control prior to being transmitted to the Epidemiology/Biostatistics Core where additional quality control checks are completed (Johns Hopkins University, Baltimore, Maryland).

Women were included in this analysis if they were ≥18 years old, were observed to initiate ART, had at least 6 months of observation time from 1996 through 2016, had no history of any type of cancer at the start of observation, and had at least two viral load measurements (one at the time of ART initiation, and at least one more within two years while under observation) (**Figure 4-1**).

Outcome Ascertainment

The outcome, first incident, invasive breast cancer diagnosis, was ascertained and validated using a standardized abstraction protocol that has previously been reported.[73] This abstraction includes manual review of medical charts and pathology reported for cancer site and histopathology, and/or linkages with cancer registries, depending on the cohort. We included a 6-month washout period to exclude prevalent breast cancer diagnoses.

Exposure: Cumulative Viral Load on ART

I hypothesized that the effect of HIV on progression to breast cancer is a cumulative exposure of HIV over time which interferes with tumor development. Copy-years viremia, or cumulative viral load, is a metric that integrates viral copies/mL per year over the total duration of infection, much like pack-years for smoking.[172] This estimates total burden of HIV disease progression, capturing fluctuations in viral load, and has been used to predict cancer risk in previous studies.[173–176] This metric was adapted to assess cumulative viral load beginning at ART initiation rather than at HIV infection. This adaptation was made because data are not systematically collected from HIV infection, and date of infection is rarely known. ART initiation was selected because it is a biologically meaningful timepoint for women with HIV. Also, with time since ART as our timescale, this controlled for differences in duration of treatment.

ART initiation was defined as the date of the first recorded ART prescription for at least 30 days. The viral load measurement closest to ART initiation (within a window of 9 months prior to 3 months after ART initiation) was the starting point from which cumulative viral load was estimated and was time-updated. Cumulative viral load was assessed at the end of follow-up and annually lagged up to 5 years. Lagging refers to assessing the exposure of interest at earlier time points during follow-up. A longitudinal model estimating a cumulative viral load trajectory for each woman (described below in *Statistical Analyses*) was used to ascertain cumulative viral load at the prespecified time points; therefore, all women meeting our inclusion criteria contributed to all analyses. This was done to characterize the etiologically relevant time window for the association between HIV viral load and breast cancer. Given breast cancer can take many years to develop, it is possible that cumulative viral load prior to diagnosis is more meaningful than at diagnosis. For interpretability, cumulative viral load was also $\log_{10}$ transformed.

Covariates

Covariates that were assessed included age, race/ethnicity, smoking status, body mass index (BMI), AIDS diagnosis prior to, and viral load at, ART initiation. Age was determined at ART initiation. Race/ethnicity was defined as: non-Hispanic white, non-Hispanic Black, Hispanic, and other. Smoking status was defined as ever self-reported smoking versus never reported smoking. BMI (using measured weight and height) was assessed at ART initiation entry (+/- 24 months). Missing values for BMI (749 women, [13%]) and smoking (1,736 women [30%]) were imputed using multiple imputation by fully conditional specification (details in **Appendix 4-1**). AIDS diagnosis prior to ART was a composite variable defined as one or both of the following prior to the first observed ART prescription: a measured CD4 count <200 cells/mm^3 or an AIDS-defining clinical diagnosis. Viral load at ART initiation was categorized into quartiles based on the distribution within the sample (<330 copies/mL, 330-7500 copies/mL, 7500-61000 copies/mL, and ≥61000 copies/mL).

Statistical Analyses

Observation in this study began at 6 months following the date of ART initiation. Because we included a six-month washout period to exclude prevalent breast cancer cases, we also excluded the first 6-months of follow-up for every women to avoid immortal person-time bias (attributing time under observation where a subject could not be at risk for breast cancer by design).[137] ART initiation date had to be preceded by the latest date of the following: January 1, 1996, date of enrollment into the NA-ACCORD, or when cohorts began capturing cancer diagnoses. Women were followed from 6 months after ART initiation until the first of the following events: an incident breast cancer diagnosis, death, administrative censoring on December 31, 2016, cohort-specific cancer observation end date, or loss-to-follow-up. Loss-to-follow-up was defined as the

date of the last HIV viral load measurement prior to a two-year gap in HIV viral load laboratory measurements. The timescale for this analysis was time since 6 months post-ART initiation.

We used joint longitudinal survival models to assess the association between cumulative viral load on ART and breast cancer risk. Joint longitudinal survival models are a compilation of two models that estimate both longitudinal processes (cumulative viral load on ART) and a time-to-event model (time to breast cancer diagnosis) simultaneously.[177] This statistical technique is advantageous because it can account for measurement error of the longitudinal process of HIV RNA. Joint longitudinal survival modelling, using a time-to-event model, can handle loss-to-follow-up and can account for informative censoring of the longitudinal process as a result of the time-to-event model outcome (breast cancer).

The longitudinal model assessing change in $\log_{10}$ cumulative viral load was evaluated using linear mixed-effects models. This model estimates cumulative viral load on ART using the covariates described, independent of breast cancer status. The final longitudinal submodel was selected using Akaike Information Criteria (AIC). Covariates for consideration in candidate models included: time since ART + 6 months (assessed continuously and with natural cubic splines with 2 knots), race/ethnicity at enrollment, injection drug use status at enrollment, and the following covariates assessed at ART initiation: history of an AIDS diagnosis, HIV viral load (quartiles), calendar year, age (assessed continuously and with natural cubic splines with 2 knots), in addition to varying interactions with these variables and time since ART + 6 months. The final model included random intercept and random slopes for time since ART + 6 months in addition fixed effects for: time since ART + 6 months, HIV viral load, history of an AIDS diagnosis, race/ethnicity, injection drug use status, age (centered at 40 years), calendar

year (centered at 2007), and an interaction term between time since ART initiation and viral load at ART.

The joint longitudinal survival model estimated longitudinal $\log_{10}$ cumulative viral load on ART using the final model described above and a proportional piecewise hazard model (i.e. the hazard is assumed to be proportional within 6 evenly distributed percentiles of event times) for time to breast cancer diagnosis. The survival submodel included linear $\log_{10}$ cumulative viral load on ART (estimated in the longitudinal submodel) and additionally adjusted for AIDS diagnosis prior to ART, age at ART initiation (per 5-year increase) and calendar year of ART initiation (per 1-year increase). Separate joint longitudinal models evaluated cumulative viral load on ART at the end of follow-up and lagged 1 to 5 years.

Sensitivity analyses were conducted adjusting for race/ethnicity, BMI, and smoking status in addition to the factors listed above. Subgroup analyses were also conducted excluding women with a viral load of <200 copies/mL at ART initiation to mitigate including women whose ART initiation date was possibly misclassified (N=4,549). Lastly, among women with a CD4 count prior to ART (N=5,716), subgroup analyses were conducted incorporating nadir CD4 count and clinical AIDS diagnosis prior to ART as two separate variables in the longitudinal submodel and survival model instead of our previously defined composite definition of AIDS-defining diagnosis prior to ART.

Results

Characteristics of the Study Population

There were 5,839 women included in this analysis contributing 30 breast cancer diagnoses with median follow-up of 5.0 years (IQR 2.4, 9.2 years) (**Table 4-1**); 621 women died during follow-up. The median age at ART initiation was 40 years (IQR 33.2,

48.3 years), and did not substantially differ by tertiles of $\log_{10}$ cumulative viral load on ART. Median age at breast cancer diagnosis was 53.3 years (IQR 45.6, 57.6 years). Women in this analytic sample were predominantly Black (61%), were ever smokers (64%), and had median BMI of 27.3 kg/m^2 (IQR 23.3, 32.5 kg/m^2). Forty-five percent had an AIDS diagnosis prior to ART initiation. AIDS diagnosis prior to ART initiation was highest among women in the highest tertile of cumulative viral load (64%). Stratified by tertiles of cumulative viral load, women in the highest tertile more frequently had higher viral load at ART initiation. For instance, 56% of women in the highest tertile of cVL had a viral load at ART of ≥61,000 copies/mL, compared to only 19% of women in the second tertile of cVL. No women in the lowest tertile of cVL had a viral load at ART of ≥61,000 copies/mL.

Longitudinal Characterization of Cumulative Viral Load on ART

The mean $\log_{10}$ cumulative viral load on ART at analysis entry (6 months following ART initiation) among white women, entering at age 40 in 2007 with no AIDS diagnosis prior to ART or injection drug use with a viral load <330 copies/mL was 2.04 $\log_{10}$ copies*years/mL (**Table 4-2**). There were no significant differences in cumulative viral load by race/ethnicity comparing non-Hispanic white women to each other race/ethnicity group. Cumulative viral load on ART was 0.15 $\log_{10}$ copies*years/mL higher in women with an AIDS diagnosis compared to women without and AIDS diagnosis. Women who reported injection drug use had a 0.19 $\log_{10}$ copies*years/mL higher cumulative viral load compared to women with no injection drug use. Increasing age and calendar year (per 10-year increase) were associated with lower cumulative viral load. There was a significant interaction between time since ART initiation and viral load at ART initiation on cumulative viral load: with increasing viral load at ART initiation, the rate of increase for cumulative viral load was steeper.

Figure 4-2 depicts longitudinal $\log_{10}$ cumulative viral load on ART (not model estimated) for each woman by outcome status (breast cancer or censoring) with the lowess-smoothed median at each time point. Cumulative viral load increased steadily over time among women who did not have breast cancer, with the steepest incline observed in the first 5 years following ART initiation. A similar trend is observed for women who were diagnosed with breast cancer, though at approximately 7 years following ART, the median cumulative viral load stabilizes.

Association between Cumulative Viral Load on ART and Breast Cancer

Estimates from the survival portion of the joint longitudinal survival model suggest a null association between cumulative viral load on ART and breast cancer (**Table 4-3**). There was a non-significant 10% decrease in the risk of breast cancer for every $\log_{10}$ increase in cumulative viral load (adjusted hazard ratio (aHR): 0.90, 95% CI 0.62, 1.30). The inverse association between cumulative viral load and breast cancer strengthened with increasing exposure lag. When lagged by 1 year, a $\log_{10}$ increase in cumulative viral load was associated with a non-significant 13% decrease in the risk of breast cancer (aHR: 0.87, 95% CI 0.61, 1.25). When lagged by 5 years, there was a non-significant 22% reduced risk of breast cancer per $\log_{10}$ increase in cumulative viral load (aHR: 0.78, 95% CI 0.56, 1.10). Assessed visually, the association between cumulative viral load and breast cancer risk declined with every 1-year increase in the lag but remained within the 95% confidence intervals (**Figure 4-3**). Age (per 5-year increase) was significantly associated with increasing breast cancer risk across all models (30-32% increase in breast cancer risk per 5-year increase in age). There was no association between calendar year or having an AIDS diagnosis prior to ART and breast cancer across all models.

Sensitivity analyses additionally adjusting for BMI, race/ethnicity, and smoking yielded similar estimates to the main analysis (**Figure 4-4**). Subgroup analyses removing women with viral load at ART initiation <200 copies/mL also yielded comparable estimates. Lastly, subgroup analyses among women with a CD4 count prior to ART were conducted in which nadir CD4 count and clinical AIDS diagnosis prior to ART initiation were adjusted for instead of our composite variable for AIDS defining illness. These estimates were also comparable to the main analysis (**Figure 4-4**).

Discussion

In this sample of women with HIV, initiating ART, and no history of any cancer diagnosis, I found no association between cumulative viral load on ART and breast cancer risk adjusting for AIDS diagnosis prior to ART, and age and calendar year of ART initiation. When I lagged cumulative viral load on ART 1-5 years, estimates trended in a protective direction, though remained non-significant, demonstrating that viral load assessed in the years prior to diagnosis may be the most biologically relevant assessment of HIV viremia in relation to breast cancer.

This is a novel assessment of the association between HIV viral load measured as a cumulative exposure and breast cancer risk. One prior case-control study found that, among women with HIV with viral load ≥500 copies/mL, there was no association between the unadjusted odds of breast cancer and a $\log_{10}$ increase in HIV viral load, (odds ratio=0.60, 95% CI 0.30, 1.20).[93] In the same study, CXCR-4 tropism was strongly inversely associated with lower breast cancer risk (adjusted odds ratio, 0.10, 95% CI 0.002–0.84), which remained after adjustment for HIV clinical factors like viral load. This adds further plausibility to a potential biological mechanism related to tropism, but not necessarily viral load, which merits further investigation. Another study that

assessed NADCs overall found no association between time spent with HIV viral load >500 copies/mL and risk of any NADC.[174] Our observation that the association between cumulative viral load and breast cancer trended in a protective direction, increasing in strength with increasing lag of the exposure, corresponds with our existing understanding of the latent period associated with breast cancer development of at least 1-2 years, though there is substantial variation by subtype and individual-level factors and our estimates were not statistically significant.

Despite having a large analytic sample, this analysis was limited by the small number of breast cancer cases observed (n=30) from 1996-2016. As a result, there was limited power to detect significant associations. I was not able to account for factors that may be relevant to breast cancer risk including traditional important risk factors such as reproductive factors (e.g. age at menarche/menopause, parity) because these are not collected in the NA-ACCORD. Given the association observed between CXCR-4 tropic HIV and breast cancer, it would also have been ideal to additionally stratify by tropism; however, this was not feasible due to lack of data availability. I also did not account for the competing risk of death, which could inform breast cancer risk, especially earlier in the HIV epidemic when mortality was at its highest in this population. This was deemed not feasible for this aim due to the already limited precision of the current analysis. That said, this analysis was among women who initiated treatment, where the risk of death is likely lower relative to the larger population of women with HIV. Moreover, in aim 1, among women in the NA-ACCORD from 1997-2016, there were no changes in breast cancer over time accounting for the competing risk of death. Taken in sum, accounting for the competing risk would have been ideal; but given the lack of an association between breast cancer risk over time in aim 1, and the limited precision of the present analysis, it was deemed that such refinements should be considered in future works.

The assessment of cumulative viral load was measured from ART initiation onward, which does not capture time prior to treatment (when HIV viral load is higher), though it is more likely to be more routinely ascertained after ART initiation. Cumulative viral load on ART is an underestimation of cumulative viral load since HIV infection, and the association with breast cancer may differ prior to ART initiation. It is possible that women with high cumulative viral load prior to ART could have developed breast cancer and died before getting on to treatment; however, this is unlikely given the risk of breast cancer in the pre-ART era was still low. Still, in this aim, I am characterizing a group of women with HIV with potentially modified breast cancer risk. Given these limitations, the findings should be interpreted cautiously.

Nonetheless, this aim represents a novel assessment of cumulative HIV viral load after ART initiation and breast cancer risk in the United States, accounting for measurement error of cumulative viral load and informative censoring related to longitudinal measurements of viral load. The findings of this study serve as a hypothesis generating step in understanding the impact of HIV on breast cancer mechanisms in women.

Though I found no statistically significant association between cumulative viral load on ART and breast cancer risk, the finding of an increasing reduction in breast cancer risk with increasing exposure lag merits further investigation. In addition to incorporating viral load assessments prior to breast cancer diagnosis, future studies on breast cancer in women with HIV should explore this association in larger, contemporary populations of women with HIV, with long duration of follow-up. Accounting for the competing risk of death and traditional risk factors for breast cancer is also needed to better characterize this association. Further monitoring of breast cancer in this population may also inform our understanding of the possible mechanisms involved in pathogenesis. As the age distribution of women with HIV shifts to older ages, and

women are living longer, I would expect to see more breast cancer diagnoses in this population. Given this, and the null association between viral load and breast cancer, age-appropriate breast cancer screening remains an important aspect of care for women with HIV.

Chapter 4 Tables

Table 4-1. Demographic and clinical factors among women with HIV in the NA-ACCORD, N=5,839 (1996-2016)

	Overall		cVL on ART <3.63 log_{10} copies*years/mL		cVL on ART 3.63-4.77 log_{10} copies*years/mL		cVL on ART 4.77+ log_{10} copies*years/mL	
	N=5839		N=1910		N=2001		N=1928	
	median	IQR	median	IQR	median	IQR	median	IQR
Age at entry	40.3	33.2, 48.3	42.6	34.9, 50.8	39.2	32.3, 47.4	39.4	32.6, 46.4
Age at exit	47.0	39.0, 55.0	48	39.0, 56.0	46.5	38.0, 56.0	47	40.0, 55.0
Calendar year entry	2007	2002, 2011	2010	2006, 2013	2006	2002, 2010	2003.0	2000, 2008
Calendar year exit	2015	2010, 2017	2016	2012, 2017	2015	2010, 2017	2013.0	2008, 2017
Follow-up time	5	2.4, 9.2	3.7	1.8, 6.7	5.3	2.5, 9.5	6.5	3.3, 11.5
Body mass index (BMI, kg/m^2)[a]	27.3	23.3, 32.5	28.1	24.0, 33.4	27.7	23.7, 32.8	26.3	22.7, 31.3
	N	%	N	%	N	%	N	%
Race								
NH Black	3581	61.3	1231	64.5	1195	59.7	1155	59.9
NH white	1159	19.8	360	18.8	393	19.6	406	21.1
Hispanic	531	9.1	152	8.0	176	8.8	203	10.5
Other	568	9.7	167	8.7	237	11.8	164	8.5
Smoking status[a]								
Never	2083	35.7	754	39.5	754	37.7	575	29.8
Ever	3756	64.3	1156	60.5	1247	62.3	1353	70.2
AIDS diagnosis prior to ART initiation								
Yes	2638	45.2	592	31.0	811	40.5	1235	64.1
No	3201	54.8	1318	69.0	1190	59.5	693	35.9
Viral load at ART initiation (copies/mL)								
≤330	1461	25.0	1053	55.1	252	12.6	156	8.1
>330-≤7500	1457	25.0	755	39.5	379	18.9	323	16.8
>7500-≤61000	1465	25.1	102	5.3	992	49.6	371	19.2

>61000	1456	24.9	0	0.0	378	18.9	1078	55.9
	N	IR per 100 PY (PY)	N	IR per 100 PY (PY)	N	IR per 100 PY (PY)	N	IR per 100 PY (PY)
Breast cancer diagnosis								
Yes	30	16.7 (179.7)	11	23.9 (46.1)	9	13.9 (64.9)	10	14.6 (68.7)
No	5,809	15.9 (36649.0)	1899	20.9 (9075.5)	1992	15.4 (12961.3)	1918	13.1 (14612.1)
Death								
Yes	621	19.4 (3201.2)	76	27.1 (280.1)	149	18.4 (808.6)	396	18.7 (2112.4)
No	5,218	15.5 (33627.5)	1834	20.7 (8841.4)	1852	15.2 (12217.6)	1532	12.2 (12568.4)

Abbreviations: cumulative viral load on antiretroviral therapy, cVL on ART; interquartile range, IQR; non-Hispanic, NH; antiretroviral therapy, ART; incidence rate, IR; person-years, PY.

[a] Based on one imputation set

Table 4-2. Differences in longitudinal cumulative viral load since ART by selected covariates

	Estimate	95% CI*
Intercept	2.04	1.99, 2.09
Race/ethnicity		
Non-Hispanic white	ref	
Non-Hispanic Black	0.01	-0.04, 0.05
Hispanic	0.02	-0.04, 0.08
Other	-0.05	-0.11, 0.01
AIDS diagnosis prior to ART		
No	ref	
Yes	**0.15**	**0.12, 0.19**
IDU		
No	ref	
Yes	**0.19**	**0.14, 0.23**
Viral load at ART initiation (copies/mL)		
≤280	ref	
>280-≤7400	**0.87**	**0.81, 0.93**
>7400-≤60600	**1.73**	**1.67, 1.79**
>60600	**2.56**	**2.50, 2.62**
Calendar year of ART initiation (per 10-year increase)	**-0.30**	**-0.33, -0.27**
Age at ART initiation (per 10-year increase)	**-0.06**	**-0.07, -0.04**

Abbreviations: antiretroviral therapy, ART; 95% confidence interval, 95% CI.
*Excludes interactions with time since ART initiation, viral load at ART and immune injury before ART
Statistically significant estimates are bolded

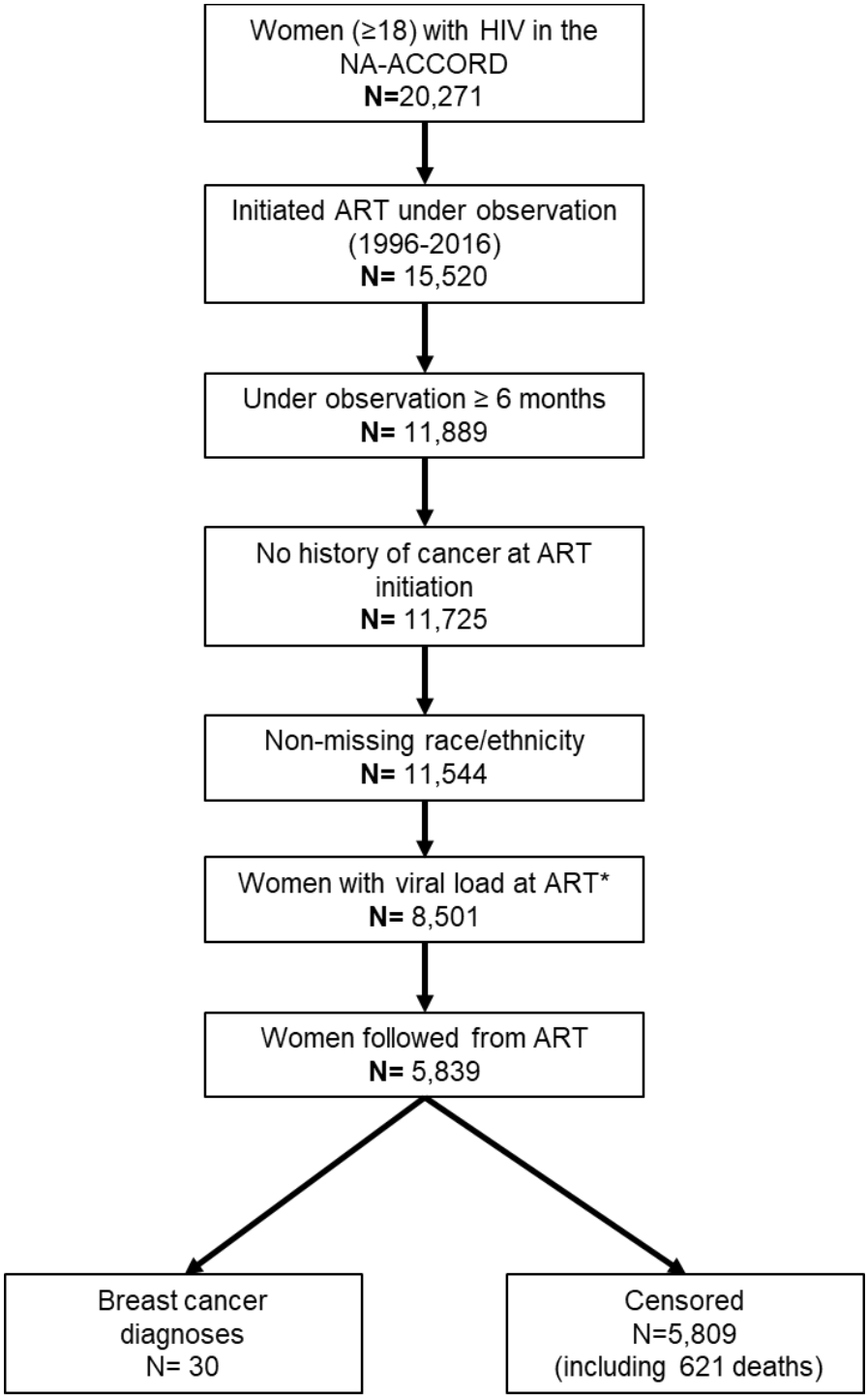
Table 4-3. Association between treated cumulative viral load and breast cancer (survival submodel)[a]

	cVL At time t		cVL One-year lag		cVL Two-year lag		cVL Five-year lag	
	aHR	95% CI	aHR	95% CI	aHR	95% CI	aHR	95% CI
Cumulative viral load	0.90	0.62, 1.30	0.87	0.61, 1.25	0.81	0.57, 1.15	0.78	0.56, 1.10
AIDS diagnosis prior to ART initiation								
No	ref	ref	ref	ref	ref	ref	ref	ref
Yes	1.13	0.54, 2.35	1.15	0.55, 2.41	1.18	0.57, 2.47	1.23	0.59, 2.58
Calenda year (per 1-year increase)	0.94	0.86, 1.03	0.94	0.86, 1.02	0.94	0.86, 1.02	0.93	0.85, 1.02
Age (per 5-year increase)	**1.32**	**1.11, 1.56**	**1.32**	**1.11, 1.57**	**1.30**	**1.10, 1.55**	**1.32**	**1.11, 1.56**

Abbreviations: cVL, cumulative viral load; adjusted hazard ratio, aHR; 95% confidence interval, 95% CI.
[a] using piecewise constant baseline risk function
Statistically significant estimates are bolded

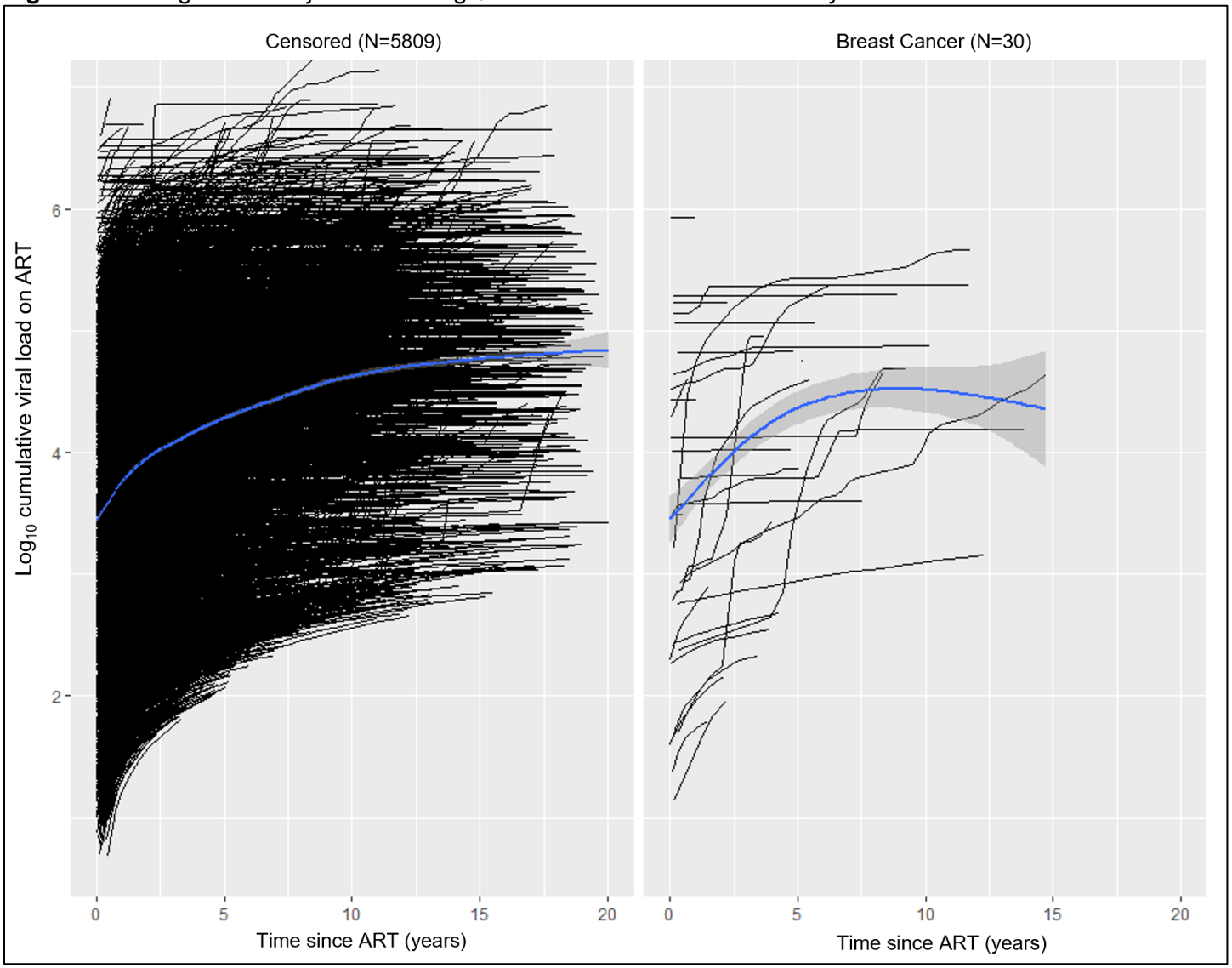
Chapter 4 Figures

Figure 4-1. Flow diagram for inclusion into the analytic sample, NA-ACCORD, 1996-2016



Abbreviations: antiretroviral therapy, ART.

Figure 4-2. Longitudinal trajectories of log_{10} cumulative viral load on ART by outcome status



Abbreviations: antiretroviral therapy, ART.

Figure 4-3. Association between cumulative viral load on ART and breast cancer risk with 0-5 year lag

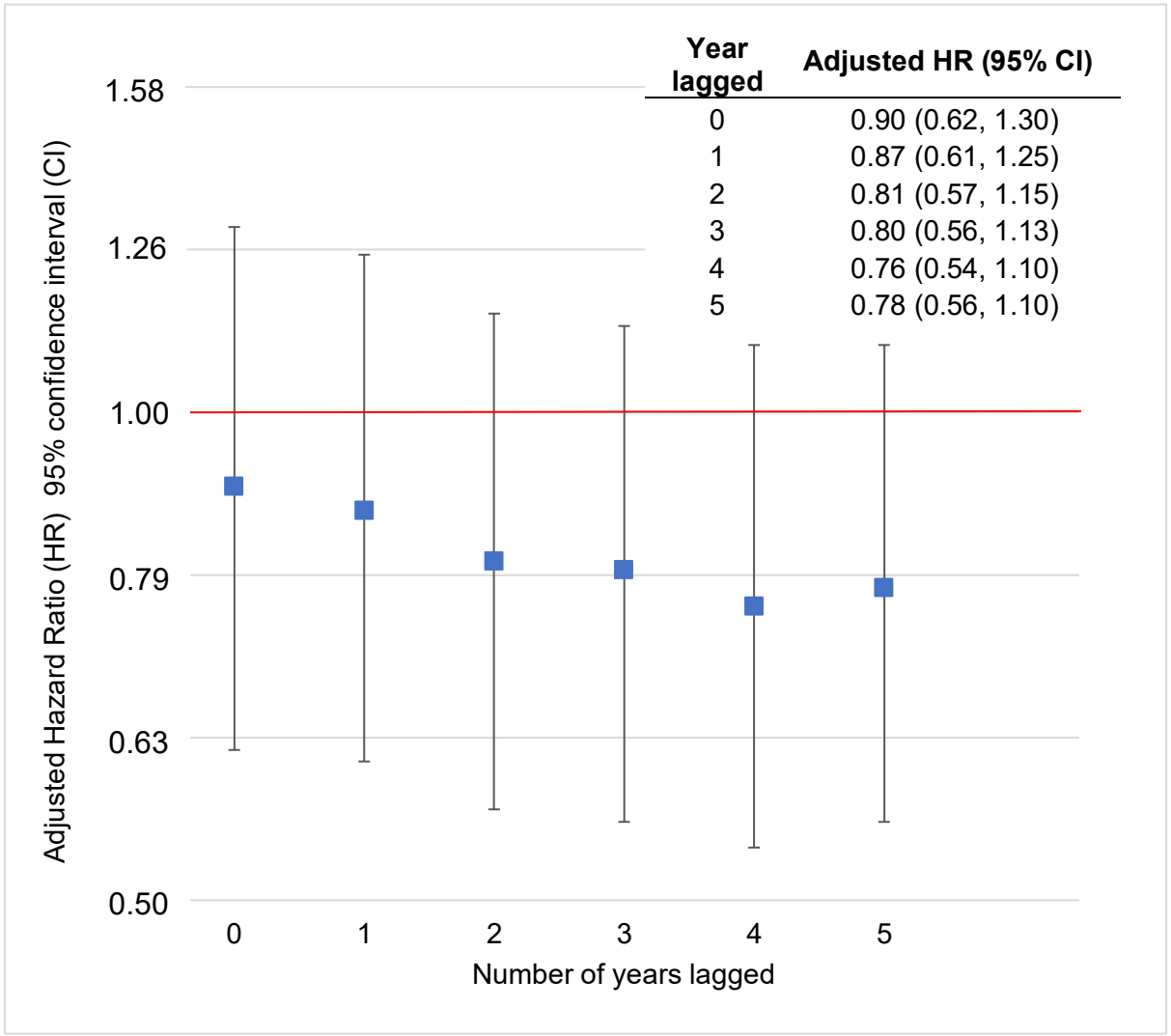
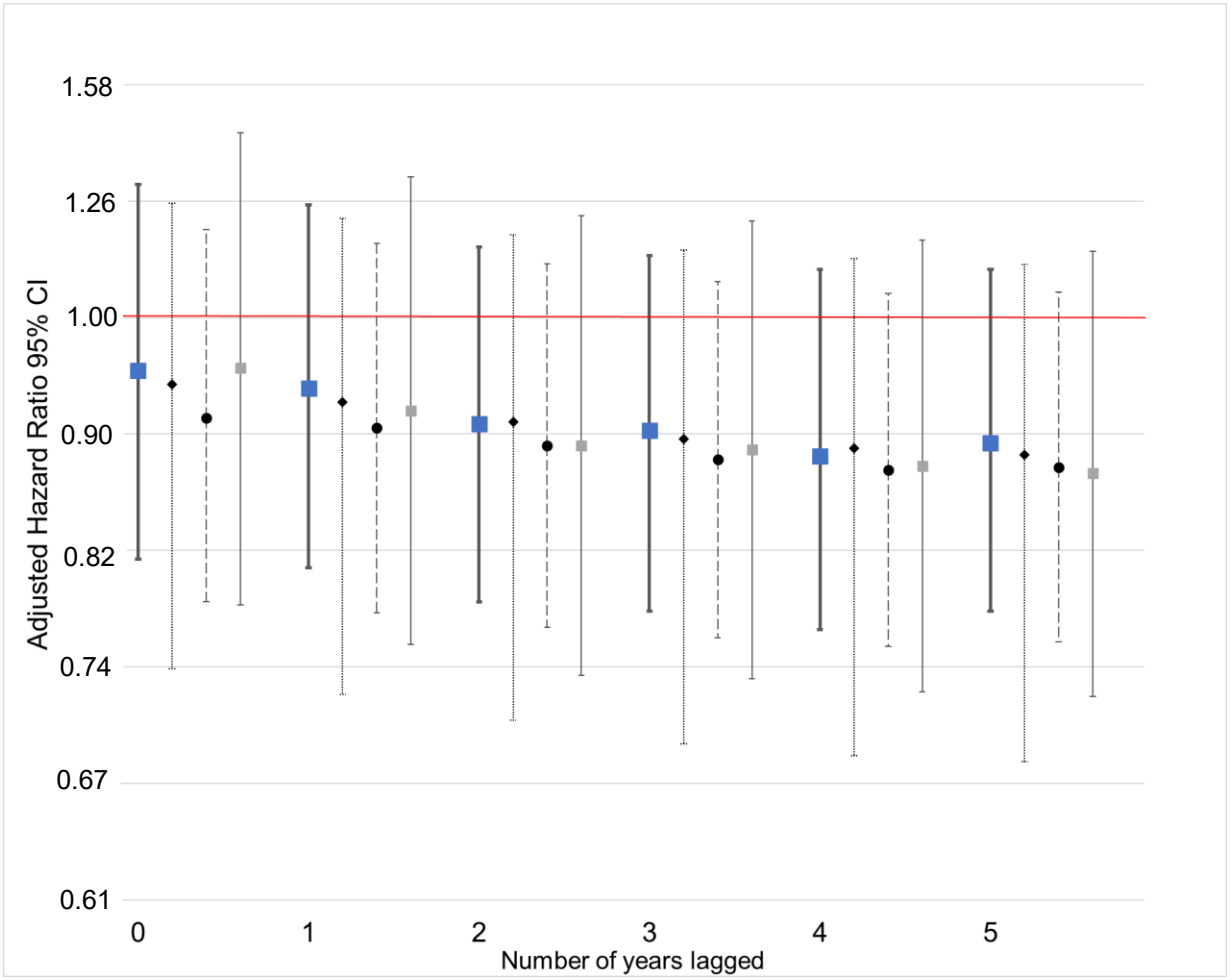


Figure 4-4. Association between cumulative viral load on ART and breast cancer risk with 0-5 year lag comparing main analysis to all sensitivity/subgroup analyses



- Main analysis
- Additionally adjusting for body mass index, race/ethnicity, and smoking status
- Adjusting for nadir CD4 count and clinical AIDS diagnosis prior to antiretroviral therapy initiation
- Removing women with viral load < 200 copies/mL at antiretroviral therapy initiation

Chapter 4 Appendix

Appendix 4-1 Supplemental Tables

Table 4-1. Variables utilized to impute body mass index and smoking

Variable	Definition
Smoking	Ever reported smoking
BMI	BMI at analysis entry +/- 2 years
BMI 25	For each woman, the 25th percentile of all her BMI measurements while in NA-ACCORD
BMI 50	For each woman, the 50th percentile of all her BMI measurements while in NA-ACCORD
BMI 75	For each woman, the 75th percentile of all her BMI measurements while in NA-ACCORD
HIV risk group	Injection drug use, hemophilia, heterosexual contact, receipt of blood transfusion, worked in healthcare/laboratory setting, perinatal, other, unknown
Race	Asian, black, indigenous, multiracial, white, other, missing
Hispanic	Hispanic ethnicity yes, no
HAART	Ever received a prescription for HAART >30 days
AIDS diagnosis prior to ART	Clinical AIDS diagnosis or CD4 count <200 cells/mm^3 prior to ART initiation date
D-drug exposure	Ever received a prescription for one of the following medications >30 days: didanosine, Stavudine, Zalcitabine, or azidothymidine
Breast cancer diagnosis	Censored in analysis due to breast cancer diagnosis
Death	Censored in analysis due to death
Cause-specific hazard breast cancer	Cause-specific cumulative baseline hazard for breast cancer diagnosis
Cause-specific hazard death	Cause-specific cumulative baseline hazard for death using nelson-aalen estimator
Cohort/subsite	Cohort, or for multisite cohort subsite within cohort using nelson aalen estimator
Age at start	Age (years) at analysis entry
At risk alcohol use	Ever reported >7 drinks/week or binge drinking
Diabetes diagnosis	Ever had Patient had HgA1c >6.5% OR diabetes-specific medication OR diabetes diagnosis AND diabetes-related medication OR random glucose measured
Hypertension	Ever prescribed anti-hypertensive medication and received hypertension diagnosis

Prior hypertension	Ever prescribed anti-hypertensive medication and received hypertension diagnosis prior to cohort-specific capture of hypertension
Dyslipidemia diagnosis	Ever prescribed lipid-lowering medication
Prior dyslipidemia diagnosis	Prevalent lipid-lowering medication at the beginning of cohort-specific observation
Statin use	Ever prescribed a statin
Prior statin use	Prevalent statin medication at the beginning of cohort-specific observation
High total cholesterol	Ever had TC measurement ≥ 240 mg/dL
Prior high total cholesterol	Had high cholesterol prior to cohort's observation
High LDL	Ever had LDL measurement ≥ 130 mg/dL
Prior high LDL	Had high LDL prior to cohort's observation
Low HDL	Ever had HDL <50 mg/dL
Prior low HDL	Had low LDL prior to cohort's observation

Abbreviations: body mass index, BMI; highly active antiretroviral therapy, HAART; antiretroviral therapy, ART; hemoglobin A1c; total cholesterol, TC; low density lipoprotein, LDL; high density lipoprotein, HDL.

Chapter 5: Conclusions

Key Findings, Implications, and Future Research

The goal of this dissertation was to pursue a more in-depth understanding of breast cancer risk and etiology among women with HIV. To do this, this dissertation first characterized trends in breast cancer risk and cumulative incidence over time among women with HIV in the last twenty years. This dissertation then sought to explore differences in estrogen, a strong risk factor for breast cancer, comparing women with versus without HIV. Last, this dissertation assessed the direct association between HIV viral load, using a cumulative metric of HIV burden, and breast cancer risk among women with HIV. Overall, the findings from these aims inform the larger question: what factors specific to women with HIV could drive the observed lower risk of breast cancer comparing women with versus without HIV? Clarifying the mechanisms underlying this observed association between HIV and breast cancer may provide additional insight into breast cancer etiology and should inform clinical care among women with HIV regarding screening and surveillance of breast cancer.

Trends in Breast Cancer Hazard and Cumulative Incidence Over Time Among Women with HIV

The first aim sought to characterize trends in breast cancer over time among women with HIV who had initiated ART from 1997-2016 in the North American Cohort Collaboration on Research and Design (NA-ACCORD). I observed no trends in the hazard (instantaneous risk) or the cumulative incidence of breast cancer from 1997-2016 accounting for age, race/ethnicity, and cohort. The hazard and cumulative incidence of death did consistently decline over time overall (1997-2016) and within/across calendar periods. The overall cumulative incidence of breast cancer among women with HIV initiating ART with no history of cancer was 3.2% over 19 years (median follow-up 4.5 years). This aim also found no differences in breast cancer cumulative incidence by

race/ethnicity or calendar period. The substantial decline in mortality is well established in PWH.[8,72,139] The findings related to trends in breast cancer are supported by two prior works in the HIV/AIDS Cancer Match Study, which found: 1) breast cancer incidence rates did not change from 1996-2010,[71] and 2) projected rates of breast cancer through 2030 demonstrated no change in risk.[70]

The findings from this aim indicate a limited role of changes in declining mortality on breast cancer risk. Although there were declines in mortality over time, breast cancer risk did not change. If changes in mortality were to influence breast cancer risk, there would have been an increase in breast cancer cumulative incidence over time. Therefore, additional explanations for the reduced risk of breast cancer comparing women with versus without HIV should continue to be investigated.

This novel assessment of breast cancer over time accounting for the competing risk of death adds further support to the literature indicating that changes in mortality do not drive the lower risk of breast cancer observed comparing women with versus without HIV. Nonetheless there are limitations to this work which should inform future assessments of breast cancer trends in women with HIV. The age distribution of our source population was younger than the general population of women in the United States, and there was limited follow-up observed beyond age 65. Given the median age of breast cancer diagnosis in the general population is 62, case under-ascertainment is possible, and limited the ability to estimate lifetime breast cancer risk. Women with HIV should continue to be tracked with respect to breast cancer in observational cohort studies, with long duration of follow-up and as women continue aging.

Differences in Estradiol by HIV Status, and Among Women with HIV, by Viral Suppression Status

For the second aim, I quantified differences in total estradiol, free estradiol and sex hormone binding globulin (SHBG) by HIV status, and in women with HIV by viral load among premenopausal women participating in the Women's Interagency HIV Study (WIHS). Overall, there were no clinically relevant differences in total or free estradiol comparing women with versus with HIV, though both were generally modestly lower in women with compared to without HIV. There were higher SHBG concentrations comparing women with unsuppressed viral load to women without HIV, with differences ranging from 10-12 nmol/L across the percentiles assessed. There were also no clinically meaningful differences in total, free estradiol or SHBG among women with HIV by suppression status. Higher concentrations of SHBG inform the amount of free estradiol in circulation, and can result in lower concentrations. This was not observed in this aim where only modestly lower free estradiol was observed at the 75th percentile of the estradiol distribution in women with suppressed viral load compared to women without HIV, and in women with HIV, by suppression status.

Research on the association between HIV status and estradiol is equivocal, with studies finding lower, comparable, or higher total estradiol comparing women with and without HIV.[62,111,116–119] The lack of an association between total or free estradiol in this analysis may indicate that estradiol is comparable by HIV status, and viral suppression status. Alternatively, by limiting the study sample to women reporting at least one period within the six months prior to the study visit, the comparison groups are more homogenous with respect to amenorrhea than what would be observed in the real world. This could obscure potential hormonal differences.

This aim provided unique insight into the most biologically active form of estrogen, estradiol, as well as SHBG, among premenopausal women with HIV and how

it compared to a similar sample of women without HIV in the United States. This work built on the limited research on estradiol in women with HIV by assessing a large sample of women with and without HIV and by implementing regression techniques to account for demographic and clinical characteristics that could partly explain differences by HIV status, and among women with HIV by viral suppression status. Though there were minimal differences in total or free estradiol, and the sample size at extreme percentiles was limited, seeing varying differences depending on whether the 25^{th}, 50^{th} or 75^{th} percentile was assessed demonstrates the importance of considering the entire distribution of these biomarkers as opposed to measures of central tendency alone.

Further research is needed to characterize potential differences in estradiol by HIV status and viral suppression status. Given the differences observed in SHBG, this should continue to be accounted for and investigated, as it may alter free estradiol in circulation. Additional research is also needed to understand the temporality of the association between HIV status and estradiol (e.g. does viral load impact estradiol or vice versa). Among women with HIV, a differential impact of HIV on estradiol by treatment status should also be explored, as certain classes of HIV medications have known metabolic side effects. The sample size of this source population could not afford such an analysis in this aim. With respect to breast cancer etiology, the strongest relationship between estrogen and breast cancer is observed in postmenopausal women. Therefore, studies of estradiol should include longitudinal measurements tied to biologically meaningful time points. Further research is needed in contemporary observational settings among both pre and postmenopausal women to fully understand estradiol over the life course of women with HIV.

Association Between Cumulative Viral Load on ART and Breast Cancer Risk

For the final aim, I assessed the association between cumulative viral load on ART and breast cancer risk among women with HIV in the NA-ACCORD using joint longitudinal survival models. There was no association between cumulative viral load on ART and breast cancer risk adjusting for AIDS diagnosis prior to ART, and calendar year/age at ART initiation. Cumulative viral load on ART was lagged 1-5 years in additional analyses. Though the estimated association between cumulative viral load on ART and breast cancer remained null, the trend in the estimates suggested an increasingly protective association with increasing exposure lag. This was consistent in sensitivity analyses additionally adjusting for race/ethnicity, BMI, and smoking, as well as in subgroup analyses restricting to women with a viral load at ART initiation greater than 200 copies/mL, and utilizing nadir CD4 and AIDS diagnosis prior to ART initiation as adjustment factors instead of our composite definition of AIDS-defining illness prior to ART.

There are very few studies that have looked at the association between HIV viremia and breast cancer risk. These findings are similar to one previously conducted case-control study assessing HIV viral load and breast cancer odds among women with a viral load >500 copies/mL, where there was a null association that trended protective (odds ratio=0.60, 95% CI 0.30, 1.20).[93]

Though this analysis was limited by the small case count, there are important implications of these findings. The increasing strength of the association between cumulative viral load on ART and breast cancer with increasing exposure lag suggests that if there is an effect of HIV viral load on breast cancer, it is likely acting in the years prior to diagnosis and should continue to be explored. Further research is also warranted where women with HIV are observed for longer periods of time at older ages to allow for additional case ascertainment. Larger number of cases would allow for adjustment for

traditional risk factors for breast cancer to isolate the association between HIV viral load and breast cancer and to account for the competing risk of death.

Conclusions

This dissertation sought to shed light on potential mechanisms driving the observed reduced risk of breast cancer comparing women with versus without HIV. Taken in sum, the findings from these aims provide support that among women with HIV, factors beyond sociodemographic characteristics, such as race/ethnicity and age, may be associated with breast cancer risk. This work underscores the need to continue investigating breast cancer and risk factors for breast cancer among this population and obstacles in studying a lower frequency and/or risk of breast cancer in women with compared to without HIV.

To investigate a potential HIV effect on breast cancer, a larger number of breast cancer diagnoses is necessary. Despite utilizing one of the largest samples of adults with HIV in the US with validated cancer diagnoses, only 30 breast cancer diagnoses met the inclusion criteria for the third aim of this dissertation. Women with HIV in the US are different from women in the general US population with respect to sociodemographic, lifestyle and clinical factors. To make inferences regarding breast cancer risk comparing women with and without HIV, the ideal control group of women without HIV should be as similar as possible with respect to these factors. The WIHS, the source population for aim 2, is an apt observational cohort for such a comparison, but again, the sample size is insufficient for the rare outcome of breast cancer in this context.

Our understanding of breast cancer in the general population is deeply nuanced, with characterizations by stage, grade, molecular subtype and more. As the intersection of HIV and cancer is a burgeoning field of interest, these data are only just beginning to

be collected systematically among PWH. Such data could provide additional insight into the etiology of breast cancer among women with HIV. For instance, understanding estrogen receptor status could inform whether hormonal mechanisms of tumor development are relevant in women with HIV. Given this, surveillance of breast cancer among populations of women with HIV, and comparable women without HIV, is needed to accrue enough cases to describe and compare breast cancer occurrence by HIV status.

Women with HIV in the US as a population are living longer, with most entering mid-life/menopause, the age group at highest risk for breast cancer.[1] Therefore, the number of diagnoses will likely continue rising, despite the observed lower risk compared to women without HIV. Moreover, as women with HIV live longer on treatment, their risk profile may become increasingly comparable to women without HIV. This is particularly concerning given the risk of all-cause and breast cancer specific mortality is higher comparing women with versus without HIV.[92,94,100] Thus, age appropriate screening for breast cancer should continue to be incorporated into clinical care among women with HIV, and additional research into breast cancer etiology among women with HIV should be explored.

References

1. Centers for Disease Control and Prevention. HIV Surveillance Report, 2018 (Updated). 2020;31. https://www.cdc.gov/hiv/pdf/library/reports/surveillance/cdc-hiv-surveillance-report-2018-updated-vol-31.pdf. Accessed May 14, 2020.
2. Centers for Disease Control (CDC). Current Trends Update: Acquired Immunodeficiency Syndrome -- United States. *MMWR Morb Mortal Wkly Rep*. 1986;35(2). https://www.cdc.gov/mmwr/preview/mmwrhtml/00000667.htm. Accessed April 22, 2021.
3. Anderegg N, Johnson LF, Zaniewski E, et al. All-cause mortality in HIV-positive adults starting combination antiretroviral therapy: Correcting for loss to follow-up. *AIDS*. 2017;31(Suppl 1):S31-S40. doi:10.1097/QAD.0000000000001321
4. Boulle A, Schomaker M, May MT, et al. Mortality in Patients with HIV-1 Infection Starting Antiretroviral Therapy in South Africa, Europe, or North America: A Collaborative Analysis of Prospective Studies. *PLoS Med*. 2014;11(9). doi:10.1371/journal.pmed.1001718
5. Trickey A, May MT, Vehreschild JJ, et al. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. *Lancet HIV*. 2017;4(8):e349-e356. doi:10.1016/S2352-3018(17)30066-8
6. Adih WK, Selik RM, Hall HI, Babu AS, Song R. Associations and Trends in Cause-Specific Rates of Death Among Persons Reported with HIV Infection, 23 U.S. Jurisdictions, Through 2011. *Open AIDS J*. 2016;10(1):144-157. doi:10.2174/1874613601610010144
7. Adih WK, Selik RM, Hu X. Trends in Diseased Reported on US Death Certificates that mentioned HIV Infection, 1996-2006. 2011. doi:10.1177/1545109710384505
8. Antiretroviral Therapy Cohort Collaboration A, May MT, Vehreschild J-J, et al. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. *lancet HIV*. 2017;4(8):e349-e356. doi:10.1016/S2352-3018(17)30066-8
9. Morlat P, Roussillon C, Henard S, et al. Causes of death among HIV-infected patients in France in 2010 (national survey). *AIDS*. 2014;28(8):1181-1191. doi:10.1097/QAD.0000000000000222
10. Bosh KA, Johnson AS, Hernandez AL, et al. Vital Signs: Deaths Among Persons with Diagnosed HIV Infection, United States, 2010–2018. *MMWR Morb Mortal Wkly Rep*. 2020;69(46):1717-1724. doi:10.15585/mmwr.mm6946a1
11. Wandeler G, Johnson LF, Egger M. Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population. *Curr Opin HIV AIDS*. 2016;11(5):492-500. doi:10.1097/COH.0000000000000298
12. Samji H, Cescon A, Hogg RS, et al. Closing the Gap: Increases in Life Expectancy among Treated HIV-Positive Individuals in the United States and Canada. Okulicz JF, ed. *PLoS One*. 2013;8(12):e81355. doi:10.1371/journal.pone.0081355
13. Marcus JL, Chao CR, Leyden WA, et al. Narrowing the Gap in Life Expectancy Between HIV-Infected and HIV-Uninfected Individuals With Access to Care. *J Acquir Immune Defic Syndr*. 2016;73(1):39-46. doi:10.1097/QAI.0000000000001014
14. Althoff KN, Chandran A, Zhang J, et al. Life-Expectancy Disparities Among Adults With HIV in the United States and Canada: The Impact of a Reduction in Drug- and Alcohol-Related Deaths Using the Lives Saved Simulation Model. *Am J*

Epidemiol. 2019;188(12):2097-2109. doi:10.1093/aje/kwz232
15. Panel on Clinical Practices for Treatment of HIV Infection. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. *Dep Heal Hum Serv*. 2000. https://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL01282000010.pdf. Accessed May 13, 2020.
16. Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV*. https://files.aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf. Accessed May 15, 2020.
17. Triant VA. Cardiovascular disease and HIV infection. *Curr HIV/AIDS Rep*. 2013;10(3):199-206. doi:10.1007/s11904-013-0168-6
18. Drozd DR, Kitahata MM, Althoff KN, et al. Increased Risk of Myocardial Infarction in HIV-Infected Individuals in North America Compared With the General Population. *JAIDS J Acquir Immune Defic Syndr*. 2017;75(5):568-576. doi:10.1097/QAI.0000000000001450
19. Islam F, Wu J, Jansson J, Wilson D. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *HIV Med*. 2012;13(8):n/a-n/a. doi:10.1111/j.1468-1293.2012.00996.x
20. Boyd MA, Mocroft A, Ryom L, et al. Cardiovascular disease (CVD) and chronic kidney disease (CKD) event rates in HIV-positive persons at high predicted CVD and CKD risk: A prospective analysis of the D:A:D observational study. *PLoS Med*. 2017;14(11):e1002424. doi:10.1371/journal.pmed.1002424
21. Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA. Loss of Bone Mineral Density After Antiretroviral Therapy Initiation, Independent of Antiretroviral Regimen. *JAIDS J Acquir Immune Defic Syndr*. 2009;51(5):554-561. doi:10.1097/QAI.0b013e3181adce44
22. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS*. 2006;20(17):2165-2174. doi:10.1097/QAD.0b013e32801022eb
23. Wyatt CM. Kidney Disease and HIV Infection. *Top Antivir Med*. 2017;25(1):13-16. http://www.ncbi.nlm.nih.gov/pubmed/28402929. Accessed December 9, 2018.
24. Fine DM, Perazella MA, Lucas GM, Atta MG. Renal Disease in Patients with HIV Infection. *Drugs*. 2008;68(7):963-980. doi:10.2165/00003495-200868070-00006
25. Palella FJ, Baker RK, Moorman AC, et al. Mortality in the Highly Active Antiretroviral Therapy Era. *JAIDS J Acquir Immune Defic Syndr*. 2006;43(1):27-34. doi:10.1097/01.qai.0000233310.90484.16
26. Price JC, Thio CL. Liver disease in the HIV-infected individual. *Clin Gastroenterol Hepatol*. 2010;8(12):1002-1012. doi:10.1016/j.cgh.2010.08.024
27. Joshi D, O'Grady J, Dieterich D, Gazzard B, Agarwal K. Increasing burden of liver disease in patients with HIV infection. *Lancet*. 2011;377(9772):1198-1209. doi:10.1016/S0140-6736(10)62001-6
28. Hernandez-Romieu AC, Garg S, Rosenberg ES, Thompson-Paul AM, Skarbinski J. Is diabetes prevalence higher among HIV-infected individuals compared with the general population? Evidence from MMP and NHANES 2009-2010. *BMJ Open Diabetes Res Care*. 2009;5:304. doi:10.1136/bmjdrc-2016
29. Nansseu JR, Bigna JJ, Kaze AD, Noubiap JJ. Incidence and Risk Factors for Prediabetes and Diabetes Mellitus Among HIV-infected Adults on Antiretroviral Therapy. *Epidemiology*. 2018;29(3):431-441. doi:10.1097/EDE.0000000000000815
30. Nix L, Tien PC. Metabolic Syndrome, Diabetes, and Cardiovascular Risk in HIV.

doi:10.1007/s11904-014-0219-7
31. Deeken JF, Tjen-A-Looi A, Rudek MA, et al. The Rising Challenge of Non-AIDS-Defining Cancers in HIV-Infected Patients. *Clin Infect Dis*. 2012;55(9):1228-1235. doi:10.1093/cid/cis613
32. Shiels MS, Pfeiffer RM, Gail MH, et al. Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst*. 2011. doi:10.1093/jnci/djr076
33. Guaraldi G, Stentarelli C, Zona S, Santoro A. HIV-associated lipodystrophy: Impact of antiretroviral therapy. *Drugs*. 2013;73(13):1431-1450. doi:10.1007/s40265-013-0108-1
34. Kravcik S. HIV Clinical Trials HIV Lipodystrophy: A Review. 2015. doi:10.1310/3HHB-59UP-93MM-AAXY
35. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS*. 1998;12(7):F51-F58. doi:10.1097/00002030-199807000-00003
36. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: A cohort study. *Lancet*. 1999;353(9170):2093-2099. doi:10.1016/S0140-6736(98)08468-2
37. Sension M, Deckx H. Lipid metabolism and lipodystrophy in HIV-1-infected patients: the role played by nonnucleoside reverse transcriptase inhibitors. *AIDS Rev*. 2015;17(1):21-36.
38. Eckard AR, McComsey GA. Weight gain and integrase inhibitors. *Curr Opin Infect Dis*. 2020;33(1):10-19. doi:10.1097/QCO.0000000000000616
39. Bourgi K, Wanjalla C, Koethe JR. Inflammation and Metabolic Complications in HIV. *Curr HIV/AIDS Rep*. 2018;15(5):371-381. doi:10.1007/s11904-018-0411-2
40. Mirza FS, Luthra P, Chirch L. Endocrinological aspects of HIV infection. *J Endocrinol Invest*. 2018;41(8):881-899. doi:10.1007/s40618-017-0812-x
41. Willig AL, Overton ET. Metabolic consequences of HIV: Pathogenic insights. *Curr HIV/AIDS Rep*. 2014;11(1):35-44. doi:10.1007/s11904-013-0191-7
42. Kibirige D, Ssekitoleko R. Endocrine and metabolic abnormalities among HIV-infected patients: A current review. *Int J STD AIDS*. 2013;24(8):603-611. doi:10.1177/0956462413479895
43. Srinivasa S, Grinspoon SK. Metabolic and body composition effects of newer antiretrovirals in HIV-infected patients. *Eur J Endocrinol*. 2014;170(5). doi:10.1530/EJE-13-0967
44. Nasi M, De Biasi S, Gibellini L, et al. Ageing and inflammation in patients with HIV infection. doi:10.1111/cei.12814
45. Miles DRB, Bilal U, Hutton HE, et al. Tobacco Smoking, Substance Use, and Mental Health Symptoms in People with HIV in an urban HIV clinic. *J Health Care Poor Underserved*. 2019;30(3):1083-1102. doi:10.1353/hpu.2019.0075
46. Mdodo R, Frazier EL, Dube SR, et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: Cross-sectional surveys. *Ann Intern Med*. 2015;162(5):335-344. doi:10.7326/M14-0954
47. Jamal A, Phillips E, Gentzke AS, et al. Current Cigarette Smoking Among Adults — United States, 2016. *MMWR Morb Mortal Wkly Rep*. 2018;67(2):53-59. doi:10.15585/mmwr.mm6702a1
48. Tesoriero JM, Gieryic SM, Carrascal A, Lavigne HE. Smoking among HIV positive New Yorkers: Prevalence, frequency, and opportunities for cessation. *AIDS Behav*. 2010;14(4):824-835. doi:10.1007/s10461-008-9449-2
49. Gritz ER, Vidrine DJ, Lazev AB, Amick BC, Arduino RC. Smoking behavior in a low-income multiethnic HIV/AIDS population. *Nicotine Tob Res*. 2004;6(1):71-77.

doi:10.1080/14622200310001656885
50. Frazier EL, Sutton MY, Brooks JT, Shouse RL, Weiser J. Trends in cigarette smoking among adults with HIV compared with the general adult population, United States - 2009–2014. *Prev Med (Baltim)*. 2018;111:231-234. doi:10.1016/j.ypmed.2018.03.007
51. Crothers K, Griffith TA, McGinnis KA, et al. The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans. *J Gen Intern Med*. 2005;20(12):1142-1145. doi:10.1111/j.1525-1497.2005.0255.x
52. Farzadegan H, Hoover DR, Astemborski J, et al. Sex differences in HIV-1 viral load and progression to AIDS. *Lancet*. 1998;352(9139):1510-1514. doi:10.1016/S0140-6736(98)02372-1
53. Boodram B, Plankey MW, Cox C, et al. Prevalence and correlates of elevated body mass index among HIV-positive and HIV-negative women in the women's interagency HIV study. *AIDS Patient Care STDS*. 2009;23(12):1009-1016. doi:10.1089/apc.2009.0175
54. Amorosa V, Synnestvedt M, Gross R, et al. A tale of 2 epidemics: The intersection between obesity and HIV infection in Philadelphia. *J Acquir Immune Defic Syndr*. 2005;39(5):557-561. doi:10.1097/01.qai.0000171638.65830.9b
55. Jones CY, Hogan JW, Snyder B, et al. Overweight and Human Immunodeficiency Virus (HIV) Progression in Women: Associations HIV Disease Progression and Changes in Body Mass Index in Women in the HIV Epidemiology Research Study Cohort. *Clin Infect Dis*. 2003;37(s2):S69-S80. doi:10.1086/375889
56. Hoover KW, Hu X, Porter SE, et al. HIV Diagnoses and the HIV Care Continuum among Women and Girls Aged ≥13 Years-39 States and the District of Columbia, 2015-2016. *J Acquir Immune Defic Syndr*. 2019;81(3):251-256. doi:10.1097/QAI.0000000000002023
57. Gold EB, Bromberger J, Crawford S, et al. *Factors Associated with Age at Natural Menopause in a Multiethnic Sample of Midlife Women*. Vol 153.; 2001. https://academic.oup.com/aje/article/153/9/865/124589.
58. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. *Prospective Study of the Determinants of Age at Menopause*. Vol 145.; 1997. https://academic.oup.com/aje/article/145/2/124/90838.
59. McKinlay SM, Brambilla DJ, Posner JG. "Reprint of" The normal menopause transition. *Maturitas*. 2008;61(1-2):4-16. doi:10.1016/j.maturitas.2008.09.005
60. Yalamanchi S, Dobs A, Greenblatt RM. Gonadal function and reproductive health in women with human immunodeficiency virus infection. *Endocrinol Metab Clin North Am*. 2014;43(3):731-741. doi:10.1016/j.ecl.2014.05.002
61. Cejtin HE, Kalinowski A, Bacchetti P, et al. Effects of Human Immunodeficiency Virus on Protracted Amenorrhea and Ovarian Dysfunction. *Obstet Gynecol*. 2006;108(6):1423-1431. doi:10.1097/01.AOG.0000245442.29969.5c
62. Seifer DB, Golub ET, Lambert-Messerlian G, et al. Biologic markers of ovarian reserve and reproductive aging: application in a cohort study of HIV infection in women. *Fertil Steril*. 2007;88(6):1645-1652. doi:10.1016/j.fertnstert.2007.01.122
63. Seifer DB, Golub ET, Lambert-Messerlian G, et al. Variations in serum müllerian inhibiting substance between white, black, and Hispanic women. *Fertil Steril*. 2009;92(5):1674-1678. doi:10.1016/j.fertnstert.2008.08.110
64. Fantry LE, Zhan M, Taylor GH, Sill AM, Flaws JA. Age of Menopause and Menopausal Symptoms in HIV-Infected Women. *AIDS Patient Care STDS*. 2005;19(11):703-711. doi:10.1089/apc.2005.19.703
65. Chirgwin KD, Feldman J, Muneyyirci-Delale O, Landesman S, Minkoff H. Menstrual function in human immunodeficiency virus-infected women without

acquired immunodeficiency syndrome. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996;12(5):489-494. doi:10.1097/00042560-199608150-00008
66. Clark RA, Mulligan K, Stamenovic E, et al. Frequency of Anovulation and Early Menopause among Women Enrolled in Selected Adult AIDS Clinical Trials Group Studies. *J Infect Dis*. 2001;184(10):1325-1327. doi:10.1086/323999
67. Greenblatt RM, Ameli N, Grant RM, Bacchetti P, Taylor RN. Impact of the Ovulatory Cycle on Virologic and Immunologic Markers in HIV-Infected Women. *J Infect Dis*. 2000;181(1):82-90. doi:10.1086/315207
68. King EM, Albert AY, Murray MCM. HIV and amenorrhea. *AIDS*. 2019;33(3):483-491. doi:10.1097/QAD.0000000000002084
69. Shiels MS, Engels EA. Evolving epidemiology of HIV-associated malignancies. *Curr Opin HIV AIDS*. 2017;12(1):6-11. doi:10.1097/COH.0000000000000327
70. Shiels MS, Islam JY, Rosenberg PS, Hall HI, Jacobson E, Engels EA. Projected Cancer Incidence Rates and Burden of Incident Cancer Cases in HIV-Infected Adults in the United States Through 2030. *Ann Intern Med*. 2018;168(12):866. doi:10.7326/M17-2499
71. Robbins HA, Shiels MS, Pfeiffer RM, Engels EA. Epidemiologic contributions to recent cancer trends among HIV- infected people in the United States HHS Public Access. *AIDS March*. 2014;27(286):881-890. doi:10.1097/QAD.0000000000000163
72. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet (London, England)*. 2014;384(9939):241-248. doi:10.1016/S0140-6736(14)60604-8
73. Silverberg MJ, Lau B, Achenbach CJ, et al. Cumulative Incidence of Cancer Among Persons With HIV in North America: A Cohort Study. *Ann Intern Med*. 2015;163(7):507-518. doi:10.7326/M14-2768
74. Robbins HA, Pfeiffer RM, Shiels MS, Li J, Hall HI, Engels EA. Excess cancers among HIV-infected people in the United States. *J Natl Cancer Inst*. 2015;107(4). doi:10.1093/jnci/dju503
75. Chaturvedi AK, Pfeiffer RM, Chang L, Goedert JJ, Biggar RJ, Engels EA. Elevated risk of lung cancer among people with AIDS. *AIDS*. 2007;21(2):207-213. doi:10.1097/QAD.0b013e3280118fca
76. Shiels MS, Cole SR, Kirk GD, Poole C. A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals. *J Acquir Immune Defic Syndr*. 2009;52(5):611-622. doi:10.1097/QAI.0b013e3181b327ca
77. Parka LS, Hernandez-Ramirez RU, Silverberg MJ, Crothers K, Dubrow R. Prevalence of non-HIV cancer risk factors in persons living with HIV/AIDS: A meta-analysis. *AIDS*. 2016. doi:10.1097/QAD.0000000000000922
78. Patel P, Hanson DL, Sullivan PS, et al. Incidence of Types of Cancer among HIV-Infected Persons Compared with the General Population in the United States, 1992–2003. *Ann Intern Med*. 2008;148(10):728. doi:10.7326/0003-4819-148-10-200805200-00005
79. Goedert JJ, Schairer C, McNeel TS, Hessol NA, Rabkin CS, Engels EA. Risk of breast, ovary and uterine corpus cancers among 85 268 women with AIDS. *Br J Cancer*. 2006;95(5):642-648. doi:10.1038/sj.bjc.6603282
80. Coghill AE, Engels EA, Schymura MJ, Mahale P, Shiels MS. Risk of breast, prostate, and colorectal cancer diagnoses among hiv-infected individuals in the United States. *J Natl Cancer Inst*. 2018;110(9):959-966. doi:10.1093/jnci/djy010
81. Marcus JL, Chao CR, Leyden WA, et al. Prostate Cancer Incidence and Prostate-Specific Antigen Testing Among HIV-Positive and HIV-Negative Men. *JAIDS J Acquir Immune Defic Syndr*. 2014;66(5):495-502.

doi:10.1097/QAI.0000000000000202
82. Shiels MS, Goedert JJ, Moore RD, Platz EA, Engels EA. Reduced risk of prostate cancer in U.S. Men with AIDS. *Cancer Epidemiol Biomarkers Prev*. 2010;19(11):2910-2915. doi:10.1158/1055-9965.EPI-10-0741
83. Dutta A, Uno H, Holman A, Lorenz DR, Gabuzda D. Racial differences in prostate cancer risk in young HIV-positive and HIV-negative men: a prospective cohort study. doi:10.1007/s10552-017-0896-9
84. *Cancer Facts and Figures 2021*.
85. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(6):438-451. doi:10.3322/caac.21583
86. N Howlader, AM Noone, M Krapcho, et al eds. Trends in SEER Incidence and U.S. Mortality Using the Joinpoint Regression Program,1975-2016 With up to Five Joinpoints, 2000-2016 With up to Three Joinpoints,All Ages by Race/Ethnicity. SEER Cancer Statistics Review, 1975-2016. Based on the November 2018 SEER data submission, posted to the SEER website April 2019. Surveillance, Epidemiology, and End Results Program, National Cancer Institute, National Institutes of Health; 2019. https://seer.cancer.gov/archive/csr/1975_2016/results_merged/sect_04_breast.pdf. Accessed March 29, 2021.
87. Richardson LC, Henley SJ, Miller JW, Massetti G, Thomas CC. Patterns and Trends in Age-Specific Black-White Differences in Breast Cancer Incidence and Mortality – United States, 1999–2014. *MMWR Morb Mortal Wkly Rep*. 2016;65(40):1093-1098. doi:10.15585/mmwr.mm6540a1
88. Anderson GL, Barrington WE. Narrowing of racial disparities in breast cancer incidence: insights from menopausal hormone therapy study findings. *J Natl Cancer Inst*. 2016;108(4):djv393. doi:10.1093/jnci/djv393
89. Clarke CA, Glaser SL, Uratsu CS, Selby J V., Kushi LH, Herrinton LJ. Recent declines in hormone therapy utilization and breast cancer incidence: clinical and population-based evidence. *J Clin Oncol*. 2006;24(33). doi:10.1200/jco.2006.08.6504
90. Ravdin PM, Cronin KA, Howlader N, et al. The Decrease in Breast-Cancer Incidence in 2003 in the United States. *N Engl J Med*. 2007;356(16):1670-1674. doi:10.1056/nejmsr070105
91. Centers for Disease Control and Prevntion/ National Center for Health Statistics. Use of Hormone Replacement Therapy Among Postmenopausal Women in the United States, 1988–94. http://www.cdc.gov/nchs/nhanes.htm. Published 2003. Accessed May 8, 2021.
92. Chhatre S, Schapira M, Metzger DS, Jayadevappa R. Association between HIV infection and outcomes of care among medicare enrollees with breast cancer. *EClinicalMedicine*. 2019;17. doi:10.1016/j.eclinm.2019.11.001
93. Hessol NA, Napolitano LA, Smith D, et al. HIV Tropism and Decreased Risk of Breast Cancer. *HIV Trop Decreased Risk Breast Cancer PLoS ONE*. 2010;5(12). doi:10.1371/
94. Coghill AE, Han X, Suneja G, Lin CC, Jemal A, Shiels MS. Advanced stage at diagnosis and elevated mortality among US patients with cancer infected with HIV in the National Cancer Data Base. *Cancer*. 2019;125(16):2868-2876. doi:10.1002/cncr.32158
95. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J Cancer*. 2008;123(1):187-194. doi:10.1002/ijc.23487
96. Hessol NA, Seaberg EC, Preston-Martin S, et al. Cancer risk among participants

in the women's interagency HIV study. *J Acquir Immune Defic Syndr*. 2004;36(4):978-985. http://www.ncbi.nlm.nih.gov/pubmed/15220706. Accessed April 9, 2018.
97. Long JL, Engels EA, Moore RD, Gebo KA. Incidence and outcomes of malignancy in the HAART era in an urban cohort of HIV-infected individuals. *AIDS*. 2008;22(4):489-496. doi:10.1097/QAD.0b013e3282f47082
98. Gallagher B, Wang Z, Schymura MJ, Kahn A, Fordyce EJ. Cancer incidence in New York State acquired immunodeficiency syndrome patients. *Am J Epidemiol*. 2001;154(6):544-556. doi:10.1093/aje/154.6.544
99. Engels EA, Pfeiffer RM, Goedert JJ, et al. Trends in cancer risk among people with AIDS in the United States 1980–2002. *AIDS*. 2006;20(12):1645-1654. doi:10.1097/01.aids.0000238411.75324.59
100. Coghill AE, Suneja G, Rositch AF, Shiels MS, Engels EA. HIV Infection, Cancer Treatment Regimens, and Cancer Outcomes Among Elderly Adults in the United States. *JAMA Oncol*. 2019;5(9):e191742. doi:10.1001/jamaoncol.2019.1742
101. Gant Z, Hess K, Johnson AS, et al. *Diagnoses of HIV Infection in the United States and Dependent Areas, 2014 HIV Surveillance Report 2 Vol. 26*.; 2014. http://www.cdc.gov/hiv/library/reports/surveillance/.PublishedNovember2015.Accessed[date].OntheWeb:http://www.cdc.gov/hiv/library/reports/surveillance/http://www.cdc.gov/cdc-info/requestform.html. Accessed December 9, 2018.
102. Daly B, Olopade OI. Race, ethnicity, and the diagnosis of breast cancer. *JAMA*. 2015;313(2):141-142. doi:10.1001/jama.2014.17323
103. Oliver NT, Chiao EY. Malignancies in women with HIV infection. *Curr Opin HIV AIDS*. 2017;12(1):69-76. doi:10.1097/COH.0000000000000332
104. Shiels MS, Althoff KN, Pfeiffer RM, et al. HIV Infection, Immunosuppression, and Age at Diagnosis of Non-AIDS-Defining Cancers. *Clin Infect Dis*. 2017;64(4):468-475. doi:10.1093/cid/ciw764
105. Shiels MS, Althoff KN, Pfeiffer RM, et al. HIV Infection, Immunosuppression, and Age at Diagnosis of Non-AIDS-Defining Cancers. *Clin Infect Dis*. 2016;64(4):ciw764. doi:10.1093/cid/ciw764
106. Preston-Martin S, Kirstein LM, Pogoda JM, et al. Use of Mammographic Screening by HIV-Infected Women in the Women's Interagency HIV Study (WIHS). *Prev Med (Baltim)*. 2002;34(3):386-392. doi:10.1006/PMED.2001.1003
107. Simonsen SE, Kepka D, Thompson J, Warner EL, Snyder M, Ries KM. Preventive health care among HIV positive women in a Utah HIV/AIDS clinic: A retrospective cohort study. *BMC Womens Health*. 2014;14(1):37. doi:10.1186/1472-6874-14-37
108. Momplaisir F, Mounzer K, Long JA. AIDS Care Psychological and Socio-medical Aspects of AIDS/HIV Preventive cancer screening practices in HIV-positive patients. doi:10.1080/09540121.2013.802276
109. Kendall CE, Walmsley S, Lau C, et al. A cross-sectional population-based study of breast cancer screening among women with HIV in Ontario, Canada. *C Open*. 2017;5(3):E673-E681. doi:10.9778/cmajo.20170038
110. Szotek EL, Narasipura SD, Al-Harthi L. 17β-Estradiol inhibits HIV-1 by inducing a complex formation between β-catenin and estrogen receptor α on the HIV promoter to suppress HIV transcription. *Virology*. 2013;443(2):375-383. doi:10.1016/j.virol.2013.05.027
111. Karim R, Mack WJ, Kono N, et al. Gonadotropin and sex steroid levels in HIV-infected premenopausal women and their association with subclinical atherosclerosis in HIV-infected and -uninfected women in the Women's Interagency HIV Study (WIHS). *J Clin Endocrinol Metab*. 2013. doi:10.1210/jc.2012-3195

112. Brown SB, Hankinson SE. Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers. *Steroids*. 2015;99:8-10. doi:10.1016/j.steroids.2014.12.013
113. Key T. Sex hormones and risk of breast cancer in premenopausal women: A collaborative reanalysis of individual participant data from seven prospective studies. *Lancet Oncol*. 2013;14(10):1009-1019. doi:10.1016/S1470-2045(13)70301-2
114. Fuhrman BJ, Schairer C, Gail MH, et al. Estrogen metabolism and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst*. 2012;104(4):326-339. doi:10.1093/jnci/djr531
115. Greenblatt RM, Ameli N, Grant RM, Bacchetti P, Taylor RN, Greenblatt R. *Impact of the Ovulatory Cycle on Virologic and Immunologic Markers in HIV-Infected Women*. Vol 181.; 1999. https://academic.oup.com/jid/article-abstract/181/1/82/896843. Accessed September 12, 2018.
116. Weinberg A, Enomoto L, Marcus R, Canniff J. Effect of menstrual cycle variation in female sex hormones on cellular immunity and regulation. *J Reprod Immunol*. 2011;(89):70-77. doi:doi:10.1016/j.jri.2010.11.009
117. Murphy K, Keller MJ, Anastos K, et al. Impact of reproductive aging on the vaginal microbiome and soluble immune mediators in women living with and at-risk for HIV infection. Landay A, ed. *PLoS One*. 2019;14(4):e0216049. doi:10.1371/journal.pone.0216049
118. Santoro N, Lo Y, Moskaleva G, et al. Factors affecting reproductive hormones in HIV-infected, substance-using middle-aged women. *Menopause J North Am Menopause Soc*. 2007;14(5):859-865. doi:10.1097/gme.0b013e31802f7369
119. Cu-Uvin S, Wright DJ, Anderson D, et al. Hormonal Levels among HIV-1-Seropositive Women Compared with High-Risk HIV-Seronegative Women during the Menstrual Cycle. *J Womens Health Gend Based Med*. 2000;9(8):857-863. doi:10.1089/152460900750020883
120. Berger EA, Doms RW, Fenyo EM, et al. A new classification for HIV-1. *Nature*. 1998;391(6664):240. doi:10.1038/34571
121. Paiardini M, Müller-Trutwin M. HIV-associated chronic immune activation. *Immunol Rev*. 2013;254(1):78-101. doi:10.1111/imr.12079
122. Schmid BC, Rudas M, Rezniczek GA, Leodolter S, Zeillinger R. *CXCR4 Is Expressed in Ductal Carcinoma in Situ of the Breast and in Atypical Ductal Hyperplasia*. Vol 84.; 2004.
123. Mueller A, Homey B, Soto H, et al. *Involvement of Chemokine Receptors in Breast Cancer Metastasis*.; 2001. www.nature.com. Accessed March 19, 2021.
124. Endo M, Inatsu A, Hashimoto K, Takamune N, Shoji S, Misumi S. Human immunodeficiency virus-induced apoptosis of human breast cancer cells via CXCR4 is mediated by the viral envelope protein but does not require CD4. *Curr HIV Res*. 2008;6(1):34-42. http://www.ncbi.nlm.nih.gov/pubmed/18288973. Accessed April 22, 2019.
125. Griffin DO. The Challenge of Malignancies in HIV-1, Beyond Immune Activation and Back to Decreased Immune Surveillance. *J AIDS Clin Res*. 2016;7(1):535. doi:10.4172/2155-6113.1000535
126. Pati S, Pelser CB, Dufraine J, Bryant JL, Reitz MS, Weichold FF. *Antitumorigenic Effects of HIV Protease Inhibitor Ritonavir: Inhibition of Kaposi Sarcoma*.; 2002. www.bloodjournal.org. Accessed December 9, 2018.
127. Monini P, Sgadari C, Toschi E, Barillari G, Ensoli B. Antitumour effects of antiretroviral therapy. *Nat Rev Cancer*. 2004;4(11):861-875. doi:10.1038/nrc1479
128. Brüning A, Burger P, Gingelmaier A, Mylonas I. The HIV reverse transcriptase

inhibitor tenofovir induces cell cycle arrest in human cancer cells. *Invest New Drugs*. 2012;30(4):1389-1395. doi:10.1007/s10637-011-9704-7
129. Gange SJ, Kitahata MM, Saag MS, et al. Cohort profile: the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD). *Int J Epidemiol*. 2007;36(2):294-301. doi:10.1093/ije/dyl286
130. Adimora AA, Ramirez C, Benning L, et al. Cohort Profile: The Women's Interagency HIV Study (WIHS). *Int J Epidemiol*. 2018;47(2):393-394i. doi:10.1093/ije/dyy021
131. Shiels MS, Pfeiffer RM, Gail MH, et al. Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst*. 2011. doi:10.1093/jnci/djr076
132. Simard EP, Pfeiffer RM, Engels EA. Cumulative incidence of cancer among individuals with acquired immunodeficiency syndrome in the United States. *Cancer*. 2011;117(5):1089-1096. doi:10.1002/cncr.25547
133. Crane HM, McCaul ME, Chander G, et al. Prevalence and Factors Associated with Hazardous Alcohol Use Among Persons Living with HIV Across the US in the Current Era of Antiretroviral Treatment. *AIDS Behav*. 2017;21(7):1914-1925. doi:10.1007/s10461-017-1740-7
134. Galvan FH, Bing EG, Fleishman JA, et al. The prevalence of alcohol consumption and heavy drinking among people with HIV in the United States: results from the HIV Cost and Services Utilization Study. *J Stud Alcohol*. 2002;63(2):179-186. doi:10.15288/jsa.2002.63.179
135. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin*. 2017;67(6):439-448. doi:10.3322/caac.21412
136. Shiels MS, Copeland G, Goodman MT, et al. Cancer stage at diagnosis in patients infected with the human immunodeficiency virus and transplant recipients. *Cancer*. 2015;121(12):2063-2071. doi:10.1002/cncr.29324
137. Lash TL, Cole SR. Immortal Person-Time in Studies of Cancer Outcomes. *J Clin Oncol*. 2009;27(23):e55-e56. doi:10.1200/JCO.2009.24.1877
138. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology: Third Edition*.; 2011.
139. Palella FJ, Delaney KM, Moorman AC, et al. Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection. *N Engl J Med*. 1998;338(13):853-860. doi:10.1056/NEJM199803263381301
140. French AL, Gawel SH, Hershow R, et al. Trends in Mortality and Causes of Death among Women with HIV in the US: A Ten-year Study. *J Acquir Immune Defic Syndr*. 2009;51(4):399-406. doi:10.1097/QAI.0b013e3181acb4e5
141. Centers for Disease C, Prevention. QuickStats: Age-Adjusted Death Rates* for Human Immunodeficiency Virus (HIV) Infection, by Sex - United States, 1987-2003. *Morb Mortal Wkly Rep*. 2005;54:1188. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5446a7.htm. Accessed March 24, 2020.
142. Howlader N, Noone A, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2017. Table 4.17. Cancer of the female breast (invasive)-Lifetime risk of being diagnosed with cancer given alive and cancer-free at current age. National Cancer Institute. Bethesda, MD. http://seer.cancer.gov/csr/1975_2017/. Accessed June 9, 2020.
143. Cejtin HE. Gynecologic issues in the HIV-infected woman. *Infect Dis Clin North Am*. 2008;22(4):709-739. doi:10.1016/j.idc.2008.05.006
144. Rochira V, Guaraldi G. Hypogonadism in the HIV-Infected Man. *Endocrinol Metab Clin North Am*. 2014;43(3):709-730. doi:10.1016/j.ecl.2014.06.005
145. Klein SL, Roberts CW. *Sex Hormones and Immunity to Infection*. Springer-Verlag

Berlin Heidelberg; 2010. doi:10.1007/978-3-642-02155-8
146. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, et al. Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: Long-term results of a prospective study. *Br J Cancer*. 2004;90(1):153-159. doi:10.1038/sj.bjc.6601517
147. *Endogenous Sex Hormones and Breast Cancer in Postmenopausal Women: Reanalysis of Nine Prospective Studies*. https://academic.oup.com/jnci/article/94/8/606/2520148.
148. Rebecca EJ, Lukanova A, Dossus L, et al. Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: A nested case-control study. *Cancer Prev Res*. 2011;4(10):1626-1635. doi:10.1158/1940-6207.CAPR-11-0090
149. Cauley JA, Lucas FL, Lewis ;, et al. *Elevated Serum Estradiol and Testosterone Concentrations Are Associated with a High Risk for Breast Cancer*.; 1999. http://www.acponline.org.
150. Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. *J Clin Endocrinol Metab*. 2008;93(1):131-138. doi:10.1210/jc.2007-1738
151. Merz CNB, Johnson BD, Delia Sharaf BL, et al. Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: A report from the NHLBI-sponsored WISE study. *J Am Coll Cardiol*. 2003;41(3):413-419. doi:10.1016/S0735-1097(02)02763-8
152. Ellerbrock T V, Wright TC, Bush TJ, Dole P, Brudney K, Chiasson MA. Characteristics of menstruation in women infected with human immunodeficiency virus. *Obstet Gynecol*. 1996;87(6):1030-1034. doi:10.1016/0029-7844(96)00047-6
153. Shah P, Smith J, Wells C, Barton S, Kitchen V, Steer PJ. Menstrual Symptoms in Women Infected by the Human Immunodeficiency Virua. *Obs Gynecol*. 1994;83(3):397-400.
154. Cejtin HE, Evans CT, Greenblatt R, et al. Prolonged Amenorrhea and Resumption of Menses in Women with HIV. *J Women's Heal*. 2018;27(12):1441-1448. doi:10.1089/jwh.2018.7046
155. Thomas MP, Potter BVL. The structural biology of oestrogen metabolism. *J Steroid Biochem Mol Biol*. 2013;137:27-49. doi:10.1016/j.jsbmb.2012.12.014
156. Södergard R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. *J Steroid Biochem*. 1982;16(6):801-810. doi:10.1016/0022-4731(82)90038-3
157. Lee JS, Cole SR, Richardson DB, et al. Incomplete viral suppression and mortality in HIV patients after antiretroviral therapy initiation. *AIDS*. 2017;31(14):1989-1997. doi:10.1097/QAD.0000000000001573
158. Department of Health and Human Services editor. Panel of Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 2013:C5-undefined.
159. Alf EF, Grossberg JM. The geometric mean: Confidencelimits and significance tests. *Percept Pyshophysics*. 1979;26(5):419-421.
160. Burgette LF, Reiter JP, Miranda ML. Exploratory Quantile Regression With Many Covariates. *Epidemiology*. 2011;22(6):859-866. doi:10.1097/EDE.0b013e31822908b3
161. Wei Y, Kehm RD, Goldberg M, Terry MB. Applications for Quantile Regression in Epidemiology. *Curr Epidemiol Reports*. 2019;(6):191-199. doi:9 https://doi.org/10.1007/s40471-019-00204-6

162. Sato T, Matsuyama Y. Marginal Structural Models as a Tool for Standardization. *Epidemiology*. 2003;14:680-686. https://www-jstor-org.proxy1.library.jhu.edu/stable/pdf/3703427.pdf. Accessed July 31, 2020.
163. Eliassen AH, Missmer SA, Tworoger SS, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. *J Natl Cancer Inst*. 2006;98(19):1406-1415. doi:10.1093/jnci/djj376
164. Fortner RT, Eliassen AH, Spiegelman D, Willett WC, Barbieri RL, Hankinson SE. *Premenopausal Endogenous Steroid Hormones and Breast Cancer Risk: Results from the Nurses' Health Study II*.; 2013. doi:10.1186/bcr3394
165. Cramer DW, Barbieri RL, Fraer AR, Harlow BL. Determinants of early follicular phase gonadotrophin and estradiol concentrations in women of late reproductive age. *Hum Reprod*. 2002;17(1):221-227. doi:10.1093/humrep/17.1.221
166. Randolph JF, Sowers M, Gold EB, et al. Reproductive Hormones in the Early Menopausal Transition: Relationship to Ethnicity, Body Size, and Menopausal Status. *J Clin Endocrinol Metab*. 2003;88(4):1516-1522. doi:10.1210/jc.2002-020777
167. Cofrancesco J, Shah N, Ghanem KG, et al. The effects of illicit drug use and HIV infection on sex hormone levels in women. 2009. doi:10.1080/09513590600687603
168. Marcus JL, Leyden WA, Alexeeff SE, et al. Comparison of Overall and Comorbidity-Free Life Expectancy Between Insured Adults With and Without HIV Infection, 2000-2016. *JAMA Netw Open*. 2020;3(6). doi:10.1001/jamanetworkopen.2020.7954
169. Wong C, Gange SJ, Moore RD, et al. Multimorbidity Among Persons Living with Human Immunodeficiency Virus in the United States. *Clin Infect Dis*. 2018;66(8):1230-1238. doi:10.1093/cid/cix998
170. Salters KA, Cescon A, Zhang W, et al. Cancer incidence among HIV-positive women in British Columbia, Canada: Heightened risk of virus-related malignancies. *HIV Med*. 2016;17:188-195. doi:10.1111/hiv.12290
171. Park LS, Tate JP, Sigel K, et al. Association of Viral Suppression With Lower AIDS-Defining and Non–AIDS-Defining Cancer Incidence in HIV-Infected Veterans. *Ann Intern Med*. 2018;169(2):87. doi:10.7326/M16-2094
172. Cole SR, Napravnik S, Mugavero MJ, et al. Copy-years viremia as a measure of cumulative human immunodeficiency virus viral burden. *Am J Epidemiol*. 2010;171(2):198-205. doi:10.1093/aje/kwp347
173. Zoufaly A, Stellbrink HJ, an der Heiden M, et al. Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. *J Infect Dis*. 2009;200(1):79-87. doi:10.1086/599313
174. Bruyand M, Thiébaut R, Lawson-Ayayi S, et al. Role of Uncontrolled HIV RNA Level and Immunodeficiency in the Occurrence of Malignancy in HIV-infected Patients during the Combination Antiretroviral Therapy Era: Agence Nationale de Recherche sur le Sida (ANRS) CO3 Aquitaine Cohort. *Clin Infect Dis*. 2009;49(7):1109-1116. doi:10.1086/605594
175. Hernández-Ramírez RU, Qin L, Lin H, et al. Association of Immunosuppression and Human Immunodeficiency Virus (HIV) Viremia with Anal Cancer Risk in Persons Living with HIV in the United States and Canada. *Clin Infect Dis*. 2020;70(6):1176-1185. doi:10.1093/cid/ciz329
176. Kowalkowski MA, Day RS, Du XL, Chan W, Chiao EY. Cumulative HIV Viremia and Non–AIDS-Defining Malignancies Among a Sample of HIV-Infected Male Veterans. *JAIDS J Acquir Immune Defic Syndr*. 2014;67(2):204-211. doi:10.1097/QAI.0000000000000289

177. Wulfsohn MS, Tsiatis AA. A Joint Model for Survival and Longitudinal Data Measured with Error. *Biometrics*. 1997;53(1):330. doi:10.2307/2533118

Curriculum Vitae

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EDUCATION

Doctor of Philosophy in Epidemiology (GPA: 3.95) — Baltimore, MD
Bloomberg School of Public Health, Johns Hopkins University — August 2017-present
Thesis Advisory Committee: Dr. Keri Althoff, Dr. Bryan Lau, Dr. Kala Visvanathan, Dr. Todd Brown

Master of Public Health in Epidemiology (summa cum laude) — Washington, DC
George Washington University — August 2013-August 2015

Bachelor of Science in Public Health (summa cum laude) — Washington, DC
Minor in Music — August 2009-May 2013
George Washington University

AWARDS

Distinguished Scholar Award — May 2015
Department of Epidemiology and Biostatistics
Milken Institute School of Public Health, George Washington University

Merit Scholarship — August 2013
Milken Institute School of Public Health, George Washington University

Presidential Scholar of the Arts — August 2009
George Washington University
(Four-year scholarship in recognition of musical talent-Jazz Drumming)

TRAINING

T32 Pre-Doctoral Trainee — July 2018-April 2020
Clinical Research & Epidemiology in Diabetes and Endocrinology Training Grant
Johns Hopkins University

GRANTS

Ruth L. Kirschstein National Research Service Award (NRSA) — April 2020-present
Individual Predoctoral Fellowship
National Cancer Institute, F31 CA247610

RESEARCH EXPERIENCE

Graduate Research Assistant for Dr. Bryan Lau — Baltimore, MD
Department of Epidemiology — August 2019-present
Bloomberg School of Public Health Johns Hopkins University

- Conducting analysis on colorectal cancer screening effectiveness by HIV status among Medicaid recipients through the Johns Hopkins MediCan research group
- Collaborates with faculty and students on development of manuscripts for publication

Graduate Research Assistant for Dr. Keri Althoff Baltimore, MD
Department of Epidemiology July 2018-present
Bloomberg School of Public Health Johns Hopkins University

- Conducts data analyses for several research projects in nationwide HIV cohorts
- Conducts data analyses for seroprevalence of SARS-CoV-2 among decedents undergoing autopsy in Maryland
- Drafts manuscripts and abstracts for publication and works in tandem with faculty to develop new research questions and potential analyses

Epidemiologic Research Analyst Bethesda, MD
Metabolic Epidemiology Branch June 2015-July 2017
Division of Cancer Epidemiology and Genetics, National Cancer Institute

- Directed several analyses and drafted accompanying manuscripts
- Conducted data analyses across the spectrum of epidemiologic study designs including cross-sectional, case-control, nested case-control, and cohort analyses utilizing multiple statistical analysis platforms (e.g., SAS, STATA, R)
- Collaborated with investigators on drafting manuscript for publication, including writing results from statistical analyses I conducted
- Responsible for data management and data cleaning for numerous ongoing research projects, including developing an algorithm to monitor quality control data in real-time, managing databases of more than 1 million participants

Research Assistant-DC Cohort Washington, DC
(Cohort of People with HIV in care in Washington, DC) January 2012-June 2015
Department of Epidemiology and Biostatistics
Milken Institute School of Public Health, George Washington University

- Examined participant enrollment and participant characteristics through data entry and analysis
- Collaborated with investigators on potential analyses
- Drafted the annual patient newsletter to inform participants on study progress
- Attended site visits to ensure protocol compliance and completed Institutional Review Board documentation

Research Intern Washington, DC
Elizabeth Glaser Pediatric AIDS Foundation May 2014- September 2014

- Wrote the scientific manuscript for a secondary analysis investigating mother-infant pair retention to PMTCT HIV care in Rwanda
- Constructed tables and figures for publication utilizing Microsoft excel and R

Summer Intern Bethesda, MD
Protection of Participants, Evaluation and Policy Branch May 2011-August 2011;
Office for Policy in Clinical Operations May 2012-August 2012
Division of Acquired Immunodeficiency Syndrome
National Institute of Allergy and Infectious Diseases

- Co-authored policy posters presented at DHHS Office of Research Integrity Conference
- Assessed research protocols for human protections violations for continued funding
- Designed training course to improve Institutional Review Board practices in Sub-Saharan Africa

TEACHING EXPERIENCE

Problems in the Design of Epidemiologic Studies: Baltimore, MD
Proposal Development and Critique Teaching Assistant February 2020-May 2020;
Johns Hopkins University February 2021-present

- Single teaching assistant for second-year doctoral student course to develop and complete their dissertation proposals and conduct mock NIH study sections
- Integral in moving course coordination completely online during the COVID-19 pandemic
- Gave lecture on the scientific grant review process including scoring and study section
- Meets with students one-on-one to refine written work

Epidemiologic Methods (753) Teaching Assistant Baltimore, MD
Johns Hopkins University January 2019-March 2019

- Lead laboratory sessions twice per week for approximately 40 students to practice and expand on concepts learned in class and held office hours

Epidemiologic Methods (752) Teaching Assistant Baltimore, MD
Johns Hopkins University November 2018-Decemeber 2018

- Lead laboratory sessions twice per week for approximately 40 students to practice and expand on concepts learned in class and held office hours

Writing in the Disciplines Graduate Teaching Assistant Washington, DC
George Washington University January 2015-May 2015

- Guest lectured on how to create budgets in the context of grant writing
- Graded written assignments throughout the semester
- Advised students individually on final projects for the Public Health Senior Seminar course

Graduate Teaching Assistant for the Use of Statistical Packages Washington, DC
for Data Management and Data Analysis August 2014-December 2014
George Washington University

- Conducted weekly laboratory sessions for 15-20 graduate-level students to provide guidance on data analyses focused on interpretation of output and programming proficiency
- Graded homework assignments and exams

ACADEMIC SERVICE

Working Group Member *June 2019-present*
Epi-IDEAS

Department of Epidemiology
Bloomberg School of Public Health Johns Hopkins University

Co-President *June 2019-May 2020*
Epidemiology Student Organization
Department of Epidemiology
Bloomberg School of Public Health Johns Hopkins University

Epidemiological and Statistical Methods for Epidemiologists Journal Club *August 2019-May 2020*
Journal Club Coordinator
Department of Epidemiology
Bloomberg School of Public Health Johns Hopkins University

Student Chair *August 2018-June 2019*
Admissions and Credentials Committee
Department of Epidemiology
Bloomberg School of Public Health Johns Hopkins University

General Epidemiology & Methodology Track *August 2018-June 2019*
Journal Club Coordinator
Department of Epidemiology
Bloomberg School of Public Health Johns Hopkins University

PROFESSIONAL AFFILIATIONS

Society for Epidemiologic Research
2018-present

PRESENTATIONS

Poster Presentations

Coburn SB, Silverberg MJ, Rabkin CS, Castilho JL, Horberg MA, Klein MB, Gill MJ, Napravnik S, Lee J, Shiels M, Crane H, Althog KN, Lau B, for the the NA-ACCORD of IeDEA. “Cumulative viral load on antiretroviral therapy and breast cancer risk among women with HIV in North America)”. International AIDS Society Conference on HIV Science, 2021 (virtual).

Coburn SB, Silverberg MJ, Moore RD, Mathews WC, Marcus JL, Castilho JL, Kitahata MM, Crane HM, Klein M, Napravnik S, Brown TT, Visvanathan K, Lau B, Althoff KN, for the NA-ACCORD of IeDEA. “Breast Cancer Risk Among Women with HIV in North America (2000-2015)”. Conference on Retroviruses and Opportunistic Infections, 2020 (virtual).

Coburn SB, Brown TT, Moore RD, Silverberg MJ, Horberg MA, Marcus JL, Castilho JL, Visvanathan K, MJ Gill, Kitahata MM, Mayor AM, Lau B, Althoff KN, for the NA-ACCORD of IeDEA. “Cumulative Incidence of Breast Cancer Among Women with HIV in North America from 2000-2015”. International Conference on Malignancies in HIV/AIDS, 2020 (Bethesda, MD).

Invited Presentations (not for publication)

MACS/WIHS Combined Cohort Study 2021 Annual Meeting, Early-Stage Investigator Presentation, May 13th, 2021 (virtual). "Cumulative HIV viremia and breast cancer risk in women with HIV"

- Included practice presentations with MACS/WIHS faculty and other early stage investigators

International epidemiology Databases to Evaluate AIDS 2021 Global Annual Meeting, Early-Stage Investigator Presentation, April 7th, 2021 (virtual). "Underutilization of statins in adults with HIV: The treatment gap and predictors of statin initiation"

Harvard HIV Working Group: February 19th, 2021, Harvard University (virtual). "Cumulative viral load on ART and breast cancer risk among women with HIV"

HIV Aging Mentored-Research Group Meeting: October 14th, 2019, Johns Hopkins University (Baltimore, MD). "What is the pathogenesis of breast cancer in the context of HIV?"

PUBLICATIONS

First author publications

Coburn SB, Shiels MS, Silverberg MJ, Horberg MA, Gill MJ, Brown TT, Visvanathan K, Connor AE, Napravnik S, Marcus JL, Moore RD, Mathews WC, Mayor AM, Sterling TR, Li J, Rabkin CS, D'Souza G, Lau B, Althoff KN; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of the International Epidemiology Databases to Evaluate AIDS. Secular Trends in Breast Cancer Risk Among Women With HIV Initiating ART in North America. J Acquir Immune Defic Syndr. 2021 May 1;87(1):663-670. doi: 10.1097/QAI.0000000000002627. PMID: 33492023; PMCID: PMC8026587.

Coburn SB, Stanczyk F, Falk RT, McGlynn KA, Brinton LA, Sampson J, Bradwin J, Xia X, Trabert B. Comparability of serum, plasma, and urinary estrogen and estrogen metabolite measurements by sex and menopausal status. Cancer Causes Control. 2019 Jan;30(1):75-86. doi: 10.1007/s10552-018-1105-1.

Coburn SB, Graubard BI, Trabert B, McGlynn KA, Cook MB. Associations between circulating sex steroid hormones and leukocyte telomere length in men in the National Health and Nutrition Examination Survey. *Andrology*. 2018 May 11. doi: 10.1111/andr.12494.

Coburn SB, Sherman ME, Bray F, Trabert B. International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. *Int J Cancer*. 2017 Jun 1;140(11):2451-2460. doi: 10.1002/ijc.30676.

Additional publications

Althoff KN, Laeyendecker O, Li R, **Coburn SB**, Klock E, Baker OR, Quinn TC, Michael J, Shields WC, Ehsani J, Thomas FD, Graham LA, Ali Z, Manabe YC, Li L. Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Status in Decedents Undergoing Forensic Postmortem Examination in Maryland, May 24 to June 30, 2020. Open

Forum Infect Dis. 2020 Dec 15;8(1):ofaa611. doi: 10.1093/ofid/ofaa611. PMID: 33506069; PMCID: PMC7798730.

Trabert B, **Coburn SB**, Falk RT, Manson JE, Brinton LA, Gass ML, Kuller LH, Rohan TE, Pfeiffer RM, Qi L, Stefanick ML, Wentzensen N, Anderson GL, Xu X. Circulating estrogens and postmenopausal ovarian and endometrial cancer risk among current hormone users in the Women's Health Initiative Observational Study. Cancer Causes Control. 2019 Nov;30(11):1201-1211. doi: 10.1007/s10552-019-01233-8. Epub 2019 Sep 21.

Falk RT, Manson JE, Barnabei VM, Anderson GL, Brinton LA, Rohan TE, Cauley JA, Chen C, **Coburn SB**, Pfeiffer RM, Reding KW, Sarto GE, Wentzensen N, Chlebowski RT, Xu X, Trabert B. Estrogen metabolism in menopausal hormone users in the Women's Health Initiative Observational Study: Does it differ between estrogen plus progestin and estrogen alone? Int J Cancer. 2018 Sep 5. doi: 10.1002/ijc.31851.

Li X, Kleeman S, **Coburn SB**, Fumagalli C, Perner J, Jammula S, Pfeiffer RM, Orzolek L, Hao H, Taylor PR, Miremadi A, Galeano-Dalmau N, Lao-Sirieix P, Tennyson M, MacRae S, Cook MB, Fitzgerald RC. Selection and application of tissue microRNAs for non-endoscopic diagnosis of Barrett's Esophagus. *Gastroenterology*. 2018 Jun 12. pii: S0016-5085(18)34643-2. doi: 10.1053/j.gastro.2018.05.050.

Trabert B, Waterboer T, Idahl A, Brenner N, Brinton LA, Butt J, **Coburn SB**, Hartge P, Hufnagel K, Inturrisi F, Lissowska J, Mentzer A, Peplonska B, Sherman ME, Wills GS, Woodhall SC, Pawlita M, Wentzensen N; Antibodies against chlamydia trachomatis and ovarian cancer risk in two independent populations. *J Natl Cancer Inst*. 2018 May 21. doi: 10.1093/jnci/djy084.

Trabert B, **Coburn SB**, Mariani A, Yang HP, Rosenberg PS, Gierach GL, Wentzensen N, Cronin KA, Sherman ME. Reported incidence and survival of fallopian tube carcinomas: a population-based analysis from the North American Association of Central Cancer Registries. *J Natl Cancer Inst*. 2017 Dec 21. doi: 10.1093/jnci/djx263.

Playdon MC, **Coburn SB**, Moore SC, Brinton LA, Wentzensen N, Anderson G, Wallace R, Falk RT, Pfeiffer R, Xu X, Trabert B. Alcohol and oestrogen metabolites in postmenopausal women in the Women's Health Initiative Observational Study. *Br J Cancer.* 2018 Feb 6;118(3):448-457. doi: 10.1038/bjc.2017.419. Epub 2017 Dec 12.

Oh H, Arem H, Matthews CE, Wentzensen N, Reding KW, Brinton LA, Anderson GL, **Coburn SB**, Cauley JA, Chen C, Goodman D, Pfeiffer RM, Falk RT, Xu X, Trabert B. Sitting, physical activity, and serum oestrogen metabolism in postmenopausal women: the Women's Health Initiative Observational Study. *Br J Cancer*. 2017 Sep 26;117(7):1070-1078. doi: 10.1038/bjc.2017.268. Epub 2017 Aug 17.

Zhou CK, Young D, Yeboah ED, **Coburn SB**, Tettey Y, Biritwum RB, Adjei AA, Tay E, Niwa S, Truelove A, Welsh J, Mensah JE, Hoover RN, Setherhenn IA, Hsing AW, Srivastava S, Cook MB. ERG expression in prostate cancer of West African men and a meta-analysis of racial differences in *TMPRSS2-ERG* gene fusions. *Am J Epidemiol*. 2017 Jun 12. doi: 10.1093/aje/kwx235.

Oh H, **Coburn SB**, Matthews CE, Falk RT, LeBlanc ES, Wactawski-Wende J, Sampson J, Pfeiffer R, Brinton LA, Wentzensen N, Anderson GL, Manson JE, Chen C, Zaslavsky O, Xia X, Trabert B. Anthropometric measures and serum estrogen metabolism in postmenopausal women: the Women's Health Initiative Observational Study. *Breast Cancer Res*. 2017 Mar 11;19(1):28. doi: 10.1186/s13058-017-0810-0.

Cook MB, **Coburn SB**, Lam JR, Taylor PR, Schneider J, Corley DA. Cancer incidence and mortality risks in a large US Barrett's Esophagus cohort. *Gut*. 2017 Jan 4. pii: gutjnl-2016-312223. doi: 10.1136/gutjnl-2016-312223.

Woelk GB, Ndatimana D, **Behan S**, Mukaminega M, Nyirabahizi E, Hoffman HJ, Mugwaneza P, Ribakare M, Amzel A, Phelps BR. Retention of mothers and infants in the prevention of mother-to-child transmission of HIV programme is associated with individual and facility-level factors in Rwanda. *J Int AIDS Soc*. 2016 Jul 20;19(5 Suppl 4):20837. doi: 10.7448/IAS.19.5.20837. eCollection 2016.

Non-peer reviewed, commentary, and editorials

Althoff KN, **Coburn SB**, Nash D. Contact tracing: Essential to the public health response and our understanding of the epidemiology of COVID-19 [published online ahead of print, 2020 Jun 11]. Clin Infect Dis. 2020;ciaa757. doi:10.1093/cid/ciaa757

Zhang M, Jarrett BA, Althoff KN, Burman FS, Camarata L, **Coburn SB**, Dickerson AS, Foti K, Kaur M, Leifheit KM, Malone J, Moore EA, Mouslim MC, Menezes NP, Robsky K, Tang O, Wallace AS, Dean LT. Recommendations for the Society for Epidemiologic Research to further promote diversity and inclusion at the annual meeting and beyond, *Am J Epi.* kwaa100, doi.org/10.1093/aje/kwaa110