STATISTICAL ANALYSIS PLAN

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Statistical Analysis Plan (SAP)

FULL TITLE	Public health epidemiology of HIV/tuberculosis in a lower income compared to a high-income country (SHCS no. 905 / IeDEA-SA no. 272)
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2. Introduction

This document describes the proposed presentation and analysis for the main paper(s) reporting results from a study comparing the public health epidemiology of people living with HIV presenting with and without tuberculosis (TB) in South Africa and Switzerland. To address this research question, we will analyze data from two cohorts, namely the Western Cape Cohort of the International epidemiology Databases to Evaluate AIDS (IeDEA; South Africa) and the Swiss HIV Cohort Study (SHCS; Switzerland). We will compare the outcomes of patients on different ART and TB regimens, including those who are co-infected with TB and those who are not. We will also explore the differences in outcomes between TB patients in the two countries.
Any deviations from the statistical analysis plan will be described and justified in the final report. The analysis will be carried out by identified, appropriately qualified and experienced statisticians, who will ensure the integrity of the data during their processing.

3. Background

3.1. Objectives of the study

The primary objective of the study is to compare the use of antiretroviral therapy (ART) regimens and the immune recovery in HIV patients with and without tuberculosis (TB) in South Africa and Switzerland. The following comparisons are of interest:

- Compare incident TB in both countries.
- Compare the use of ART regimens between Switzerland and South Africa
    - For all HIV
    - For HIV/TB co-infected
    - Time trends
- Assess CD4 cell recovery and HIV viral load decline in patients presenting and not presenting with TB after starting ART.

3.2. Definitions

3.2.1. Incident TB

"Incident TB" will be defined as TB diagnosis after two months of starting ART.

3.2.2. Prevalent TB

"Prevalent TB" will be defined as TB diagnosis two months before and within two months after ART start.

3.3. Primary outcome

a. Incidence of patients developing TB after starting ART, stratified by country.

3.4. Secondary outcomes

a. Immune recovery (slope of viral load and CD4+ count) after starting ART, with or without TB, stratified by country.

b. Time to viral suppression (2x viral load <50, 400 or 1,000 copies of HIV RNA per mL; CD4 cell count < 50, 200, 350, or 500 cells/mm3) from ART start (as a proxy for adherence and emergence of drug resistance).
c. Time to viral non-suppression (2x viral load >50, 400 or 1,000 copies of HIV RNA per mL; CD4 cell count >50, 200, 350, or 500 cells/mm3) from ART start (as a proxy for adherence and emergence of drug resistance).
d. Timing of HIV and TB treatment (time interval between initiation of TB treatment and ART initiation) (1)
e. Proportion of TB drug resistance
f. Prevalent TB
g. TB treatment outcomes
h. All-cause mortality

3.5. Variables

a. Prevalent and incident TB in the population
b. Dates of HIV/TB treatment start, diagnosis and relapse
c. Patient characteristics (age, sex, comorbidities, country of birth, duration of follow-up)
d. Viral load (copies of HIV RNA per mL)
e. CD4 cell count (cells/mm3)
f. Different ART regimens among people with and without TB
g. Different TB regimens among people on TB treatment
h. TB drug resistance profile

3.6. Subgroup analysis

The primary outcome will be analyzed within the following subgroups:

a. Type of TB ("prevalent" and "incident" TB)
b. Group of TB (newly diagnosed, relapse)
c. Site of TB (Pulmonary, Extrapulmonary)
d. WHO clinical stage (I, II, III, IV)
i. Other opportunistic infections (e.g., cryptococcal meningitis, *Pneumocystis jirovecii* pneumonia, Kaposi's sarcoma)

The analysis will help identifying risk groups of and risk factors for TB and poor immune recovery in HIV patients.

3.7. Hypothesis framework

The null hypothesis will be that there is no true difference in TB incidence between countries. The minimum detectable effect (MDE) size in terms of the absolute difference in proportions of those with TB between the two countries is 0.47 %, given a two-sided significance level of 0.05 and statistical power of 80% (2).

4. Study populations

The target population includes all HIV treatment-naïve people starting ART between 2010 and 2022 (SHCS, Switzerland) and 2017 and 2022 (Western Cape, South Africa). We will also compare all HIV patients diagnosed with TB between 2010-2022 (SHCS, Switzerland) and 2017-2022 (Western Cape, South Africa) and 2010-2022 (SHCS), regardless of the ART initiation date. The study included patients as outlined in Figures 1 and 2 in supplementary Appendix.

"Prevalent TB" will be defined as TB diagnosis two months before and within two months after ART start, and "incident TB" as diagnosis after two months of starting ART.

5. Statistical analyses

5.1. Comparative analysis

We will employ descriptive statistics to summarize patient data. Categorical variables will be presented as the number and percentage of patients, while continuous variables will be reported as mean and Standard deviation (SD) or median and interquartile range (IQR). We will investigate group differences using the chi-square test (or a related variant) for categorical variables and the Wilcoxon rank-sum test for continuous variables.

Create summary tables and figures to describe and compare patient characteristics, type of TB, site of TB, group of TB, WHO clinical stage, other opportunistic infections, timing of HIV and TB treatment, TB drug resistance, and TB treatment regimens in HIV patients diagnosed with TB in the study period (regardless of ART initiation date) between Switzerland and South Africa.

5.2. Primary analysis

a. Incident TB
    - We will compare incident TB using a Poisson model with a log(offset) term to account for the time at risk and estimate the relative difference in rates using incidence rate ratios (3).

5.3. Secondary analysis

a. Incident TB
    - Mixed effect logistic regression with random effects for patients and fixed effects for cohorts, in order to estimate risk factors for TB (4).
    - Kaplan-Meier estimate for the survival probability accounting for competing risk (death), will be used to describe the time to incident TB over time (5).
    - The log rank test will be used to compare the difference in the Kaplan-Meier curves.
    - The Cox Proportional-hazards model accounting for competing risk (death) will be used to compute the hazard ratio between countries and risk factors for TB.
b. Immunological recovery (slope of viral load and CD4+ count
    - Linear mixed-effect regression with random effects for patients and fixed effects for cohorts will be used to estimate the slopes of viral load and CD4+ count separately. Non-linear slopes will be estimated with regression splines as required. Viral load and CD4+ count are first checked for normality and transformed if necessary (commonly log10 for viral load and square root for CD4+ count is applicable).

c. Time to viral suppression from ART start
    - Same methods as in a.

This analysis may be adjusted for baseline CD4 count and viral load as well as patient characteristics (age, sex, country of birth etc).

5.4. Supplementary Analyses

5.4.1. Bayesian analysis

- We will repeat the above analyses of immune recovery and incident TB with a Bayesian analysis.
- Priors may be informed by published estimates from the literature, or the posterior estimates from the larger South African Cohort may be used as priors for the smaller Swiss Cohort.
- We will Impute missing data in models that are estimated with a fully Bayesian approach. Here missing data will be imputed during model estimation to optimize the model fit, rather than before analysis during the data preprocessing.

5.4.2. Multiverse analysis

Address the issue of researcher degrees of freedom (6). This will involve testing different assumptions, data preprocessing, and modeling choices. Each choices represents an analysis and the results are compared between and summarized across analyses. By exploring the range of plausible outcomes, we aim for better quantification of uncertainty and more robust findings.

5.5. Missing data

We will generate imputed datasets using both the MICE and SmcfcS packages in R. Multiple imputations will be performed to create differently imputed datasets, each of which will replace the missing values with plausible values based on the observed data under the missing at random assumption. We will then use Rubin's rules to combine the results from these imputed datasets taking into account the variability due to uncertain imputations in the missing data. The results from the complete case analysis and following imputation will be compared, and reported accordingly. Informative missingness will be investigate using appropriate sensitivity analysis approaches.

5.6. Statistical software employed

The statistical software R (version 4.2.2 or later) with RStudio will be used for all statistical analyses. Data preprocessing will be performed in the tidyverse package (version 2.0.0). Survival models will be estimated with the survival (version 3.5-5) and rms packages (version 6.5-0). Missing data imputation will be performed in the MICE (version 3.15.0) and SmcfcS package (version 1.7.1). Bayesian analysis will be performed in the brms package (version 2.19.0), which is an interface to the probabilistic programming language Stan (version 2.31). The lme4 package (version 1.1-32) will be used for fitting linear mixed models. We will use the multiverse package (version 0.6.1) to perform the multiverse analysis.

5.7. Multiple testing and reporting of results

Allowance for multiple treatment comparisons will be made, as appropriate. Results from frequentist analyses will be reported with confidence intervals and/or p-values. Results from Bayesian analyses will be reported with credible intervals.

6. Concept approvals

The data to be used for these analyses have been approved:

- Swiss HIV Cohort Study (SHCS) no. SHCS 905
- International epidemiology of Evaluating AIDS in Southern Africa (IeDEA-SA) no. SA272

6.1. References

1. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray AL, et al. Integration of Antiretroviral Therapy with Tuberculosis Treatment. N Engl J Med. 2011 Oct 20;365(16):1492–501.

2. Sealed Envelope Ltd. 2012. Power calculator for binary outcome superiority trial. [Internet]. [cited 2023 May 1]. Available from: https://www.sealedenvelope.com/power/binary-superiority/

3. University of Zurich, Switzerland, Winkelmann R. Counting on count data models. IZA World Labor [Internet]. 2015 [cited 2023 Apr 17]; Available from: http://wol.iza.org/articles/counting-on-count-data-models

4. Sperandei S. Understanding logistic regression analysis. Biochem Medica. 2014 Feb 15;24(1):12–8.

5. Rich JT, Neely JG, Paniello RC, Voelker CCJ, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. Otolaryngol Neck Surg. 2010;143(3):331–6.

6. Steegen S, Tuerlinckx F, Gelman A, Vanpaemel W. Increasing Transparency Through a Multiverse Analysis. Perspect Psychol Sci. 2016 Sep 1;11(5):702–12.