Refill Non-Adherence and Immunologic Failure as Identifiers of HIV Virologic Treatment Failure among Adolescents in Botswana

Leah Genn
REFILL NON-ADHERENCE AND IMMUNOLOGIC FAILURE AS IDENTIFIERS OF HIV VIROLOGIC TREATMENT FAILURE AMONG ADOLESCENTS IN BOTSWANA

By

LEAH GENN

Examiner Committee:

Neil Abell, Thesis Director
College of Social Work

Lisa Johnson, Member
College of Medicine

Mark J. Kasper, Member
College of Human Sciences

Lynn Panton, Member
College of Human Sciences

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The members of the Defense Committee approve the thesis of Leah Genn defended on April 11, 2016.

\[\text{Signature}\]

Dr. Neil Abell
Thesis Director

\[\text{Signature}\]

Dr. Lisa Johnson
Outside Committee Member

\[\text{Signature}\]

Dr. Mark J. Kasper
Committee Member

\[\text{Signature}\]

Dr. Lynn Panton
Committee Member
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Abstract

Background

Adolescents worldwide are the only age group for which AIDS-related deaths have risen between 2001 and 2012 and medication non-adherence is a major cause. In low resource settings, low-cost, simple methods of tracking adherence are desirable and in adults, pharmacy refill data have been shown to reflect virologic treatment outcomes better than immunologic response to treatment. The utility of pharmacy refill data in treatment-experienced adolescents is unknown and may differ from adults because they may not be responsible for picking up medications, but have autonomy over their medication-taking.

Methods

We evaluated data from 291 adolescents on antiretroviral therapy (ART) in Botswana. The main outcome measure was virologic treatment failure, defined as two consecutive viral load measurements $\geq 400$ copies/mL during 24 months of follow-up. Pharmacy refill non-adherence was defined as two consecutive refill adherence measurements <95% during 24 months of follow-up. Immunologic failure was defined as a worsening WHO/CDC immune status classification from baseline category during 24 months of follow-up. The associations between virologic failure and both comparator measures were assessed using receiver operating characteristic (ROC) analysis. The ROC curves were compared through a non-parametric comparison of AUCs using STATA software.

Results

Fifty-three (18%) adolescents experienced virologic failure. One hundred nine adolescents had immunologic failure. One hundred twenty-eight adolescents had refill non-adherence. Immunologic failure and refill non-adherence had similarly poor discriminative ability for indicating virologic failure (AUCs of 0.66 and 0.60, respectively ($p = 0.17$)). Sensitivity and specificity for the strength of immunologic failure in predicting virologic treatment failure were 64% (95% CI: 46-74%) and 68% (95% CI: 53-66%); the same measures for refill non-adherence were 60% (95% CI: 50-77%) and 60% (95% CI: 62-74%).

Conclusion

Both immunologic failure and refill non-adherence had low sensitivity and specificity for identifying virologic treatment failure in adolescents in Botswana. The lack of viable alternatives highlights the urgent need for more access to virologic testing.
I. Introduction

The human immunodeficiency virus (HIV) affects more than 33.3 million people worldwide. Over 3.1 million of these individuals are adolescents aged 10-19 years living in low- and middle-income countries (UNAIDS, “Global Report”). Botswana has one of the highest HIV prevalence rates in the world at 18.5% (UNAIDS, “Progress Report”). In African countries such as Botswana, AIDS is the leading cause of death among adolescents. In 2013, more than 500 adolescents out of the roughly 11,000 adolescents living with HIV in the country died due to an AIDS-related illness (UNAIDS, “Global Report”). In comparison, 126 young people (age 13-24) died from AIDS in the United States in 2012 (“HIV Among Youth”). Adolescents are the only age group around the world in which AIDS deaths have risen between 2001 and 2012 (UNAIDS, “Global Report”).

The infants perinatally infected with HIV during the height of disease transmission, mainly between the years 2000 and 2006, are reaching adolescence now. Some have been taking antiretroviral therapy (ART) for the better part of ten years. During adolescence, youth are more likely to participate in risky behaviors and to deviate from their normal routines (Guiella, Georges, and Madise, 182). Teenagers with HIV may be less inclined to be adherent to their medications and their overall health could be at risk for premature death. It is therefore very important to have the ability to measure adherence to medications, especially in low resource settings.

Adherence is strongly associated with virologic response in a dose-dependent manner (Gross, et al, 187). Continued adherence to an effective regimen can allow a patient to achieve and sustain undetectable levels of the HIV virus in his or her body. Poor adherence is the most common cause of virologic treatment failure, which can lead to immunologic decline, or a weakening of the immune system. It has been found that adults who are less than 80% adherent to their ART treatment have a three times greater risk for mortality than
those who are more than 80% adherent (Nachega, et al, 82). Additionally, at least 95% ART adherence is required to achieve durable viral suppressions. If medications are not taken as prescribed, the virus can also mutate and become resistant to the treatment; drug resistance is a major threat to the effectiveness of ART (Ndubuka and Ehlers, 1325). Viral load and resistance test data can be used to determine whether a new line of ART therapy is necessary to suppress the virus.

There are multiple ways to measure medication adherence in an HIV-infected adolescent population. Several examples include patient self-report, parent-reported adherence, pill counts, medication event monitoring system (MEMS) caps, and pharmacy refill records. MEMS caps are considered the gold standard in measuring medication adherence and MEMS data have been shown to have a strong association with virologic treatment failure (Eby, et al.). A microelectronic chip contained in the cap registers every bottle opening and it is assumed that each opening is indicative of the patient ingesting one dose of his or her medication (Farmer, 1076). In low resource settings, however, low-cost, simple methods of tracking adherence are necessary. For this reason, researchers have increasingly turned to pharmacy refill records. Using the dates that prescriptions are refilled, a patient’s adherence can be determined based on whether or not he or she returns to get medications before the prior prescription should have been completed if all medicines were being taken as prescribed. These data are known as the patient’s “medication: possession ratio.” An additional advantage of pharmacy refill data is the objective nature of the data. These records have also been found to be more strongly associated with virologic response outcome than self-reported adherence data (Grossberg and Gross, 188).

In low-resource settings, immunologic criteria are often used in lieu of expensive viral load testing to identify individuals with treatment failure (Bisson, et al, 778). CD4 cell counts measured at multiple times during ART treatment can provide an idea of the progression of
the HIV virus in individual patients. In adults, however, CD4 cell count criteria miss nearly half of all virologic treatment failure cases due to the fact that an immunologic decline tends to follow, instead of precede, an increase in viral load for some patients (Rawizza, et al, 1287). The high number of false positives for treatment failure based on CD4 data is also unacceptably high, causing many researchers to study whether other variables are more predictive of virologic treatment failure (Davies, et al, 1369).

A 2008 study found that pharmacy refill data were more accurate predictors of virologic treatment failure than CD4 changes among adults newly starting antiretroviral treatment in Botswana (Bisson, et al, 777). This association has yet to be studied in a treatment-experienced population or among adolescents, for whom pharmacy refill might be less predictive if the adolescents have autonomy over medication-taking but frequently do not obtain their own refills. An additional issue that has yet to be studied is the association between refill adherence records and MEMS adherence data.

II. Terminology

**Acquired immunodeficiency syndrome (AIDS):** a disease of the immune system caused by the HIV virus which destroys CD4+ T-lymphocyte cells; most advanced stage of HIV infection that leaves the body vulnerable to life-threatening infections and cancers

**Antiretroviral therapy (ART):** a combination of several antiretroviral medications taken to slow the rate at which the HIV virus multiplies in the body

**Autonomy Over Medication-Taking:** when an individual chooses to independently take his or her medication as prescribed; in this data set, ranges of autonomy include “I took my medicines by myself with nobody reminding me or watching me”, “I took my medicines by myself, but somebody reminded me to take them”, “somebody gave me my medicines when
it was time to take them but I took them by myself” and “somebody gave me my medicines and watched me take them”

**Autonomy Over Obtaining Refills:** when an individual has the freedom and takes the responsibility to pick up his or her medication refills at the pharmacy or clinic each month; in this data set, ranges of autonomy include “I went to the clinic alone and picked up my medicines from the clinic myself”, “I went to the clinic with someone else to get my medicines” and “somebody picked up my medicines for me”

**Clinical failure:** new or recurrent clinical event indicating severe immunodeficiency after six months of effective treatment

**CD4 Count:** laboratory test that measures the amount of CD4+ T-lymphocytes present in the bloodstream at any given time

**CD4+ T-lymphocyte:** a type of white blood cell that is destroyed by the HIV virus; an important indicator of the overall strength of a patient’s immune system and a strong predictor of overall HIV progression

**Human immunodeficiency virus (HIV):** retrovirus (a virus that integrates its viral DNA into the DNA of the host cell, allowing it to replicate) that is transmitted through direct contact with HIV-infected body fluids and destroys a patient’s immune system; causes acquired immunodeficiency syndrome (AIDS)

**Immunologic failure:** a worsening immune status classification from baseline World Health Organization (WHO) and/or Centers for Disease Control (CDC) category (see Table 1 on page 14)

**Likelihood ratio:** the likelihood of that a given test result would be expected in a patient with a target disorder compared to the likelihood that the same result would be expected in a patient without the target disorder
Medication adherence: the extent to which a patient’s behavior, with respect to taking medication, corresponds to agreed recommendations from a healthcare provider

Mother-to-Child Transmission of HIV: when an HIV-infected mother passes the HIV virus to her child during pregnancy, labor and delivery, or breastfeeding

Negative predictive value (NPV): probability that participants with a negative screening test truly do not have the disease

Positive predictive value (PPV): probability that participants with a positive screening truly have the disease

Refill non-adherence: less than 95% adherence to prescribed medication regimen; refill adherence percentage is calculated according to the following equation: \( \frac{\text{number of pills remaining from previous refill}}{\text{(number of days between current visit and previous refill) \times \text{number of prescribed pills per day}} \times 100\% \)

Sensitivity: the probability that a medical test will detect correctly the condition being tested for in people who actually have the disease

Specificity: the probability that a medical test will produce correctly a negative result in people who do not have the disease

Treatment-experienced: when an HIV-infected person is currently taking or has previously taken ART

Treatment failure: when an antiretroviral regimen (ARV) is incapable of controlling a patient’s HIV infection; can be clinical failure, immunologic failure, virologic treatment failure, or any combination of the three; factors that contribute include drug resistance, drug toxicity, or poor treatment adherence

Viral load: the amount of HIV virus in a sample of blood; reported as the number of HIV RNA copies per milliliter of blood
**Viral suppression:** when a patient’s ART successfully reduces his or her viral load to an undetectable level (generally below either 400 or 1000 copies of virus per mL of blood)

**Virologic treatment failure:** when ART fails to suppress and sustain a patient’s viral load to undetectable levels

III. **Reasons for Study**

The accuracy of prescription refill data identifying patients with virologic treatment failure has yet to be studied in an adolescent population. This study is necessary to ensure that it is not just assumed that adolescents act or respond in the same way as adults. Currently, in clinics, adolescent and adult patients tend to be monitored in the same ways. Adolescents around the world, including the population in this study, are afforded differing levels of autonomy over decisions that impact their health. Some of the patients in this study are unaware of their HIV status and simply are told to take their medication daily by their caregiver(s). Others are largely independent and are capable of picking up their medication at the pharmacy and following their prescription regimen as prescribed. Therefore, because adolescents are often different from adults in terms of the autonomy they have over obtaining their medication refills and taking their medications as prescribed, the accuracy of monitoring strategies likely differs in this population.

The dearth of studies for this specific HIV-positive population is troubling, especially since youth have rising mortality rates, despite decreasing mortality in all other age groups (UNAIDS, “Global Report”). In addition, very few studies have been conducted on individuals who are treatment-experienced. These patients have been on ART therapy for years and most are largely healthy with undetectable viral loads. Due to an increase in risky behaviors during these years, it is imperative to measure adolescents’ adherence and to determine the best methods for detecting poor adherence and supporting adherence in those
at risk of treatment failure (Guiella, Georges, and Madise, 182). HIV-positive adolescents face unique challenges and should therefore be studied more diligently to ensure they are receiving the best care for their illness.

IV. Expectations for Study

It is expected that the results for this study will be different from the 2008 adult study (Bisson, et al.). It is assumed that adult patients will pick up their medications from pharmacies themselves and proceed to follow the regimen for the prescription time period. With adolescents, however, the case may be different. For example, caregivers may pick up the prescription with or without the adolescent present and the adolescent may therefore not take ownership of the responsibility to take the medication as prescribed. It is expected that pharmacy refill records will overestimate the participant’s adherence and ultimately fail to identify virologic treatment failure as accurately as they do for an adult population.

It is also expected that refill adherence records will be relatively similar to adherence values measured from MEMS caps. However, it is hypothesized that pharmacy refill records will overestimate the patient’s adherence due to the common occurrence of patients throwing away unused doses (Okatch, et al.).

V. Objectives of Study

In treatment-experienced HIV-infected adolescents, this study plans to:

Aim 1: Study the accuracy of prescription refill adherence records and CD4 counts to identify virologic treatment failure

Aim 1a. Determine if a significant association exists between refill non-adherence and virologic treatment failure.
**Null Hypothesis 1a:** There is no association between refill non-adherence and virologic treatment failure

**Alternative Hypothesis 1a:** Adolescents with refill non-adherence are more likely to have virologic treatment failure

**Aim 1b.** Determine if a significant association exists between immunologic failure and virologic treatment failure.

**Null Hypothesis 1b:** There is no association between CD4 decline and virologic treatment failure

**Alternative Hypothesis 1b:** Adolescents with a decline in CD4 category are more likely to have virologic treatment failure than those who remained in the same CD4 category as their baseline

**Aim 1c.** Determine whether pharmacy refill records or CD4 counts better identify virologic treatment failure.

**Null Hypothesis 1c:** There is no difference between either refill non-adherence or CD4 decline in identification of virologic treatment failure

**Alternative Hypothesis 1c:** Pharmacy refill adherence records are not as accurate as CD4 counts for detecting current virologic treatment failure

**Aim 2:** Study the relationship between prescription refill adherence records and medication adherence as measured by Medication Event Monitoring System (MEMS) caps

**Null Hypothesis 2:** There is no association between refill adherence records and MEMS-measured medication adherence

**Alternative Hypothesis 2:** There is a significant association between refill adherence records and MEMS-measured medication adherence
VI. Methods

Study Participants: This study will evaluate data from 291 adolescent patients currently enrolled in ART at the Botswana-Baylor Children’s Clinical Centre of Excellence in Gaborone, Botswana with the support of the Botswana National HIV Treatment program. This program began in January 2002 and works to improve the lives of HIV-infected children, adolescents, and adults. Adolescents included in this study are participating in an ongoing longitudinal study that began in 2012 and is conducted by a research team at the Children’s Hospital of Philadelphia (CHOP) and the University of Pennsylvania. Study participants are admitted to the study on a rolling admission basis.

The population is primarily perinatally infected, although patients are not excluded based on other modes of infection. The population has a median age of 13.4 years old (IQR: 11.8-15.6 years) and has been on ART for a median of 7.5 years (IQR: 5.3-8.8 years) at study entry. The study was designed so that half of the participants are currently taking non-nucleoside reverse transcriptase inhibitors (NNRTI)-based regimens and the other half are taking protease inhibitors (PIs)-based regimens. The time period for this particular study is the first twenty-four months after a patient’s entry into the ongoing longitudinal study.

Data Collection and Relevant Definitions: Botswana treatment guidelines recommend that CD4+ T-lymphocyte counts and HIV viral load be measured every 3-6 months. All results of laboratories taken during the study period are recorded in the electronic study database Research Electronic Data Capture (REDCap).

For this study, virologic treatment failure is defined as 2 consecutive viral load measurements ≥400 copies/mL (a “detectable” level) during the study follow-up period. We defined refill non-adherence as both a dichotomous and a continuous variable. The dichotomous variable is defined as two consecutive adherence measurements <95% according to prescription refill adherence records. The continuous variable is defined as the
percentage of refill adherence measurements <95% during the follow-up period. For Aim 2, MEMS non-adherence was defined as two consecutive adherence measurements <95% according to MEMS adherence records.

Immunologic failure is defined as a worsening immune status classification from baseline category. For these adolescents, categories were established in the manner described in Table 1 based on recommendations by the World Health Organization (WHO) and the Centers for Disease Control (CDC). The WHO standards are based on absolute CD4 counts and include 4 categories. The CDC standards are based on the percent of CD4 cells present and include 3 categories. If a participant moved to a higher numbered category, he or she will be determined to be failing immunologically.

If a participant started at category 4 for absolute CD4 count or category 3 for percent CD4 count at month 0 of the study, he or she will be determined to be in immunologic failure if his or her absolute CD4 count or CD4% drops by ≥25% of baseline. For these twelve individuals, their first follow-up CD4 count acted as their initial baseline CD4 count due to the fact that their initial CD4 count categorized them already at the lowest level making detection of a subsequent drop in category impossible.

<table>
<thead>
<tr>
<th>Category</th>
<th>Absolute CD4 Count</th>
<th>Percent CD4 Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 500</td>
<td>&gt; 28%</td>
</tr>
<tr>
<td>2</td>
<td>350-499</td>
<td>15-28%</td>
</tr>
<tr>
<td>3</td>
<td>200-349</td>
<td>&lt; 15%</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 200</td>
<td>N/A</td>
</tr>
</tbody>
</table>

_Institutional Review Board (IRB) Approval Timeline:_ IRB approval from FSU was necessary due to the analysis of adolescent data in this study. Approval was provided on October 19, 2015.
**Data Analysis:** We utilized logistic regression analysis to measure if associations were present between immunologic failure and virologic treatment failure and between refill non-adherence and virologic treatment failure. Since associations were found to be present (Aims 1a and 1b), additional analyses were completed to determine which independent variable was more accurate at identifying those with treatment failure (Aim 1c). We utilized receiver operating characteristic (ROC) curves to further determine the statistical association between the comparator measures and the outcome measure (Table 2). Test characteristics were then calculated to measure the sensitivity and specificity of both refill non-adherence and immunologic failure. Sensitivity was defined as the accuracy in predicting the status (or proportion) of patients with virologic treatment failure who had immunologic failure or had refill non-adherence, and specificity is defined as the accuracy in predicting the status (or proportion) of patients without virologic treatment failure who did not exhibit immunologic failure or who achieved refill adherence.

MEMS data were analyzed using the application known as MedAmigo that records the dates and times of every cap opening during the study period. We utilized the data from MedAmigo to calculate percent adherence during our time period of interest. Logistic regression analysis was completed to determine the association between refill non-adherence and MEMS adherence data (Table 2). Test characteristics were calculated to further determine the strength of refill non-adherence in identifying MEMS non-adherence.

For each aim, a secondary analysis was completed to determine if there is any difference in the accuracy of refill adherence records or CD4 counts in identifying patients with virologic treatment failure based on whether or not they are currently in school or have dropped out of school during the study period. After analysis was completed, it was determined that there were too few adolescents out-of-school in our cohort for this study to
be properly powered to draw any significant conclusions. Results for this analysis have therefore been edited out of the final draft of this study.

An additional secondary analysis was completed to see how gender impacts the accuracy of the CD4 counts or refill adherence records to identify patients whose treatment is failing to suppress the virus in their body. A final secondary analysis will be conducted to determine if ART regimen also impacts the relationship between the comparator measures and virologic treatment failure.

Statistical analyses will be performed using Stata, version 14 as defined below. Statistical significance will be set at \( p < 0.05 \) (Table 2).
<table>
<thead>
<tr>
<th>Hyp.</th>
<th>Hypothesis Stated</th>
<th>Exposure (Independent Variable)</th>
<th>Outcome (Dependent Variable)</th>
<th>Test(s) to be Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Rx refill non-adherence is associated with VF</td>
<td>Refill non-adherence (&lt;95% adherence)</td>
<td>Virologic treatment failure: ≥2 consecutive VL &gt;400 copies/mL</td>
<td>Logistic regression</td>
</tr>
<tr>
<td>1b</td>
<td>A worsening WHO/ CDC immune status is associated with VF</td>
<td>Immunologic failure: worsening CD4 category</td>
<td>Virologic treatment failure: ≥2 consecutive VL &gt;400 copies/mL</td>
<td>Logistic regression</td>
</tr>
<tr>
<td>1c</td>
<td>Rx refill records are &lt; accurate than CD4 counts in identifying VF</td>
<td>Rx refill records, CD4 counts</td>
<td>Virologic treatment failure</td>
<td>Comparison of ROC curves, Calculation of Test Characteristics</td>
</tr>
<tr>
<td>2</td>
<td>Rx refill records are predictive of MEMS adherence data</td>
<td>Rx refill records</td>
<td>MEMS adherence data</td>
<td>Logistic Regression</td>
</tr>
</tbody>
</table>

**Missing Data:** Before any data analysis began, a thorough investigation of the quality of the data was conducted. Based on these results, data for nine adolescents were taken out of the final analysis because these participants left the study before 24 months of data could be collected.
VII. Results

Prevalence of Failure

Participant characteristics are presented in Table 3. There were slightly more females than males with most of the sample still attending school. The median number of refills during the twenty-four month period for which data were collected for each participant in this study was eighteen, with a range of 7 to 25. Two hundred forty-two participants, or 83% of the study population, had at least one refill adherence measurement less than 95%. Following the dichotomous definition of refill non-adherence, 128 participants, or 44% of the study population, experienced refill non-adherence during the same time period. These adolescents had at least two consecutive adherence measurements less than 95% (Table 3).

Table 3. Characteristics of Adolescent Study Population (N = 291)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median [IQR] Time on ART</td>
<td>7.5 [5.3-8.8] years</td>
</tr>
<tr>
<td>Sex</td>
<td>47% male, 53% female</td>
</tr>
<tr>
<td>Schooling Status*</td>
<td>270 in school, 21 out of school</td>
</tr>
<tr>
<td>ART Regimen</td>
<td>147 NNRTI, 144 PI</td>
</tr>
<tr>
<td>Number (%) of Patients with Refill Non-Adherence</td>
<td>128 (44.0%)</td>
</tr>
<tr>
<td>Median [IQR] Number of Refills During Study Period</td>
<td>18 [16-20]</td>
</tr>
<tr>
<td>Number (%) of Patients with Immunologic Failure</td>
<td>109 (37.5%)</td>
</tr>
<tr>
<td>Number (%) of Patients with Virologic Failure</td>
<td>53 (18.2%)</td>
</tr>
</tbody>
</table>

IQR= interquartile range; NNRTI= non-nucleoside reverse transcriptase inhibitors; PI= protease inhibitors
*at any point during the 24-month study period

Twelve participants began the study at the highest immunologic category designation of 4, meaning that their CD4+ T-cell count was below 200. The majority of the study population, 241 adolescents, began the study at the lowest immunologic category designation of 1, meaning that they had a CD4 count above 500 at their study initiation point. Over the course of the study, 71 participants worsened in WHO absolute CD4 count and 83 participants worsened in CDC CD4 count.
percent CD4 count. One hundred nine adolescents, or 37.5% of the study population, worsened at least once according to either standard (Table 3).

Ninety-nine adolescents had a “detectable” viral load greater than 400 copies/mL of the HIV virus in their bloodstream at least once during the study period. Sixty-two participants had at least two detectable viral loads during the same time period. According to this study’s definition, participants experienced virologic failure if they had two consecutive viral loads above 400 copies/mL. Fifty-three participants, or eighteen percent of the study population, experienced virologic failure at least once during the 24-month study period (Table 3).

Strength of Refill Non-Adherence to Identify Virologic Failure

One hundred twenty-eight participants were refill non-adherent during the 24-month study period. Thirty-two of these individuals also experienced virologic failure during the same time period; because virologic failure is our “gold standard” for treatment success, these individuals are considered “true positives.” Therefore, 96 of these individuals did not have at least two consecutive detectable viral loads during the study period; these individuals are considered “false positives.” There were 163 participants who sustained excellent refill adherence. One hundred forty-two of these adolescents also did not experience virologic failure; these participants are considered “true negatives.” Finally, the twenty-one adolescents who achieved refill adherence but who experienced virologic failure are considered “false negatives” (Table 4).
Table 4. Diagnostic Test Evaluation for Refill Non-Adherence in Adolescent Cohort (N = 291)

<table>
<thead>
<tr>
<th>Test: Refill Non-Adherence</th>
<th>Disease: Virologic Failure</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>32 True Positive</td>
<td>96 False Positive</td>
</tr>
<tr>
<td></td>
<td>21 False Negative</td>
<td>142 True Negative</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>238</td>
</tr>
</tbody>
</table>

There is a statistically significant association between refill non-adherence and virologic failure. The odds of having treatment failure is 2.25 times higher among individuals with refill non-adherence (95% CI: 1.23-4.14; p<0.01) (Table 5) (Immunologic failure is further discussed below). Test characteristics for refill non-adherence as an exposure to the outcome of virologic treatment failure are outlined in Table 6. Ninety-five percent confidence intervals for sensitivity and specificity are 46.0-74.0% and 53.1-66.0%, respectively.

Table 5. Odds Ratio and Statistical Significance of Comparator Measures to Identify Virologic Failure in Adolescent Cohort (N = 291)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunologic Failure (Absolute)</td>
<td>5.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Immunologic Failure (Percentage)</td>
<td>3.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Immunologic Failure (Absolute or Percentage)</td>
<td>3.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Refill Non-Adherence</td>
<td>2.25</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Table 6. Test Characteristics for Identification of Virologic Failure in Adolescent Cohort (N = 291)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refill Non-Adherence</td>
<td>60.4%</td>
<td>59.7%</td>
<td>25.0%</td>
<td>87.1%</td>
<td>1.50</td>
<td>0.66</td>
</tr>
<tr>
<td>Immunologic Failure (Absolute or Percentage)</td>
<td>64.2%</td>
<td>68.5%</td>
<td>31.2%</td>
<td>89.6%</td>
<td>2.04</td>
<td>0.52</td>
</tr>
</tbody>
</table>

PPV= positive predictive value; NPV= negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio

ROC analysis resulted in an area under the curve (AUC) of 0.60 for the dichotomous refill non-adherence variable and an AUC of 0.69 for the continuous refill non-adherence variable (Appendix Figure 1). There was no difference between the AUCs resulting from ROC curves for male and female participants (0.66 and 0.72, respectively; p > 0.4) (Appendix Figure 3). Similarly, there was no significant difference between the AUCs resulting from ROC curves for participants on NNRTI and PI ART regimens (0.72 and 0.68, respectively; p > 0.6) (Appendix Figure 4).

Strength of Immunologic Failure to Identify Virologic Failure

One hundred nine participants had immunologic failure during the 24-month study period. Thirty-four of these individuals also experienced virologic failure during the same time period; these individuals are considered “true positives.” Therefore, 75 of these individuals did not have at least two consecutive detectable viral loads during the study period; these individuals are considered “false positives.” There were 182 participants who did not experience immunologic failure. One hundred sixty-three of these adolescents also did not experience virologic failure; these participants are considered “true negatives.” Finally, the nineteen adolescents who did experience virologic failure are considered “false negatives” (Table 7).
There were statistically significant associations between the three distinct categories of immunologic failure and virologic failure. The odds of having treatment failure is 5.64 times higher among individuals with immunologic failure based on WHO absolute CD4 count (95% CI: 3.00-10.6; p<0.001). The odds of having treatment failure is 3.38 times higher among individuals with immunologic failure based on CDC percent CD4 count (95% CI: 1.82-6.25; p<0.001). Finally, the odds of having treatment failure is 3.89 times higher among individuals with immunologic failure based on either standard (95% CI: 2.08-7.26; p<0.001) (Table 5).

Test characteristics for immunologic failure as an exposure to the outcome of virologic treatment failure are outlined in Table 6. Ninety-five percent confidence intervals for sensitivity and specificity are 49.8-77.0% and 62.2-74.3%, respectively.

ROC analysis resulted in an area under the curve (AUC) of 0.66 (Appendix Figure 5). There was no difference between the AUCs resulting from ROC curves for male and female participants (0.64 and 0.69, respectively; p > 0.5) (Appendix Figure 7). Similarly, there was no significant difference between the AUCs resulting from ROC curves for participants on NNRTI and PI ART regimens (0.63 and 0.66, respectively; p > 0.7) (Appendix Figure 8).
Comparison of Refill Non-Adherence and Immunologic Failure as Identifiers of Virologic Treatment Failure

When comparing both dichotomous definitions of refill non-adherence and immunologic failure, there is no statistical difference in the AUCs of the resulting ROC curves for identifying virologic treatment failure (0.60 and 0.66, respectively; difference = 0.06; \( \chi^2 = 1.87; p=0.17 \)) (Appendix Figure 9).

Comparison of Adherence based on Pharmacy Refill Data and MEMS Data

There is a strong statistically significant association between refill and MEMS adherence data. The odds of having MEMS adherence <95% is 12.9 times higher among individuals with refill adherence <95% (95% CI: 5.91-28.0; p<0.001). Test characteristics for the comparison between adherence as measured by pharmacy refill and adherence measured by MEMS is outlined in Table 8. Ninety-five percent confidence intervals for sensitivity and specificity are 40.0- 55.4% and 87.4- 97.1%, respectively.

Table 8. Relationship between Refill Adherence Data and MEMS Adherence Data

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Odds Ratio</th>
<th>p-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refill Non-Adherence</td>
<td>12.9</td>
<td>&lt;0.001</td>
<td>47.7%</td>
<td>93.4%</td>
<td>91.0%</td>
<td>56.0%</td>
<td>7.21</td>
<td>0.56</td>
</tr>
</tbody>
</table>

VIII. Discussion

These results demonstrate that both refill non-adherence and immunologic failure have low sensitivity and specificity for identifying virologic treatment failure in treatment-experienced adolescents. The two comparator measures were similarly suboptimal for indicating virologic failure in our cohort. A diagnostic test with an AUC between 0.6 and 0.7, the range in which ours fell, is widely considered to be a poor indicator for an outcome measure (“Diagnostic Tests”).
Both refill non-adherence and immunologic failure misclassified a wide number of adolescents. This is in contrast to the 2008 treatment-naïve adult study based in Botswana that found that pharmacy refill data reflected virologic treatment outcomes better than immunologic response to treatment. Both comparator measures also had larger AUCs (0.85 for refill non-adherence and 0.75 for immunologic failure), illustrating that they were both stronger in identifying virologic failure in treatment-naïve adults than in our treatment-experienced adolescent cohort. Both studies had similar prevalence rates of virologic treatment failure and refill non-adherence, while the rate of immunologic failure was much smaller in the adult study (10% vs. 37%) (Bisson, et al, 780).

Pharmacy refill data could be a poorer indicator of virologic treatment failure in adolescents than in adults for several reasons. First, adolescents may have autonomy over taking their medications, but not over obtaining their own pharmacy refills. Discordance between responsibility over medication-taking and responsibility over obtaining refills may lead to misclassification of good refill adherence as indicative of good medication adherence. Adolescents may fail to consistently take ownership over their medication adherence and may fail to follow their treatment regimen as prescribed. In a setting such as ours where refill adherence is tracked by the clinical team, adolescents may also be motivated to make it appear that they are taking their pills well by obtaining refills at the appropriate time, even if they have not completed their medications. Adolescents sometimes fail to bring their pill bottles to the pharmacy, throw out pills, or otherwise “lose” them in order to make it seem like the correct number of pills have been ingested (Okatch, et al). Refill adherence rates for this adolescent cohort may thus over-represent doses taken. This certainly would decrease the sensitivity of our refill adherence variable in identifying virologic failure and doses not taken. Not surprisingly, refill adherence was relatively specific as an indicator of doses not taken since patients who do
Several secondary analyses were completed to determine if specific study population characteristics had an effect on the strength of either of the comparator measures to identify adolescents with virologic failure. It was hypothesized that schooling status may have an impact because a particularly vulnerable life event for adolescents occurs when they are aging out of the school system but still lack necessary job training. We cannot draw specific conclusions on this relationship, however, simply due to the fact that our cohort had such a small percentage (7%) of adolescents out-of-school at any point during the 24-month follow-up. No effect was found when considering the characteristics of gender and ART regimen as well.

The relationship between adherence measurements provided by pharmacy refill data and MEMS data is important to study to determine if the cheaper, easy-to-use refill data could be a more cost-effective method of measuring adherence than the MEMS technology. Based on our results, when the adolescent is non-adherent based on refill records, there is a very good chance that his/her MEMS data also show non-adherence. Also, when an adolescent is adherent based on MEMS data, there is a very good chance that his/her refill data also show adherence. However, refill data have a similar chance of correctly identifying refill non-adherence when an adolescent is MEMS non-adherent to flipping a coin; the resulting specificity value is less than 50%. If we only had refill adherence data to measure adherence for our population, we would be misclassifying 89 individuals (31%) as adherent who really are not adherent based on their MEMS data. Therefore, in our adolescent cohort, MEMS adherence data should continue to be considered the gold standard and used as the primary adherence measurement whenever possible.

This study is important and particularly timely as public health researchers and clinicians are currently debating the need for frequent CD4 monitoring among stable patients. Our adolescent cohort is fairly stable as a whole, with fewer than one-in-five individuals experiencing
virologic failure during the study follow-up period. Since both pharmacy refill data and CD4 counts are poor identifiers of possible treatment failure for treatment-experienced adolescents, there is a current need for alternative monitoring methods or increased access to viral load tests. Adolescents continue to have worse adherence to their medication than adults and even among our relatively stable cohort, almost half of all individuals failed to adhere to their medication at least once in the 24-month follow-up. Public health experts must understand that adolescents behave differently from adults and guidelines and recommendations for their treatment should be determined separately from the adult HIV-infected population.

A clinical implication of these results is that systematic monitoring of pharmacy refill adherence may not be considered a reasonable alternative to CD4 count monitoring for identification of possible virologic treatment failure in an adolescent cohort. In low-resource settings, pharmacy refill data may not be the best approach to use in lieu of CD4 count or viral load monitoring. That is not to say, however, that there is no need to continue pharmacy-based adherence monitoring, especially for an adolescent population. During regular clinic appointments, clinicians must balance stressing the importance of medication adherence with ensuring that adolescents do not simply come back at the right times with the right number of pills to appear adherent.

IX. Delimitations of Study

This study included only young men and women aged 10-19 years living with HIV in and around Gaborone, Botswana. All other populations were excluded.

MEMS is considered a “gold standard” for adherence monitoring. However, we cannot be sure that when a patient opens a medication bottle, he or she ingests the medication. Similarly, the refill adherence percentage, which is calculated based on the assumption that the pills not
returned to the pharmacy were ingested, is taken to accurately reflect the number of pills that were ingested between refills.

We determined to include in our immunologic classification of participants a portion of patients for whom initial status could not be absolutely determined. This distinction is due to the fact that they were already classified at the bottom of their respective categorizations. Therefore, in order to determine absolute baseline immunologic values, values were extrapolated retroactively from subsequent viral load data points and replaced the valid baseline statuses.

Due to time limitations, sensitivity analyses using different definitions of virologic treatment failure were not conducted in order to better determine the strength of refill non-adherence and immunologic failure as identifiers of virologic failure.

X. Limitations of Study

These findings are only related to adolescents living with HIV in Botswana. Therefore, the results from this study cannot be generalized to the others. However, >90% of the adolescents living with HIV in the world live in sub-Saharan Africa. Botswana is similar in many ways to other sub-Saharan African nations living at the center of the epidemic.

In addition, capturing immunological failure may be subject to overestimation due to the potential for health care providers to encourage participants who they expect to be failing to return to the clinic more often for follow-up. It may also be subject to underestimation due to the possibility that adolescents who fear they may be identified as failing may somehow avoid viral load testing to minimize the risk of shame or other negative reinforcements due to their viral status.

The assumption that when patients open the MEMS bottle, they ingest the medication may lead to an overestimation in their medication adherence. Refill adherence percentages may be overestimated as well due to the assumption that the amount of pills not returned to the
pharmacy have been ingested, when in fact participants may have discarded them or left them at home.

In addition, the initial status of those particular individuals with extrapolated baseline statuses may have been misclassified, resulting in an incorrect estimation of the true rates of failure within this cohort. If, for instance, the extrapolated baseline status was lower than the actual status of the adolescent, then there was less opportunity for him or her to decrease in CD4 count. On the other hand, if the extrapolated baseline status was higher than the actual status of the adolescent, then he or she had more of a chance to be misclassified as having had immunologic failure during the follow-up period. Due to this potential misclassification, the association data may overestimate or underestimate the degree to which these patients experience virologic failure.

XI. Areas of Future Study

Future research concerning this particular patient cohort should examine alternative definitions of refill non-adherence and immunologic failure to determine if the sensitivity and specificity of the comparator measures change in strength for identifying adolescents with treatment failure. Sensitivity analyses with different definitions for virologic treatment failure should also be conducted.

Future studies could also look at possible new methods for clinicians to support and encourage desired outcomes, such as increased adherence and treatment success. Several new techniques, such as cell phone reminders, have not been thoroughly studied in adolescent populations, especially in low-resource settings, to determine the efficacy in improving medication adherence.

Additionally, as previous research (Okatch, et al) has shown that “over-adherence” (refill adherence >100% for at least 1/3 of 1-year follow-up period) is more common in adolescents
with virologic treatment failure than those who have undetectable viral loads, future research can focus on devising possible interventions to decrease the amount of “pill-dumping” in our patient cohort. Other areas of research can also include further qualitative studies that aim to understand the particular reasons that drive adolescents to be refill non-adherent or over-adherent.
IX. References


XII. Appendix

Receiver Operating Characteristic Analysis

Figure 1.

Figure 2.
Figure 3.

Figure 4.
Figure 5.

[Graph showing the strength of immunologic failure to identify virologic failure, with different lines representing different methods and their AUC values.]  

Significance Probability: 0.4103

Figure 6.

[Graph showing the strength of immunologic failure to identify virologic failure by schooling status, with different lines representing different schooling statuses and their AUC values.]  

Significance Probability: 0.7447
Figure 7.

Strength of Immunologic Failure to Identify Virologic Failure by Gender

- Male Participants AUC: 0.6437
- Female Participants: 0.6855
- Reference

Significance Probability: 0.5757

Figure 8.

Strength of Immunologic Failure to Identify Virologic Failure by ART Regimen

- NNRTI Regimen AUC: 0.6343
- PI Regimen AUC: 0.662
- Reference

Significance Probability: 0.7188
Figure 9.