The CASTLE study

Computer Aided Screening for Tuberculosis in Low Resource Environments

# Statistical Analysis Plan

NCT number: NCT04545164

Version: 0.9 **Draft**
Date: 2021-05-25

## 1. Administrative Information

**Title:** Statistical Analysis Plan for Computer Aided Screening for Tuberculosis in Low Resource Environments (CASTLE) trial.

**Trial registration:** NCT04545164

**SAP version:** v0.9 2021-05-25

**Protocol version:** v5.1 2020-10-30

| **SAP revision history** | |
|---|---|
| V0.9 2021-05-25 | Initial version |
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| **Signatures** | | | |
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## 2. Introduction

**Background and rationale:**

The CASTLE study is a randomised trial to assess impact of digital chest X-ray with computer aided diagnosis (DCXR-CAD) on TB treatment initiation and inpatient mortality among people living with HIV admitted to hospital. Please refer to the study protocol for further information.

This document outlines how data will be analysed.

## 3. Study Methods

**Trial design**

CASTLE is a cluster randomised trial with two trial arms. Clusters are day of admission to hospital.

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| **Arm 1**<br>**Usual Care alone** | Any TB diagnostic tests standardly available at Zomba central hospital, on treating clinician request.<br>Includes sputum Xpert, urine AlereLAM and conventional plain film CxR, if requested. |
| **Arm 2**<br>**Interventions (DCXR-CAD + FujiLAM + usual care)** | FujiLAM + AlereLAM + DCXR-CAD for everyone (regardless of symptoms). Study team members will arrange sputum Xpert if CAD score >=60. This is in addition to usual care (as above) |

CASTLE has a third arm which is a nested observational diagnostic cohort ("diagnostic cohort"). People admitted on days assigned to diagnostic cohort arm do not contribute to trial outcomes.

The overall allocation ratio is 4:4:1 to usual care : interventions : diagnostic cohort. Accordingly, there is equal allocation (4:4) to the two trial arms.

**Randomisation**

Randomisation codes were generated randomly using block randomisation with varying randomly assigned block sizes (blocks could be 9, 18 or 27 days).

R statistical software with package "RandomizerR" was used to generate sequences using the following code:

```
a <- rpbrPar(300,rb=c(9,18,27), K=3, ratio=c(4,4,1), groups=c("usual","CXR+LAM","cohort"))
b <- genSeq(a,50,220188) # includes a seed
c <- getRandList(b)
d <- t(c)
```

Fifty separate sequences were generated using above code (by RMB) and exported to a spreadsheet. A second academic researcher (not affiliated with the CASTLE study) chose one of the fifty sequences and co-ordinated the printing of allocation codes and putting each cluster (day) allocation into sequentially numbered, sealed, opaque envelopes.

Each morning, the CASTLE trial team open the next sealed envelope in the sequence to reveal the allocation for that day.

**Sample size**

We plan to recruit 102 clusters per trial arm.

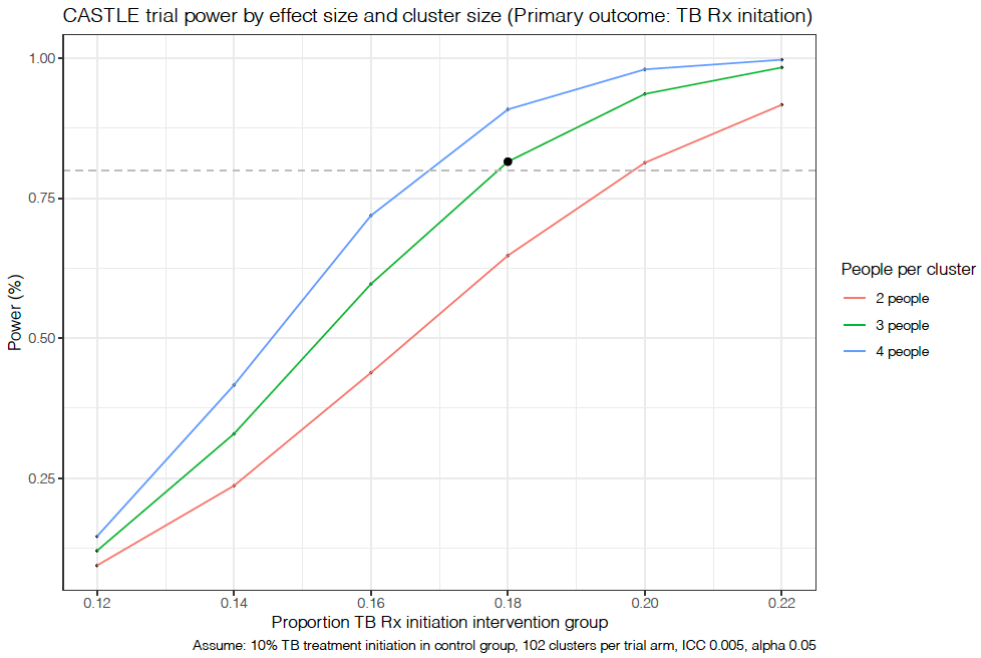
Sample size was calculated for primary outcome (TB treatment initiation). We assumed 10% people in usual care arm would initiate TB treatment by the time of hospital discharge, and 18% of people in intervention arm would initiate TB treatment. We assumed clusters with three participants per cluster and minimal clustering of outcome (ICC of 0.005).

Sample size for primary outcome was calculated in R using 'clusterPower' package

*library(clusterPower)*

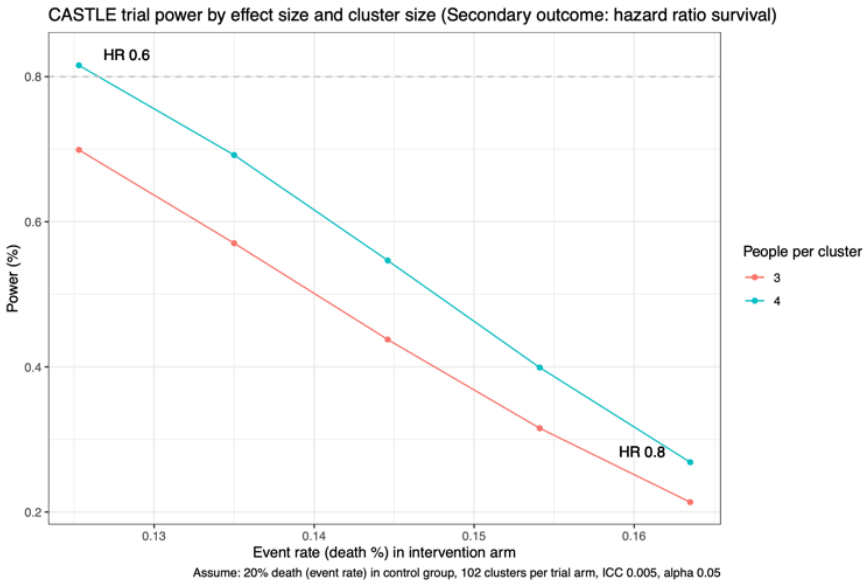
*p_int <- c(0.12,0.14,0.16,0.18,0.20,0.22)*

*pwr_3 <- crtpwr.2prop(alpha = 0.05, power = NA, m = 102, n = 3, cv = 0.1, p1 = p_int, p2 = 0.1, icc = 0.005, pooled = FALSE, p1inc = TRUE)*



Sample size for secondary outcome was calculated in stata using below command (assuming 80% survival by 56 days):

*power logrank 0.8, k1(102) m1(3,4) hratio(0.6,0.65,0.7,0.75,0.8) rho(0.005) cvcluster(0.05)*




If there were three participants per cluster, K 0.005 and TB treatment initiations 10% in usual care and 18% in intervention arm, then with 102 clusters per arm we will have 81% power to detect a difference between arms at least that large.

For secondary (survival) outcome, if there are four people per cluster and a hazard ratio of 0.6 (ICC 0.005 and survival probability 80% in usual care group) then with 102 clusters per arm we could have 81.6% power to detect an effect at least that large. With three people per cluster the power drops to 69.9%.

**Framework**

The hypothesis for CASTLE is that DCXR-CAD plus FujiLAM plus usual care is superior to usual care alone.

**Statistical interim analyses and stopping guidance**

There are no planned interim analyses.

**Timing of final analysis**

Final analysis will be conducted when the final participant has completed 56 days from time of enrolment.

**Timing of outcome assessments**

The primary outcome (TB treatment initiation) will be assessed at the time the participant is discharged from hospital (including TB treatment started on the day of discharge), participant dies or at 56 days from enrolment, whichever is the earlier.

The secondary outcomes are: TB treatment initiation within 24 hours from enrolment in trial, measured up to 24 hours from enrolment; undiagnosed TB at the time of discharge from hospital, measured at the time participant is discharged from hospital or dies or 56 days from enrolment whichever is earlier; and time-to-death, measured up to 56 days from enrolment.

## 4. Statistical Principles

### Confidence intervals and *p* values

All applicable statistical tests (p values) will be two-sided. All confidence intervals will be 95% and will be two sided.

There is a single primary outcome and three secondary outcomes. No adjustments for multiplicity are planned.

### Adherence and protocol deviations

Adherence to intervention is defined as receiving a DCXR and urine LAM test (for those in intervention arm). Analysis is intention-to-treat and is not affected by adherence to intervention.

Protocol deviations are defined in LSHTM-SOP-012-02 (LSHTM Research Governance and Integrity Office, 2019-11-28). Major and minor protocol deviations and any protocol violations will be summarised in an appendix to main trial paper.

### Analysis populations

CASTLE will be analysed on an intention to treat basis.

The analysis population will include all randomised participants according to the intervention their cluster was randomised to receive, regardless of whether the intervention was received or not. Participants enrolled into a randomised cluster who were subsequently found to be ineligible for the CASTLE trial will be removed from analysis.

Should there be a major protocol deviation / violation involving large numbers of participants not receiving the intervention to which their cluster was assigned, we would consider a per protocol additional analysis.

## 5. Trial Population

### Screening data

The following summaries will be presented for all screened potential participants:

> Number of days recruiting, number of potential participants screened, number of screened potential participants not recruited and the reason for non-recruitment.

### Eligibility

Eligibility for CASTLE is detailed in the protocol.

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| **Inclusion**: | Adult (age >= 18 years), admitted to medical ward at Zomba Central Hospital (for any reason), HIV positive (new diagnosis or known diagnosis) |
| **Exclusion:** | Unable or unwilling to consent, already on TB treatment or has received TB treatment in past six months, admitted for longer than 18 hours at the time of start of assessment for CASTLE enrolment. |

### Recruitment

A "CONSORT" diagram will be used to summarise the number of people screened, eligible, consented, randomised, receiving their allocated intervention and withdrawing / lost to follow up.

### Withdrawal / follow up

Should any participant withdraw from the study, this will be described, including the level of consent withdrawal (i.e. consent withdrawal for any or all of intervention, for follow up or for data retention). The reasons for withdrawal (where stated by participant) will be captured.

For participants who are lost to follow up by 56 days, they will be censored from follow up at the last time they were observed alive by the study time (this will usually be date of discharge from hospital) or the last time a reliable witness reports observing the person alive.

### Baseline participant characteristics

Baseline participant characteristics for categorical variables will be summarised by frequencies and percentages. Continuous data (age, CD4 count) will be summarised by median and interquartile range. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

The primary analysis will be unadjusted for baseline characteristics. If there is a major imbalance in baseline characteristics, we would consider a secondary sensitivity analysis adjusted for the imbalanced variable.

## 6. Analysis

### Outcome definitions

| | Definition | Timing | Notes |
|---|---|---|---|
| **Primary outcome** | | | |
| 1. Proportion of people starting TB treatment | Participant started on TB treatment. Numerator is all those starting TB treatment and denominator is all those enrolled. | From time of enrolment in CASTLE trial to end of the day in which a participant was discharged from hospital, or time participant died, or 56 days from enrolment in CASTLE whichever is sooner. | TB treatment as recorded in Zomba Central hospital TB register (paper-based ledger). Every participant's name checked against TB register within a week of discharge. Includes people started on TB treatment even if TB treatment subsequently stopped. Includes TB treatment started on the same day as discharge from hospital. |
| **Secondary outcomes** | | | |
| 2. Mortality (time to event) | Death from any cause | From time of enrolment in CASTLE trial to 56 days from enrolment. For those who are not reached at or after 56 days, their time in trial will be censored at the time of death or time they were last known to be alive by a reliable witness. | Includes in hospital deaths and deaths after discharge from hospital up to 56 days from enrolment. Deaths reported by relatives, mainly phonecall based tracing and home visits if unable to contact by phone.<br><br>If a person dies on the same day as enrolment they will be assigned 0.5 of a day survival time. |
| 3. Proportion starting same day TB treatment. | TB treatment started within 24 hours of enrolment in CASTLE trial. | Measured from time of enrolment in CASTLE for further 24 hours. | Time of starting TB treatment is time that guardian picks up TB medicines from TB office at Zomba Central Hospital (or ward nurse picks up medicines if no guardian). Time of picking up TB medications determined by observation of study team and guardian / relative / patient / nurse report (as time is not recorded in paper ledger, only date). |
| 4. Proportion with undiagnosed TB. | Sputum culture at mycobacterial lab in Blantyre grows *M. tuberculosis complex* organisms **and** participant did not start TB treatment at or before time of discharge from hospital or death or 56 days from enrolment (whichever is sooner). | TB treatment initiation as defined in primary outcome. | People whose sputum grows non-tuberculous mycobacteria are not included as having undiagnosed TB. People who start TB treatment but who subsequently interrupt TB treat are not treated as having undiagnosed TB. Denominator is all people in CASTLE trial, not just those who produce sputum. |
| **Other outcomes / measurements** | | | |
| Inpatient mortality | Death from any cause | From time of enrolment until time of hospital discharge or 56 | Determined through observation, notes review, asking healthcare staff and the ward paper ledger. |

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| | | days from enrolment, whichever is sooner. | |
| 56 day mortality (measured as a proportion) | Death from any cause | From time of enrolment in CASTLE trial to 56 days from enrolment. Analysed as binary data. | In hospital deaths determined as above, deaths after discharge from hospital ascertained from phone-call with nominated next of kin. |
| Microbiologically confirmed TB. | Proportion of all TB diagnoses that are microbiologically confirmed. | Refers to TB diagnoses made before discharge from hospital or before 56 days from enrolment, whichever is sooner. | Microbiologically confirmed means at least two positive Acid Fast Bacilli (AFB) smears or one or more Xpert Mtb/Rif positive or one or more culture positive for M. tb on any specimen or a positive urine LAM result. Includes samples collected by CASTLE team and those by usual care team. |
| Intervention fidelity | Proportion of people recruited in intervention clusters who have a CxR with CAD score and urine LAM result recorded | We will record any instance where intervention not delivered on the same day as enrolment. | Reasons for not receiving interventions will also be detailed. |

## Analysis methods

| | Metric to be reported | Method of calculation |
|---|---|---|
| **Primary outcome** | | |
| 1. TB treatment initiation | Risk ratio of TB treatment initiation by allocation arm.<br><br>We will also calculate and report absolute risk difference. | Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.*<br>Absolute risk difference will be obtained from binomal GLM with identity link function. |

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| **Secondary outcomes** | | |
| 2. Mortality | Hazard ratio for death (time to event) | Cox regression using robust standard errors to account for clustering. |
| 3. Same day TB treatment initiation | Risk ratio of TB treatment initiation within 24 hours from enrolment by allocation arm. | Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.* |
| 4. Undiagnosed TB | Risk ratio of undiagnosed TB by allocation arm. | Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.* |

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| **Other outcomes** | | |
| 5. Inpatient mortality | Risk of death prior to discharge from hospital. | Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.* |
| 6. 56 day mortality (measured as a proportion) | Risk of death within 56 days from enrolment (i.e. analysed as a proportion rather than time to event) | Regression using robust standard errors to account for clustering. GLM with log-link and binomial distribution to approximate risk ratio.* |
| 7. Microbiologically confirmed TB. | Proportion of TB | Numerator is those with microbiologically confirmed TB and denominator is all who had TB diagnosed. |
| 8. Intervention fidelity | Proportion | Numerator is all those who received DCXR with valid CAD score and a FujiLAM urine result, denominator is all those recruited in clusters allocated to intervention arm. |

**Notes on analysis methods**

* If GLM with log-binomial link function fails to converge then we will use an alternative method to calculate risk ratio, or report an odds ratio. *Sensitivity analyses*

If there are large numbers (>5%) of participants who do not receive their allocated intervention, then we will perform a sensitivity analysis on a per-protocol basis, where outcomes are based on intervention actually received rather than intervention each cluster was allocated to.

*Subgroup analyses*

For the primary outcome (TB treatment initiation) and for time-to-death outcome, we will analyse the intervention effect (RR) on outcomes by subgroups of those with and without TB in the differential diagnosis at admission ("TB suspects") and by CD4 count. CD4 count measurement isn't a study specific activity, but where it is measured by hospital we will capture data, and we will analyses outcomes in subgroups CD4 =<100 cells/$mm^3$, CD4 > 100 cells/$mm^3$ and CD4 not measured. Where a person has more than one CD4 count measured in hospital, the CD4 count closest to admission will be used. P values for interaction will be reported.

**Missing data**

No imputation will be performed for missing data. There will be no missing data for day recruited or cluster (i.e. arm) allocation. If an outcome is missing then it will be reported as such and not imputed.

No adjustment by baseline variables is planned, therefore missing data other than randomisation arm and outcome does not affect analysis. There is no imputatoion for missing covariates at baseline.

For the mortality secondary outcome (time to event), participants will be censored at the time they were last known to be alive.

**Additional analyses**

The diagnostic cohort (which does not contribute to trial outcomes) will be analysed mainly using descriptive statistics.

Within trial arms, as an exploratory analysis, we will compare risk of death by 56 days from enrolment among those with HIV virological failure and those without HIV virologic failure. This will be in a subset of participants who have HIV viral load measured (a convenience sample based on when protocol amendment to collect HIV viral load and time of day of admission so that lab staff are available to process samples).

**Harms**

Adverse events are defined in study protocol.

**Statistical software**

The analysis will be carried out using R statistical software, and robust standard error calculated using 'sandwich' package and 'lmtest' package to adjust confidence intervals of glm. Packages 'survival' and 'survminer' will be used for survival analysis (secondary outcome).

Stata may also be used if necessary.

## 7. References


Reich NG, Myers JA, Obeng D, Milstone AM, Perl TM (2012) Empirical Power and Sample Size Calculations for Cluster-Randomized and Cluster-Randomized Crossover Studies. PLoS ONE 7(4): e35564. https://doi.org/10.1371/journal.pone.0035564

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Zeileis A, Hothorn T (2002). "Diagnostic Checking in Regression Relationships." *R News*, 2(3), 7–10. https://CRAN.R-project.org/doc/Rnews/. **lmtest package.**

Zeileis A (2006). "Object-Oriented Computation of Sandwich Estimators." *Journal of Statistical Software*, 16(9), 1–16. doi: 10.18637/jss.v016.i09. **Sandwich package.**

Therneau T (2021). *A Package for Survival Analysis in R*. R package version 3.2-10, https://CRAN.R-project.org/package=survival. **Survival package**

Pantelli, N. LSHTM-SOP-012-02 Protocol / trial violations and deviations. LSHTM Research Governance and Integrity Office, 2019-11-28.


In addition, at the time of writing these were the versions of following relevant trial documents;

**CASTLE protocol:** v5.1 2020-10-30
**Data Management Plan:** v1.0 2019-10-30
**Data and Safety Monitoring Committee charter:** v1.0 2020-08-27