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Clinical Risk Assessment Tools for the Characterization of Early Warning Indicators of HIV Virologic Failure on First-Line Antiretroviral Therapy in an Urban Clinic in KwaZulu-Natal, South Africa

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Abstract

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By Maneesha Chitanvis

Objective:

HIV virologic failure (VF), the inability achieve or maintain suppression of viral replication, is associated with increased morbidity, early mortality, and has been causally-linked to the acquisition of HIV drug resistance. In addition to institutional and community-level influences, there are a number of individual-level risk factors associated with a patient's likelihood of failing virologically. Here, a series of risk indices were developed in order to provide a practical risk characterization tool, implementable in clinical settings.

Design and Methods:

Findings from the Risk Factors for Virologic Failure (RFVF) study conducted in an urban clinic in KwaZulu-Natal, South Africa were utilized to develop indices characterizing individual risk of VF at initiation of ART as well as after at least five months on treatment. Indices were based on multivariate models where statistically significant demographic, socioeconomic, psychosocial, and clinical/laboratory-related predictors of VF were identified. Baseline, RFVF (full), and Restricted RFVF (parsimonious) Indices were comprised of point values derived from model output. Assessments were conducted of the fit statistics and predictive discrimination of the models from which the point-based indices were derived as well as of models from which index-derived patient scores were the sole predictors of VF.

Results:

Patients' risk of VF was characterized as a "score" expressed as a percentage of the total theoretical maximum risk of VF for each index. Model assessments revealed that both the RFVF and Restricted Indices provided more robust predictions of VF as compared to the Baseline Index. Univariate analysis of risk scores also revealed that while both the Restricted and RFVF Indices outperformed the Baseline Index, the Restricted Index was comparable to the RFVF (ROC curve AUC of 0.848 versus 0.847, respectively).

Conclusions:

The Baseline Index provides a means for characterizing an individual's risk of VF based on measurable factors present at ART initiation. The Restricted RFVF Index provides risk characterization with equal utility to that of the full, RFVF Index but with a limited, and therefore more feasible, number of predictors. These prognostic indices and their methodologic derivations may serve as useful foundational work for the application and implementation of such rapid, risk quantification tools in future research and clinical care settings.

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Table of Contents

Glossary of Terms

AIC	Akaike Information Criterion
ART	Antiretroviral Therapy
ARV	Antiretroviral drugs
CD4	CD4 (cluster designation 4) + T-lymphocyte cell
CRF	Case Report Form
DOH	Department of Health
EWI	Early Warning Indicators
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
HIVDR	HIV- Drug Resistance
KZN	KwaZulu-Natal, South Africa
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor-Based (ART regimen)
PEPFAR	(United States) President's Emergency Plan for AIDS Relief
RFVF	Risk Factors for Virologic Failure
ROC, AUC	Receiver Operator Characteristic curve, Area Under the Curve
VACS	Veteran's Aging Cohort Study (index)
VF	Virologic Failure
VL	Viral Load

Chapter I: Background and Literature Review

Introduction

With an estimated 36.9 million infected individuals worldwide by the end of 2014, human immunodeficiency virus/acquired-immune deficiency syndrome (HIV/AIDS) persists globally as a leading cause of morbidity and mortality. Nearly 1.2 million people died from AIDS-related illnesses in just 2014 [1]. Sub-Saharan Africa has the highest global burden with over 25.8 million people infected with HIV, while South Africa alone was attributed with approximately 18% of its population, or 6.8 million, HIV-positive individuals in 2014 [1, 2]. According to the 2012 South African National HIV Prevalence, Incidence and Behavior Survey, the province of KwaZulu-Natal, located on the southeastern coast of South Africa, has for the last decade retained the highest proportion of any province in the nation with a prevalence of approximately 16.9% [3]. While efforts have improved to increase the proportion of individuals who know their HIV status and are subsequently initiated and retained in antiretroviral treatment programs, the disproportionate burden of HIV in this region remains a pressing public health concern [3].

Historical Context of the HIV Epidemic in South Africa

The national response to the initial outbreak of HIV in South Africa was illustrative of the impact of political influence and the extent to which social stigmatization contributed to detrimental health outcomes for both infected and at-risk populations [4]. Following the trends witnessed in other African countries in the late 1980s, the prevalence of cases in South Africa had begun to reach epidemic proportions

by the early 1990s [5]. The South African Department of Health published results from an antenatal survey of women attending health clinics that reported approximately 320,000 individuals were infected with HIV by 1993—a 60% increase in incidence since 1990 [6]. Despite this massive and growing disease burden, the initial governmental response was minimal. With the end of apartheid in 1994, national attention was diverted from public health issues. Political focus was fixed on efforts to restructure civil society in an attempt to address and rectify the institutionalized gender and race-associated inequalities that characterized the nation's recent past [6].

The predominant political party, the African National Congress (ANC), established the National Advisory Group in 1994 and developed the National AIDS Plan which detailed preventive interventions. Its scope, however, was extremely limited by minimal available information and proved ineffective at reducing disease incidence. As a result of lack of effective interventions, South Africa experienced a seemingly inexorable rise in new cases of HIV [5]. The national prevalence of HIV rapidly ascended from 4% to 22.8% in just the post-apartheid period between 1994 and 1998 [6]. Furthermore, of the nearly 3 million HIV-positive individuals thin South Africa in 1998, over 700,000 of were infected in just 1997 [5].

As the epidemic progressed and intensified in the mid-to late-1990s, the lack of action taken by the South African national government played an increasingly important role. Perpetuating the negative impact of HIV was the atmosphere of denialism personified by then President Thabo Mbeki's administration, as it publicly refuted even the most rudimentary medical evidence—such as that identifying HIV as the causative agent of AIDS. The increase in contentious discourse surrounding HIV mirrored the

persistent incline in incident cases into the late-1990s, despite the growing availability of evidence-based, effective interventions. This political climate created an unstable environment within which it proved difficult to promote, effectively implement, and maintain meaningful advances towards combating HIV [7].

Circulating misconceptions within the general population regarding the nature of HIV causes, risk factors, and transmission only served to further facilitate underestimations of the true threat of HIV and contributed to the increasing stigmatization of the disease [7]. Furthermore, the shifting national political dynamics of the post-apartheid period contributed to the inability of the public to gain widespread access to antiretroviral drugs including: nucleoside analog reverse-transcriptase inhibitor—Zidovudine (ZDV), or azidothymidine (AZT), and non-nucleoside reverse transcriptase inhibitor (NNRTI)—Nevirapine (NVP) [8]**.** A 2008 study modeled projections estimating over 330,000 lives were lost due to the inability to provide readily accessible antiretroviral therapy (ART) for vulnerable populations in South Africa during the first decade of the epidemic [9]. Thus, both the international and regional scientific communities were at odds with the socio-political sphere of South Africa.

However, in a response to persistent pressures from the international medical community and domestic civilian dissidents, the South African national government eventually initiated a formal rollout of ART programs and established the Joint Health and Treasury Task Team in 2002 [6]. This was meant to significantly and meaningfully expand provisions of HIV treatment and prevention—specifically related to the prevention of mother-to-child transmission and post-exposure prophylaxis. Ushering in a new era of diligent and resolved commitment towards mitigating acquisition,

transmission, and HIV-associated morbidity and mortality, The United States President's Emergency Plan for AIDS Relief (PEPFAR) was rolled out in 2003. The initiation of PEPFAR funding in South Africa helped enable large-scale rollouts of both treatment and preventive services—significantly increasing access to treatment for large proportions of vulnerable populations [10]. Supported by the World Bank, the Global Fund, the Clinton Foundation and a number of other international partners, the South African government had set forth the Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa by the conclusion of 2003 [6]. To further combat the epidemic, South Africa launched their HIV/ADS and Sexually Transmitted Infection Strategic Plan in 2007.

As reiterated by Minister of Health Dr. Aaron Motsoaledi in his 2009 speech to South Africa's Parliament, the nation had spent the previous 10 years "pedaling backwards" [6]. He urged the continued sourcing of evidence-based, scientific information to inform national best practices in order for the country to keep moving forward from its fractious past [6]. The explicit as well as implicit denial that previously resulted in a refusal to meaningfully respond to the HIV epidemic had diminished significantly by the mid-2010s. In December of 2007, approximately 424,000 individuals were receiving ART in South Africa, and by 2015, there were over 3.1 million [8, 11]. The Deputy President and Chair of the South African National AIDS Council Kgalema Motlanthe emphatically expressed the need for sustainable approaches in 2012 stating, "Fundamentally, we must endeavor to change the perception of viewing our response as an emergency that needs to be controlled and managed to positioning this response as an investment in the health of our people and our new democracy" [12].

Since the official initiation of HIV prevention and ART programs, a multitude of indicators pointed to the improved health in the region. By 2012, there was a 4.2 year increase in life expectancy, a 25% decrease in child and infant mortality rates, significant declines in maternal mortality, and marked decreases in HIV transmission rates. From 2003 to 2014, South there was a 48% reduction in deaths, 43% of people known to be living with HIV were enrolled in treatment, and South Africa boasted its lowest incidence rate since the official declaration of the epidemic in 1992 [6].

Despite this progress in apparent number of individuals receiving care, South Africa still has the highest number of patients on ART compared to any other nation in the world [6]. Furthermore, a critical gap persists beyond this. Patients receiving antiretrovirals are still dramatically impacted by HIV-induced immunodeficiency and the associated comorbidities. This has been attributed to a multitude of factors contributing to their inability to achieve virologic suppression, defined as the inability to limit the reproduction of the virus to immunologically manageable levels [13]. Individuals who enter and intend to remain on therapy face individual, interpersonal as well as institutional and structural barriers that prevent them from retaining adequate adherence to prescribed treatment regimens [14]. This points to the need for continued diligence regarding not only prevention of acquisition and transmission, but adequate case-management of HIV-positive individuals as well. The challenges that remain are particularly apparent in the context of hyperendemic areas such as KwaZulu-Natal [15].

HIV in KwaZulu-Natal, South Africa

KwaZulu-Natal (KZN) is one of nine provinces in South Africa and is formally composed of 11 districts [16]. The settings surrounding its largest city, Durban, can be categorized into two distinct districts: eThekwini (the metropolitan area of Durban) and the district of uMkhanyakude, which encompasses the vast majority of the surrounding peri-urban and rural areas of the province [16].

Given the heterogeneity of the HIV epidemic within South Africa, regional statistics highlight the differences in HIV prevalence among geographic regions of the nation. Since 2005, KZN has led the nation with the highest prevalence of HIV infections at approximately 40% among women in pre-natal screening surveys [17]. Furthermore, the eThekwini metropolitan area has the highest HIV prevalence of any municipality at approximately 14.5%, with the prevalence among 15 to 19 year old pregnant women at approximately 35% [18]. Attributed as the epicenter of the HIV epidemic in South Africa, there are a number of factors reported to have fueled the disproportionate burden of HIV occurring in KZN, specifically. A 2003 report by Lurie et al. contends that the large proportion of migrant laborers commuting from their residence in rural areas, to urban areas where they stay for varying periods of time for work significantly contributed to bridging the populations in these two regions [19]. More explicitly, it was suggested that the high number of migrant workers with multiple, concurrent sexual partners in different communities significantly contributed to the spread of HIV [19]. Essuon et al. makes note that the re-entry of transient populations into communities significantly increased HIV transmissions because these individuals were also more likely to engage in risky behaviors such as injection drug use, substance abuse, and lack of condom use

during sexual encounters [20]. The unique sexual behavior patterns that characterize a large proportion of migrant workers in the region had been identified as intensifying the spread of HIV by linking transmission between high and low risk populations [20].

KZN HIV Epidemiology by Race, Gender, and Age

The Black South African population constitutes approximately 80% of the national population. Stratified demographic analysis revealed an overwhelmingly higher prevalence among Black South Africans as compared to other races. Blacks ages 20 to 34 have the highest incidence rate (over 15%) as compared with other racial groups and also make up over 30% of the proportion of those living with HIV on ART nationwide. This disparity is, in part, thought to be attributed to more unstable living conditions. More than 85% of those living in informal urban settlements are Black [3]. Among other structural disadvantages, those in informal settlements have limited financial resources, less access to reliable health care facilities, and suboptimal quality of housing and overall living conditions. Additionally, individuals in these areas are often subjected to high crime rates, which predispose them to violence-related injuries and drug use—risk factors for contracting HIV [21]. HIV prevalence remains notably highest in under-resourced, predominantly Black areas in both urban and rural informal settlements in KwaZulu-Natal [3].

A growing body of literature also points to the gender differences in HIV trends in the region. While males over 50 years old have a notably higher prevalence as compared to females of the same age group, the South African Nation HIV Prevalence, Incidence

and Behavior Survey 2012 found that, overall, there is a higher prevalence of HIV among South African women (14.4%) as compared to men (9.9%) [3]. A 2012 study led by the Centre for the AIDS Programme of Research in South Africa (CAPRISA) sought to explore the HIV incidence of adolescent females in KwaZulu-Natal, specifically, and reported that the HIV infection rate in females ages 15-24 years old was 3-6 times higher than that of their male counterparts [22]. 2012 national survey results report a statistically significant difference of an 11.4% prevalence in females compared to a 2.9% prevalence in males among youths ages 15-24. These statistics are of critical importance as 2010 HIV incidence rates in South Africa show that greater than 60% of all HIV positive adults become infected before the age of 25 [3]. This holds true when looking specifically at KwaZulu-Natal where reports have estimated prevalence ranges of 36% in women between the ages of 30-34, 40.7% prevalence among females ages 15-49, and 52% among women ages 18-50, —prevalences higher than the national estimates [3, 23].

Furthermore, similar demographic trends are seen among females when analyzing geographic differences between populations. A 2010 prospective cohort study conducted by Karim et al. sought to determine the incidence rates of HIV among both urban and rural women in KwaZulu-Natal, as they experience higher HIV prevalence than their male counterparts [24]. The study reported that HIV prevalence was highest in urban women less than 20 years old (17.2/100 person-years) as well as in rural women over the age of 20 (10.2/100 person-years) [22].

The hyperendemic nature of the HIV epidemic and its disproportionate effect on certain sub-populations in KwaZulu-Natal highlights the necessity for sustained, adequate, preventions as well as case-management strategies. As both financial and

human capital resources remain limited in the region, identifying specific at-risk populations is of paramount importance for tailoring targeted approaches.

At Risk Populations

The South African National AIDS Council's National Strategic Plan on HIV, Sexually Transmitted Infections and Tuberculosis 2012-2016 detailed a five-year strategy to combat the challenges posed by the dual burden of both tuberculosis and HIV with the hopes of outlining a clear long-term goal of zero new infections. This report identified key affected populations. Those who are considered at-risk and highly vulnerable to HIV exposure, infection, and transmission included: men who have sex with men (MSM), women between the ages of 15-24 years, road/highway workers, people living in informal settlements, migrant populations, adolescents not attending school, people with physical and mental disabilities, sex workers, substance and injection drug users, transgender persons, and orphans and vulnerable children [12].

A 2015 geospatial cluster analysis of risk factors for HIV infection in KwaZulu-Natal utilized clinical trial data from two medical units (Umkomaas and Botha's Hill, KZN). Geoadditive models investigating the spatial characteristics of the study population focused on a cohort of women enrolled in a preventive biomedical HIV intervention trial. This study reiterated findings of previous literature noting that there were a number of significant HIV infection risk factors, including low levels of education, early sexual debut age, multiple sexual partners, exchanging sexual acts for

monetary or other incentives, and being unmarried while cohabitating with a sexual partner [25].

Understanding the risk factors associated with the relative likelihood of becoming infected with HIV is critical to informing preventive interventions. Case management of infected individuals, however, is another essential component of reducing the impact of HIV-associated morbidities and mortality [26]. Enrollment and adherence to established treatment guidelines is among the most important elements to significantly reducing the impact of HIV in this region.

Global Treatment Guidelines

Treatment guidelines and antiretroviral therapy (ART) prescription protocols have progressed significantly since the initiation of formal ART programs in South Africa. According to 2010 World Health Organization (WHO) guidelines, initiation of ART in HIV-positive adults, including all pregnant women as well as adolescents, was to begin with a confirmed diagnosis of infection and a CD4+ T-lymphocyte cell (CD4) count of less than 350 cells/mm^3 [27]. This was recommended despite the presence or absence of HIV-related clinical symptoms categorized as stage 1 (asymptomatic) or 2 (mild symptoms). However, these guidelines also stipulate that ART should be initiated irrespective of CD4 count for cases in which presentation of illness is classified as clinical stage 3 (advanced symptoms) or 4 (severe symptoms), as clinical events generally reflected immune deterioration . These recommendations reflect the discretionary advice of WHO to minimize HIV-associated mortality, mitigate the risk of

transmission, and minimize the financial burden imposed upon national health systems [27].

In 2013, WHO guidelines recommended that all individuals living with HIV, regardless of clinical stage, with CD4 counts at or below 500 cells/mm^3 be initiated on lifelong ART [28]. These policy guidelines relied upon evidence-based findings suggesting that relaxing the threshold for CD4 cell count could markedly reduce the risk of developing AIDS-defining comorbidities. Furthermore, these guidelines included recommendations that ART be initiated, regardless of clinical stage or CD4 count for individuals coinfected with hepatitis virus, diagnosed with TB disease, who are pregnant and/or breastfeeding, and or HIV negative with seropositive sexual partners. WHO contended that revising the threshold and altering ART initiation criteria could potentially avoid the deaths of approximately 21 million individuals as well as prevent 28 million new infections globally [28].

WHO ART and Pre-exposure Prophylaxis Guidelines were revised in September 2015 to include superseding recommendations that stipulate any adult (older than 19 years of age) who is HIV-seropositive should receive ART regardless of CD4 cell count and clinical stage diagnosis in order to further limit the clinical manifestation of disease [29].

South African National Department of Health (DOH) guidelines, as of November 2015, were compliant with WHO guidelines indicating that any adult who tests positive for HIV, regardless of CD4 count, should be initiated on an ART regimen. These guidelines also include the initiation of treatment for all individuals diagnosed with tuberculosis, pregnant and breastfeeding HIV-positive women, and/or positive for

hepatitis B co-infection regardless of CD4 count. These recommendations were made in order to ensure that national guidelines reflect the absolute benefit observed when ART is initiated at higher CD4 counts [30].

The potential positive impact of establishing safe and effective treatment protocols was reflected in the documented success of the Treatment 15 Initiative of the Joint United Nations Program on HIV and AIDS (UNAIDS). The "15 by 15" initiative describes the target of providing ART to 15 million individuals globally by 2015. The achievement of this target represents one of the first documented proof that a global health goal was achieved prior to the projected deadline [31]. However, the limited scope of this initiative also illuminates the need for more comprehensive global public health goals that account for not only treatment coverage, but also retention in care with consistent monitoring of treatment efficacy [32].

WHO in partnership with numerous United Nations agencies and national governments, including South Africa, has adopted the ambitious 90-90-90 Goals [33]. These guidelines enumerate that by 2020, 90% of all individuals living with HIV will be diagnosed as such, 90% of those diagnosed will be enrolled and retained on ART, and 90% of all those on therapy will remain virally suppressed (levels of HIV RNA lower than the assay's limit of detection). The overarching goal of such targets is to end the AIDS epidemic—to eliminate the progression of life-threatening secondary infections—in immunosuppressed, HIV-positive individuals by 2030 [33]. Estimates suggest that if these goals are to be achieved by 2030, nearly 73% of those living with HIV globally must be virally suppressed, and as a result, will be able to re-establish and maintain immune function within a normal range [34]. Identifying and acknowledging the risks

and associated consequences of failing on treatment is critical to assessing the feasibility of achieving such goals [34].

Virologic Failure

The maintenance of an undetectable viral load is essential to significantly decreasing an individual's HIV-associated adverse health outcomes. By reducing viral loads to an immunologically manageable level, ART has the potential to significantly decrease HIV-associated comorbidities and mortality by normalizing immune system functionality [35, 36]

As of 2015, the preferred first line regimens, the South African DOH's guidelines for first line therapy include four fixed-dose combinations of antiretrovirals:

- Abacavir (ABC) 600mg + Lamividine (3TC) 300mg
- Tenofovir (TDF) 300mg + Emtricitabine (FTC) 200mg
- Tenofovir (TDF) 300mg + Emtricitabine (FTC) 200mg + Efavirenz (EFV) 600mg
- Zidovudine (AZT) 300mg + Lamivudine (3TC) 150mg

South Africa has relied on TDF + 3TC (or FTC) + EFV as the preferred regimen, unless there is contraindication, based on the patient's clinical profile, to one or more of the drugs [37].

However, when a patient is unable to remain virally suppressed while on ART and HIV RNA replication rebounds to detectable levels, virologic failure (VF) occurs. Virologic failure is defined as the inability to maintain virologic suppression and viral replication persists. The 2010 WHO criteria for virologic failure, defined as plasma viral

levels above 1000 copies/mL after at least six months of treatment, was reflected by *immunologic failure*—CD4 counts below baseline (or below 100 cells/mm^3), and *clinical failure*—the occurrence or persistence of WHO stage 4 clinical events after six months of antiretroviral treatment [38].

A systematic review of 25 studies on HIV positive adults performed by Rutherford et al. examined the adequacy of the aforementioned indicators to predict virologic failure. Their assessment revealed that these clinical and immunologic criteria may be inadequate to characterize virologic status, as they are insufficiently sensitive and have a low positive predictive value for identifying VF [39]. These findings were utilized to inform WHO guideline revisions. As of 2013, national recommendations call for routine plasma viral load monitoring to detect virologic failure and to serve as the gold standard for guiding the determination of the need for drug regimen changes from first-line to second-line therapy, after adherence to first-line regimens has been confirmed. Recommended laboratory monitoring for ART efficacy from the Southern African HIV Clinicians Society entails viral load testing at baseline, one to two months after treatment initiation, and thereafter every six months [40, 41].

A 2012 meta-analysis assessed 5,812 studies reporting data from resource-limited settings around the world, including five studies conducted in South Africa, concluded that the proportion of adults who fail on first-line therapy ranges from 21.8% to 38.0% [42]. 2013 District Health Information System (DHIS) reports that of the 37% of ART patients who had viral load testing completed, 80% were virally suppressed—illustrating the 20% proportion that is potentially and presumably failing on treatment. Highlighting the importance of retaining patients on first-line therapy, findings from a systematic

review of 19 studies on virologic failure estimated that the 15% prevalence of virologic failure in first-line ART was significantly less than the prevalence of virologic failure seen in second-line users [43]. Retaining patients on first-line therapy is of particular concern in regions of high endemicity, such as KZN, where the prevalence of virologic failure for those on more expensive, more toxic second-line therapy has been measured to range from 13% to over 60% in certain cohorts [44, 45].

The South African National Department of Health's (DOH) recommendations state that after the initiation of ART, routine viral load testing should be completed first at six months, and then every 12 months, subsequently [46]. According to the 2015 national guidelines, it is not recommended that a patient be switched from first- to second-line therapy unless a viral load greater than 1000 copies/mL is measured. In this scenario, patients are to be assessed for their adherence and compliance, drug tolerability and drug-to-drug interactions, as well as psychologically assessed. Repeat viral loads should be done two months following the initial high viral load reading, and if similar levels persist, it is recommended individuals be switched to second-line treatment [46].

Viral Load Monitoring in KZN

The National DOH reported data from health systems throughout the country in an endeavor to capture viral load monitoring capabilities as measured through the reporting of patient information to the National Health Laboratory Services (NHLS) laboratory database. The Analysis of Big Data for Better Targeting of ART Adherence Strategies sought to explore health systems-level tracking of patients' viral loads to better understand the overall epidemiology of viral suppression at the national, provincial,

district, and sub-district levels. Provincial level analysis revealed that of the over 600,000 patients with VL testing completed in the 12-month period prior to record collection, KwaZulu-Natal has one of the highest proportions of virologic suppression with 82% with viral loads less than 400 copies/ML. Furthermore, of the nearly 300,000 patients on ART in the eThekwini metropolitan area (encompassing the largest city, Durban), 86% of patients were virally suppressed, with VL less than 400 copies/mL. However, only 59% of these patients actually know of their suppression status. Furthermore, while these results seem to indicate that certain health clinics, particularly in urban KwaZulu-Natal, may be on track to reach the national goal of 90% viral load suppression among HIV patients enrolled in ART, there are still critical gaps in complete, routine viral load monitoring and the communication of virologic status as well as its significance by clinicians to the patients themselves. According to NHLS reports, there are still nearly 100,000 patients (15%) in KwaZulu-Natal with VL greater than 1000 copies/mL [47].

These gaps are further substantiated in research studies conducted in resource-constrained settings, where routine viral load monitoring is often intermittent at best [48]. A study published in 2014 described the findings from modeling 31,450 patients from seven cohorts of patients on ART in South Africa. The study aimed to describe clinical/CD4-based indicators to serve as optimal cutoffs for targeted viral load testing. CD4-based risk charts developed here were intended to guide virologic testing in order to monitor ART in a targeted way in settings in which routine VL testing is not possible. The results enabled researchers to develop and validate a CD4 count-based risk chart to inform targeted viral load testing [49]. Rawizza et al. sought to determine the efficacy of

using CD4-based monitoring as a proxy for VL-based strategies for switching therapy. Study results demonstrate that three-year rates of clinical failure and loss of treatment options were not significantly different between CD4 and VL monitoring. Although study limitations do allude to the inability to measure the longer-term consequences of CD4 monitoring [50].

Despite its perceived benefit in resource-constrained settings where means to utilize laboratory-based methods are unavailable, there are still a number of studies that point to the insufficiency as well as potential danger of using CD4 cell counts and clinical monitoring as proxies for VL monitoring. A 2011 study conducted in Nigeria examined 9,690 patients for a median of 33.2 months and illustrated the inability to rely on immunologic criteria. Results demonstrated the low sensitivity of immunologic criteria based on the large proportion of virologic failures missed and the potential for this to manifest into a large number of patients with acquired resistance as well as poor health outcomes. Furthermore, this study indicated that this could result in increased costs to health care systems linked to the likelihood of the increased number of individuals who will need to switch to more expensive, second-line ART as a results of the accumulation of acquired drug resistant mutations [51].

Results previously noted in the NHLS district and subdistrict analysis reveal the gap in viral load testing that still remains. This has proven to be particularly important for determining when patients need to be switched from first- to second-line therapy. Furthermore, a retrospective cohort study published in February 2016 detailed findings after monitoring CD4 counts of patients in two HIV programs in South Africa. Results indicate that utilizing CD4 counts alone may lead to delayed revisions of therapy for

patients, especially those with drug resistance [52]. As such, the recommended gold standard for preventing drug resistance remains routine viral load testing [53].

The necessity of timely revisions of therapy was further emphasized by Petersen et al. in their 2014 study. Assessing the health outcomes of patients from four cohorts in both Uganda and South Africa, telling evidence revealed the impact that delayed switching of therapy has on mortality among patients with virologic failure. The study examined 823 patients with laboratory-confirmed virologic failure on first-line NNRT-based therapy regimens and modeled the estimated impact of delayed switching to second-line drug regimen on mortality. After adjusting for CD4 count and HIV viral RNA levels, mortality was found to be higher (odds ratio of 2.1 [95% confidence interval 0.99-5.8]) among individuals who remained on first-line therapy than for those who switched to second line therapy. The study findings also suggest that in the absence of resistance testing, switching to second-line therapy based on viral load levels alone has the potential to substantially improve health outcomes and decrease mortality associated with progression of disease as well as the emergence of drug resistance [54].

Acquired HIV Drug Resistance

Suppressing viral replication to below the assay's limit of detection is also essential to limiting the emergence of acquired drug resistance to HIV [55]. Due to the high rate of viral production and sequence evolution, HIV is prone to errors in transcription—causing high rates of spontaneous genetic mutations. The development of HIV drug resistance (HIVDR) results from drug-induced mutations or persistence of favorable spontaneous mutations of wild type strains of the virus. Generally, drug-

induced mutations provide a competitive advantage over wild type strains that would otherwise not exist in an ecological environment without selective drug pressures. This relative biological fitness allows the resistant strains to persist and predominate if pharmacologic pressures from drugs are not sufficient to substantially limit viral replication [55].

The development or acquisition of HIVDR significantly thwarts the efficacy of ARV treatment [55]. Evidence suggests that achieving and retaining low loads of viral RNA prevents the selection of drug-resistant mutations from emerging [56]. A study seeking to understand the appearance of drug-induced mutations in HIV-1 infected patients showed that viral evolution related to the emergence of resistance mutations was often associated with individuals with viral RNA levels greater than 200 copies/mL [57]. Moreover, a subsequent study pointed to an increase in the strength of this association when viral loads are above 500 copies/mL [58]. As such, the inability to maintain low rates of virologic replication, a consequence of virologic failure, is often causally linked to the acquisition of drug-resistant mutations [59].

Assessing the prevalence of HIV-1 drug resistance among patients in South Africa, Marconi et al. provided a comparative analysis of drug resistance testing among patients who attended two clinics in KwaZulu-Natal [45]. This study performed resistance testing on 124 patients with confirmed virologic failure. 83.5% of the participants had at least one drug-resistance mutation, 64.3% had dual-class drug resistance, while there were 2.6% with triple-class drug resistance. The detection of drug resistance in over 83% of the South African study cohort who experienced virologic

failure on first-line ART points to the significance of drug resistance as a consequence of first-line virologic failure [45].

Causes and Risk Factors of Virologic Failure

While virologic failure is often, but not always, associated with resistance, mitigating the risks of failing virologically is an ideal means of substantially decreasing the likelihood of patients needing to be enrolled in higher-cost, limitedly-available second-line therapy. As reflected by the position of the South African Clinicians Society, the predominant causes of virologic failure in the literature include previous use of a single-dose of NVP (usually issued to pregnant mothers as a means of preventing mother-to-child transmission), directly transmitted drug-resistance, as well as the most the most common indicator: suboptimal adherence to treatment [13].

Adhering to recommended antiretroviral protocol is associated with a number of behavioral, psychosocial, and environmental challenges [60]. Evidence in the literature points to the significant role that suboptimal adherence to drug regimens plays in influencing the likelihood of experiencing virologic failure [61-63].

A study conducted in 2008 followed 456 patients on NNRTI ART in a retrospective cohort study based on Soweto, South Africa [61]. Analysis revealed that after 15 months of ART, 19% of patients had failed both virologically and immunologically. Virologic failure was defined as two repeated viral loads measuring greater than 1000 copies/mL after at least three months of ART. Patients who failed immunologically were defined as those whose CD4 cell count was less than 100 cells/mm^3 after six months of treatment, CD4 cell count was less than or equal to pre-

ART readings after six months of therapy, or those who had greater than a 50% reduction from the highest measured CD4 count measured at any point during ART. This study concluded that for patients with less than 95% adherence to drug-refill visits, there was an association with both virologic and immunologic failure. Furthermore, projected survival analysis modeling revealed that the virologic failure rate at 48 months post-initiation of ART was 37% among non-adherent patients [61].

In addition, a secondary analysis of the data from a clinic in urban KZN points to the indicators by which suboptimal adherence to ART was assessed. Re-analyzed data from the 2012 case-control study revealed that a combination of pill count (derived from a pill count to adherence ratio—PCAR) and self-reported adherence questions were highly predictive of virologic failure [63]. In addition to previously recognized indicators including WHO clinical stage, viral load, as well as CD4 count, the study also noted a statistically meaningful association between treatment interruptions (potentially serving as a proxy for poor adherence) and virologic failure [63].

A study published in 2014 by Court et al. reiterated the known high likelihood of virologic failure on second line (protease inhibitor) antiretrovirals in a study of adults at McCord Hospital in Durban, KZN [64]. This observational cohort study explored patient pharmacy refill data to determine clinical risk factors for VF. For a median follow-up time of 27 months, the study, during a four-month follow-up period, assessed 243 patients with at least one viral load. Results showed that pharmacy refill adherence when assessed four months prior to a V, was able to predict virologic suppression. The study also found that identifying poorly adherent individuals by short-term pharmacy refills before they fail virologically is especially critical, as the availability of third-line drug

options after failing on second-line therapy is often limited in many clinical settings in South Africa [64].

Risk Factors

The measurable risk factors that contribute to a patient's likelihood of failing virologically are well-explored in the literature. As an overview of the breadth of contributing factors, a study published in 2014 described findings from data collected from a cross-sectional study providing virologic testing for patients. This study focused its scope on adult patients who had been on ART for longer than six months in an effort to describe their clinical as well as demographic risk factors associated with VF [62]. This cohort of 1,488 patients in Lesotho had a VF prevalence of 6.9% among the study population. Socio-demographic factors that were identified as contributing risk factors included age less than 30 years old, lower wealth quintile, no primary education, history of treatment interruption, history of drug substitution, lack of disclosure of HIV status to persons, long travel-to-clinic time (classified as more than two hours), and previous Nevirapine and Zidovudine-based backbone ART [62].

Demographic Risk Factors

The gender differences associated with failing on treatment are well documented in Druyts' et al. meta-analysis assessing treatment outcomes across 23 cohort studies in Africa. Pooled proportional hazard ratios estimated a risk of mortality for males as 1.37 times that of females [65]. These findings were echoed in studies specific to sub-Saharan Africa which have found men to be significantly more vulnerable to virologic failure. A

case control study conducted in Burkina Faso estimated that among cases, male gender remained strongly associated with virologic failure (an estimated odds ratio of 2.52) after controlling for age, length of treatment, and CD4 count [66].

Additionally, other studies representative of patient populations in southern Africa also showed similar findings. Studies have pointed to the higher proportion of men ages 18-28 years being non-adherent, while males over the age of 48 were attributed with the highest rates of adherence [66]. A 2012 survival analysis conducted on over 46,000 adult patients in South Africa revealed that men had significantly higher mortality than women in the eight South Africa ART programs assessed [67]. This disparity was best attributed to later onset of disease at time of diagnosis and treatment initiation, higher loss-to-follow-up among male participants, differences in response to treatment, as well as external factors outside the scope of clinical classification [67].

In an attempt to explore these external contributing factors, Hare et al. examined an array of socioeconomic indicators related to gender-associated risk factors for virologic failure in the province of KZN, specifically [68]. In assessing individual-level predictors as well as attempting to account for the structural barriers patients face, this study analyzed a patient population enrolled in therapy at an urban clinic at McCord Hospital in Durban, KZN. Findings suggested that younger age, financial stability, as well as personal ownership of vehicles were statistically significant predictors associated with virologic failure among males when compared to females [68].

Clinical Risk Factors

The same study previously mentioned which described risk factors for virologic failure among patients in Lesotho [62] also found a number of associated clinical risk factors that were identified as contributing to the likelihood of treatment failure (according to WHO guidelines). Identified factors included the presence of PPE (pruritic papular eruption—typical HIV-associated rash) and immunologic failure. Further multivariate analysis revealed that age, history of treatment interruption, PPE, immunologic failure, and history of drug substitution were predictors of virologic failure [62].

A similar cross-sectional study assessed immunovirologic, adherence, and pharmacologic outcomes in patients enrolled on ART for a duration of 12 months as well as 24 months [69]. Patient information was gathered through the administration of a questionnaire that entailed a combination of clinical reports and two self-reporting indicators of adherence (percentage of pills taken in the previous four days, and the percentage of adherence in the previous 30 days). Statistical analysis revealed that at 12 and 24 months, 25% and 28%, respectively, had viral loads greater than 400 copies/mL. Among those considered to be failing virologically (classified as those with viral loads greater than 1000 copies/mL), significant risk factors included tuberculosis diagnosis after ART initiation, subtherapeutic NNRTI concentrations, appearance of general clinical symptoms, lower weight at baseline, and poor adherence [69].

Psychological/Social Risk Factors

Adherence to ART has been noted to be influenced by the social pressures and mental health effects that patients face. Recognized psychosocial determinants of adherence include social stigma, abuse of drugs and alcohol, extent of social/familial support, and depression [70-72]. Stigma has the potential to influence delays in seeking and enrolling in treatment programs. The literature also points to its potential effect on treatment interruptions. Substance abuse has been attributed with affecting the ability of patients to maintain healthy lifestyles, and thus, negatively impacts their likelihood of habitually taking medications. Mental health challenges related to depression have been noted to decrease patient retention in care and serve as an emotional barrier for routinely adhering to treatment protocol [71, 73].

Early Warning Indicators

WHO has an established set of early warning indicators (EWI) of HIV drug resistance as a key component of their strategy to mitigate the emergence of drug resistance resulting from virologic failure [74]. Such preventive measures are particularly pertinent in countries, such as South Africa, that are scaling up the availability of ART and thereby increasing the number of patients enrolled in therapy and therefore potentially at risk of failing on treatment. This enumerated list established in 2010 includes each indicator with its associated target:

WHO Early Warning Indicators for Virologic Failure	Target Coverage
1. Prescribing practices in accordance with WHO guidelines (100%)	100%
2. Loss to follow-up (% of patients lost to follow-up at 12 months on ART) (<10%)	<10%*
3. Retention on first-line ART (% of patients retained on first-line therapy) (>90%)	>90%*
4. On time pill pick-up (% of patients with 100% on-time drug pickups during the first 12 months of ART, or during specified time period)	>80%
5. On-time clinic appointment keeping (% of patients who attended all appointments on time during the first 12 months of ART, or during a specified time period)	>80%
6. Drug supply continuity (% of clinics with antiretroviral drug supply continuity during a 12-month period)	100%
7. Adherence as measured by pill count (% patient adherence to antiretroviral therapy by pill count or other standardized measure)	>90%
8. Viral load suppression 12 months after ART initiation (% of patients with viral load <1000 copies/ML at 12 months of ART)	>90%*

**Updated to reflect 2015 "90-90-90" targets*

These guidelines reflect system-level indictors related to the prevention of drug resistance and are utilized in efforts to improve delivery of health care in settings with low EWI scores. While these markers serve as a guide for clinical practices and community-level interventions, recent literature reflects the need to identify the emergence of drug resistance at an earlier time point by honing in on the mitigation of virologic failure, specifically [75]. Furthermore, recent studies performed in Sub-Saharan Africa have demonstrated the need for a focus on individual-level indictors in resource-limited settings [76, 77].

Study Justification

Risk factors that dramatically impact patients interact in a complex way. The socioeconomic barriers and burdens faced by patients in resource-constrained settings are common among those in South Africa. Kagee et al. have characterized the structural barriers to adhering to treatment that individuals in southern Africa face. Their categorizations include economic, institutional, cultural, as well as political factors that all interact in dynamic ways to collectively interfere with the ability to adequately follow treatment regimens, particularly in resource-constrained settings [14]. More specifically, these barriers include difficulty attaining routine transport to clinic appointments, food insecurity, and the minimally-available provisions for individuals with disabilities or other physical, mental, or social disadvantages. The difficulty of overcoming these obstacles is only compounded by the institutional barriers related to limited availability of adequate and sufficient patient counseling, overburdened health facilities, and limited mental health resources. Largely, these can be considered consequences related to financial limitations for ART treatment and counseling provisions, day/short-term labor migration, institutionalized gender inequalities that limit social mobility, and traditional or cultural barriers to accessing routine care [14, 70].

The nuanced intricacies of the interconnected relationship between these multilevel issues was well-acknowledged in the Risk Factors for Virologic Failure (RFVF) study conducted by the KwaZulu-Natal HIV Drug Resistance Surveillance Study team [78]. In the 2013 publication, the RVFV study assessed a multitude of clinical, behavioral, psychosocial, and structural factors that influence the relative likelihood of virologic failure among a cohort of patients at McCord Hospital in Durban, KwaZulu-

Natal. The analyses of these data elucidated a number of individual-level risk factors considered to be early warning indicators for virologic failure among the population of interest.

Study Setting

Marconi et al. provided a comprehensive assessment of indicators to reflect a patient demographic in the HIV clinic at McCord Hospital in Durban, KZN. The Sinikithemba outpatient HIV/AIDS clinic of McCord hospital was operational from 1998 until it closed in June 2012. The facility, as of 2016, is operational as a district DOH hospital. Sinikithemba, meaning “we bring hope” in Zulu, was subsidized by PEPFAR in 2004 until greater than three fourths of the funding was withdrawn in 2012 [79]. While operational, the clinic provided clinical care, adherence counseling, and routine CD4 cell count and viral load monitoring for ART enrolled patients [79].

Study Summary and Findings

All HIV-positive McCord clinic attendees over the age of 18 years old who were on ART for greater than five months were offered enrollment in the RFVF study. In this unmatched (2:1) case-control study, cases (n=158) were defined as those experiencing VF (defined as >1000 copies of/mL) and controls (n=300) were those with viral loads <1000 copies/mL. Utilizing univariate analysis as well as multivariable logistic regression models of VF, the final, full model (controlling for the measure of access and adherence to treatment) revealed that lack of a religious faith, male gender, lack of satisfaction with clinical experience, experiencing symptoms of depression, rash, fatigue,

low CD4 count, utilizing television and radio as ART reminders as compared to mobile phones, and someone aside from a "friend" recommending ART initiation contributed to VF. This study elucidated a number of telling risk factors for this patient population related to demographics, socioeconomic, clinical, psychosocial, and transportation limitations [78].

Use of Predictive Risk Indices in Clinical Practice

Particularly in time- and resource-limited settings with minimal health care personnel and/or laboratory testing capabilities, risk scores serve as an efficient and effective predictive analytic tool to inform physician decision-making in clinical practice [80].

Risk score indices have been routinely implemented and widely adopted in clinical practice—one of the most notable of which is the Framingham risk score assessment tool. The Framingham index utilizes a multivariate risk assessment approach to categorize patients' overall risk of developing coronary heart disease [81]. While this is only one of a set of risk categorization tools used to predict the development of cardiovascular disease, this particular assessment approach is readily implemented and used in clinical settings [81]. Furthermore, the validation of this tool provides a justification for utilizing other systematic indicator-based risk characterization tools and associated scoring systems to inform preventive medical approaches to care [81-83].

Specifically related to HIV health outcomes, the Veteran's Aging Cohort Study (VACS) risk index serves as a validated system for characterizing the risk of mortality among HIV infected individuals. Seeing a gap in the ability of traditional HIV

biomarkers such as CD4 cell count, hepatitis C diagnosis, and age alone to predict an individual's mortality risk, the VACS index was initially designed to improve the prediction of mortality for individuals who have been on ART [84].

Study participants were limited to adults (individuals ages 18 years or older) and who initiated ART between 2000 and 2007 [84]. Predictors of mortality were derived from data obtained from men in the VACS cohort of over 33,000 patients—which includes all HIV-infected veterans receiving Veterans Administration care in the United States. Inclusion of predictors was determined by their relative ability to be ascertained from clinical and research databases, precisely and reliably measured, as well as their ability to predict mortality as derived from the five-year, all-cause mortality multivariate models for patients after one year on antiretroviral treatment. Predictors in the original index were quantified in terms of hazard ratios. To decrease the necessity of numerous transformations necessary for interpreting hazard ratios for continuous variables, all variables were categorically measured in the creation of the original VACS index [84].

The VACS index (based on age, CD4 count, viral RNA level, hemoglobin, alanine and aspartate transaminase, platelets, hepatitis status, and creatinine) as well as the Restricted Index (based on a smaller subset of indicators including age, CD4 cell count, viral RNA, and an outcome of death within six years following ART initiation) were composed by scaling the hazard ratios obtained from Cox regression models to interpretable values. Point values were derived from an arbitrary transformation (obtaining the log of each hazard ratio and subsequently scaling the values by multiplying by 25 or 30 for the VACS and Restricted index, respectively) in order to obtain a range of

indices values between 0-100. Individual patient risk scores were calculated by summing their cumulative scores, and these data sets were used for the remaining analysis [84].

The prognostic accuracy of each index was determined using concordance (c-statistics)—a quantity related of Somers' D (a measure of association between two variables, particularly in nonparametric statistical models) [85]. Results of the analysis showed that the VACS index provided more predictive power than the Restricted Index. A sensitivity analysis and external validation of the findings was also completed. In order to create an internationally generalizable tool, the VACS Index was validated on six external, independent cohorts from both Europe and the United States. These cohorts were among studies included in the Antiretroviral Therapy Cohort Collaboration, a collection of data on HIV-positive patient in North America and Europe [84].

Chapter II: Manuscript

Introduction

Due to the high burden of disease in KwaZulu-Natal, the scaling up of ART availability, delivery, and patient enrollment increases the proportion of individuals who can potentially fail on treatment—particularly those who are asymptomatic, and therefore, are likely harder to retain in long-term care. The results of the Risk Factors for Virologic Failure (RFVF) study are of particular relevance, as virologic response to ART, one of the earliest indicators of ART effectiveness, is of increasing importance in KwaZulu-Natal [45, 54, 78].

The RFVF acknowledges the complexity of the array of risk factors for virologic failure at the individual, patient-level. Given this, there is a need to compile the findings from this study into a readily available tool to enable physicians to rapidly identify those at risk of virologic failure prior to initiation and while on treatment. This tool should characterize the complex factors that contribute to an individual's likelihood of experiencing virologic failure both at the initiation of ART as well as after at least six months of treatment. Leveraging the multivariate, predictive logistic regression models presented in the RVFV study 2013 publication, associated risk scores are to be assigned and a baseline, overall, and "restricted" index composed. The indices will be comprised of the predictive variables of statistical significance and their relative predictive capacity assessed.

This work seeks to utilize the indicators identified in the RFVF results and provides a secondary analysis that is hypothesized to meaningfully quantify a patient's risk of virologic failure based on the indicators identified from the most parsimonious, while highly predictive model of both clinical and statistical significance. This risk

assessment methodology will be utilized in future studies in which the best model will be applied to prospective cohort VF studies conducted in both rural and peri-urban settings KZN.

Methodology

RFVF Study Methods:

The Risk Factors for Virologic Failure (RFVF) study was conducted in an urban clinic in Durban, South Africa with the intention of examining virologic failure, typically associated with drug resistance, among HIV-positive adult clinic attendees. Through a variety of measured indicators, this study sought to elucidate individual-level risk factors linked with a patient's likelihood of failing virologically. Domains of variables measured included demographic, socioeconomic, psychosocial, clinical (symptoms/exams, medical history, laboratory results), and medications, as well as both access and adherence to treatment regimens [78].

Clinical Setting

The RFVF study took place at McCord Hospital in Durban, KwaZulu-Natal, South Africa at the HIV/AIDS outpatient clinic, Sinikithemba. McCord Hospital has served patients on ART since 2002 and functioned as a regional referral center until it closed in 2012. McCord transitioned to become the Comprehensive Centre of Excellence Provincial Eye Hospital in 2014 [86]. With operations subsidized by PEPFAR funds as well as the South African national government, in 2011

Sinikithemba reported serving approximately 5,000 patients per month, while McCord Hospital provided services accommodating over 13,000 patient visits per month [79]. Clinical services provided at Sinikithemba cost $15 per month per patient. Services included routine viral load laboratory monitoring five months after the initiation of ART, as well as adherence counseling and education at ART initiation [78].

Study Participants

Participants in the study included all HIV-positive individuals receiving care at Sinikithemba between October 2010 and June 2012. Inclusion criteria restricted study participants to adult patients, over the age of 18 years, who had been enrolled on their first ART regimen for five months or more.

Study Design

Because the overall rate of virologic failure was relatively low among the study participants, an unmatched case-control design was chosen for this study. Cases of virologic failure, the primary outcome of interest, were defined as having an initial viral load of greater than 1000 copies/mL after at least five months on ART. Among the HIV-positive patient demographic seeking care at Sinikithemba, enrollment of cases was generally conducted one to two weeks after viral load testing, when patients were notified of the study and patient consent for enrollment was received. As such, study enrollment dates were generally within two to three

weeks of the most recent pharmacy refill pickup. Controls were defined as individuals who were categorized as virologically suppressed (viral loads less than 1000 copies/mL) after five or more months on ART. Controls (2:1) were randomly selected clinic attendees who met viral load eligibility criteria and consented to participate.

Data Collection

Data was collected through semi-structured interviews conducted by research coordinators blinded to patient case-control status. The interview was administered in the participant's preferred language (either Zulu or English) and included a questionnaire (containing demographic, educational, employment, socioeconomic, psychosocial, and clinical satisfaction assessments), a neurocognitive assessment, depression scale assessment (Kessler 10), an unannounced pill count, and previously validated questions relating to ART adherence practices and clinical attendance. Neurocognitive assessments were performed by coordinators trained by a licensed psychiatrist who had previously modified and validated the (Trail Making Test A/B and Digit Span Forwards/Backwards tests) for the setting (see Appendix A). The research coordinators conducted the remainder of the interview after formal training from anthropologists, a social worker, a psychologist and the clinicians involved as principle investigators of the study.

Case report forms (CRFs) were completed for all patients and contained information regarding patient pharmacy refill dates/pill quantities, laboratory test results, and clinical history information abstracted from medical records. REDCap

electronic data manager, hosted through Emory University, was used as the primary data capture and storage tool.

Primary Statistical Analysis

All variables, both from the questionnaire and the CRF, were univariately analyzed to determine their association with the dichotomized assigned outcome of either failing virologically (case) or not (control). Variables of statistical significance (p <0.05) and, based on previous literature, clinical relevance were further analyzed after being categorized into domains: demographic, socioeconomic, psychosocial, symptoms/exam, medical history, access—a calculated measure passed on the medication possession ratio determined by pharmacy refill dates and dispensed amounts from the preceding 180 days, and adherence— a measure combining pill counts versus quantity dispensed in the preceding 180 days (see Appendix A). Univariate analyses within domains were completed to assess for correlations and interactions to inform subsequent multivariate analyses.

Primary multivariate analyses yielded five logistic regression models. Models were constructed in SAS version 9.4 using the PROC LOGISTIC procedure in which stepwise model selection was employed. Model 1 identified baseline factors, those present at the initiation of ART, associated with VF. Model 2 described all time-updated variables aside from access and adherence, while Model 3 included the socioeconomic and psychosocial variables in an effort to describe the extent to which they impacted likelihood of VF after controlling for the access variable. Model 4 expanded upon the previous two models and incorporated psychosocial, symptoms,

and other clinically relevant variables likely to be associated with VF after controlling for the adherence variable. Model 5, the full model, controlled for both adherence and access measures as well as all time-updated variables. However, access had to be removed due to high collinearity with the adherence variable.

Risk Score Index Methods:

Analytic methodology for risk score index derivations similar to that of the VACS study was applied. Predictive models from the original RFVF study for patients on first-line ART for 12 months were adapted for utilization here. Three indices were created: a Baseline, RFVF, and Restricted RFVF Index.

The Baseline Index, derived from Model 1 from the original RFVF study, includes all significant indicators that were measured at treatment initiation. This allowed for an index to enable quantification of a patient's predicted risk of VF based on the set of characteristics measured at the start of ART. The RFVF Index included all indicators identified in the full model, Model 5, of the original study. This included all variables from each domain with adherence forced in the model, and access removed due to high collinearity with adherence. Variable significance level was not taken into consideration for inclusion into the index, as this set of predictors was selected in order to provide the most comprehensive predictive collection possible, given available measured risk factors upon which to build a prognostic risk index. While the Baseline Index provided a way to assess a patient's initial risk of VF at the start of treatment, the RFVF and Restricted RFVF indices

were compared to one another in order to identify whether or not a more parsimonious index could provide the same predictive/descriptive risk characterization of VF after six months or more of ART.

The predictors included in the Restricted Index were selected by noting the level of significance at which variables in the full model remained associated with VF. The inclusion criteria for the Restricted Index was limited to variables from the full model with *p* values significant at the <0.05 level. This limited set of predictors, now with approximately half the quantity of variables included in the full model, served as a parsimonious index to be directly compared to the comprehensive RFVF Index for both explanatory and predictive capabilities.

Before constructing the indices, logistic regression model outputs were adjusted. To ensure that the risk categorization was characterized and attributed appropriately, a number of variables were either recoded and/or their reference group changed. The inverse of variables with odds ratios less than 1.00 (showing a protective effect) was computed and their interpretation changed accordingly in order to ensure the indices only included indicators associated with an increased risk of VF. The variable age in the original model was modeled such that the odds ratio for every 5-year increase in age was associated with an odds of 0.87, 95% CI 0.71-1.06 of failing virologically—demonstrating that younger patients were at a higher risk for VF. This was inverted such that those in the youngest 5-year age bracket had the highest risk (1/0.860 = 1.2) and the oldest 5-year age group was associated with the lowest risk (reference group). Similar transformations were done for the time-updated variables log of CD4 cell count and adherence.

Similarly, reference groups for bivariate predictors, as well as for multi-level categorical variables, were adjusted for variables including family members who were HIV positive, feeling pleased versus neutral in regards to clinical experience, having lipodystrophy versus not having this non-AIDS defining condition, who recommended the patient to attend an ARV clinic (friend versus family, healthcare provider, or other), and current ARV regimen.

The subset of variables identified to be included in the Restricted Index were identified and run in a separate logistic regression model. Odds ratios from this were utilized to create the associated points for the restricted index.

Construction of Indices

Subsequently, risk score indices were derived similarly to VACS-based indices in that point values were assigned to the selected predictive indicators. It was important to create an index with a scale with an estimated working range that was easily interpretable, for which readability and delimitation between the respective contributions of each distinct variable on an individual patient's likelihood of failing virologically was clear.

Point values were determined by scaling the odds ratios obtained from the logistic regression modeling outputs. Obtaining the natural logarithm of the odds ratio provided the regression coefficients. These were then scaled up by a factor of 10, for the composition of each index, for ease of interpretability. Point values were rounded to the nearest whole number. Individual patient scores were quantified by

summing the total values of their attributable risk factor scores to create index score data tables. A theoretical maximum score for each index was summed such that an individual's cumulative risk of VF could be expressed in terms of a percentage of the total potential risk of failure, given that particular set of indicators. Three risk scores were calculated for each patient based on each of the three indices. These scores were presented as percentages of the total theoretical maximum risk of each respective index.

Assessment of Indices

The predictive and explanatory capabilities of the three indices were assessed and explored through model characteristics and fit statistics. The model quality and prognostic accuracy of all three indices was measured using the Akaike information criterion (AIC), Somers' Delta (Somers' D) statistic, and the area under the receiver operator characteristic curve (AUC of ROC curves).

AIC provided information allowing for the comparison of models relative to one another, in that it quantifies the tradeoffs between goodness of fit of a model and the number of predictors included. This is particularly relevant for direct comparisons of the RFVF versus the Restricted Index. The predictive capabilities of the models were measured through the use of ROC curve AUC and Somers' D. The AUC of an ROC is a graph generated from measuring the true positive rate (sensitivity) versus the false positive rate (1-specificity) in order to assess the threshold for which an increase in number of predictors yields the maximum sensitivity and specificity [87]. Somers' D essentially provides a quantification not

of the area under the ROC curve, but rather a measure of the difference between the areas above and below this curve [85]. While as performance indicators, Somers' D and ROC AUC are equivalent, both are presented here.

A set of three cumulative scores for individual patients, derived from point values associated with all indices were calculated. These "risk scores" were presented as percentages of the total theoretical maximum score for each index in order to express individuals' risk of VF in a way that was comparable between all three indices. Quintiles delineating risk categorization into "very low, low, moderate, high, and very high" VF risk characterization were composed to further describe the study cohort and to allow another means by which to compare the characterization capabilities of the three indices.

In order to assess the relative utility of the indices themselves, univariate logistic regressions were modeled where patient risk scores were the sole, continuous predictor of VF. Similar to the assessment of the original models, fit statistics and predictive probabilities of the index models were quantified and compared based on their AIC, Somers' D, and AUC of their respective ROC curves.

Instruments

All data was abstracted from REDCap data management tool and all subsequent analyses utilized SAS version 9.4.

Ethical considerations

The original RFVF study was approved by the McCord Hospital ethics committee in Durban, KwaZulu-Natal, as well as the Emory University institutional review board in Atlanta, Georgia.

Results

Cohort description-Univariate Analysis

Measured characteristics of interest (variables included in the indices) are listed in Table 1. Of the 458 study participants, there were 158 cases and 300 controls. The median age among cases was 36.6 years and 39.4 years among controls while there were 75 (47.5%) male cases and 87 (29.0%) males in the control group. Univariate logistic regression revealed that age alone was not associated with an increased odds of VF (odds ratio of 0.95, CI 0.9-1.0). The odds of VF for males was 2.2, CI 1.5-3.3, times greater than in females. Socioeconomic indicators assessed included education level (a median of 11 years of education for cases and 11.5 for controls) and transportation (personal vehicle versus all other means). Both years of education and personal transportation alone were associated with an increased odds of VF. Psychosocial risk factors assessed included not being religiously active, practicing safe sex less than always, having one to four family members who were HIV positive, having a treatment supporter, and having a Kessler depression score greater than 12.

All of these psychosocial risk factors were univariately-associated with increased odds of VF. Univariate increased odds of VF were also associated with the presence of clinical symptoms including fatigue, diarrhea, feeling sad, and the presence of skin

lesions. Lack of presence of lipodystrophy was also associated with an increased odds of VF. Similarly, decreased levels of CD4 cell count was associated with an increased odds of VF. An increased odds of VF was also seen among patients for whom ART initiation was recommended by someone other than a friend.

Assessing the impact of adherence counseling and pre-ARV training sessions alone on VF revealed that more adherence counseling sessions were associated with increased odds of VF, while having greater than three pre-ARV sessions was protective. As compared with "other" regimens, d4T alone was associated with an increased odds of VF, while ZDV was not. Use of television/radio as a reminder to take ARVs as well as the use of fluconazole or ethambutol showed an increased odds of VF, while increased adherence to medication was protective.

Multivariate Model Assessment

Discriminatory model characteristics shown in Table 2 compiled the fit statistics and quantification of predictive characteristics of the three models. While the baseline model had notably higher AIC relative to the full RFVF and Restricted models (529.5 versus 379.2 and 381.8, respectively), this was to be expected as this model did not include any time-updated variables and had the fewest number of predictors.

As for the direct comparison between fit statistics of the RFVF and the Restricted models upon which the point-based indices were derived, the Restricted Index had slightly higher AIC as well as slightly lower ROC AUC and Somers' D reflected the decrease in predictive accuracy given a limited number of variables. The amount of

decreased predictive power, however, associated with the Restricted model was very minimal, 0.02, and arguably negligible. As such, the Restricted RFVF Index, the parsimonious model, was considered to provide a quantification of overall risk of VF comparable to that of the full RFVF model.

Risk Indices Comparisons

Adjusted odds ratios and associated points for respective models are shown in Table 3. Point values were derived from taking the log of each odds ratio (in order to obtain the regression coefficients) and scaling that value up by a factor of 10. After this transformation, the point values reflect the relative contribution each predictor variable had on an individual patient's total risk score. Scores for dichotomous variables (gender, practice safe sex, mode of clinical transport, depression score, religious faith activity, number of family members with HIV, presence of a treatment supporter, experiencing clinical symptoms (fatigue, diarrhea, sadness, skin lesions, and using TV/radio as a reminder for taking ARVs) were either assigned a point value for the outcome associated with increased risk of VF, or a 0. For example, males have increased odds of VF compared to females and were thus given a point of 7 or 9 for the Baseline or time-updated Indices, respectively. Categorical variables with multiple levels (provider recommendation, number of adherence counseling sessions, and current regimen) had point values assigned similarly, with reference groups as obtaining a point value of 0. Continuous variables (age [per every 5 year increment], education [per every one year increment], ARV duration [per one month increment] log CD4 cell count [per every one unit increase] and adherence [per every one month increase]) were coded such that

individuals were given a point value from a range dependent on the value of the given variable. The cumulative total risk of the Baseline, RFVF, and Restricted Indices was 89, 283, and 207 points, respectively.

By scaling the odds ratios of all variables in all models in the same way, it was possible to directly compare the point values. The differences in odds ratios and associated point values, derived from the RFVF and Restricted Indices further illustrate the similarities between the two in terms of their risk characterization. Despite having substantially fewer variables than the full model, the Restricted Index had point values that provided the same single variable risk characterization for six of the indicators. Three indicators in the Restricted Index had point values that differed from those derived from the RFVF Index by only one point, while the remaining three variable in the Restricted Index do differ from the full model by more than one point.

Model characteristics of the univariate logistic regressions using patient risk scores as the predictor of VF are provided in Table 4. Corresponding ROC curves are illustrated in Figure 4. The Baseline Index, as was noted in the Baseline model, had notably higher AIC (508.1) indicating worse model fit as compared to the RFVF and Restricted Indices (414.8 and 420.9, respectively). The ROC curve AUC illustrate marginally higher, more predictive, values for the Restricted as compared to the full RFVF model (0.847 versus 0.848, respectively), while the Baseline Index had a markedly lower AUC at 0.75.

Table 5 provides a risk characterization of the RFVF study cohort. The index classification system revealed the total number of study participants whose percent risk of VF falls into the “Very Low” category (below the 20th percentile), “Low” category

(between the 20th and 40th percentile), the "Moderate" risk category (40th to 60th percentile), "High" risk (60th to 80th percentile), and "Very High" (above the 80th percentile). The distribution of these categorizations is easily visualized in the histograms in Figure 5.

In comparing the use of the Baseline, RFVF, and Restricted Indices in their categorization of the risk of VF, the Baseline Index classified a larger proportion (122, 26%) of the study population with moderate risk, whereas the RFVF classified 108 (23.6%) and the Restricted classified 95 (20.7%). As for the extremes of the characterization range, the Baseline Index categorized fewer patients as having both "very low" risk (77, 16.8%) and also "very high" risk (89, 19.4%) as compared to both the RFVF ("very low"—(99, 21.6%) and "very high"—(90, 19.7%) and Restricted ("very low"—(95, 20.7%) and "very high"—(96, 21.0%). The Restricted Index categorized fewer patients in the "very low" and more patients in the "low" category, as well as more patients in the "very high" and "high" (as opposed to the "moderate" category) compared to the RFVF index.

Discussion

Overall, the risk indices derived from the RFVF study presented here sought to characterize the risk of VF among a study cohort of 458 patients. Determinants for VF will potentially be applied for the identification and delineation of the extent of VF risk of individuals.

Calibration refers to the ability of the model to resolve the predicted outcomes with the observed outcomes, while *discrimination* refers to the relative ability of the models to distinguish those with VF (the outcome of interest) and those without VF [88]. The characteristics of the adapted logistic regression models from the original RFVF study upon which the indices were derived were measured in terms of the model fit statistics and predictive capabilities by means of the AIC and the AUC of the model's ROC curves, respectively. The analysis of the original VACS index presented the c-statistic, or concordance statistic, in order to assess the relative discriminative ability of patients' indices-derived scores. This is a quantified, unit-less measure of the predicted probabilities of the outcome of interest (VF) occurring versus not occurring [87]. It should be noted here that for logistic regression methods, the c-statistic is theoretically equivalent to and has the same value as the AUC for ROC curves [87].

Results indicate that compared to the Baseline Index (the set of predictors for estimating a patient's overall risk for VF at the initiation of therapy), both the Restricted RFVF and the full RFVF Index provided improved calibration and discrimination with lower, better fit AIC statistics, as well as higher, more predictive ROC curve AUC values.

The Restricted RFVF Index, a parsimonious model of the full RFVF Index, provided a comparably informative risk assessment of an individual's risk of VF as the full RFVF Index. While the model upon which the Restricted Index was built was associated with a decrease in AUC of the ROC curve—lower predictive capabilities—this difference was marginal. As such, this difference was not considered sufficient to

justify the use of the full RFVF model over the restricted, parsimonious model based on these model characteristics alone.

Furthermore, the point values (derived from the scaling of the odds ratios of the model outputs) between the Restricted and full RFVF Indices were very similar. This suggested that there was not a meaningful difference between the two. As such, it was hypothesized that the Restricted Index would be able to provide comparably accurate predictions of VF when assessing patients' cumulative risk scores as the single predictor of VF.

Interestingly, although the Restricted Index did have a higher AIC (indicating a slightly worse model fit due to the decrease in the number of predictors), results indicate that the Restricted Index not only provided a comparable AUC/c-statistic to the full RFVF Index, but was actually a slightly higher value (0.848 versus 0.847, respectively). These statistics are presented in Table 4. This serves as further evidence to support the assertion that the Restricted Index actually provided an equally useful means of quantifying cumulative patient risk of VF. Concluding that the parsimonious index provides comparable utility to the full RFVF Index is of particular significance because the marked reduction in the quantity of factors that must be measured by health care providers has implications for the ease of implementation and use of this tool in practice.

In regards to the index classifications of the study cohort, there are several points of interest. As compared to the other two indices, the Baseline Index appears to categorize more patients in the "moderate" category as compared to the extreme ends of the spectrum ("very low" or "very high"). This indicates that given its lower predictive certainty, it provides more modest categorizations of risk. In comparing the Restricted to

the RFVF Index, the subtle differences in quantity of patients in each risk category indicate that the Restricted Index is more likely to categorize patients as "very high" and fewer in the "very low" categories. This implies that the use of this index could lead to potentially more conservative, protective clinical interpretations, diagnoses, and recommendations of patient risk.

Strengths

The results presented here represent important findings. The creation of both the Baseline and Restricted RFVF Indices provides a novel approach for characterizing the risk of virologic failure. Utilizing and applying similar methodologic approaches to that employed in the creation of the validated and widely-used VACS index, this work has provided a means and a justification to quantify an individual's risk of failing virologically, both at the initiation and while on treatment. Providing a "score," expressed as a percentage of one's cumulative risk based on a set of predictors associated with VF attainable at routine clinical visits, enables this rapid characterization of a patient's likelihood of VF.

Virologic failure remains a critically important characteristic and often precursor of the acquisition of HIV drug-resistance [40-43, 51]. Furthermore, VF is also associated with adverse clinical outcomes, irrespective of the presence of drug resistance. As such, the ability to rapidly assess, quantify, and categorize a patient's likelihood of failing virologically with a modest, measurable degree of certainty has the potential to serve as a meaningful supplemental diagnostic tool for health care professionals and contribute to improved clinical outcomes. The implementation of such a tool in clinical settings is

indicative of the potential for this to actively contribute to decreasing the number of individuals who may either fail virologically, develop HIVDR, and/or experience negative health outcomes.

The utility of this clinical assessment tool is rooted in its ability to characterize risk in such a way that public health professionals can more easily devise targeted interventions to address specific barriers once particularly at risk populations are identified, at the initiation and throughout the duration of treatment, in order to best promote positive health outcomes. Furthermore, it provides a usable, easily implementable, and practical translation of the findings from the original RFVF study. Upon further validation of this tool, it also has the potential to serve as a prognostic surrogate for VF in resource-constrained settings in which routine viral load testing is not readily accessible.

Limitations

There are, however, several notable limitations of this work. One concern is that this was a cross-sectional study as opposed to a prospective cohort design where patients could have been followed-up such that indicators would be measured at multiple time points. Given this limitation of the study design, a time horizon was unable to be specified for which the quantifications of risk of VF would be relevant, aside from that indicated by the Baseline Index (treatment initiation).

An additional note of concern is the limited generalizability of the index. This information on individual-level risk factors related to VF is derived from a single cohort

of ART-initiated adult patients receiving care from a particular clinic in a particular setting. Although the fundamental methodology (assigning point values to multivariate model output values) used here to derive the indices is mirrored off that of other validated indices, there are presently no validation data sets, with similar measured characteristics, available to verify the external validity of these findings. Given that the characteristics that remained significant in the best-suited (Restricted) index are context-specific (specifically related to patients' clinical experiences and patient behaviors), these finding are not necessarily applicable to external populations.

It is also important to conceit that while these prognostic indices provide a useful means of categorizing and characterizing an individual's risk of VF, there are aspects of patient care, personal experiences, and clinical manifestations of disease that are not able to be measured or quantified in such a way, but that are still elemental in the determination of health outcomes. Specific to this patient demographic, a 2015 study by Appelbaum et al. qualitatively demonstrated the importance and potential implications of concurrent use of traditional African medicine and allopathic medicine among HIV-positive patients in KwaZulu-Natal. The 26 patients included in the focus groups and who completed in-depth interviews revealed that these two approaches to health care are often viewed as complimentary in this setting [89]. Such evidence demonstrates the inability to quantify all relevant patient information in a way that truly encompasses the entirety of the array of influences, determinants, and approaches that comprise patient care and, ultimately, play a critical role in health outcomes.

In addition, it is also difficult to draw direct comparisons between these results and that of the VACS study. The VACS index was derived from a sample size of

approximately 4,500 compiled from six different studies. As such, findings were able to be cross-validated across a number of cohorts. This was not possible given that the RFVF indices were based solely upon one dataset. The predictive power of the RFVF is, naturally, higher and these indices' model fit statistics outperform that of the VACS study (ROC curve AUC/c-statistic of 0.85 versus 0.82, respectively).

It should further be noted that expectations of the perceived benefit, practicality and potential utility of the RFVF due to the fact that it outperforms the VACS index should be tempered. The methods applied in the VACS study, especially given the large set of data points and multiple validation data sets, indicate their intention to provide an index that measures *absolute* risk, as opposed to *relative* risk [90].With the aforementioned strengths of the VACS study, the patient scores derived from their index provide an estimate of *absolute* risk, or the likelihood of mortality given a set number of criteria. Contrarily, the RFVF indices, particularly the risk classification schema ("very low" to "very high"), is only able to provide an estimate of *relative* risk, or an individual's degree of likelihood of VF relative to other patients in that study cohort.

Due to the fact that the RFVF indices were derived from a single data set, interpreting the level of predictive power should be done conservatively, as it is highly likely that these models have been overfit—the presence of overly optimistic results that, in actuality, prove difficult to replicate [91]. There are limited number observations in the RFVF data set (information for only the 458 study participants), and as such, there is an upper limit to the complexity (directly related to the number of degrees of freedom, which increases with the number of model predictors) of a model that can be expected to explain the given data [91]. As such, the overfitting of the RFVF model-derived indices

will, as mentioned previously, likely lead to limited ability to generalize these results to external dataset.

Future Studies

Ultimately, the utility and the likelihood of this tool being adopted in practice is largely dependent on extending the generalizability of these findings. As such, validating these findings on external data sets that have measured similar characteristics of a similar patient demographic (adult HIV-positive individuals enrolled in ART) is an essential next step before the implementation and use of this tool as a clinical diagnostic supplement in practice. In light of the previous mention of overfitting these model-derived indices, generalizing these results to external patient populations will only be possible once the quantity of data points upon which the models for the indices are built is increased substantially. As such, similar studies that measure similar predictors in other study populations are essential in order to fit more realistically predictive, robust models and their associated risk indices.

In addition, these findings would notably benefit from an analysis of longitudinal studies measuring similar patient characteristics over time in order to enable the characterization of a timeline for which such risk characterization is prudent. Applications of this tool and similar adaptations will include the availability of an open source, free-of-cost, online site. Similar to that related to the VACS study, this site would enable clinicians, health care professionals, as well as any individual with access to it to manually input values for the listed predictors and be provided with an immediate "score"—percent prediction of their risk of VF. This tool would theoretically be

accessible and easily usable via mobile devices, which are typical in health care clinics even in rural/remote settings.

Public Health Implications

Clinical care, in regards to the direct interaction between health care providers and patients, remains a critically important component of patients' treatment, as the ability to identify and appropriately characterize the status of HIV progression on a routine basis is key to achieving positive health outcomes. Furthermore, improving quality of clinical outcomes entails utilizing knowledge gleaned from population-level risk identification studies through practical applications in which research findings are translated and incorporated into functional, implementable, and usable assessment tools for intervention strategies. Particularly in time- and resource-constrained settings, risk scores serve as a feasible, efficient, rapid, and predictive analytic assessment tool to guide physician decision-making in clinical settings [83].

Further illustrating the utility of scoring indices in clinical practice, a 2015 publication detailed the combined utilization of both the Framingham index as well as the VACS index to assess the addition of new biomarkers, abnormal coagulation and inflammation, as potential predictors of higher scores in both indices. This work demonstrated the extent of the utility of these tools, and illustrated the range of applicability of such scoring indices for other areas of research [92].

The benefits of utilizing risk scores to characterize patients' status in clinical settings are well-documented with regard to both the Framingham and VACS indices [83,

92, 93]. Serving as surrogate measures of clinical outcomes, both indices provide exemplary models of risk characterization tools.

With comparable c-statistics to the widely accepted and used Framingham index, the strengths of the VACS index justify the application of this methodology of risk score derivations to the RFVF study data—a study of which the goals of the use of the multivariate models had a similar scope of clinical applicability.

Conclusion

Rooted in these findings is the ethical imperative of communicating this type of health categorization ("very low-very high" risk) directly to the patients themselves. Providing a means to expressly communicate to a patient—particularly those who may have limited education and/or understanding of medical terminology—their likelihood of experiencing a largely preventable negative health outcome in straight-forward, quantified (percentage risk), and easy-to-understand manner is a valuable tool for health care providers to have. Enabling patients to better understand their own health status helps bridge a gap in the critical intersection of improving clinical outcomes from a provider-perspective and increasing autonomy and promoting ownership of health status from the patient-perspective.

The composition and demonstrated cohort characterization of the Baseline and Restricted RFVF Indices presented here represent an important and notably necessary example of the potential for research translation into preventive health practice. Despite the limitations of this work, the methodology and results presented here provide an adaptable framework upon which to base future work. Extending the predictive

capabilities and expanding upon the versatility of this tool, future adaptations will aim to build upon these findings to provide more complex designs that can more appropriately reflect the complexity of the multiple institutional, community, as well as individual-level contributors of virologic failure.

Appendix A:

Adapted Quantitative Measures Used in RFVF Study

Domain	*Measure*	*Reference*
Psychosocial	Depression scale	Kessler RC, Andrews G, Colp LJ et al, Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychol Med 2002; 32: 959-976.
Symptoms and exam	Neurocognitive testing	Karnofsky DA. *Criteria of Performance Status* (P. S.). New York: McGaw-Hill; 1954.
Access	Pharmacy refill dates and dispensed amounts over the preceding 180 days	Leslie RS, et al. Calculation medication compliance, adherence, and persistence in administrative pharmacy claims databases. Pharmaceut Program 2008; 1: 13-19. Ndubuka NO, Ehlers VJ. Adult patients' adherence to antiretroviral treatment: A survey correlating pharmacy refill records and pill counts with immunologic and cirological indices. Int L Nurs Stud 2001; 48: 1323-1329.
Adherence	Pill counts at enrolment visit	Lee JK, Grace KA, Foster TB, et al. How should we measure medication adherence in clinical trials and practice? Ther Clin Risk Manag 2007; 3: 685-690.

Appendix B

Tables and Figures

Table 1: Cohort characteristics of 458 RFVF participants

Domain/Characteristic	Case (N=158) N (%)	Control (N=300) N (%)	Unadjusted Odds Ratios (95%CI)
Demographic			
Age at enrollment (median(SD))	36.6 (8.4)	39.4 (9.1)	0.95 (0.9, 1.0) *per 5 year increase*
Gender (male)	75 (47.5)	87 (29.0)	2.2 (1.5, 3.3)
Socioeconomic			
Education (median(SD))	11 (1.8)	11.5 (2.7)	1.2 (1.2, 1.3) *per 1 year increase*
Transportation (personal)	31 (19.6)	29 (9.7)	2.3 (1.3, 3.9)
Psychosocial			
Religious activity (none)	96 (60.8)	125 (41.7)	2.2 (1.5, 3.2)
Practice safe sex (<always)	25 (15.8)	15 (5.0)	3.6 (1.8, 7.0)
Family members HIV+ (1-4)	79 (50.0)	114 (38.0)	1.6 (1.1, 2.4)
Treatment supporter (yes)	35 (22.2)	34 (11.3)	2.2 (13, 3.7)
Clinic feel pleased (neutral)	55 (34.8)	36 (12.0)	3.9 (2.4, 6.3)
Depression (12+)	107 (67.2)	145 (48.3)	2.2 (1.5, 3.4)
Symptoms and Exam			
Fatigue (yes)	75 (47.5)	72 (24.0)	2.9 (1.9, 4.3)
Diarrhea (yes)	28 (17.7)	25 (8.33)	2.4 (1.3, 4.2)
Sad (yes)	72 (45.6)	86 (28.7)	2.1 (1.4, 3.1)
Skin lesions (yes)	73 (46.2)	76 (25.3)	2.5 (1.7, 3.8)

Medical History/Lab Values			
Lipodystrophy (%no)	134 (84.8)	189 (63.0)	3.3 (2.0, 5.4)
Log CD4 (cells/mL) (median)	2.3 (0.5)	2.6 (0.3)	0.08 (0.04, 0.2) *per 1 unit increase*
Medications			
Recommended ART			
Provider (doctor/nurse)	71 (44.9)	128 (42.7)	1.7 (0.9, 3.3)
Family	49 (31.0)	58 (19.3)	2.6 (1.3, 5.2)
Friend	16 (10.2)	50 (16.7)	ref
Other	22 (13.9)	64 (21.3)	1.1 (0.5, 2.3)
Pre-ARV training sessions			
(0-2)	14 (8.9)	8 (2.7)	3.5 (1.5, 8.7)
(3+)	144 (9.1)	292 (97.3)	ref
Adherence counseling			
2-4	90 (57.3)	234 (78.0)	4.0 (2.1, 7.7)
5+	39 (24.8)	48 (16.0)	1.9 (1.1, 4.0)
0-1	28 (17.8)	18 (6.0)	ref
Current regimen			
d4T	44 (27.9)	52 (17.33)	1.6 (1.0, 2.6)
ZDV	24 (15.2)	74 (24.7)	0.6 (0.4, 1.1)
Other	90 (57.0)	174 (58.0)	ref
Recall ARVs (TV/Radio) (%yes)	22 (13.9)	16 (5.3)	2.9 (1.5, 5.6)
Fluconazole (%yes)	14 (8.9)	3 (1.0)	9.6 (2.7, 34.0)
Ethambutol (%yes)	9 (5.7)	4 (1.3)	4.5 (1.4, 14.8)
Adherence (median)	1.1 (0.1)	1.1 (0.1)	0.01 (0.002, 1.06) *per 0.1 unit increase*

Figure 1: Diagram illustrating the methodological flow of patient-based information from which the risk indices are derived

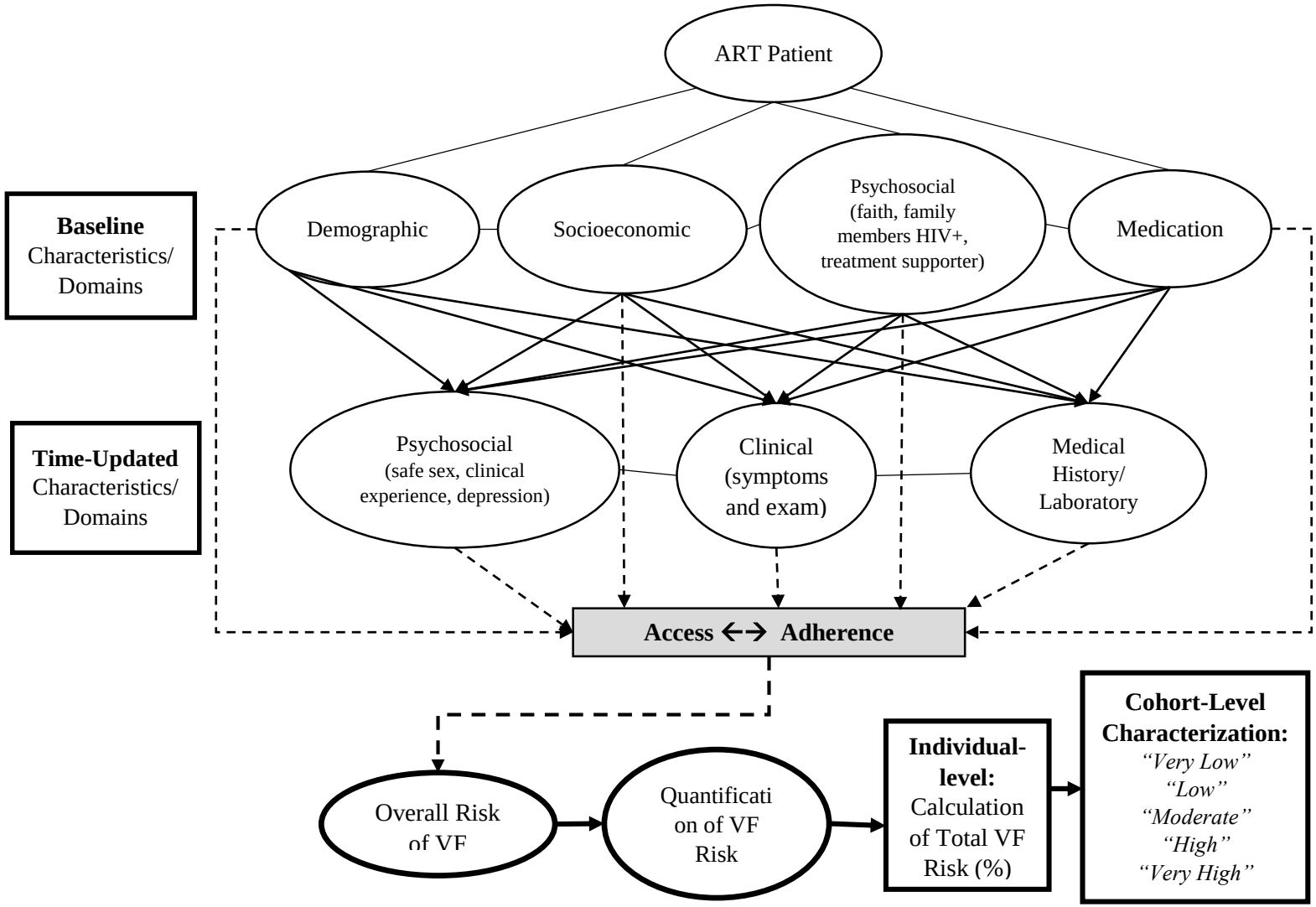


Figure 2: Schema depicting the social, behavioral, and clinical factors that contribute to individuals' virologic repose while on ART. Socioeconomic factors (transportation and education), comorbidities (clinical manifestation of disease), medication (regimen and adherence), and psychosocial components (related to mental health) have more direct influence on access to ART. These combined influences on access assist in determining a patient's adherence, which, cumulatively, impacts an individual's viral load level. This all functions within the broader political, social, and economic context within which institutional, community and society-level factors influence, control, and even determine aforementioned individual-level factors.

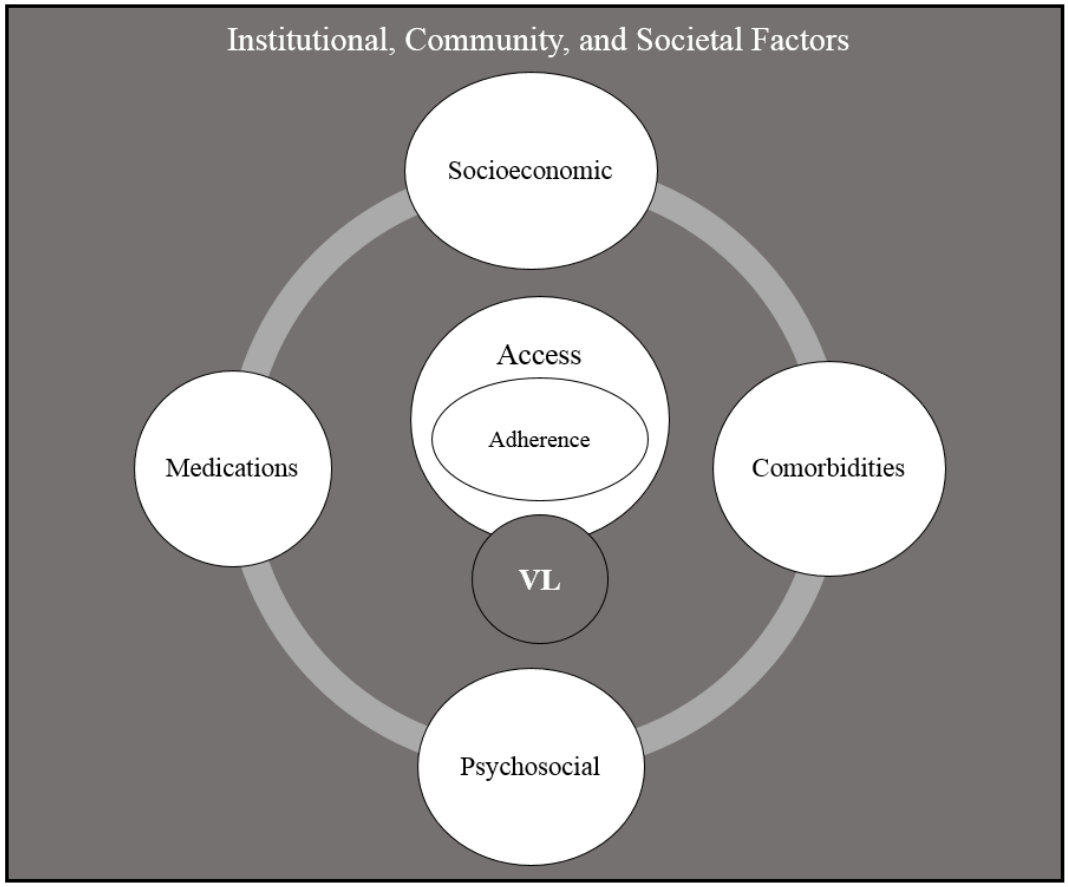


Table 2: Baseline, RFVF, and Restricted predictive model characteristics

Model Fit Statistics and Prediction Characteristics			
	Baseline Model	**RFVF Model**	**Restricted Model**
AIC	529.5	379.2	381.8
Somers' D	0.50	0.79	0.75
ROC AUC/ c-statistic	0.75	0.89	0.87

Figure 3a Baseline Model ROC curve

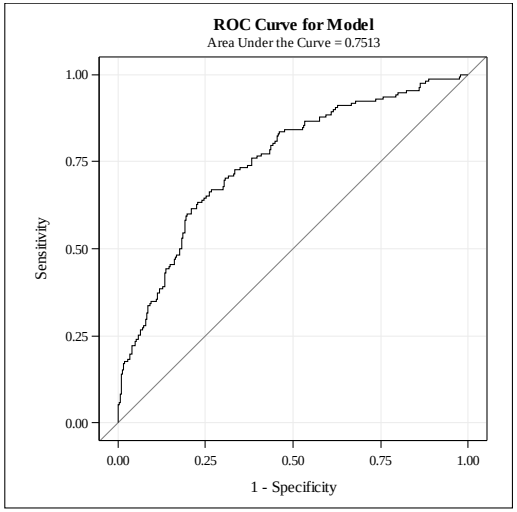


Figure 3b RFVF ROC curve

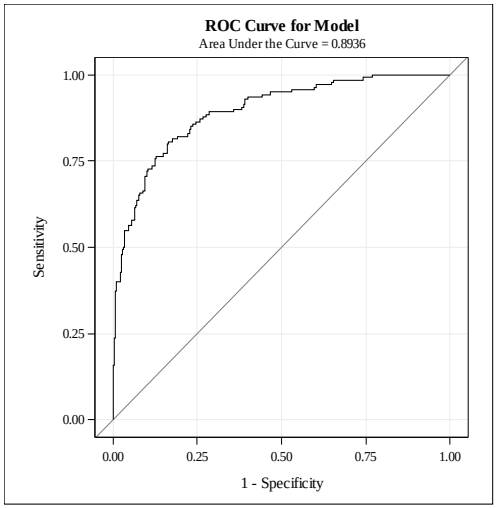


Figure 3c Restricted Model ROC curve

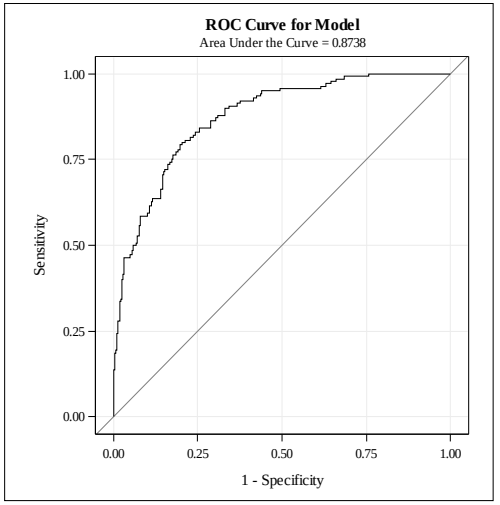


Table 3: Adjusted odds ratios and point values for Baseline, RFVF, and Restricted Indices derived from logistic regression models

	Odds Ratios (95% Wald CL)			**Points**		
	Baseline	**RFVF**	**Restricted**	**Baseline**	**RFVF**	**Restricted**
Age (years)	1.3 (1.1, 1.4)	1.2 (0.9, 1.4)		2 *range: 0-24*	1 *range: 0-12*	
Male (vs. female)	2.1 (1.3, 3.4)	2.4 (1.3, 4.7)	2.6 (1.5, 4.7)	7	9	9
Education (per 1year increase)		1.1 (0.9, 1.3)			1	
Practice safe sex (<always)		5.3 (1.9, 15.1)	5.8 (2.3, 15.8)		17	18
Transport to clinic personal (vs. all other)		2.1 (0.8, 5.4)			8	
Depression score (12+)		3.1 (1.6, 6.1)	2.7 (1.5, 4.9)		11	10
Faith activity (none)	1.6 (1.0, 2.4)	1.8 (1.0, 3.3)	1.8 (1.1, 3.2)	4	6	6
Family members HIV+ (1-4)	1.5 (1.0, 2.4)	2.0 (1.1, 3.7)	2.2 (1.3, 3.9)	4	7	8
Treatment support (yes)	2.1 (1.1, 3.7)	1.8 (0.8, 4.1)		7	6	
Clinic Experience (neutral)		2.0 (1.0, 3.9)	2.2 (1.2, 4.2)		7	8
Fatigue (yes)		2.4 (1.3, 4.5)	2.5 (1.4, 4.4)		9	9
Diarrhea (yes)		2.1(0.8. 5.3)			7	
Sadness (yes)		1.4 (0.8, 3.4)			3	
Skin lesion (yes)		2.0(1.1, 3.8)	2.1 (1.2, 3.7)		7	7
Recommended ART						

Family vs Friend	2.8 (1.3, 6.2)	4.1 (1.5, 12.4)	2.8 (1.1, 7.7)	10	14	10
Other vs Friend	1.1 (0.5, 2.6)	1.6 (0.8, 3.4)	1.0 (0.4, 2.9)	1	5	0*
Provider (nurse/doctors) vs Friend	2.4 (1.2, 5.1)	3.4 (1.3, 9.6)	2.2 (0.9, 5.6)	9	12	8
Lipodystrophy (no)		1.7 (0.8, 3.4)			5	
Log CD4 (per 1 unit increase)		13.8 (5.0, 43.3)	12.9 (5.6, 32.8)		26 *range: 0-78*	26 *range: 0-78*
ARV duration (per 1 month)		1.0 (0.97, 1.01)			0	
D4T vs Other	1.7 (1.0, 2.9)	2.3 (1.1, 4.7)		5	8	
ZDV vs Other	0.9 (0.5, 1.6)	1.6 (0.7, 3.6)		0*	4	
Adherence Counseling Sessions						
0-1 vs 2-4		2.6 (0.9, 7.2)			9	
5+ vs 2-4		1.1 (0.5, 2.2)			1	
Recall ART (TV/radio)		4.0 (1.6, 10.5)	3.9 (1.7, 9.4)		14	14
Fluconazole	5.7 (1.7, 25.9)	3.4 (0.5, 25.5)		17	12	
Ethambutol	3.0 (0.8, 12.6)	3.4 (0.5, 23.4)		11	12	
Adherence (per 0.1 increase)		1.6, (1.2, 2.1)	1.6 (1.2, 2.1)		4 *range: 0-24*	5 *range: 0-30*
			Theoretical Maximum Scores	89	283	207

**Scores have been adjusted. When using the same reference groups as that which was used in the full model, indicators were actually partially protective (odds ratios (<1). As such, these variables were no longer considered "risk factors" and were, therefore, assigned point values of 0.*

Table 4: Baseline, RFVF, and Restricted predictive characteristics of VF as an outcome for every one unit increase in cumulative score percentage

Model Fit Statistics and Prediction Characteristics			
	Baseline Index	**RFVF Index**	**Restricted Index**
AIC	508.1	414.8	420.9
Somers' D	0.50	0.769	0.696
ROC AUC/ c-statistic	0.75	0.847	0.848

Figure 4a Baseline Index ROC curve

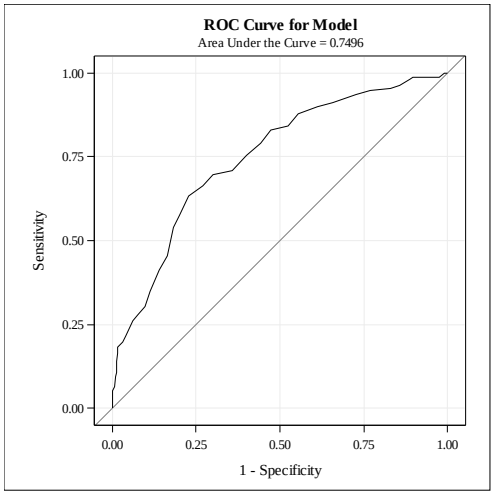


Figure 4b RFVF Index ROC curve

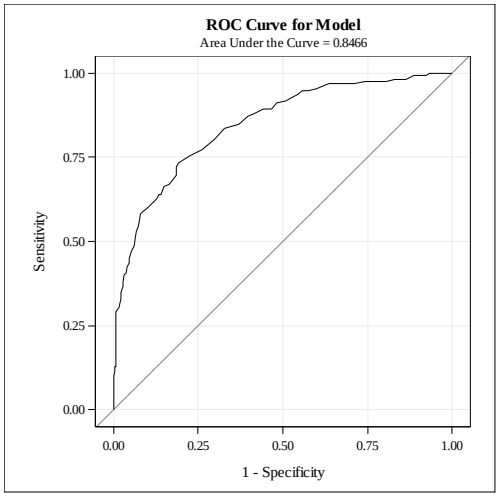


Figure 4c Restricted Index ROC curve

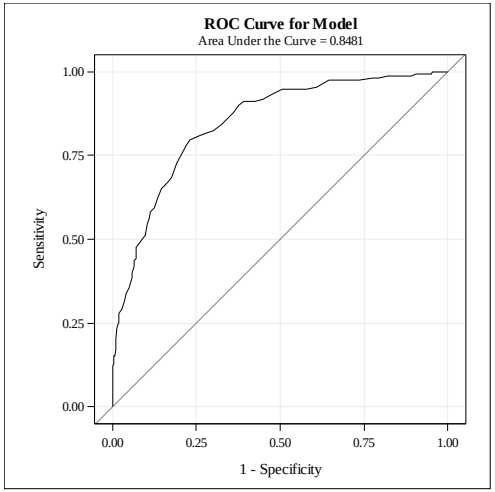
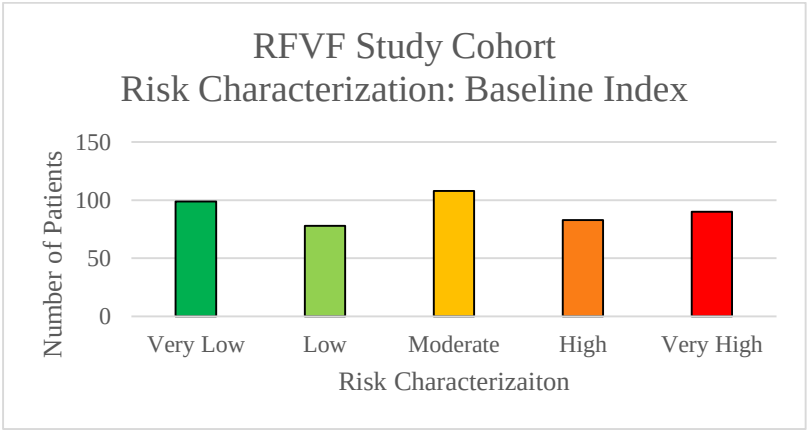


Table 5: RFVF study cohort risk characterization classifications using score quintiles derived from the Baseline, RFVF, and Restricted Indices

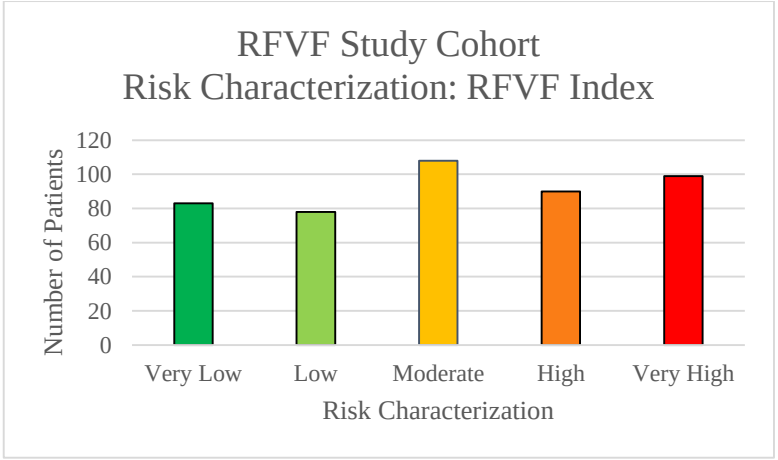
Index Classification	Baseline Index N (%)	RFVF Index N (%)	Restricted Index N (%)
Very Low	77 (16.8)	99 (21.6)	95 (20.7)
Low	91 (19.9)	78 (17.0)	83 (18.1)
Moderate	122 (26.6)	108 (23.6)	95 (20.7)
High	79 (17.3)	83 (18.1)	89 (19.4)
Very High	89 (19.4)	90 (19.7)	96 (21.0)

Figure 5: RFVF study population risk characterization distributions derived from Baseline, RFVF, and Restricted Indices, respectively

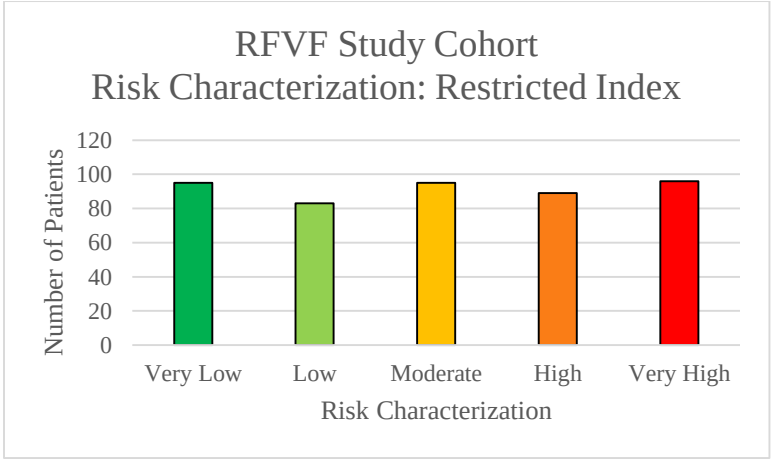
5a



5b



5c



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