

**An autopsy study exploring the spectrum of
disease in individuals with advanced HIV in
primary care clinics in South Africa**

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Chapter 0. Abstract and administrative information

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0.1. Declaration

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0.2. Abstract

0.2.1. Background and methods

Tuberculosis (TB) remains the leading reported cause of mortality among HIV-positive individuals in low- and middle-income countries (LMIC), but disease prevalence and cause-specific mortality data are based on estimates; reliable data are needed to track progress towards targets for reductions in TB and HIV-related deaths. The aim of this work was to estimate disease prevalence and causes of death (CoD) among people dying after enrolment to three large studies of TB and HIV in South Africa.

Literature was systematically reviewed for autopsy studies estimating disease prevalence in HIV-positive adults; studies validating verbal autopsy (VA) for HIV and TB deaths; and studies directly estimating CoD in HIV-positive adults in LMIC. Primary data were collected concerning HIV-positive decedents from three parent studies and HIV-negative controls. Minimally-invasive autopsy (MIA), involving tissue biopsy, fluid aspiration, and bronchoalveolar lavage was conducted in a subset of HIV-positive decedents. CoD were assigned, through structured review of clinical and research data (“reference-standard”) and VA data, collected using the World Health Organization (WHO) 2012 instrument and interpreted using physician-certified (PCVA) and computer-coded VA (CCVA) methods (InterVA-4 and SmartVA-Analyze). VA-assigned and reference-standard CoD were compared; agreement was measured at individual- and population-level.

0.2.2. Results

MIA was conducted for 34 HIV-positive adults: 16 (47%) had evidence of TB at autopsy, 14/16 (88%) had evidence of extrapulmonary disease, and 6/16 (38%) had not been started on TB treatment; 23/34 (68%) had evidence of bacterial pneumonia and 20/34 (59%) had evidence of two or more concomitant infections. Most (94%) individuals who underwent MIA were assigned HIV-associated reference-standard CoD; this was underestimated by all three VA methods (PCVA 74% [chance-corrected concordance {CCC} 0.71], InterVA-4 47% [CCC 0.42], and SmartVA-Analyze 41% [CCC 0.31]).

Reference-standard CoD were assigned, without MIA data, to 259 HIV-positive adults: 183 (71%) were assigned HIV-associated causes. Only the PCVA estimate was similar, at 80% (CCC 0.78); InterVA-4 and SmartVA-Analyze underestimated the HIV-associated mortality fraction (estimates 48% and 29%; CCC 0.48 and 0.20, respectively).

Agreement between VA methods and the reference-standard was poor at individual level (overall CCC ≤ 0.22) and slightly better at population level (cause-specific mortality fraction [CSMF] accuracy 0.43–0.79). Only PCVA could estimate the HIV-associated TB mortality fraction, underestimating it when compared with a reference standard that included autopsy data (reference 41% vs. PCVA 32%; CCC 0.23) and overestimating it when compared with a reference standard without autopsy data (reference 27% vs. PCVA 42%; CCC 0.42).

Among 356 HIV-positive and 103 HIV-negative adults with confirmed HIV status, the VA instrument was sensitive (84.3%) and specific (94.2%) in assigning HIV status; VA methods showed high specificity (all methods $>89\%$) in assigning HIV-associated CoD; and both CCVA methods underestimated the likely true HIV-associated mortality fraction among confirmed HIV-positive decedents (InterVA-4 44.7% and SmartVA-Analyze 22.5%).

0.2.3. Conclusions

TB remains a leading CoD among HIV-positive adults in LMIC. Changes are needed to disease classification systems and automated VA methods to allow for better estimation of HIV-associated mortality overall and mortality due to HIV-associated TB. Structured guidelines for assigning CoD in HIV-positive people in clinical settings and the use of MIA at sentinel surveillance sites may be useful additions to current methods.

0.3. List of abbreviations

AFB	Acid-fast bacilli
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
CCC	Chance-corrected concordance
CCCSMFa	Chance-corrected cause-specific mortality fraction accuracy
CCVA	Computer-coded verbal autopsy
CDA	Complete diagnostic autopsy
CI	Confidence interval
CoD	Cause/s of death
CrAg	Cryptococcal antigen
CRVS	Civil registration and vital statistics
CSMF	Cause-specific mortality fraction
CSMFa	Cause-specific mortality fraction accuracy
HDSS	Health and demographic surveillance system
HIV	Human immunodeficiency virus
ICD	International Statistical Classification of Diseases and Related Health Problems
IFA	Immunofluorescence assay
IHME	Institute of Health Metrics and Evaluation
IQR	Interquartile range
LAM	Liporabinomannan
LMIC	Low- and middle-income countries
LSHTM	London School of Hygiene & Tropical Medicine
MeSH	Medical subject headings
MGIT	Mycobacterium growth indicator tube
MIA	Minimally-invasive autopsy
MTB	<i>Mycobacterium tuberculosis</i>
NHLS	National Health Laboratory Service
NTM	Non-tuberculous mycobacteria
PCP	<i>Pneumocystis pneumonia</i>
PCVA	Physician-certified verbal autopsy
PHMRC	Population Health Metrics Research Consortium
SD	Standard deviation
SE	Standard error
SOP	Standard operating procedure
TB	Tuberculosis
UN	United Nations
UNAIDS	Joint United Nations Programme on HIV/AIDS
VA	Verbal autopsy
WHO	World Health Organization
XTEND	Xpert for TB: Evaluating a New Diagnostic

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~

For my brother

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1.1. Background

1.1.1. Tuberculosis, HIV, and death

There were 10.4 million new cases of tuberculosis (TB) and 1.8 million TB deaths in 2016 [1]. Among human immunodeficiency virus (HIV)-positive individuals, in whom TB is the leading cause of death, there were 1.2 million new cases and an estimated 390,000 deaths. *Mycobacterium tuberculosis* (MTB) is an ancient organism; there is evidence to suggest that it may have originated around 70,000 years ago [2] and signs of TB disease have been found in 9,000-year-old human remains [3,4]. Effective chemotherapy against MTB first emerged in the 1940s, with additional agents and various combinations trialled in the subsequent decades [5,6]. TB-related mortality, which had been in steady decline for most of the 20th century, likely due to improvements in hygiene & living conditions, reduced more sharply between 1950 and 1980 [7]. The emergence of the HIV epidemic in the mid-1980's, however, created an enormous pool of individuals with greatly increased susceptibility to TB disease, which led to higher numbers of TB cases and a dramatic spike in TB-associated mortality [8–10]. The last 30 years have seen increasingly coordinated efforts by members of the global health community working on TB; the establishment of specific funding streams, including the Global Fund to Fight AIDS, Tuberculosis, and Malaria; and the establishment of national TB programmes in almost all high-burden countries [1]. In the same period, the incidence of TB has fallen by almost 20% and mortality by almost 50%, and an estimated 43 million deaths have been averted [11]. Regardless, in 2015, TB remained the world's leading infectious cause of death [1].

Any discussion of TB mortality in the 21st century must pay equal attention to HIV and the synergistic relationship between the two diseases (see Chapter 1.2.2). Throughout the 1990s and early 2000s, HIV and the acquired immune deficiency syndrome (AIDS) became increasingly prominent underlying causes of death in adults; in sub-Saharan Africa, HIV was responsible for the reversal of some of the positive trends established over the previous decades, with life-

expectancy falling or remaining static from 1990 until the mid-2000s [12,13]. In 2001, the United Nations (UN) General Assembly declared the HIV epidemic a 'global crisis' [14]; by 2003, HIV/AIDS was the leading cause of death in individuals aged 15–49 years, worldwide [15]. In addition to the increased vulnerability to TB seen in HIV-positive individuals, the conditions that allowed the rapid spread of the virus in low- and middle-income countries (LMIC) also helped set the scene for a TB co-epidemic. People in LMIC of lower socio-economic status, with less access to food, education, sanitation, and health services, are disproportionately affected by both diseases, TB in particular, and are at higher risk of death [16,17].

In the early stages of the HIV epidemic, the absence of effective therapy for HIV itself meant that the treatment of an opportunistic infection simply delayed the inevitable; patients invariably experienced a progressive decline in immune function and eventually died. In LMIC, death was very often from TB [18,19]. The advent of antiretroviral therapy (ART), together with sustained international efforts to improve access to treatment, has led to a dramatic reduction in all-cause mortality among people living with HIV [20,21], although mortality remains high among those with advanced disease in LMIC, particularly pre- and early on ART [22–25]. Assuming adherence to treatment, an individual living in a high-income country who is today diagnosed as HIV-positive can expect a lifespan much the same as an HIV-negative individual in the same context [26,27]. The same cannot be said for people living with HIV in LMIC, and the reasons for the marked differences in outcomes are likely structural, rather than biological [28–31]; despite substantial progress in providing access to care, a number of economic, societal, and political factors mean that many of those who need care are unable to or do not access it, or do so when their disease is at an advanced stage, increasing their risk of morbidity and death [32–34].

1.1.2. Ending the epidemics

The World Health Organization (WHO) has published a set of targets as part of the 'End TB' strategy, launched in 2015 [35]. A central aim is to reduce TB-related mortality to 75% and 95% of 2015 levels by 2025 and 2035, respectively. Equally ambitious is the aim of the Joint United Nations Programme on HIV/AIDS (UNAIDS), to reduce the absolute numbers of AIDS-related deaths from 1.1 million in 2015 to under 0.5 million by 2020 [36,37]. Both programmes have aligned their targets with the UN Agenda for Sustainable Development, Goal 3.3 of which is to end the TB and AIDS epidemics by 2030 [38].

Aside from the considerable logistical and financial implications of trying to achieve these goals, measuring progress towards them will be a critical part of the process, providing essential information on the success, or otherwise, of specific activities, and determining the character, focus, and intensity of any subsequent actions. There is, therefore, a need to establish robust methods that will accurately and consistently monitor the impact of interventions to reduce TB and HIV deaths and track progress towards these ambitious targets [35,36].

This chapter will provide context for some of the issues discussed in this thesis, including a description of some of the challenges faced in measuring disease-specific outcomes; the interactions between HIV and TB; the difficulties of diagnosing TB disease in individuals with advanced HIV; the methods currently used to estimate global disease burden and cause-specific mortality patterns, with a particular focus on HIV- and TB-associated mortality in LMIC; the current situation with other methods that could be used to estimate cause-specific mortality; and the setting in which a mortality study, the results of which make up the bulk of this thesis, was conducted. This last section will include a description of the status of the TB and HIV epidemics in South Africa, the organisation of the South African health system at the time of the study, and a brief overview of the parent studies within which the mortality-study was nested. Finally, this chapter will list the overall aims and objectives of the thesis, outline how it intends to meet those objectives, and give a detailed account of my contributions to the work presented here.

1.2. Context

1.2.1. Measuring cause-specific mortality

Most health outcomes are context-specific. They may be dependent on the perspective of the health professional or patient involved, or specific to a single disease in a particular sub-group. Even supposedly standardised metrics, such as disability-adjusted life-years (DALYs) and quality-adjusted life-years (QALYs), are complex, compound measures, constructed by health economists; context and understanding shape the ways in which they are presented and interpreted [39–41]. The occurrence of a death, in contrast, is easier to define and should be easy to quantify, hence the frequency with which it is used as the base unit of measurement to estimate the effectiveness of health interventions.

As this thesis will explore, counting deaths may appear easy, but it is often an imprecise and challenging process.

Similar difficulties are encountered when trying to estimate disease prevalence in a population, either among living

individuals or at death. Importantly, the limitations of methods currently used to estimate morbidity and mortality are often not well understood by many of those who depend on these estimates to make major health policy decisions [42,43].

The simplest way to measure mortality is to count the overall number of deaths, a metric referred to as all-cause mortality. Estimates of all-cause mortality, often stratified by age, gender, ethnicity, and socio-economic status, can be extremely useful when used in a well-defined population, for example, within a clinical trial or epidemiological study, where rigorous inclusion and exclusion criteria have already been applied. Outside of research settings, among diverse groups of individuals in unpredictable environments, all-cause mortality is often too non-specific a method with which to measure the effect of an intervention. Estimates of cause-specific mortality, in general, provide a better means for impact evaluation: for better or worse, most interventions are disease-specific, as are the structures of many health systems and governing bodies [44], and funding practices in the last two decades have led to a predominance of vertical programmes [45]. Disease-specific estimates also play a central role in a number of decision-making processes, including allocating funding, structuring health systems, and determining research frameworks [11,38,46,47]. As such, reductions in cause-specific mortality are prominent on the agendas of governing bodies at global and national levels [35,36,48] and success in global health is often measured by the ‘numbers of lives saved’ [49].

While efforts to unify funding streams and create holistic interventions are much needed, these changes will take time to implement. In the meantime, estimates of disease burden and cause-specific mortality remain essential in measuring the effect(s) of many targeted public health interventions aiming to reduce mortality.

1.2.2. HIV-associated tuberculosis

Immunosuppressed HIV-positive individuals are up to 20–30 times more susceptible to active TB disease compared with HIV-negative, immunocompetent individuals [50]. Vulnerability to TB, and other infections, increases as immune function decreases. TB disease often presents atypically in individuals with advanced HIV disease, and can be difficult to differentiate from other HIV-associated infections or even from non-specific symptoms caused by severe immunosuppression [51–53]. Microbiological investigations used to diagnose TB disease in immunocompetent individuals are also less useful in those who are immunosuppressed: the sensitivity of sputum microscopy (‘smear’) is markedly reduced and Xpert® MTB/RIF, which detects MTB deoxyribonucleic acid (DNA) and is more sensitive than smear, performs less well in HIV-positive individuals with low CD4 counts or smear-negative disease or both [54,55].

Liquid mycobacterial culture is the most sensitive and specific investigation in these patients, but MTB is a slow-growing organism and culture may take several weeks to yield a positive result or to provide a negative result to help rule out TB disease [56]. In individuals with advanced HIV disease, at very high risk of death, long delays in starting treatment (either anti-TB treatment or ART) can be life-threatening [23–25,57–59]. At present, there is not a cheap, sensitive, point-of-care test that can be used to diagnose active TB disease in individuals with advanced HIV attending primary care in resource-limited settings [60–62]. As a result, there is a paucity of data on the prevalence of TB disease in these individuals. Studies investigating this have found missed opportunities for investigation [63]; a lack of adequate investigation for individuals who initially test negative, even in the presence of persistent symptoms [64]; and a high prevalence of undiagnosed TB disease, including among individuals who initially test negative for TB [65–67].

More reliable estimates of TB prevalence come from autopsy studies, though many of these involve only small numbers of individuals dying in hospitals [68,69]. The burden of TB at autopsy is typically higher than in studies of living individuals. This difference is likely to be artificially inflated, at least in part due to the increased difficulty of ascertaining TB disease in the living, and because the higher risk of TB disease and mortality in those with advanced HIV disease and higher risk of mortality in those with TB [23,70] may lead to the over-representation of TB among those who die. However, as discussed above, the relative inadequacy of TB diagnostics in living individuals, particularly in those who are HIV-positive, suggests that current methods do underestimate the prevalence of disease in living patients; a systematic review of autopsy studies describing the autopsy prevalence of TB in HIV-positive adults dying in LMIC found that almost half of the active TB prevalent at autopsy had not been diagnosed ante mortem [71].

As discussed further below, national and international estimates of TB prevalence are derived, in part, from numbers of reported cases; these numbers are also used to generate estimates of TB mortality, particularly in HIV-positive individuals. The potential underestimation of the true burden of TB and, by proxy, mortality due to TB, has implications not just for individual patients in whom the diagnosis is missed, but for the at-risk populations in high-burden countries, the healthcare workers treating them, and the surveillance and governing bodies that generate statistics and determine resource allocation [72,73].

1.2.3. Measuring mortality

1.2.3.1 Causes of death and the ICD

The processes that lead to the death of an individual are often multiple and complex, but their distillation to a single ‘underlying’ cause has been central to the approach taken to cause of death monitoring since its inception [74].

Considering that methods have been in development for over a century, it can be argued this reductive process has led to data, at a national and international level, that are of poorer quality than are desirable.

Modern classification systems have their origins in the ‘International List of Causes of Death’, first collated in the late 19th century [75]. There was a recognition, from its earliest days, that any such list was never going to be comprehensive. The intention was for it to be functional, to allow for structured observation and quantification of natural phenomena; rather than a list of “terminal causes”, it was intended to describe “the morbid condition that initiated the train of events ultimately resulting in death” [75]. Regular revisions were built in to the process to incorporate new evidence and knowledge to improve the accuracy of the list. Revisions were initially scheduled to occur every 10 years; after the 9th revision in 1975, however, it was decided that cycles were too short, and the 10th revision was delayed until 1989. The 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) was endorsed by WHO in 1990 and has undergone several minor updates in the intervening period [76], but the next major revision, ICD-11, is not due for release until 2018, a gap of almost thirty years [77].

In the early stages of the HIV epidemic there were few data available on its aetiology and clinical presentation. As more knowledge emerged, a working case-definition was developed that allowed epidemiologists to identify and count cases of HIV-associated illnesses at a time when confirmatory diagnostics were not necessarily available [78,79]. In 1993, the Center for Disease Control (CDC) definitions of AIDS were developed and have been used, broadly, ever since [80].

Codes for HIV-associated diseases were incorporated into the ICD system in 1990 and have been changed only slightly since then [81].

At present, all HIV-associated causes of death are classified under five, three-character codes: HIV disease resulting in infectious and parasitic diseases (B20); resulting in malignant neoplasms (B21); resulting in other specified disease (B22); resulting in other conditions (B23); and unspecified HIV disease (B24) [76]. More specific causes are specified with a digit after a point, i.e., a five-character code. For example, B20.4, “HIV disease resulting in candidiasis” and B21.1, “HIV disease resulting in Burkitt lymphoma”. Of particular relevance to this thesis is the way TB is classified by

ICD-10. TB as an underlying cause of death is classified under ICD-10 codes A15, “respiratory TB, bacteriologically or histologically confirmed”; A16, “respiratory TB, not confirmed bacteriologically or histologically”; A17, “TB of nervous system”; A18, “TB of other organs”; and A19, “Miliary (or disseminated) TB”. However, these definitions all specifically exclude “HIV disease resulting in tuberculosis”, which is included under HIV-associated conditions (ICD-10 code B20.0). As described in more detail below, this may seem a straightforward division, and if the full five-character ICD codes were used in all settings, it would be. In practice, however, many bodies, particularly in LMIC, use only three-character codes (B20, B21, etc.) and deaths due to HIV-associated TB are indistinguishable from other infectious and parasitic HIV-associated deaths, all of which are also included under the ‘B20’ umbrella [76].

1.2.3.2 Estimating cause-specific mortality in LMIC

Many countries rely on civil registration and vital statistics (CRVS) systems as their primary source of mortality data. In many LMIC, however, these systems are weak or non-existent, and estimates of disease burden and mortality must be compiled through other means [82,83]. For TB deaths, for example, WHO use a variety of sources to generate mortality estimates, including case notification data, results from TB prevalence surveys, estimates of HIV prevalence, and estimates of country-specific case-fatality rates [84]. Figures produced by WHO also depend heavily on mathematical models, most importantly those included in the Spectrum software suite (<http://www.avenirhealth.org/software-spectrum.php>), which is also used by UNAIDS and other groups [85].

Attempts to estimate mortality due to HIV-associated TB are doubly hampered, however, by the issues with ICD-10 described above. Even in countries with functional CRVS systems, such as South Africa, estimates of mortality must still be derived through modelling, as cause-specific mortality figures are reported to WHO only in three-character format (i.e., B20, rather than B20.0) [11,72]. As a result, there are few examples available of direct (non-modelled) estimations of mortality attributable to HIV-associated TB.

1.2.4. Alternative methods to directly estimate disease burden and mortality

1.2.4.1 Pathological autopsy

Pathological autopsy remains the most accurate method for assessing disease prevalence at death and assigning causes of death [68]. The procedure is usually conducted by a pathologist and involves exposure and visualisation of all the organs, with samples sent, at a minimum, for histological examination. Pathological autopsies provide high quality data and, as organs are directly visualised, can also provide accurate estimates of disease prevalence among the decedents

examined. However, global autopsy rates, while always low, have been in decline for several years. This is thought to be for a number of reasons, including improvements in diagnostic facilities (in high-income settings), increasing reluctance on the part of family members to provide consent, and reluctance on the part of clinicians to request the procedure or to go through the consent procedure with families [86]. Many of these reasons also apply to resource-limited settings, although, arguably, autopsies are even more important in these settings, where vital registration data are sparse and diagnostic facilities often limited [87–89].

Some of the technical strengths of pathological autopsy also count against it. A comprehensive and technically complex procedure may produce high quality data, but also requires highly trained personnel and specialist facilities, both of which increase the cost and limit the feasibility of the procedure to different contexts. As discussed in more detail in Chapter 2.2, this, at least in part, may explain why the majority of autopsy studies in LMIC have been conducted among hospitalised individuals. More recent studies have therefore used minimally-invasive techniques to circumvent some of the issues encountered with complete autopsy.

1.2.4.2 Minimally-invasive techniques

Minimally-invasive autopsy (MIA), which entails post-mortem sampling of body fluids or solid organs through aspiration or tissue core biopsy, respectively, has become increasingly popular in recent years [90]. In resource-limited settings, in particular, it compares favourably to complete autopsy, as it is cheaper and faster to conduct, more acceptable to families, does not require specialist training, and provides reasonable accuracy for estimating the prevalence of communicable diseases [91–95]. Groups in LMIC have developed standardised protocols for the use of this technique in resource-limited settings [96–99]. However, shortcomings and challenges exist, particularly in estimating the prevalence of non-communicable diseases, and in assigning causes of death from MIA data, where protocols and guidelines are not yet established.

1.2.4.3 Verbal autopsy

A verbal autopsy (VA), in its most basic form, is a conversation with the relatives or carers of a deceased individual regarding the events leading up to death. VAs have been used since the 1970s, initially to investigate maternal and child mortality [100]. The first paper describing the use of VA to quantify mortality among a more representative sample was published in 1986, reporting on a study conducted in Senegal [101]. Since then, as the scope of VA has expanded, considerable efforts have been made to refine both the interview and the methods used to assign causes of

death based on VA data [102]. In 2007, WHO convened a panel of VA experts to establish the first international standardised VA instrument [103]. Separate instruments are now used for the death of a child aged up to four weeks, a child aged four weeks to 14 years, and an adult (aged 15 years and above). Unless otherwise specified, any references to 'VA instrument(s)' in this thesis pertain only to the third, adult questionnaire.

The standard instrument consists, broadly, of four parts: first, basic details about the respondent (i.e., the person being interviewed), including their relationship to the deceased and the extent of their interaction during the final illness; second, basic details about the decedent, including demographic information, site of death, and cause(s) of death recorded on the death certificate; third, an open narrative section, allowing the respondent to describe, in their own words, the events leading up to death; and, finally, a set of closed, mostly yes/no, questions around diagnoses made by health professionals, symptoms witnessed or reported (including duration thereof), and any treatment sought or administered. The last section makes up the bulk of the questionnaire (~80% of questions in the WHO 2012 instrument [104]). Within this last section are also contained several discrete modules on specific topics. The applicability of a module to a particular decedent is often determined by a single 'entry' question; for example, section 6 of the 2012 WHO instrument is to be used only if the first question ("Did the deceased suffer from any injury or accident that led to her/his death?") is answered in the affirmative; section 7 only if the decedent was female; and section 8 only if a female decedent was pregnant, had delivered, or had miscarried in the six weeks prior to death [104]. Various groups have also added their own modules, based on particular interests or location-specific mortality patterns; the research group at Agincourt health and demographic surveillance system (HDSS) site, in Mpumalanga Province, South Africa, for example, added modules asking detailed questions about epilepsy, stroke, and a history of work underground [105].

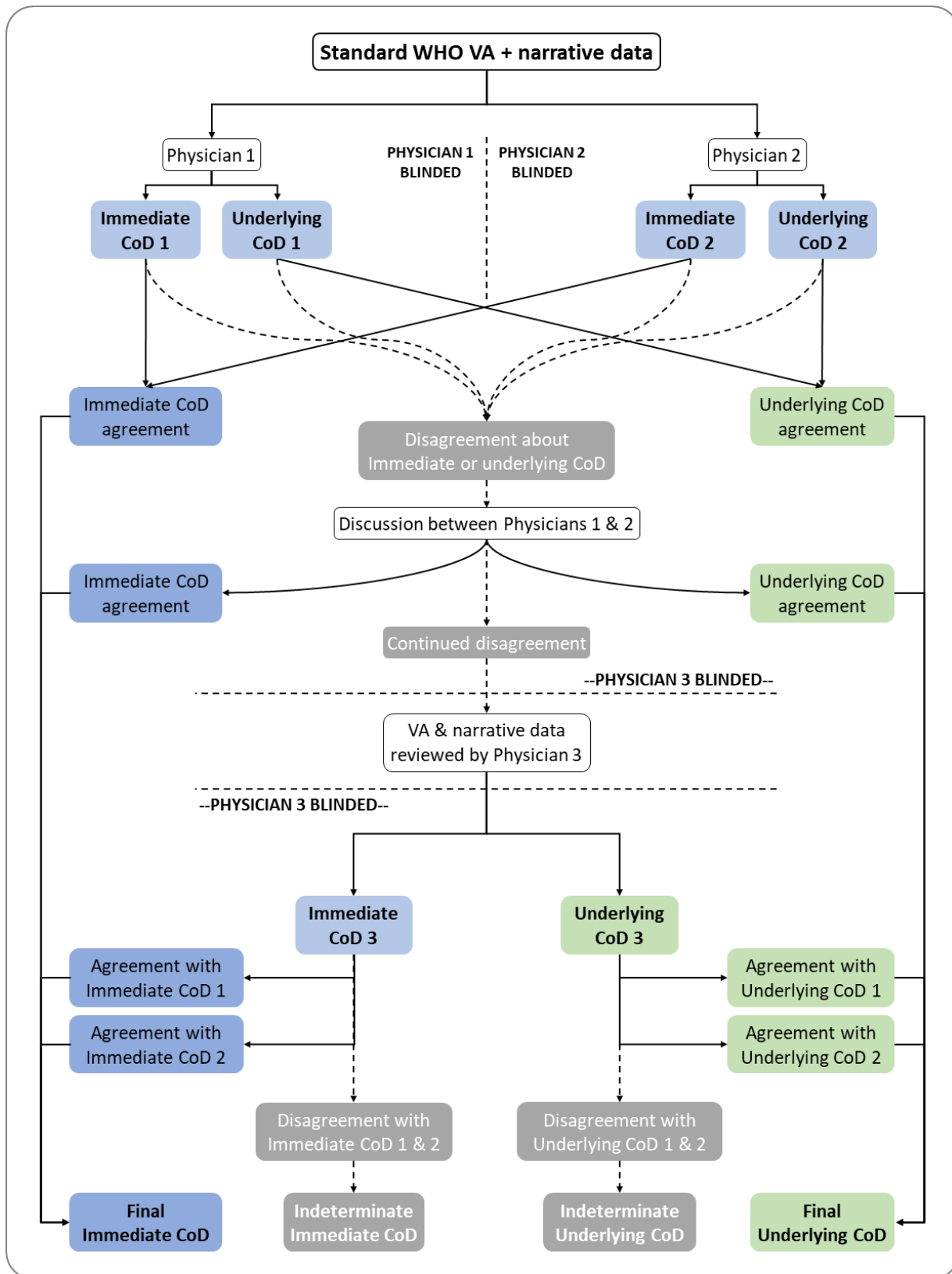
1.2.4.3.1 Using VA data to assign causes of death

WHO VA instruments were updated in 2012, 2014, and 2016, though most changes made were relatively minor [104,106,107]. More dramatic, however, have been the changes in interpretation methods made over the same period. Physician-certified verbal autopsy (PCVA) has been the most widely used method and is still widely perceived to be the most reliable in assessing cause-specific mortality fractions (CSMFs), to the extent that it is sometimes used as a reference standard in validating newer, experimental methods [108,109]. The PCVA method is described in detail later in this thesis (see Chapters 2, 5, and 6). Briefly, it involves the review of VA data by two or more physicians who independently assign causes of death per ICD guidelines; causes assigned by reviewing physicians are then compared

and, if there is disagreement, the case is either discussed by the reviewing physicians or sent to another, independent physician for review (Figure 1:1). Agreement between any two physicians is considered the final cause of death; if all three physicians are unable to agree, the deceased individual is assigned an 'unknown' or 'indeterminate' cause of death.

Although the internal validity of the process is relatively high, with some care taken to account for interactions between reviewers, the cost, logistical difficulties, and time required for the process make it an inefficient choice for use on a larger scale. In addition, there are concerns that the pre-existing beliefs or knowledge of the physicians involved may bias the assignment of certain causes of death, making it difficult to compare causes assigned in different contexts.

Figure 1:1. Illustrative overview of the PCVA process, incorporating discussion stage, per Agincourt HDSS site (adapted from upgrading appendix)



CoD: cause of death; HDSS: health and demographic surveillance system; ICD: International Statistical Classification of Diseases and Related Health Problems (10th edition); PCVA: physician-certified verbal autopsy; VA: verbal autopsy; WHO: World Health Organization

In response to the sub-optimal reliability of PCVA and to overcome some of the logistical barriers to large-scale implementation of VA, recent efforts have focused on the development of computer-coded VA (CCVA) methods to achieve the same objectives [110,111]. Automated methods are easier to standardise; less expensive to run, especially when dealing with very large numbers of deaths; and give quicker results, potentially allowing for data to be analysed by several different methods in the same time it would take for physician review. CCVA methods are therefore viewed as more suitable than PCVA for integration into civil registration systems, which is a major objective of some research and implementation groups [112–115].

The most prominent and widely used CCVA method is InterVA (<http://www.interva.net>), a programme that corresponds to the WHO 2012 VA instrument and interprets categorical VA data using a Bayesian algorithm. InterVA assigns each decedent up to three causes of death, each with an associated probability [116,117]. Like most CCVA methods, InterVA assigns causes of death using a truncated list of 34 causes, each of which is a collection of more specific ICD-10 codes [118]. InterVA was first tested in the mid-2000s and has undergone a number of revisions [119]; the most recent version, issued in January 2016 and used in this thesis, is InterVA-4.03.

The next most prominent programme, more recently made available for general use, is SmartVA-Analyze (<http://www.healthdata.org/verbal-autopsy/tools>). This was developed by the Institute of Health Metrics and Evaluation (IHME) and uses the Tariff 2.0 method [120] to assign causes of death according to a list developed by the Population Health Metrics Research Consortium [121].

InSilicoVA [122,123], a probabilistic method developed more recently, has only just been made available for public use and is therefore not evaluated in this thesis (<https://cran.r-project.org/web/packages/InSilicoVA/index.html>). As explored in more detail in Chapter 2.3, various other automated and algorithm-driven methods have been tested over the last decade, though most have now been abandoned in favour of the methods described above.

1.2.5. South Africa

1.2.5.1 General overview

South Africa has one of the most unequal societies in the world. In 2010, 53.8% of the population was living in poverty and, in 2011, the richest 10% of the population held 51% of the income share, compared with less than 1% held by the poorest 10% (Gini index 63.4) [124]. The political environment is also complex and volatile: the World Bank assigned

South Africa a 'political stability' score of -0.2 in 2015, placing it in the 38th percentile (90% confidence interval [CI] 29.4–53.1) worldwide, compared with the most and least stable (Greenland, 1.9, and Syria, -2.9 , respectively [125]). With national unemployment at 26–36% (and consistently higher in those 15–34 years of age [126]), a minority tax base [127], and a seemingly constant threat of political and civil unrest, South Africa's financial situation in recent years has been unsteady, as illustrated by the decline in the value of the Rand from 2012–2016 and the change in bond status to 'junk' in early 2017 [128].

Many of South Africa's current issues have their roots in the country's tumultuous, and well documented, racial and political history, a detailed discussion of which is beyond the scope of this thesis. It is sometimes easy to forget, in modern South Africa, that apartheid ended less than 25 years ago; the consequences of those policies continue to have a tangible impact on the daily lives of South Africans and exert considerable influence over the way national institutions, including health-related structures, are governed.

1.2.5.2 The health system, HIV, and TB

South Africa has the largest number of HIV-positive individuals of any country [129] and the second-highest number of new TB cases per year [1]. Health in the country has changed dramatically over the last twenty years, particularly in terms of HIV care: the dark days of AIDS denialism [130] are long past, in large part thanks to the tireless advocacy of civil society groups such as the Treatment Action Campaign [131] and investments made by the Zuma administration. The country now boasts the largest antiretroviral programme in the world, with an estimated 3.7 million individuals on treatment in 2016 [129]; has purchased almost as many Xpert® MTB/RIF cartridges as the rest of the world combined (over 10.5 million from 2010–2016) [132]; and, in 2013, health expenditure per-capita was among the highest in the African region [12]. Great strides have been made in improving standardisation of care, monitoring and evaluation of critical outcomes [133], and in establishing a transparent process for translating evidence to policy [134].

Since the late 1990's, there has been a steady move towards the decentralisation of healthcare in South Africa [135]. Primary health clinics and community health centres are now a central pillar of the health system: among individuals aged five years or older, there were over 120 million visits to the 3,427 facilities operating in 2015/16 [136]. Health coverage of the population is varied, in part due to the wide variation in population density in different parts of the country (for example, Rustenburg municipality has a density of 160 persons/km² and the City of Johannesburg 2,600 persons/km² [population 0.5 million and 4.4 million, respectively]) [137,138]. In 2013, WHO estimated that South

Africa had 5.9 health posts and 0.1 provincial hospitals per 100,000 population [12]. All public health facilities are served by a network of centrally coordinated laboratories (the National Health Laboratory Service [NHLS]) which allows for standardisation of protocols and testing nationwide [139]. The NHLS has also developed a national database, with online access available to practitioners and other healthcare professionals, allowing for greater accessibility of test results [140].

ART and TB treatment are available free of charge at all public facilities, a programme now majority financed by the South African government (79% of HIV spending and 91% of the national TB programme funding came from domestic sources in 2014 and 2016, respectively [1,129]). Numbers of individuals on ART, in particular, have increased since the adoption of the nurse-initiated management of ART (NIMART) programme in 2007 [141], allowing for most individuals to receive their care in the community. Efforts to improve adherence were given a boost by the introduction of a fixed-dose combination ART formulation in 2013 [142], reducing the pill-burden for HIV-positive people. Xpert® MTB/RIF is now the first-line diagnostic test for TB and is used at over 200 laboratories across the country [139].

1.2.5.3 Current CRVS infrastructure

South Africa is one of only three countries in the WHO African region with >70% coverage of deaths through civil registration [12]. Death certificates are completed by medical practitioners (or by tribal leaders in areas without medical practitioners) and causes of death are broadly classified as ‘natural’ or ‘unnatural’ [143]. Autopsies are generally conducted only for deaths due to unnatural causes, which include trauma, suicide, and deaths related to medical procedures. Death certificates are collected by the Department of Home Affairs and collated by the Statistics South Africa, which produces, every year, a report on cause-specific mortality [144]. To do this, Statistics South Africa codes all causes of death listed on death certificates per ICD-10 and then uses two automated methods (the Medical Mortality Data System [MMDS] [145] and Iris [146]) to generate a single, underlying cause of death, per ICD rules. Death notification data are also collected by the South African Medical Research Council, who use the South African ID (a unique identifier assigned to each citizen) to keep records of the date and location of each death and produce periodic ‘Rapid Mortality Surveillance’ reports [147].

1.2.6. Lesedi Kamoso: “Light for the future”

The results of the Lesedi Kamoso (“Light for the future”) study, a mortality sub-study nested within three large studies of TB and HIV in South Africa (described below), make up the bulk of this thesis. The study aimed to determine the

autopsy prevalence of TB and other important infections among patients who died in the TB Fast Track, XPHACTOR, and XTEND studies; to perform verbal autopsies among the same target population; and to compare causes of death assigned by the two methods. Detailed methods and findings from this study are presented throughout the thesis and are therefore not listed here. This section describes briefly the three parent studies within which Lesedi Kamoso was nested.

1.2.6.1 Parent study 1: TB Fast Track

1.2.6.1.1 TB Fast Track: Background

TB Fast Track was a pragmatic, open-label, cluster-randomised controlled trial conducted in South Africa from late 2012 to mid-2015 [148] (Table 1:1; Appendix 2 [Chapter 8.2.1]). The premise behind the study was straightforward: mortality among people with advanced HIV disease in LMIC is high, particularly pre- and early on ART; TB is thought to be a leading cause of death in these individuals, but is also more difficult to diagnose, with patients often not started on treatment due to delays in diagnosis, as described above. The hypothesis was that identifying individuals at the highest risk of TB disease and starting them on TB treatment without waiting for microbiological confirmation (therefore circumventing the delays associated with routine diagnostic algorithms), followed by ART as soon as possible, would reduce all-cause mortality at six months. The trial set out to evaluate whether a novel algorithm that incorporated point-of-care measurements would fulfil this purpose when implemented in a pragmatic way in a primary care setting.

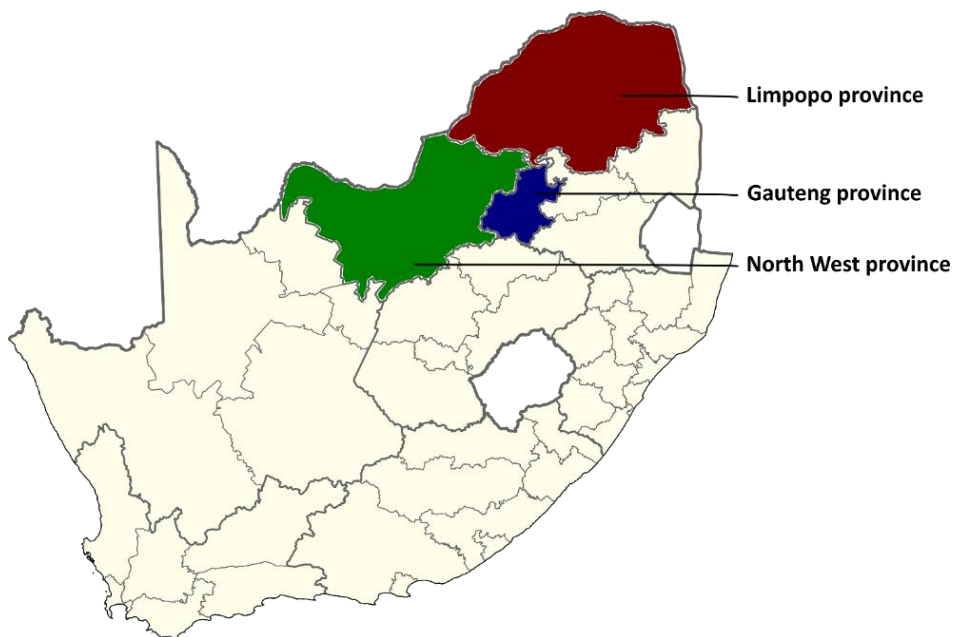
1.2.6.1.2 TB Fast Track: Setting and population

The study was conducted at 24 primary health clinics in three provinces of South Africa: Gauteng, North West, and Limpopo (Figure 1:2). Clinics were situated in diverse parts of the country, from Ekurhuleni Metropolitan municipality (Gauteng), the fourth most populous municipality in South Africa and one of the most densely populated, at 1,609 persons/km², to Moretele municipality in North West province (population density 136 persons/km²) and Greater Tubatse, in Limpopo (73 persons/km²) [149–151]. HIV prevalence among individuals aged 15–49 years, in 2012, was 20.3% (95% CI 17.5–23.4) in North West province, 17.8% (95% CI 14.6–21.6) in Gauteng, and 13.9% (95% CI 10.2–18.7) in Limpopo [152]. All study sites were primary health clinics with no access to on-site Xpert® MTB/RIF.

Individuals were eligible for inclusion in the study if, at the time of enrolment, they were ≥18 years of age; HIV-positive with a CD4 count of ≤150 cells/μL; not receiving ART or TB treatment and had not received them in the preceding six or three months, respectively; and were well enough not to require referral to a secondary care facility. To evaluate this

final criterion, all potential participants underwent a brief examination to decide if they were well enough for inclusion. Individuals were excluded, per WHO recommendations [153], if they had a heart rate of more than 120 beats per minute, respiratory rate of more than 30 breaths per minute, systolic blood pressure of less than 90 mmHg, evidence of respiratory distress or jaundice, or were otherwise judged (by a health professional) as needing referral to a secondary facility. Other exclusion criteria included a diagnosis of chronic hepatitis, a history of high alcohol intake (>112 or >84 units of alcohol per month for men and women, respectively), a previous reaction to efavirenz, and pregnancy, although the last criterion was later removed due to changes in South African guidelines [154].

Figure 1:2. Map of South African provinces in which the TB Fast Track study was conducted

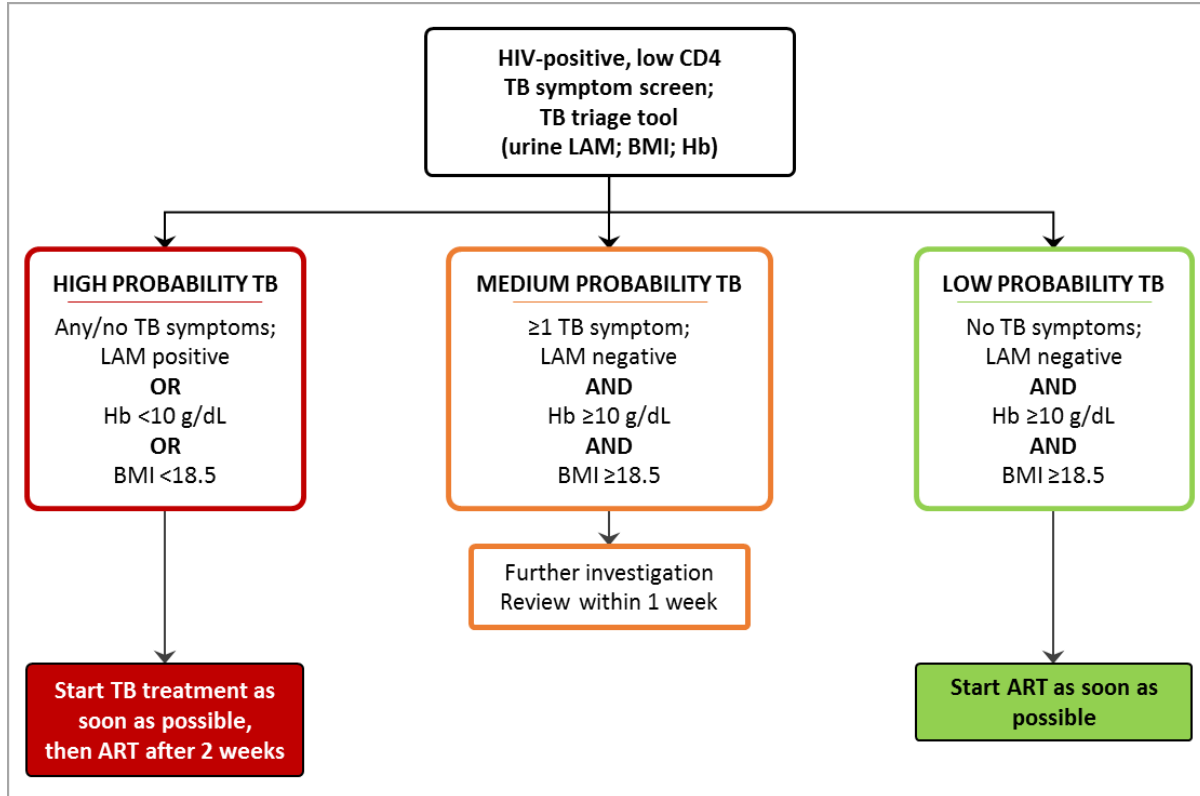


1.2.6.1.3 TB Fast Track: Study procedures

Each primary health clinic was treated as a cluster; 12 each in the intervention and control arms. At intervention sites, the body mass index (BMI) of each participant was calculated; their haemoglobin measured, using a point-of-care assay; and their urine tested, using a dipstick lateral-flow assay, for lipoarabinomannan (LAM), a marker of mycobacterial disease. A participant with any of BMI <18.5 kg/m², haemoglobin <10 g/dL, or positive (1+ or higher) urine LAM, irrespective of symptoms, was considered to have a 'high probability' of active TB and was started on TB treatment as soon as possible, followed by ART two weeks later (Figure 1:3). Participants who did not meet any of the above criteria and had no TB symptoms were considered to have a 'low probability' of active TB and were initiated on ART as soon as

possible. Those who did not meet high probability criteria but did have symptoms suggestive of TB were considered 'medium probability' and were investigated further and reassessed after a week.

Figure 1:3. The TB Fast Track study algorithm, reproduced with the kind permission of Prof Alison Grant



ART: antiretroviral therapy; BMI: body mass index; Hb: haemoglobin; LAM: lipoarabinomannan; TB: tuberculosis

1.2.6.1.4 TB Fast Track: Implementation

TB Fast Track completed enrolment in December 2014 and follow-up in June 2015. A total 3,053 participants were recruited, 31 (1.0%) of whom were subsequently excluded, leaving 3,022 in the final analysis. Some 364 individuals died after enrolment to the study, 285 (78.3%) within six months. The results of the study were presented at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI) in Boston, USA [155].

1.2.6.2 Parent study 2: XPHACTOR

The XPHACTOR study ran from mid-2012 to mid-2014 in four out-patient clinics in the Johannesburg area and evaluated a risk-based algorithm that prioritised investigation for TB (using Xpert® MTB/RIF) among HIV-positive adults attending for routine care (Table 1:1). The study enrolled individuals established on ART as well as those with low CD4 counts who had not yet initiated ART. All participants were followed up for at least three months after enrolment; a small subset of individuals with continuing symptoms suggestive of TB but without a diagnosis of TB at three months were

further investigated and followed up for an additional three months. The study enrolled 3,722 HIV-positive adults at the four sites, 125 (3.4%) of whom died. Only data from a subset of the overall cohort have been published so far [156], though other results were presented in 2015 [157].

1.2.6.3 Parent study 3: XTEND

In 2012, South Africa planned to roll-out Xpert® MTB/RIF to replace sputum smear at clinical sites across the country. Embedded within this roll-out was the XTEND study [158], a pragmatic, cluster-randomised trial to assess the effect on mortality of the implementation of Xpert® MTB/RIF in primary care facilities in South Africa (Table 1:1). The study was conducted at 20 laboratories and 40 primary health clinics, with each cluster defined as one laboratory and two clinics. Adults (≥18 years) were enrolled, irrespective of TB symptoms or HIV status, who were being investigated for TB by clinic staff, i.e., had sputum sent to a laboratory for testing. Depending on cluster, sputum samples underwent either immediate testing with Xpert® MTB/RIF or examination by smear microscopy. Laboratories using smear microscopy during the study period implemented Xpert® MTB/RIF around six months after the end of enrolment. The primary outcome was all-cause mortality at six months after enrolment.

Between June and November 2012, the study enrolled 4,656 adults, of whom 2,206 (47.4%) self-reported as HIV-positive. Some 231 individuals died after enrolment, at least 97 (42.0%) of whom were HIV-positive. Results of the study were published in 2015 [159].

Table 1:1. Summary of basic characteristics of three parent studies, including study populations and inclusion/exclusion criteria

	TB Fast Track	XPHACTOR	XTEND
Enrolment period	December 2012 to December 2014	September 2012 to March 2014	June to November, 2012
Minimum follow-up	Six months	Three months	Six months
Location	24 primary health clinics in Gauteng; North West; and Limpopo provinces.	Four out-patient clinics in Gauteng province (all in the Johannesburg area).	40 primary health clinics in Eastern Cape, Free State, Gauteng, and Mpumalanga provinces.
Population	HIV-positive adults (≥18 years) attending primary health clinics.	HIV-positive adults (≥18 years) attending out-patient clinics for HIV care.	Adults (≥18 years) attending primary health clinics
Inclusion criteria	<ul style="list-style-type: none"> • CD4 count ≤150 cells/μL 	<ul style="list-style-type: none"> • Already receiving ART or pre-ART 	<ul style="list-style-type: none"> • Being investigated for TB by clinic staff
Exclusion criteria	<ul style="list-style-type: none"> • Receiving or had received ART in the preceding 6 months • Receiving or had received TB treatment in the preceding 3 months • Any contraindication to efavirenz (until Aug 2013) • Pregnant (until Aug 2013) • Unwell at enrolment (HR >120, RR ≥30, SBP <90 mmHg, respiratory distress, jaundice, or haemoptysis) • High alcohol intake • Diagnosis of chronic hepatitis 	<ul style="list-style-type: none"> • Receiving or had received TB treatment in the preceding 3 months 	<ul style="list-style-type: none"> • Receiving TB treatment • Not resident in the catchment area • Planning to relocate within subsequent 8 months
Enrolled, n	3,022	3,722	4,656*
Female, n (%)	1,668 (55.2)	2,625 (70.5)	2,891 (62.1)
Age (years), median (IQR)	37 (32–44)	39 (33–46)	36 (28–47)
Total deaths, n (%)	364 (12.0) (until the end of the study period; 285 occurred within 6 months of enrolment)	125 (3.4)	231 (5.0)

*2,206 (47.4%) self-reported HIV-positive at enrolment

ART: antiretroviral therapy; HR: heart rate; IQR: interquartile range; RR: respiratory rate; SBP: systolic blood pressure TB: tuberculosis

1.3. Aims and objectives

1.3.1. Aims

This thesis aims to describe the burden of disease, particularly TB, in HIV-positive adults dying in resource-constrained settings; causes of death in HIV-positive adults using clinical data, with and without pathological autopsy; and causes of death in HIV-positive adults using verbal autopsies interpreted by different methods, with a particular focus on the measurement of mortality attributable to HIV-associated TB.

1.3.2. Objectives

The objectives of this thesis are to:

- 1. Synthesize the literature describing, in HIV-positive adults dying in LMIC,**
 - a) the use of pathological autopsy to estimate the burden of TB and other diseases,
 - b) the use of VA to estimate HIV- and TB-related mortality, and
 - c) direct estimations of causes of death using clinical and/or autopsy data;

- 2. Estimate the prevalence of TB and other infections, based on pathological autopsy, in adults with advanced HIV dying after enrolment to a pragmatic trial of empirical TB treatment in South Africa;**

- 3. Evaluate, among HIV-positive adults, the performance of different VA interpretation methods compared with a clinical reference standard derived from research and autopsy data, in**
 - a) assigning HIV-related causes of death at individual and population levels, and
 - b) differentiating HIV-associated TB from other HIV-associated causes of death; and

- 4. Evaluate, among adults with confirmed HIV status,**
 - a) the sensitivity and specificity of the 'HIV diagnosis' question included in the WHO 2012 VA instrument in assigning HIV status, and
 - b) the specificity of different VA interpretation methods in assigning HIV-associated causes of death.

1.4. Thesis structure

This thesis follows a 'research paper'-style format. A review of the literature is presented in two parts in Chapter 2, pertaining to (1) tissue autopsy estimates of disease burden in HIV-positive adults in LMIC and (2) the validation of VA

methods in estimating HIV- and TB-associated causes of death. The first research paper makes up Chapter 3, a systematic review of the literature around the direct estimation of causes of death in HIV-positive adults in LMIC. Chapters 4, 5, and 6 report methods and results of fieldwork conducted in South Africa, presented as three further research papers. Chapter 4 aims to address objective 2, describing the prevalence of TB and other infections at autopsy, and chapters 5 and 6 address objectives 3 and 4, respectively, describing the performance of VA in estimating TB and HIV-associated mortality, as well as the sensitivity and specificity of individual VA questions in detecting HIV status and/or ART use. Finally, Chapter 7 summarises and discusses the various elements raised in the thesis, their relation to one another and to wider policy and practice, and offers a list of recommendations and conclusions.

1.5. Contribution to the work presented in this thesis

1.5.1. Conception, study design, protocol development, and regulatory approvals

I was not involved in the conception of the idea for this study, which came from discussions between Prof Alison Grant and colleagues at the Aurum Institute (including Dr Salome Charalambous and Dr Kerrigan McCarthy) and Johns Hopkins University (Dr Chris Hoffmann). The managers of the three parent studies were also involved at an early stage (Dr McCarthy [XTEND], Dr Yasmeen Hanifa [XPHACTOR, LSHTM], and Dr Mpho Tlali [TB Fast Track, the Aurum Institute]). My first involvement was in helping develop the Lesedi Kamoso study protocol and applying for funding.

Once funding had been secured, I was closely involved in study design, including applications to regulatory bodies. I developed standard operating procedures (SOPs), hired and trained staff, and established the study among the various stakeholders (primarily mortuary owners, clinic staff, and hospital management). A particularly large undertaking was the training of TB Fast Track study staff in obtaining written informed consent for MIA, something that was done at the point of enrolment in TB Fast Track. I devised the training, led workshops, monitored progress, and worked closely with individual researchers to improve consent rates.

Various minor aspects of the protocol were modified as the study was implemented, the majority of which were at my direction, as the individual most familiar with study progress. The largest and most notable change to the study protocol, to conduct VAs among HIV-negative individuals to assess the specificity of VA in assigning HIV-associated causes of death, was suggested by Prof Daniel Chandramohan, who was not a study investigator, but is a member of my PhD advisory panel, and whose advice was sought part-way through the study.

1.5.2. Study management and data collection

1.5.2.1 Parent studies

The primary responsibilities for the management of the three parent studies were with the three trial managers. I was closely involved, however, in the day-to-day running of TB Fast Track, including in the design and piloting of case reports forms, data management, monitoring of study sites, and staff training. I was not involved in any study activities for XPHACTOR or XTEND.

1.5.2.2 Lesedi Kamoso sub-study

I oversaw all study activities and line-managed the five individuals in the study team. I wrote SOPs for MIAs, VAs, data collection from hospital files, and selection of HIV-negative deceased individuals from hospital mortuaries. I conducted the majority of MIAs and oversaw VA data collection (the VA interviews themselves were conducted by research assistants) and coordinated data collection from clinics, hospitals, and the National Health Laboratory Service (NHLS) online database. In these last two tasks, I received some assistance from Dr Natalie Wood, a student from LSHTM who volunteered to work with our team for a few months, and certain members of the TB Fast Track team. I also reviewed registers at five hospital mortuaries and traced hospital files and lab results for several hundred deceased adults to find HIV-negative individuals for whom a VA could be conducted.

The collection of VA data for decedents from the XTEND study was coordinated by a project manager at the Aurum Institute, Ms Noriah Maraba, who also led the initial analysis and write-up of those data; this was published in 2016 [160]. I was closely involved in the analysis and writing process and am listed as the second author on the paper. Although VA data from these individuals are included in this thesis (research paper 4 [Chapter 6]), I conducted all analyses presented here. This thesis also presents interpretation of these VA data by SmartVA-Analyze, something not done in the previous analysis.

1.5.3. Data management & analysis

VA data were entered directly into an online database by the interviewers; I conducted quality assurance of all VA data, including review of open narratives. Results of tests conducted on pathological autopsy samples were recorded centrally by the private laboratory at which the majority of tests were conducted. I wrote CRFs and built separate databases for remaining pathological autopsy data, hospitalisation data, secondary data from clinics, and data from the NHLS database. Data entry and management for the parent studies were coordinated by the data management

department at the Aurum Institute; I was involved in data management for TB Fast Track only in an advisory capacity and had no involvement in data management for XPHACTOR or XTEND.

I designed and managed the all physician review of verbal autopsies and clinical data. To allow for easier review of clinical cases, I collated information from several sources (clinic files, hospital files, research databases, and the NHLS database) and produced a chronological summary for each decedent (see Appendix 4 [Chapters 8.4.2 and 8.4.3] for examples). I also designed digital entry forms to allow for remote review and managed all secondary data created by reviewers.

I conducted all analyses presented in this thesis. This included the development of two large Stata do-files: the first for converting VA data from WHO 2012 format into a format suitable for SmartVA-Analyze, for which I received assistance from Prof Chandramohan, Dr Nicholas Maire, and Mr Vinit Mishra, who provided me with 'R' code that could be adapted for Stata (Appendix 5 [Chapter 8.5.4]); and the second for changing the causes of death assigned by physicians reviewing VA or clinical data into a suitable format for the Mortality Medical Data System (MMDS) to convert into a single 'underlying' cause (Appendix 5 [Chapter 8.5.5]).

1.5.4. Multi-authored papers

1.5.4.1 Research paper 1: Directly estimated causes of death among HIV-positive adults in low- and middle-income countries. A systematic review and meta-analysis (Chapter 3)

This manuscript has not yet been submitted for publication and has not yet been reviewed by all co-authors. Prof Katherine Fielding and Prof Chandramohan both provided advice regarding the systematic review protocol and Prof Grant has critically reviewed the protocol and manuscript.

1.5.4.2 Research paper 2: Autopsy prevalence of tuberculosis and other potentially treatable infections among adults with advanced HIV enrolled in out-patient care in South Africa (Chapter 4)

I carried out all analyses, wrote all drafts, and maintained overall control of the content of this paper. Dr Nicole Wolter managed and collated the results of tests carried out at the National Institute for Communicable Diseases (NICD). Prof Fielding and Drs Anne von Gottberg, McCarthy, Tanvier Omar, Wolter, and Emily Wong made important suggestions regarding the presentation of results and Drs von Gottberg, Neil Martinson, McCarthy, and Wong suggested changes to the structure and content of the discussion. All authors reviewed and approved the final manuscript prior to

submission. The peer reviewer at PLoS One had several comments; the letter written in response to her/his remarks, which also details the resultant changes made to the manuscript, is included in Appendix 6 (Chapter 8.6.1).

1.5.4.3 Research paper 3: Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: Comparing verbal autopsy, minimally-invasive autopsy, and research data (Chapter 5)

I conducted all analyses, wrote all drafts, created all the figures, and maintained overall control of the content of this paper. Prof Chandramohan facilitated the development of code for converting WHO VA data into a format suitable for SmartVA-Analyze; Prof Fielding offered guidance around statistical methods and presentation of results; and Profs Chandramohan and Kathleen Kahn provided advice around the overall direction and tone of the manuscript. All authors reviewed and approved the final manuscript prior to submission. Peer reviewers at PLoS One had only a few comments, requesting minor changes to the discussion; the letter written in response, which also details resultant changes made to the manuscript, is included in Appendix 6 (Chapter 8.6.2).

1.5.4.4 Research paper 4: Performance of verbal autopsy methods in estimating HIV-associated mortality among adults in South Africa (Chapter 6)

I conducted all analyses, wrote all drafts, created all the figures, and maintained overall control of the content of this paper. Prof Fielding made suggestions around presentation of results and table structure and Profs Chandramohan and Kahn provided advice around the overall direction and tone of the manuscript as well as around the structure and content of the discussion. All authors reviewed and approved the final manuscript prior to submission. This manuscript was rejected from PLoS Medicine (prior to peer review) and is currently being prepared for resubmission to another journal.

1.5.5. Other individuals who provided guidance and/or input

My upgrading committee, made up of Dr Corinne Merle and Profs Robin Bailey and Ron Behrens, provided advice and suggestions around methods for comparing causes of death assigned by different methods and, importantly, around structuring the review process to minimise physician learning. Prof Sebastian Lucas provided helpful advice around some of the practicalities of the MIA procedure and Prof Stephen Lawn and Dr Stephen Morris-Jones gave input on testing pathological autopsy samples for TB and other bacteria.

Dr Ed Fottrell, at the very early stages of the study, provided input around some of the logistics involved in collecting data for verbal autopsies. Dr Abraham Flaxman was very helpful in gaining access to the most recent version of SmartVA-Analyze, which, at the time, was not available from the Institute of Health Metrics and Evaluation.

Several individuals offered useful perspectives and advice at various points during the study, all of which may have had an impact on study implementation, data analysis, or ideas expressed in this thesis, though their precise effects are not necessarily quantifiable. They include Dr Kevin Cain, Dr Janneke Cox, Dr Haileyesus Getahun, Prof Judith Glynn, Dr Kobus Herbst, Dr Michael Kimerling, Dr Richard Lessells, Prof John Porter, Dr William Wells, and Prof Basia Zaba.

Prof Grant was involved at every stage of the development of this thesis and critically reviewed and provided input on most or all of the material presented here.

Chapter 2. Literature review

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2.1. Introduction

This chapter includes two separate reviews of the literature. The first review (Part 1) describes the findings of studies estimating the autopsy prevalence of TB and other infections in HIV-positive adults dying in LMIC. This does not include discussion of causes of death assigned in autopsy studies, as this is discussed in detail in Chapter 3 (research paper 1). The second review (Part 2) will describe findings from VA validation studies conducted in areas of high HIV prevalence, with a particular focus on the types and quality of reference standards used for validation, and the accuracy of VA interpretation methods in assigning HIV- and TB-associated causes of death.

2.2. Part 1: Pathological autopsy prevalence of TB and other infections among HIV-positive adults dying in low- and middle-income countries

2.2.1. Introduction

Pathological autopsy studies have been a source of information on disease processes, interactions, and epidemiology in many contexts for many decades [86]. Regarding HIV specifically, data from pathological autopsy studies conducted in the late 1980's and early 1990's were critical in the development and refinement of AIDS case definitions [78,79] and, through a better understanding of the spectrum of disease associated with HIV, allowed for more accurate estimation of the burden of HIV-associated disease in developing countries [161]. Autopsy studies have been particularly useful in better understanding the relationship between HIV and TB [68], the leading cause of death among PLHIV in LMIC. Due to the difficulties involved in diagnosing TB in living individuals with advanced HIV (see Chapter 2), autopsies have been

relied upon to provide estimates of the burden of undiagnosed TB among HIV-positive individuals dying in these settings.

As the global rollout of ART continues, pathological autopsy data remain important in allowing us to monitor progress and predict the evolution of the HIV epidemic. As they have in the past, autopsy data remain key to the maintenance of up-to-date definitions that accurately reflect real-world phenomena and allow for reliable and meaningful estimates of disease, morbidity, and mortality.

2.2.1.1 Aim

To estimate the prevalence of TB and other infections among HIV-positive adults included in pathological autopsy studies conducted in LMIC.

2.2.2. Methods

2.2.2.1 Search strategy

MEDLINE® was searched (via PubMed®) using variations of the following terms: HIV/AIDS, autopsy, and a list of LMIC (per the World Bank, 2015 [162] and the Cochrane Group, 2012 [163]; Table 2:1). Conference abstracts and unpublished data were not searched. The reference section of the recent systematic review of studies reporting the autopsy prevalence of TB in LMIC [71] was checked for additional articles.

Table 2:1. Terms used to search the MEDLINE® database (via PubMed®)

#1	(("hiv"[MeSH Terms]) OR (acquired immune deficiency syndrome[MeSH Terms]) OR (HIV) OR (human immunodef*) OR (AIDS) OR (acquired immune def*) OR (acquired immunodef*))
#2	(("autopsy"[MeSH Terms]) OR (post mortem examination[MeSH Terms]) OR (autops*) OR ((postmortem) OR (post-mortem) OR (post mortem)) OR ((needle-autops*) OR (needle autops*)) OR ((minimally invasive autops*) OR (MIA)) OR (necropsy))
#3	Filters for low- and middle-income countries as described by the Cochrane Group, 2012 [163], updated as per World Bank, 2016 [162]
Final search	
#1 AND #2 AND #3	

MEDLINE: US National Library of Medicine® (NLM) bibliographic database; MeSH: Medical subject headings;

2.2.2.2 Screening and review

I conducted all screening and review of articles. Studies were excluded, based on titles and abstract, that included only children, only individuals without confirmed HIV status, only HIV-negative adults, or only HIV-positive adults in countries considered high-income at the time of data collection, or that assigned causes of death or measured only the prevalence of non-communicable diseases without describing the prevalence of infections. The full text (where available) of the remaining articles was reviewed and the same exclusion criteria applied. Where multiple articles described the findings from a single study or single group of individuals, the article was included that best described findings relevant to this review.

2.2.2.3 Data extraction and management

Using a standardised tool, data were extracted regarding study population, inclusion criteria, and autopsy and laboratory methods in all included studies. The prevalence of TB and/or other infections found in each was recorded, as were the site of disease, autopsy method, completeness of reporting (single organ/system vs. full autopsy report), and ART status of decedents (where reported). Data were entered into EpiData v3.1 (The EpiData Association, Odense, Denmark) and Microsoft Excel; all analyses were conducted using Stata v14 (StataCorp, College Station, TX, USA); and figures were formatted using Inkscape™ software (<https://inkscape.org/>).

2.2.3. Results

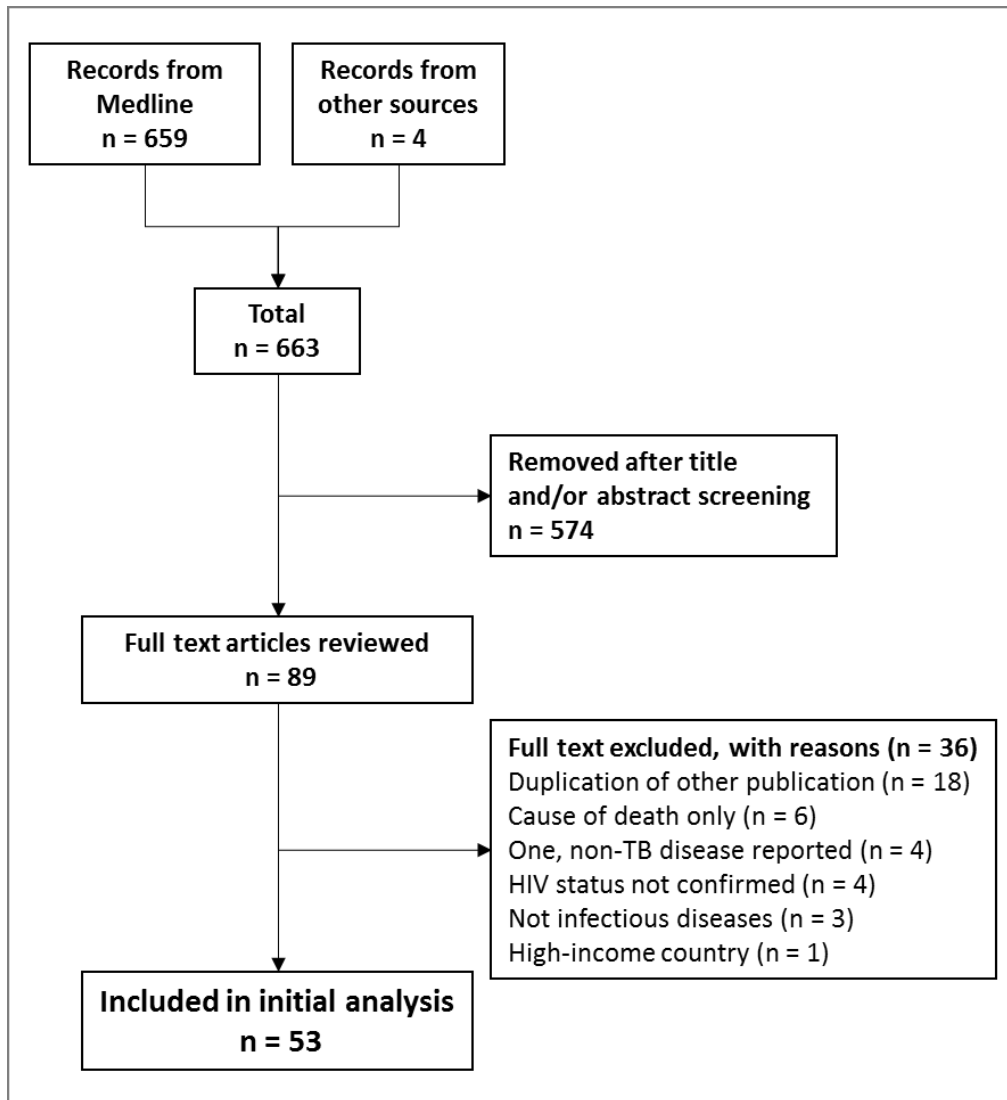
2.2.3.1 Search results and screening

The search of Medline yielded 659 results, which included all but four of the studies included in the 2015 systematic review [71]. After screening of titles and abstracts, 574/663 (86.6%) articles were excluded and 89 full-text articles, where available, were reviewed. Thirty six articles were excluded: 18 (50%) because they duplicated findings from a study already included (Table 2:2); six (17%) because they described only cause of death, not disease prevalence [95,164–168]; four (11%) because they reported findings for only one disease, other than TB [169–172]; four (11%) because they did not confirm the HIV status of decedents [97,173–175]; three (8%) because they did not report on the prevalence of infectious diseases [176–178]; and one (3%) because it was conducted in a high-income country [179]. A total 53 studies [19,87,93,94,180–228] were included in the review (Figure 2:1).

Table 2:2. Pathological autopsy studies with more than one published article; articles that were included in and excluded from this review

Study location	Data collected	First/senior author(s)	Included article(s)	Excluded article(s)
Cote d'Ivoire	1991	Lucas, SB	AIDS, 1993 [19]	BMJ, 1994 [229] Int J Cancer, 1994 [230] Tuber Lung Dis, 1994 [231]
Cote d'Ivoire	NS	Domoua, K	Revue de pneumologie clinique, 1993 [202]	Medecine tropicale: revue du Corps de sante colonial, 1995 [232]
India	1988–2007	Lanjewar, DN	Path Res Int, 2011 [188]	Clin Infect Dis, 1996 [233] Indian Heart J, 1998 [234] AIDS, 1998 [235] Indian J Pathol Microbiol, 1999 [236] HIV Med, 2001 [237] HIV Med, 2004 [238] Indian J Pathol Microbiol, 2016 [239]
Kenya	1996–1997	Rana, FS	JAIDS, 2000 [197]	J Acquir Immune Def Syndr Hum Retrovirol, 1997 [240]
Mexico	1984–1989	Mohar, A	AIDS, 1992 [205]	J Acquir Immune Def Syndr, 1990 [241]
Mozambique	2002–2004	Menendez, C	PLoS Med, 2008 [165]	PLoS Med, 2009 [242]
South Africa	2008–2009	Cohen, T	PLoS Med, 2010 [185]	Nat Med, 2016 [243]
Tanzania	1997–1999	Ng'walali, P	Forensic Sci Int, 2005 [228]	Arch Pathol Lab Med, 1999 [244]
Uganda	2009; 2013	Cox, JA	PLoS One, 2012 [183] JAIDS, 2014 [94]	J Clin Microbiol, 2015 [245] PLoS One, 2015 [246]

Figure 2:1. Flow diagram showing records found, screened, reviewed, and included in the analysis



TB: tuberculosis

2.2.3.2 Overview of studies

Between 1984 and 2013, 53 pathological autopsy studies were conducted among HIV-positive adults in LMIC, including 5,086 autopsies on HIV-positive adults (Table 2:3). The majority were carried out in sub-Saharan Africa (23 [43.4%] studies; 2,040 [40.1%] autopsies) and Latin America and the Caribbean (19 [35.9%] studies; 2,249 [44.2%] autopsies). Brazil was the country with the highest number of individual studies (n = 15), followed by South Africa (n = 6) and India (n = 5). Overall, low-income countries were under-represented, with most studies (32/53 [60.4%]) conducted in upper middle-income countries (2015 income threshold US\$ 4,036–12,475 [162]). Some of the most influential work in this area, conducted in Côte d'Ivoire and South Africa [19,211], was carried out in the 1990s, when ART was not available in most LMIC. Though the subsequent decades have seen increasing numbers of autopsy studies in PLHIV, few have

included individuals who died after initiating ART (n = 9 studies: 2/21 [9.5%] published from 2000–2009; 7/15 [46.7%] published from 2010–2016). Even in these nine studies, only half (531/1,120 [47.4%]) of the autopsies reported were conducted on individuals who had initiated ART.

The majority of individuals undergoing autopsy were recruited and died in hospitals (40 [76%] and 36 [68%] studies and 3,961 [78%] and 3,224 [63%] autopsies, respectively). Even in studies which recruited individuals from the community as well as hospitals, most recruitment and death occurred within facilities. Most studies used complete diagnostic autopsy, though three studies presented findings from only one system or organ [191,202,227]. Ten studies used partial autopsy or MIA, most (7/10) were conducted in sub-Saharan Africa. Eight studies [19,87,93,184,185,195,197,212] used mycobacterial culture to estimate the prevalence of TB, and only three [87,184,186] attempted to culture other pathogens; more recent studies used Xpert® MTB/RIF or other PCR methods on various samples to estimate TB prevalence, but most relied only on histology (38/53 [72%] used only histology and no other method).

Table 2:3. Inclusion criteria and methods used for pathological autopsy studies estimating disease prevalence in HIV-positive adults in LMIC (including deaths from 1984–2013) and stratified by geographic area (n = 53 studies; n = 5,086 autopsies)

Category	Studies, n (%)	Autopsies, n (%)
Region		
Sub-Saharan Africa	23 (43.4)	2,040 (40.1)
Latin America & the Caribbean	19 (35.9)	2,249 (44.2)
South Asia	5 (9.4)	409 (8.0)
East Asia & the Pacific	5 (9.4)	359 (7.1)
Europe & Central Asia	1 (1.9)	29 (0.6)
Country income category*		
Low-income	7 (13.2)	549 (10.8)
Lower middle-income	14 (26.4)	1,312 (25.8)
Upper middle-income	32 (60.4)	3,225 (63.4)
Recruited at		
Hospital only	40 (75.5)	3,961 (77.9)
Hospital + community	4 (7.6)	199 (3.9)
Not specified	9 (17.0)	926 (18.2)
Died at		
Hospital only	36 (67.9)	3,224 (63.4)
Hospital and community	6 (11.3)	524 (10.3)
Not specified	11 (20.8)	1,338 (26.3)
Basic inclusion criteria		
Adults only	29 (54.7)	2,568 (50.5)
Adults + children	14 (26.4)	1,422 (28.0)
Males and females	51 (96.2)	5,005 (98.4)
TB diagnosis required	5 (9.4)	341 (6.7)
Other diagnosis required	4 (7.6)	497 (9.8)
Included any individuals on ART	9 (17.0)	1,120 (22.0) [†]
Autopsy & laboratory methods		
Complete autopsy [‡]	39 (73.6)	3,996 (78.6)
Partial autopsy/MIA	10 (18.9)	717 (14.1)
Histology	46 (86.8)	4,150 (81.6)
Mycobacterial culture	8 (15.1)	937 (18.4)
Xpert [®] MTB/RIF or other TB PCR	2 (3.8)	140 (2.8)
Other bacterial/fungal cultures/PCR	3 (5.7)	172 (3.4)

* At time of study [247]

[†]Total number of individuals included in these nine studies, not all initiated ART

[‡]With or without examination of brain & spinal cord; some studies conducted complete autopsies but presented limited findings

ART: antiretroviral therapy; LMIC: low- and middle-income countries; MIA: minimally invasive autopsy; PCR: polymerase-chain reaction; TB: tuberculosis

2.2.3.3 Prevalence of TB

2.2.3.3.1 Studies that attempted to recruit a representative sample and obtained pathological specimens from only the lungs, or from the lungs and other anatomical sites

Thirty studies, conducted between 1984 and 2013, made attempts to recruit consecutive samples of HIV-positive adults dying in LMIC (Table 2:4). Most (24/30 [80%]) studies were included in a systematic review by Gupta et al., published in 2015 [71], which included post-mortem studies of HIV-positive adult and/or child deaths in LMIC published before December 2013 and used a random-effects model to generate pooled estimates of TB prevalence for each geographical region. The review excluded studies that included less than 10 individuals; that recruited individuals with a specific diagnosis (e.g., TB) or from a specific sub-group (e.g., miners); or that did not sample the lungs. Thirty-six studies were included overall, 26 (72%) involving adults; each study was assessed for quality using a pre-determined checklist (scored out of 10), which included evaluation of participant selection and autopsy methods used. Of the 26 studies, which described 2,397 autopsies, 22 (85%) had the full manuscript included and four (15%) only the abstract; 12 (46%) were from sub-Saharan Africa, seven (27%) from the Americas, four (15%) from South Asia, and three (12%) from South-East Asia; and 18 (69%) involved full autopsy (with or without brain and spinal cord), four (15%) limited autopsy, and four (15%) did not have autopsy methods specified. The median quality score for the 26 studies was 5.0 (interquartile range [IQR] 3.5–6.9). (Two studies included in the Gupta review were excluded from this review: one that was conducted in Taiwan, a high-income country [179,247] and another whose findings were replicated in a separate publication, which was included instead [241].)

Based on random effects meta-analysis, the overall pooled prevalence of TB in adults included in the Gupta et al. systematic review was 39.7% (95% CI 32.4–47.0), with 45.8% (95% CI 32.6–59.1) of those with TB at autopsy not identified prior to death. In sub-Saharan Africa, the pooled prevalence of TB was 43.2%, (95% CI 38.0–48.3; n = 9 studies of adults) compared with 27.1% (95% CI 16.0–38.1; n = 5 studies) in the Americas and 63.2% (95% CI 57.7–68.7; n = 2 studies)) in South Asia. Meta-regression analyses showed an increase in prevalence of ~5% every 10 years from 1992–2002. Twelve studies included information on dissemination: of decedents with TB, 87.9% (95% CI 82.2–93.7) had disseminated disease, with the lungs, liver, and spleen involved in 75–85% of cases (when sampled); central nervous system (CNS) involvement was seen only in ~20% of TB cases.

Two large studies of the pathological autopsy prevalence of TB among HIV-positive adults in LMIC have been published since the 2015 systematic review was conducted (Table 2:4). The first [94], conducted in a tertiary hospital in Kampala,

Uganda, recruited HIV-positive adults dying in hospital of non-maternal and non-traumatic causes in early-to-mid-2013. Complete autopsy and MIA were conducted for those who consented, with only histological examination used to assess for the presence of TB. Among the 96 adults included (57% female; median age 35 years; median CD4 count 47 cells/ μ L; 61% exposed to ART), complete autopsy found TB in 42 (44%), with the lungs (35/42 [83%]), liver (30/42 [71%]), and spleen (29/42 [69%]) most often involved. The authors did not report the proportion with disseminated disease, but at least 70% of individuals had extrapulmonary disease, suggesting high levels of dissemination; TB prevalence stratified by ART status was also not reported.

The second major study [180] was conducted in a teaching hospital in Lusaka, Zambia, and recruited HIV-positive and HIV-negative individuals; aged >16 years; with next of kin; who were admitted for any reason; and who died in one of the general medical wards from 2012–2013. Complete autopsy was conducted for those included, with histological examination and Xpert® MTB/RIF used to assess for the presence of TB. A total 125 individuals were included (36% female; median age 35 years; 65 [63%] receiving TB treatment), of whom 101 (81%) were HIV-positive (47 [48%] exposed to ART; other demographics not stratified by HIV status). TB was found in 66/101 (65%) HIV-positive adults; all 66 individuals had pulmonary TB and tested positive on Xpert® MTB/RIF and 33/66 (50%) had disseminated disease. Data are not shown for TB prevalence stratified by TB treatment or ART status, but the authors reported no correlation between exposure to or duration of ART and the presence of TB at autopsy.

A further four studies, conducted in China [187], Cote d'Ivoire [202], Mexico [205], and Brazil [206], recruited HIV-positive individuals dying in hospitals and presented either all findings from complete autopsy or findings only from the lungs (Table 2:4). The Chinese and Brazilian studies were both small (n = 7 and n = 15; TB prevalence 29% and 13%, respectively). In the two larger studies, both of which were conducted in the pre-ART era, TB was found in 31/70 (44%; Cote d'Ivoire; respiratory findings only) and 45/177 (25%; Mexico) individuals.

In the 30 studies, described in Table 2:4, that attempted to recruit representative samples of HIV-positive adults and obtained pathological specimens from at least the lungs, the prevalence of TB at autopsy ranged from 5.8% to 65.3%. Among the 2,999 individuals included, 1,111 had evidence of TB at autopsy, giving an overall crude prevalence of 37.0%.

Table 2:4. Pathological autopsy studies that attempted to recruit a representative sample and obtained pathological specimens from only the lungs, or from the lungs and other anatomical sites (n = 30 studies; n = 2,999 autopsies)

First author, year published	Data collected	Country	Population	Site of recruitment (death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments
							N (% female)	On ART, n (%)	Prevalence of TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM, n (%)	CMV, n (%)	
Cox, 2014* [94]	2013	Uganda	HIV+; died in medical ward (prospective)	Hosp (Hosp)	CDA + MIA	Histo.	96 (57)	59 (62)	42 (44)	13 (14)	12 (13)			Excluded deaths due to postpartum & trauma. Other bact. (6); KS (8); other fungi (4)
Bates, 2015* [180]	2012–2013	Zambia	HIV+ or HIV-; died in hospital (prospective)	Hosp (Hosp)	CDA	Histo.+ PCR	101 (36)	48 (48)	66 (65)	3 (3)	39 (39)	3 (3)		n = 35 on ART for median 6 (IQR 2–35) weeks; KS (6); meningitis (7)
Siika, 2012 [181]	2012	Kenya	Receiving ART	Hosp (Hosp)	CDA	NS	223 (68)	223 (100)	80 (36)		28 (13)			Abstract only; ‘sepsis’ (19)
Carrilho, 2012 [182]	2010	Mozambique	Medical i/p deaths	Hosp (Hosp)	CDA	NS	214 (NS)	0	64 (30)		19 (9)			Abstract only; adults + children; bacterial meningitis (15); KS (15)
Cox, 2012 [183]	2009	Uganda	Consecutive deaths, ID/gastro ward	Hosp (Hosp)	CDA	Histo.	35 (51)	10 (29)	16 (46)	8 (23)	1 (3)	5 (14)	1 (3)	KS (3); other bact. (4)
Wong, 2012 [184]	2009	South Africa	Consecutive i/p receiving/eligible for ART	Hosp (Hosp)	MIA	Histo. + Culture + PCR	39 (49)	25 (64)	25 (64)	4 (10)	1 (3)	6 (15)	1 (3)	Other bact. (9); NTM (2); other fungal (2)
Cohen, 2010 [185]	2008–2009	South Africa	Consecutive i/p deaths	Hosp (Hosp)	MIA	Culture	226 (56)	39 (17)	106 (47)					Samples pooled before testing for TB
Garcia-Jardon, 2010 [186]	2000–2008	South Africa	Medical i/p deaths	Hosp (Hosp)	CDA	Histo.	86 (65)	0	33 (38)	6 (7)	7 (8)	17 (20)	1 (1)	Adults & children; KS (1); other bact. (12); other fungal (1)

First author, year published	Data collected	Country	Population	Site of recruitment (death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments	
							N (%) female	On ART, n (%)	Prevalence of						
							TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM, n (%)	CMV, n (%)	T. gondii, n (%)			
Li, 2009* [187]	2005–2007	China	AIDS; died in hospital (retrospective)	Hosp (Hosp)	CDA	Histo.	7 (0)	2 (29)	2 (29)	5 (71)	2 (29)	1 (14)	Other fungal (2); other bact. (1); n = 2 on ART for less than one week		
Lanjewar, 2011 [188]	1988–2007	India	Medical i/p deaths	Hosp (Hosp)	CDA	Histo.	236 (23)	0	152 (64)	18 (8) (5)	11 (5)	48 (20)	35 (15)	KS (2); other bact. (3)	
Eza, 2006 [189]	1999–2004	Peru	I/p deaths; selected if unclear CoD	Hosp (Hosp + Comm)	CDA	Histo.	16 (38)	0	2 (13)	3 (19)	2 (13)	7 (44)	KS (1); other fungal (5)		
Souza, 2008 [190]	1996–2003	Brazil	NS	NS (NS)	NS	NS	129 (26)	0	36 (28)	7 (5)	11 (9)	22 (17)	13 (10)	Abstract only; adults & children; other bact. (6); histoplasmosis (17)	
Amarapurkar, 2005 [191]	1991–2003	India	Hosp i/p	Hosp (Hosp)	CDA	Histo.	60 (20)	0	35 (58)	1 (2)				Only liver findings reported; HCV (6); HBV (1)	
Viriyavejakul, 2002 [192]	NS	Thailand	Medical i/p deaths	Hosp (Hosp)	MIA	Histo.	17 (NS)	NS	1 (6)	2 (12)	1 (6)	2 (12)	2 (12)	Adults & children; other bact. (2); other fungal (3)	
Deshmukh, 2003 [193]	1993–2002		NS	NS (NS)	CDA	NS	60 (NS)	0	22 (37)					Adults & children	
Cury, 2003 [194]	1993–2000	Brazil	Hosp i/p	Hosp (Hosp)	CDA	Histo.	92 (25)	0	25 (27)	4 (4)	15 (16)	16 (17)	8 (9)	Other fungal (12)	
Soeiro, 2008 [195]	1990–2000	Brazil	Deaths from acute resp. failure	Hosp (Hosp)	CDA	Histo. + Culture	250 (21)	0	36 (14)	9 (4)	68 (27)	91 (36)	33 (13)	18 (7)	Adults & children; other bact. (10); 'sepsis' (34); NTM (15)
Satyanarayana, 2003 [93]	1998–1999	India	NS	NS (NS)	MIA	Histo. + Culture	44 (NS)	0	18 (41)	12 (27)				Adults & children; other bact. (12)	
Ansari, 2002 [196]	1997–1998	Botswana	Medical i/p deaths, inc. DOA	Hosp (Hosp + Comm)	CDA	Histo.	104 (46)	0	42 (40)	7 (7)	11 (11)	24 (23)	16 (15)	1 (1)	KS (11); other bact. (7); NTM (2)

First author, year published	Data collected	Country	Population	Site of recruitment (death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments	
							N (%) female	On ART, n (%)	Prevalence of						
							TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM, n (%)	CMV, n (%)	T. gondii, n (%)			
Rana, 2000 [197]	1996–1997	Kenya	Consecutive i/p deaths	Hosp (Hosp)	CDA	Histo. + Culture	75 (53)	0	38 (51)	4 (5)	3 (4)	22 (29)	3 (4)	2 (3)	≥17 with TB had non-TB 'final diagnosis'; KS (2); other bact. (12)
Liu, 1996 [198]	1996	China	NS	NS (NS)	NS	Histo.	151 (NS)	0	14 (9)						Abstract only; 25 cases of mycobacteriosis also had other infection or malignancy; NTM (20)
Marques, 1996 [199]	1996	Brazil	Medical i/p deaths	Hosp (Hosp)	NS	Histo.	40 (NS)	0	21 (53)						
Borges, 1997 [200]	1989–1996	Brazil	Medical i/p deaths	Hosp (Hosp)	CDA + MIA	Histo.	52 (NS)	0	9 (17)				16 (31)		
Ayisi, 1997 [201]	1995	Ghana	NS	NS (NS)	CDA	NS	20 (NS)	0	7 (35)						Adults & children
N'Dhatz, 1993* [202]	NS	Cote d'Ivoire	HIV+; died in respiratory ward (prospective)	Hosp (Hosp)	CDA	Histo.	70 (NS)	0	31 (44)			21 (30)			Present respiratory findings only. Only abstract available
Lucas, 1993 [19]	1991	Cote d'Ivoire	Consecutive i/p & community deaths	Hosp (Hosp + Comm)	CDA	Histo. + Culture	247 (24)	0	94 (38)	8 (3)	7 (3)	74 (30)	45 (18)		Other bact. (77); KS (22); other fungal (17); NTM (7)
Nelson, 1993 [203]	1988–1991	Zaire	Medical i/p deaths with AIDS + unknown CoD	Hosp (Hosp)	CDA	NS	64 (56)	0	26 (41)	19 (30)	2 (3)		13 (20)	11 (17)	KS (16); other fungal (35)
Abouya, 1992 [204]	1989	Cote d'Ivoire	Consecutive deaths, pulmonary ward	Hosp (Hosp)	NS	Histo.	53 (NS)	0	21 (40)		5 (9)	18 (34)			Extrapolated from CoD; KS (3)

First author, year published	Data collected	Country	Population	Site of recruitment (death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments	
							N (%) female	On ART, n (%)	Prevalence of						T. <i>gondii</i> , n (%)
						TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM, n (%)	CMV, n (%)					
Mohar, 1992* †[205]	1984–1989	Mexico	AIDS; died in hospital (prospective)	Hosp (Hosp)	CDA	Histo.	177 (7)	0	45 (25)	20 (11)	43 (24)	20 (11)	122 (69)	30 (17)	Adults & children (range 1–64 years; not stratified); other fungal (30); <i>M. avium</i> ; KS (53)
Michalany, 1987* [206]	NS	Brazil	Young males; AIDS; died in hospital (prospective)	Hosp (Hosp)	CDA	Histo.	15 (0)	0	2 (13)			3 (20)	9 (60)		Abstract only; TB not fully reported, but at least two cases; KS (2)

*Not included in Gupta et al. 2015 systematic review [71]

†Subset of these data included in Gupta et al. 2015 systematic review (Jessurun et al., 1990 [241])

AIDS: acquired immune deficiency syndrome; AM: ante mortem; ART: antiretroviral therapy; bact.: bacterial; CDA: complete diagnostic autopsy; CMV: cytomegalovirus; CoD: cause(s) of death; comm: community; crypto: cryptococcal disease; DOA: dead on arrival; i/p: in-patient; gastro.: gastroenterology; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV-: HIV-negative; HIV+: HIV-positive; Hosp: hospital; Histo.: histology; ID: infectious diseases; IQR: interquartile range; MIA: minimally-invasive autopsy; KS: Kaposi sarcoma; *M. avium*: *Mycobacterium avium*; NS: not specified; NTM: non-tuberculous mycobacteria; PCP: *Pneumocystis pneumonia*; PCR: polymerase-chain reaction (including Xpert® MTB/RIF); PM: post mortem; PNM: pneumonia; resp.: respiratory; *T. gondii*: *Toxoplasma gondii*; TB: tuberculosis

2.2.3.3.2 Studies that reported TB prevalence in non-consecutive samples, in only non-pulmonary specimens, or had other specific inclusion criteria

Seven studies had eligibility criteria that included diagnoses other than HIV (Table 2:5). Four of these, each of which required a prior diagnosis of TB, either ante or post mortem [87,207–209], were conducted in the Russian Federation, South Africa, Brazil, and Cote d'Ivoire and reported on individuals not exposed to ART. TB was found in 29/29 (100%), 37/47 (79%), 100/100 (100%), and 6/14 (43%) decedents, respectively. A further two studies excluded individuals with prior diagnoses of TB or known causes of death, but still found high prevalence of TB at autopsy (12/38 [32%], lung and lymph node biopsies, Tanzania [210] and 7/20 [35%], lung and liver biopsies, South Africa [211]).

Twelve studies reported only non-pulmonary findings (Table 2:6). Four of these, all of which made attempts to include a representative sample of inpatient deaths, reported on entire organ systems: two CNS [213,217], one gastrointestinal (GI) tract (samples from several sites, from tongue to large bowel) [214], and one bone marrow (from three anatomical sites) [215]. Prevalence of TB was low in CNS samples (4/284 [1%] and 2/138 [1%]); prevalence was higher in the GI tract (9/92 [10%]; Brazil) and even higher in bone marrow (15/50 [36%]; Uganda). Two studies conducted only liver biopsies and found TB in 19/117 (16%; Thailand [219]) and 34/100 (34%; Nigeria [218]; Table 2:6) samples; one sampled the kidneys (TB in 19/138 [14%]; Mexico [220]); and one the heart (TB in 3/32 [9%]; Puerto Rico [221]). Four further studies [222–225], all from Brazil, included adults not exposed to ART (samples sizes 100–128), sampled only exo- or endocrine glands (salivary, adrenals, thyroid, or pancreas) and reported TB prevalence from 5% (adrenals) to 23% (thyroid).

In the 12 studies that reported only non-pulmonary findings, 180 of 1,645 individuals had evidence of TB at autopsy, giving an overall crude prevalence of 10.9%. If, however, the three studies that reported only CNS findings were excluded, the overall crude prevalence increased to 17.9% (174/971 with TB; n = 9 studies).

Finally, two retrospective reviews of forensic autopsies, from Thailand [226] and Tanzania [228], and one of deaths among HIV-positive goldminers in South Africa [227] reported finding TB at autopsy in 19/67 (28%), 10/52 (19%), and 14/66 (21%) decedents, respectively (Table 2:7). Summary estimates of TB prevalence, stratified by geographic area, are presented in Table 2:8.

Table 2:5. Pathological autopsy studies that used specific diagnoses (other than HIV) as inclusion or exclusion criteria (n = 7 studies; n = 257 autopsies)

First author, year published	Data collection	Country	Population (study design)	Additional criteria	Site of recruitment (site of death)	Autopsy method	TB lab method /s	Among included HIV-positive adults							Comments	
								N (% female)	On ART, n (%)	Prevalence of				T. gondii, n (%)		
								TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM., n (%)	CMV, n (%)				
TB diagnosis																
Balabanova, 2011 [207]	2008	Russian Federation	HIV+; admitted through ED (retrospective)	Included only if PM TB CoD	H (H)	CDA	Histo.	29 (31)	NS	29 (100)				1 (3)	No CD4 data; no ART data; Diss. TB (16); TBM (5); Candida (7); Herpes (6)	
Martinson, 2007 [87]	2003–2005	South Africa	HIV+ or HIV-; died in hospital (prospective)	Included only if TB diagnosis	H (H)	CDA	Histo. + Culture	47 (55)	0	37 (79)	2 (4)	5 (11)	13 (28)	7 (15)	1 (2)	KS (3); Salmonellosis (11)
Gutierrez, 2002 [208]	1994–1996	Brazil	HIV+ or HIV-; died in hospital (retrospective)	Included only if AM or PM TB diagnosis	H (H)	CDA	Histo.	100 (20)	0	100 (100)	4 (4)	14 (14)	10 (10)	4 (4)		KS (3); other fungal (4)
Greenberg, 1995 [209]	1991–1992	Cote d'Ivoire	HIV+; autopsy in hospital (retrospective)	Included only if TB diagnosis	H+C (H)	CDA	Histo.	14 (14)	0	6 (43)		1 (7)				All patients received TB treatment; other fungal (4); NTM (1); KS (2); <i>Nocardia</i> spp. (2)
No TB diagnosis																
Kilale, 2013 [210]	NS	Tanzania	Died in hospital (prospective)	Excluded if TB diagnosis/ CoD or non-pulmonary diagnosis	H (H+C)	Lung & LN biopsies	Histo.	38 (47)	NS	12 (32)						Adults & children (range 15–44; not stratified)
Karstaedt, 1997 [211]	NS	South Africa	AIDS; died in hospital (prospective)	Included only if no CoD or no documented KS/lymphoma	H (H)	Lung & liver biopsies	Histo.	20 (NS)	0	7 (35)	2 (10)	2 (10)	4 (20)			Liver>lung for TB (n = 7 vs. n = 2); Septicaemia (1)

First author, year published	Data collection	Country	Population (study design)	Additional criteria	Site of recruitment (site of death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments
								N (% female)	On ART, n (%)	Prevalence of TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM., n (%)	CMV, n (%)	
Other															
Santosh, 1995 [212]	1989–1994	India	HIV+; died in mental health/neuroscience hospital (prospective)	Included if psychiatric/neurological presentation	H (H)	CDA/CNS only	Histo. + Culture	9 (22)	0	1 (11)	5 (56)	1 (11)		2 (22)	n = 5 CDA; n = 4 CNS samples only. Other bact. (2)

AIDS: acquired immune deficiency syndrome; AM: ante mortem; ART: antiretroviral therapy; bact.: bacterial; C: community; CDA: complete diagnostic autopsy; CMV: cytomegalovirus; CNS: central nervous system; CoD: cause(s) of death; Crypto: cryptococcal disease; diss.: disseminated; ED: emergency department; i/p: in-patient; H: Hospital; HIV-: HIV-negative; HIV+: HIV-positive; Histo.: histology; ID: infectious diseases; KS: Kaposi sarcoma; LN: lymph node(s); NTM: non-tuberculous mycobacteria; PCP: *Pneumocystis pneumonia*; PM: post mortem; PNM: pneumonia; *T. gondii*: *Toxoplasma gondii*; TB: tuberculosis

Table 2:6. Pathological autopsy studies sampling or reporting findings from a single non-pulmonary organ or system only (n = 13 studies; n = 1,645 autopsies)

First author, year published	Data collected	Country	Population (study design)	Site of recruitment (site of death)	Autopsy method	Findings reported	TB lab methods	Among included HIV-positive adults							Other results & comments
								N (% female)	On ART, n (%)	Prevalence of TB, n (%)	Crypto <i>P. jiroveci</i> , n (%)	Other bact. /fungi	CMV, n (%)	<i>T. gondii</i> , n (%)	
Systems															
Silva, 2012 [213]	1989–2008	Brazil	AIDS; autopsy at hospital (prospective)	Hosp (NS)	CDA	CNS	Histo.	284 (28)	75 (26)	4 (1)	45 (16)	1 (0.4)	11 (4)	7 (2)	85 (30)
Guimaraes, 2012 [214]	1989–1996	Brazil	Consecutive HIV+ autopsies at teaching hospital (retrospective)	Hosp (Hosp)	CDA	GI tract	Histo.	92 (17)	0	9 (10)	3 (3)		52 (57)	27 (29)	Protozoa (12); worms (8); KS (2); Three cases excluded due to poor samples
Nabadda, 2011 [215]	2005–2006	Uganda	HIV+; died in hospital from 'natural causes' (prospective)	Hosp (Hosp)	Bone marrow	Bone marrow	Histo.	50 (48)	NS	18 (36)	15 (30)		3 (6)		
Chimelli, 1992 [216]	NS	Brazil	HIV+ deaths (retrospective)	NS (NS)	CDA	CNS	Histo.	252 (NS)	0	NS	34 (13)		20 (8)	86 (34)	Abstract only; TB not reported; encephalitis (17)
Wainstein, 1992 [217]	1985–1990	Brazil	AIDS; autopsy in hospital (retrospective)	NS (Hosp)	CDA	CNS	Histo.	138 (NS)	0	2 (1)	17 (12)		2 (1)	29 (21)	Abstract only (article in Portuguese)
Solid organ(s)															
Echejoh, 2006 [218]	2003–2004	Nigeria	HIV+; died in hospital (prospective)	Hosp (Hosp)	Liver biopsies	Liver	Histo.	100 (50)	NS	34 (34)			17 (17)		KS (5)
Viriyaajakul, 2000 [219]	NS	Thailand	HIV+ (prospective)	Hosp (Hosp)	Liver biopsies	Liver	Histo.	117 (23)	NS	19 (16)	25 (21)		5 (4)	6 (5)	Adults & children (range 2–74 years; not stratified)

First author, year published	Data collected	Country	Population (study design)	Site of recruitment (site of death)	Autopsy method	Findings reported	TB lab methods	Among included HIV-positive adults							Other results & comments	
								N (% female)	On ART, n (%)	Prevalence of				T.		
								TB, n (%)	Crypto, n (%)	<i>P. jirovecii</i> , n (%)	Other bact. /fungi	CMV, n (%)	<i>T. gondii</i> , n (%)			
Soriano-Rosas, 1998 [220]	1986–1991	Mexico	AIDS; autopsy in hospital (prospective)	Hosp (NS)	CDA	Kidneys	Histo.	138 (14)	0	19 (14)	10 (7)	4 (3)	8 (6)	Adults & children (not stratified)		
Altieri, 1992 [221]	NS	Puerto Rico	“Consecutive AIDS deaths” (prospective)	NS (NS)	CDA	Heart	Histo.	32 (16)	0	3 (9)	2 (6)	5 (16)	2 (6)	Abstract only; NTM (1)		
Endo- / exocrine																
Leon, 2009 [222]	1996–1999	Brazil	AIDS; died in hospital (retrospective)	Hosp (Hosp)	CDA	Salivary glands	Histo.	105 (31)	NS	18 (17)	7 (7)		16 (15)	Adults & children		
Rodrigues, 2002 [223]	1989–1998	Brazil	AIDS; autopsy in hospital (retrospective)	Hosp (NS)	CDA	Adrenals	Histo.	128 (23)	NS	7 (5)	7 (5)	8 (6)	62 (48)	HSV (2); protozoa (7)		
Basilio-de-Oliveira, 2000 [224]	NS	Brazil	HIV+; pre-ART; 'died from AIDS complications'	NS (NS)	CDA	Thyroid	Histo.	100 (NS)	0	23 (23)	5 (5)	4 (4)	7 (7)	17 (17)	<i>M. avium</i> (5)	
Chehter, 2000 [225]	1995	Brazil	AIDS and HIV–; i/p deaths; consecutive autopsies (prospective)	Hosp (Hosp)	CDA	Pancreas	Histo.	109 (27)	50 (46)	24 (22)	10 (9)		14 (12.8)	Immunohistochemistry used for CMV, <i>T. gondii</i> , <i>P. jirovecii</i> , and mycobacteria		

AIDS: acquired immune deficiency syndrome; ART: antiretroviral therapy; bact.: bacterial; C: community; CDA: complete diagnostic autopsy; CMV: cytomegalovirus; CNS: central nervous system; CoD: cause(s) of death; Crypto: cryptococcal disease; GI: gastrointestinal; Hosp: Hospital; HIV–: HIV-negative; HIV+: HIV-positive; Histo.: histology; HSV: herpes simplex virus; i/p: in-patient; ID: infectious diseases; KS: Kaposi sarcoma; LN: lymph node(s); NTM: non-tuberculous mycobacteria; neuro: neurological; NS: not specified; *P. jirovecii*: *Pneumocystis jirovecii*; PCP: *Pneumocystis pneumonia*; PM: post mortem; PNM: pneumonia; *T. gondii*: *Toxoplasma gondii*; TB: tuberculosis

Table 2:7. Pathological autopsy studies including only forensic cases or special populations (n = 3 studies; n = 185 autopsies)

First author, year published	Data collected	Country	Population (study design)	Site of recruitment (site of death)	Autopsy method	TB lab method/s	Among included HIV-positive adults							Comments
							N (% female)	On ART, n (%)	Prevalence of TB, n (%)	Crypto, n (%)	PCP, n (%)	Bact. PNM., n (%)	CMV, n (%)	
Peonim, 2012 [226]	2000–2010	Thailand	Forensic autopsies (retrospective)	H+C (H+C)	CDA	Histo.	67 (NS)	NS	19 (28)	3 (4)		8 (12)		Salmonellosis (1); other bact. (6); meningitis (4)
Murray, 2007 [227]	1990–2002	South Africa	Miners, HIV+ and HIV- (retrospective)	H+C (H)	Cardio-pulmonary	Histo.	66 (0)	0	14 (21)	12 (18)	9 (14)			Only respiratory findings reported
Ng'walali, 2005 [228]	1997–1999	Tanzania	Forensic autopsies - 'unusual' deaths; adults only (retrospective)	H+C (H+C)	CDA	Histo.	52 (25)	NS	10 (19)	3 (6)		7 (13)	1 (2)	

ART: antiretroviral therapy; bact.: bacterial; C: community; CDA: complete diagnostic autopsy; CMV: cytomegalovirus; Crypto: cryptococcal disease; H: Hospital; HIV-: HIV-negative; HIV+: HIV-positive; Histo.: histology; PCP: *Pneumocystis pneumonia*; PNM: pneumonia; *T. gondii*: *Toxoplasma gondii*; TB: tuberculosis

Table 2:8. Crude summary estimates of the autopsy prevalence of TB in studies that sampled the lungs (n = 30) and studies that sampled only extrapulmonary sites, excluding the central nervous system (n = 9 studies), stratified by geographic area

Type of study	Crude prevalence, % (number of autopsies in which evidence of disease was sought)				
	Overall	Sub-Saharan Africa	Latin America & the Caribbean	South Asia	East Asia & the Pacific
Studies sampling the lungs (n = 30)	37.0 (2,999)	41.8 (1,653)	22.8 (771)	56.8 (400)	9.7 (175)
Studies sampling only extrapulmonary organs, excluding CNS (n = 9)	17.9 (971)	34.7 (150)	14.6 (704)	- (0)	16.2 (117)

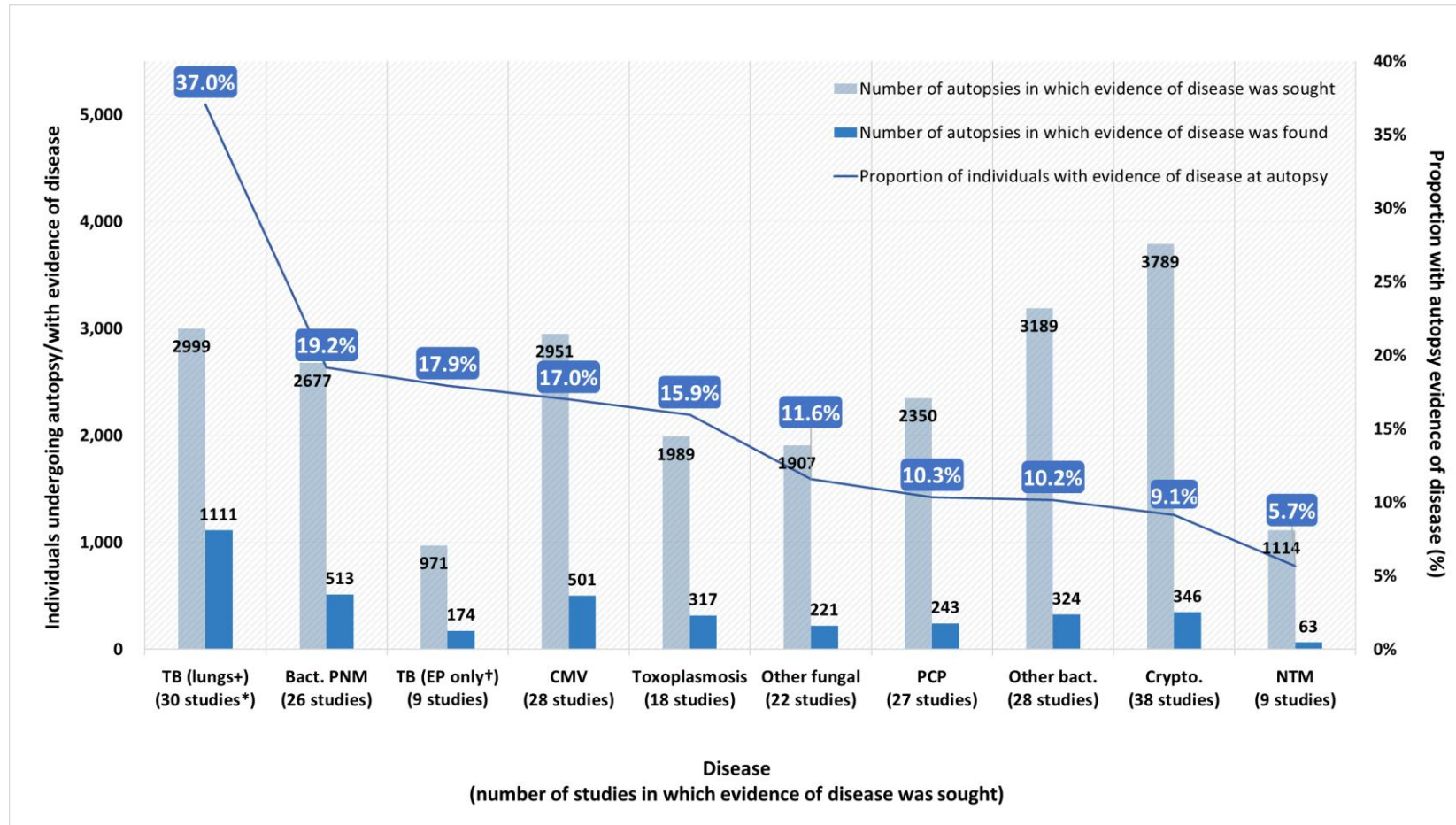
CNS: central nervous system; TB: tuberculosis

2.2.3.4 Prevalence of other infections

2.2.3.4.1 Cryptococcal disease

Thirty-eight studies reported on the autopsy prevalence of cryptococcal disease in 3,789 autopsies: 17 (45%) were conducted in the Americas; 14 (37%) in sub-Saharan Africa; four (11%) in South Asia; and three (8%) in East Asia & the Pacific. Prevalence ranged from 2% [191] to 30% [203] in studies of consecutive HIV deaths; the highest prevalence (56%) was seen in a study of nine individuals in India who presented with neurological symptoms [212]. Overall, evidence of disease was found in 346 individuals (crude prevalence 9.1%); disseminated disease was a common finding [184]. Crude estimates of the overall prevalence of TB and other infections are summarised in Figure 2:2.

Figure 2:2. Numbers of autopsies, number of individuals with evidence of disease, and overall crude autopsy prevalence of TB and other infections among pathological autopsy studies included in this review (n = 53 studies; n = 5,086 autopsies)



*Excludes studies that selected for individuals with additional diagnoses (n = 7 [87,207–212]), forensic studies (n = 2 [226,228]), and studies in special groups (n = 1 [227])

†Excludes studies that sampled only the central nervous system (n = 3 [213,216,217])

Bact.: bacterial; Crypto.: cryptococcal disease; CMV: cytomegalovirus; EP: extrapulmonary; PCP: *Pneumocystis pneumonia*; PNM: pneumonia; NTM: non-tuberculous mycobacteria; TB: tuberculosis

2.2.3.4.2 Other infections

Bacterial pneumonia was reported by fewer studies, but those that did found high proportions of decedents with evidence of disease. Of 2,677 autopsies from 26 studies (majority [58%] in sub-Saharan Africa) evidence of bacterial pneumonia was found in 513 individuals, giving an overall crude prevalence of 19.2%. Other bacterial infections, including non-specific sepsis and bacterial meningitis were reported in at least 324 individuals in 28 studies (3189 autopsies; crude prevalence 10.2%). Evidence of *Nocardia* spp. and *Salmonella* spp. were reported less often (n = 2 and n = 12 autopsies, respectively).

The study that reported bacteriology most systematically was conducted by Wong et al. in Johannesburg, South Africa [184], where antigen testing and aerobic, anaerobic, and fungal cultures were performed for a range of specimens, with additional drug sensitivity testing as needed. The authors also had pre-defined criteria that allowed them to separate 'pathologic' organisms from probable contaminants. Most decedents included in this study had several positive bacterial cultures; in 13/39 (33%), bacterial infections, most often due to *Klebsiella* spp. or multiple organisms, were considered severe enough to have been the main cause of death.

Evidence of *Pneumocystis* pneumonia was found in 243 of 2,350 autopsied individuals (crude prevalence 10.3%; n = 27 studies); non-tuberculous mycobacteria in 63 of 1,114 (5.7%; n = 9 studies); toxoplasmosis in 317 of 1,989 (15.9%; n = 18 studies); cytomegalovirus in 501 of 2,951 (17.0%; n = 28 studies); and other fungal infections in 221 of 1,907 (11.6%; n = 22 studies; Figure 2:2).

2.2.4. Discussion

2.2.4.1 Overview

Fifty-three pathological autopsy studies were included in this review, describing over 5,000 autopsies in HIV-positive adults. There was variation in methods used to obtain samples and laboratory methods used to detect TB; almost all studies used histological examination, with only a few using mycobacterial culture and even fewer using Xpert® MTB/RIF or other PCR. In the 30 studies that presented finds from lung specimens (with or without other specimens), TB was found in 1,111 of 2,999 decedents, giving a crude overall prevalence of 37%. Prevalence was also high in individual studies examining specific extrapulmonary organs, for example, bone marrow (36%) and liver (16% and 34%). Two studies that recruited only HIV-positive individuals without a diagnosis of TB still found a high autopsy prevalence of TB, at 32% and 35%.

2.2.4.1.1 Prevalence of TB

The crude estimate of TB prevalence based on the 30 studies with lung samples corresponds with the pooled estimate of 39.7% generated by random-effects meta-analysis in the 2015 review by Gupta et al. [71], which included 25 of the studies included here. The similar findings from more recent studies [94,180], including those involving individuals on ART, suggest that TB remains a major cause of morbidity, and likely mortality, in HIV-positive adults in LMIC.

A striking finding of the Gupta systematic review was the high proportion of individuals with TB who had disseminated disease (88%). However, the exclusion from the systematic review of studies that did not sample the lungs may have led to an overall underestimation of disease burden, as individuals may present with only extrapulmonary disease. For example, in the study by Wong et al. [184] (South Africa, 2009), individuals with TB were more likely to have positive histology from liver (80%) and spleen (72%) than from lung (60%) samples. This is supported by findings from the two subsequent large studies in Uganda and Zambia (70% [94] and 50% [180] extrapulmonary disease in those with TB) and from the studies assessing liver histology conducted in Uganda [218] (TB in 34% of liver samples) and South Africa (TB found in 7/20 [35%] of liver samples and not found in the lungs of 5/7 [71%] of these individuals).

2.2.4.1.2 Co-prevalent disease

The prevalence of multiple, concomitant infections was often not reported systematically in the studies included in this review, but in those studies where this was reported, it was a common finding. For example, in the study by Cox et al. [94], 26/96 (27%) decedents had autopsy evidence more than one infection; this was seen in a similar proportion of decedents in the study by Soeiro et al. (67/250 [27%]) [195]. Few studies conducted culture for organisms other than TB, but in those that did, the proportions of decedents with multiple infections were higher (Wong et al. 17/39 [44%] decedents had more than one infection and in Martinson et al. 14/37 [38%] [87] decedents with TB also had another infection).

2.2.4.1.3 Antiretroviral therapy

Only nine studies included individuals who had initiated ART, who made up less than half of all decedents included in the studies. Of these nine studies, only two presented their findings stratified by ART status. Wong et al. (South Africa [184]) found evidence of TB in 87% of individuals with less than 90 days of ART (median 32 days) and 60% of individuals with more than 90 days ART (median 326 days). Silva et al. (Brazil [213]) presented only neuropathological findings and found very low prevalence of TB overall. However, the prevalence of all infections reduced slightly in individuals who

had received ART (no exposure to ART, 59.5% [n = 163]; <3 months of ART, 46.0% [n = 76]; and >3 months of ART, 48.9% [n = 24]). Neither study attempted to estimate adherence among decedents to ART; notably, none of the studies included in this review used data from VA or other sources (other than healthcare records), making it difficult to assess if adherence to treatment, or other behavioural or non-biomedical factors, had an impact on the prevalence of TB or other diseases in the individuals examined.

2.2.4.2 Possible sources of bias

2.2.4.2.1 Publication bias

This review did not use formal systematic review methodologies and, as such, only one electronic database was searched, which may have led to the exclusion of relevant studies. Studies were also included only if they were published in English or had a detailed abstract published in English, which may have led to studies published in other languages not being represented. Finally, the grey literature was not searched, which will have biased the review towards articles published in peer-reviewed journals. This review did, however, include some conference abstracts, sourced from the Gupta 2015 systematic review.

2.2.4.2.2 Selection bias

At least 78% and 63% of decedents were recruited and died in hospitals. Hospitalised individuals are grossly over-represented among these data, reducing their generalisability; many people in LMIC receive their care and may die out of hospital, so disease patterns described in these studies may not necessarily represent those in many countries most heavily affected by the HIV and TB epidemics. This issue arises repeatedly among mortality studies in LMIC and is discussed in greater detail later in this chapter (Chapter 2.3.4.5.1).

2.2.4.2.3 Autopsy and laboratory methods

Although MIA has been shown to be very sensitive for many diseases, particularly infections [99], complete autopsy is still the most sensitive and remains the gold standard. Studies that used MIA, therefore, may have not detected TB, or other diseases, that would have been detected by complete autopsy. Estimates of disease prevalence at autopsy from studies that used MIA may therefore be lower than the true prevalence.

Variations in laboratory methods, too, may account for differences in estimates. The two studies that used PCR methods to estimate the prevalence of TB, conducted in South Africa [184] and Zambia [180], both reported very high

prevalence, at 64% and 65%, respectively. Xpert® MTB/RIF has been shown to be sensitive and specific when used with pulmonary and extrapulmonary post mortem samples [248]. However, the interpretation of a positive PCR result from an autopsy sample will vary based on the history and context of the individual decedent, particularly in the absence of confirmatory culture or histological findings. The development of a standardised protocol for the use of PCR-based diagnostics on autopsy samples in resource-limited settings, including for the interpretation of results, would facilitate comparisons between studies and allow for more accurate monitoring of the disease burden over time.

2.2.4.3 Other limitations of this review

This review has a number of other limitations. Screening of titles and abstracts and review of full text articles, as well as data extraction from included articles were conducted only by me. Although clear inclusion and exclusion criteria were defined prior to conducting the review, this may have led to systematic misapplication of these criteria, or misinterpretation of certain data. Meta-analysis was not attempted as part of this exercise, meaning that the summary estimates of disease prevalence at autopsy do not take account of differences in sample size or population and should be interpreted with caution.

2.2.5. Summary

This review of pathological autopsy studies conducted among HIV-positive adults in LMIC, most of whom were recruited and died in hospitals, found that the prevalence of TB was consistently high, particularly in studies that included pulmonary samples. A high proportion of individuals had bacterial infections, particularly pneumonia. More data are needed on the autopsy prevalence of diseases in HIV-positive individuals who receive care and may die outside of hospitals and in individuals who die after long periods on ART. There is also a need to standardise the methods used to estimate the autopsy prevalence of TB and other diseases; to obtain pathological samples; in the laboratory; and in the interpretation of results, to allow for comparison across different contexts and to adequately monitor progress towards international goals.

2.3. Part 2: Studies that have used verbal autopsy to estimate HIV-associated mortality and compared findings to reference causes of death and/or HIV serostatus

2.3.1. Introduction

VA is the primary method used to estimate cause-specific mortality at HDSS sites [102,249,250]. VA data are also used to supplement mortality statistics, particularly in countries without robust CRVS systems [251]. Many of these countries have a high prevalence of HIV and TB, but VA has not been thoroughly validated for HIV- and TB-associated deaths. At least in part, this is due to the absence of a consistent gold standard against which VA-assigned causes of death can be compared.

Several validation studies have been conducted since the early 1990s, mostly in LMIC and often in areas of high HIV prevalence, but many have used, as reference standard, causes of death assigned by unstructured review of hospital files and/or death certificates. The quality of record-keeping in health facilities is variable [252] and death certificates have consistently been shown to misclassify causes of death in LMIC [253–255]. Many studies have also assessed the accuracy of VA in assigning HIV-associated causes of death without necessarily confirming, as HIV-positive, all individuals assigned a reference HIV-associated cause. Although it may be tempting simply to exclude these studies as being of insufficient rigour, they make up a sizeable proportion of validation attempts conducted to date, and many were designed with care, with efforts taken to ensure internal validity.

Even in situations where HIV serostatus data were available, there have been few formal attempts to assess the accuracy of VA questions in detecting HIV status. The sensitivity and specificity of individual questions are important in calibrating algorithms that may be used to assign causes of death from VA data, but some questions, particularly those regarding sensitive topics such as HIV, need to be tested in a variety of settings, to allow for differences in cultural norms. Variability exists not only in the quality of data used to compile reference datasets, but also in the methods. For example, HIV-associated TB is likely responsible for a large proportion of mortality in the areas where many VA validation studies have been conducted, but few studies have made it a focus, often grouping together all HIV-associated mortality, per ICD-10.

The issues described above mean that there is uncertainty about the accuracy of VA methods in quantifying overall HIV-associated mortality and, specifically, mortality due to HIV-associated TB. The last decade has seen the development of

several automated VA interpretation methods, all of which require testing and validation using real-world data.

Without consistency in the process and reference standards used, however, it is difficult to compare the performance of different methods.

2.3.1.1 Aims

To estimate the accuracy and precision of VA interpretation methods in estimating mortality due to HIV/AIDS, TB, or HIV-associated TB compared with clinical reference standards in settings of high HIV prevalence.

2.3.1.2 Objectives

Through review of the literature, to:

5. **Estimate the accuracy of various VA methods to estimate mortality due to HIV/AIDS, TB, or HIV-associated TB when compared with**
 - a) Individually assigned reference standard causes of death based on data from individuals with all or mostly confirmed HIV status,
 - b) Individually assigned reference standard causes of death based on data from individuals without confirmed HIV status, and
 - c) Population-level estimates of cause of death for individuals with confirmed HIV status;
6. **Estimate the specificity of various VA methods in estimating HIV-associated mortality in individuals with confirmed HIV status; and**
7. **Estimate the sensitivity and specificity of the VA question, “Was there a diagnosis of HIV/AIDS?”, in individuals with confirmed HIV status.**

2.3.2. Methods

2.3.2.1 Search strategy

The MEDLINE® database was searched using variations of the following terms in various combinations: ‘verbal autopsy’; ‘HIV’; and a list of LMIC, as defined by the World Bank and as published by the Cochrane Group [163] (Table 2:9). Additionally, reference sections of other VA articles, including a systematic review by Leitao et al., published in 2014 [109], were searched for relevant titles.

Table 2:9. Terms used to search the MEDLINE database (via PubMed)

#1	((“hiv”[MeSH Terms]))
----	-----------------------

	OR (acquired immune deficiency syndrome[MeSH Terms]) OR (HIV) OR (human immunodef*) OR (AIDS) OR (acquired immune def*) OR (acquired immunodef*)
#2	((“Verbal autopsy”[MeSH Terms]) OR (verbal autopsy) OR (VA) OR (PCVA) OR (physician-certified verbal autopsy) OR (InterVA))
#3	Filters for low- and middle-income countries as described by the Cochrane Group, 2012 [163], updated per The World Bank, 2016 [162]

Final search

#1 AND #2 AND #3

AIDS: acquired immune deficiency syndrome; MeSH: Medical Subject Headings; PCVA: physician-certified verbal autopsy; VA: verbal autopsy

2.3.2.2 Inclusion and exclusion criteria

Studies of adults from LMIC, published before the end of 2016, were included that used VA data to assign causes of death and compared them with any of three types of reference standard: individually-assigned causes of death, all or mostly (≥66%) with confirmed HIV status; individually-assigned reference causes of death without confirmed HIV status; or causes of death assigned at population level based on confirmed HIV status for all included individuals. Studies were also included that did not assign causes of death but used only HIV status to compare with VA findings, allowing for estimation of the specificity of various VA interpretation methods in assigning HIV-associated causes of death and the sensitivity and specificity of VA questions in assigning HIV status.

Studies were excluded that reported only on child or maternal deaths; that required decedents to have a specific diagnosis (other than HIV); that reported only on deaths in high-income countries; that reported only VA findings, without any attempts at validation against a reference standard; or used, as a reference standard, causes of death assigned by another VA method (for example, comparing InterVA-assigned causes of death with PCVA-assigned causes of death as reference standard). Studies were further excluded that used confirmed HIV status as reference but did not present their findings separately for HIV-positive and HIV-negative individuals, therefore not allowing for estimation of VA specificity.

2.3.2.3 Data extraction and management

A standardised form was used to extract data on study setting; population size and characteristics; numbers of decedents confirmed HIV-positive; inclusion and exclusion criteria; data available to those assigning reference causes of death; methods used to assign reference causes of death; definitions of HIV/AIDS causes of death; HIV-associated mortality fraction; VA instrument used; time from death to VA; VA interpretation method(s) used; comparison methods used; and measures of agreement reported. Data entry, screening, and organisation were conducted per methods described in Part 1 of this chapter (section 2.2.2.3).

2.3.2.4 Analysis

2.3.2.4.1 Rationale

There are broadly two ways of estimating agreement between methods assigning causes of death: (1) at an individual level, i.e., comparing causes of death assigned to individuals by different methods; and (2) at a population level, i.e., comparing proportions of deaths in the entire population assigned to each cause (CSMFs) by different methods. To measure individual agreement, older studies predominantly used Cohen's kappa [256], a measure of chance-corrected agreement between two testing modalities across all causes of death (values range from 0 to 1, where 0 represents agreement no greater than chance and 1 represents perfect agreement). Kappa, however, can vary depending on the CSMF composition of the reference dataset, which has led to questions regarding its suitability for use across different validation studies. As a result, the Institute of Health Metrics and Evaluations (IHME) published recommendations on metrics for use in measuring the performance of different VA interpretation methods in validation studies [257]. For estimating individual-level agreement, they proposed that chance-corrected concordance (Equation 1) be used for each cause of death, and overall chance-corrected concordance (Equation 2) used for comparisons across several causes. These measures correct for the variability, based on CSMF distribution, seen in Cohen's kappa.

Equation 1: Cause-specific chance-corrected concordance

$$CCC_j = \frac{C_j - 1/J}{1 - 1/J}$$

C_j : cause-specific concordance for cause j ; CCC : chance-corrected concordance; J : all possible causes

Equation 2: Overall chance-corrected concordance

$$\text{Overall CCC} = \frac{1}{J} \sum_{j=1}^J \text{CCC}_j$$

CCC_j: cause-specific concordance for cause *j*; *CCC*: chance-corrected concordance; *J*: all possible causes

For estimating population-level agreement, older studies used Lin's concordance correlation coefficient [258] or intra-class correlation to quantify agreement, but the IHME team recommend CSMF accuracy (equation 3), more recently updated to chance-corrected CSMF accuracy (equation 4), which is now widely accepted as the standard method [259].

Equation 3: Cause-specific mortality fraction accuracy

$$\text{CSMF accuracy} = 1 - \frac{\sum_{j=1}^J | \text{CSMF}_j^{\text{true}} - \text{CSMF}_j^{\text{pred}} |}{2(1 - \min_j(\text{CSMF}_j^{\text{true}}))}$$

CSMF: cause-specific mortality fraction; *J*: all possible causes; *pred*: predicted

Equation 4: Chance-corrected cause-specific mortality fraction accuracy

$$\text{CCCSMF accuracy} = \frac{(\text{CSMFA} - 0.632)}{(1 - 0.632)}$$

CCCSMF: chance-corrected CSMF; *CSMFA*: Cause-specific mortality fraction accuracy

Basic measures of sensitivity and specificity are still useful if considering specific causes of death or the performance of specific questions in the VA instrument.

2.3.2.4.2 Analysis activities

Sensitivity, specificity, chance-corrected concordance, and chance-corrected CSMF accuracies were summarised for HIV/AIDS causes of death, where available; when possible, measures were derived from the data presented using the formulae listed above. To estimate overall performance of each VA method against reference standards, crude summary measures (median and IQR) were calculated with exact binomial 95% CI using measures from each study or comparison, as applicable, and stratified by type of VA interpretation method (using the `-diagt-` Stata command).

Summary measures were visualised using 'box and whiskers' plots. All analyses were conducted using Microsoft® Excel

and Stata v14 (Statacorp, College Station, TX, USA); figures were formatted using Inkscape™ software

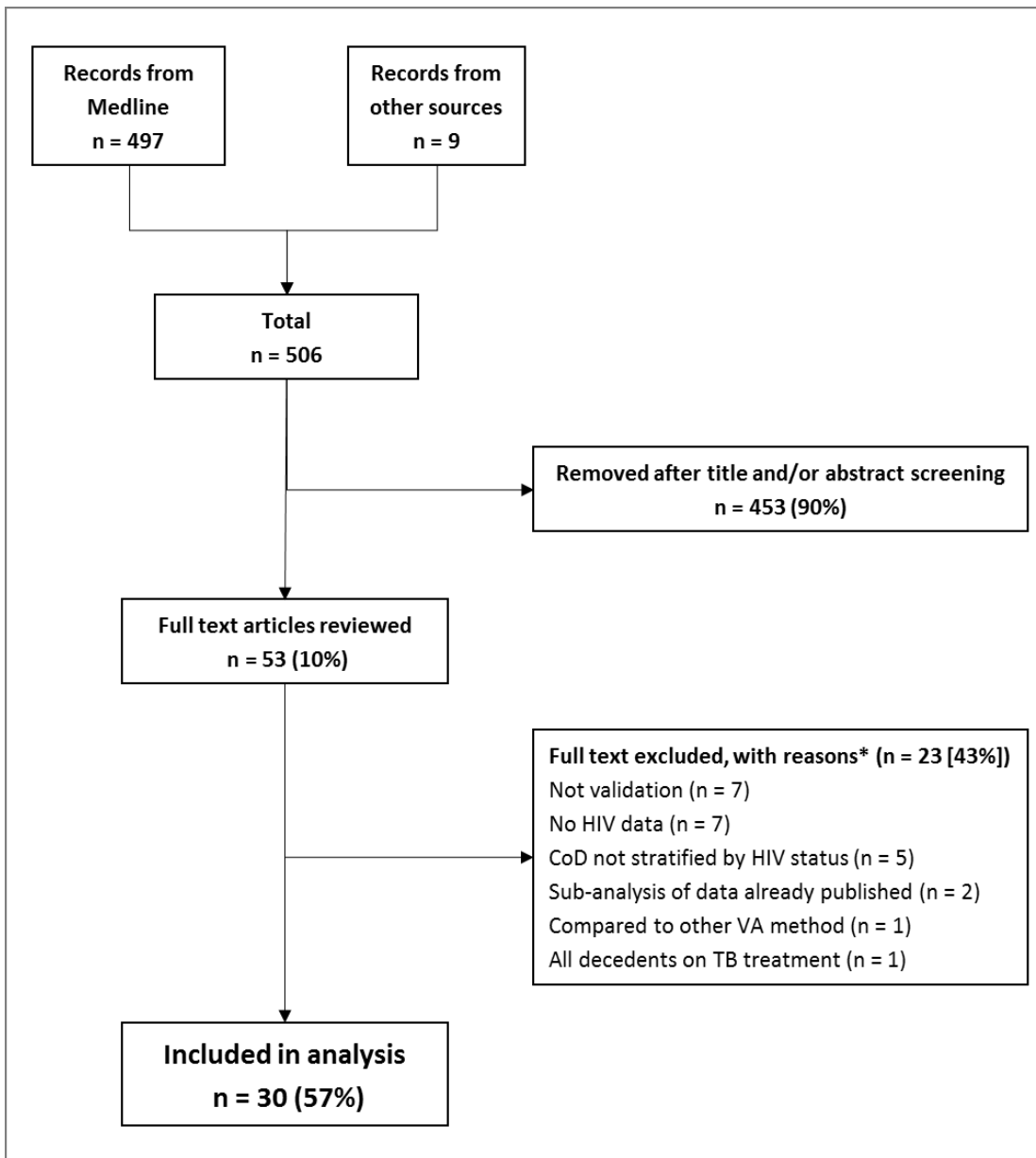
(<https://inkscape.org>).

2.3.3. Results

2.3.3.1 Search results, screening, and review

The electronic search of the Medline database yielded 497 articles; nine further records, including two PhD theses, were obtained from other sources (Figure 2:3). Of the 506 titles and/or abstracts screened, 453 (89.5%) were excluded based on the criteria described above. Fifty-three full-text articles were obtained for review, of which 23 (43.4%) were excluded: seven did not compare VA to a reference standard [260–266]; seven did not include data on HIV status [267–273]; five included HIV data, but did not show results stratified by HIV status [274–278]; two reported on sub-analyses [279,280] of data already included; and one, each, compared VA to a reference standard derived from VA data [281] or included only individuals who had received treatment for TB [282]. Several studies met more than one exclusion criterion; 30 articles were included in the analysis [120,123,283–309].

Figure 2:3. Flow diagram illustrating numbers of records found through electronic search and other sources, screened out by title or abstract, underwent full text review, and included in the analysis



*Some studies excluded for more than one reason

CoD: cause(s) of death; TB: tuberculosis; VA: verbal autopsy

2.3.3.2 Description of studies included

The 30 studies included in this review involved analysis of 22 different reference datasets and describe a total 26,890 adult deaths assessed by VA, at least 6,030 (22.4%) of which were in confirmed HIV-positive individuals (Table 2:10). Almost all datasets (20/22 [90.1%]) included adults from sub-Saharan Africa, where 61% of deaths occurred. Reference standards used for comparison were broadly divided into four categories: individual causes of death assigned where most or all individuals assigned HIV/AIDS causes of death were confirmed HIV-positive (3/22 [13.6%] datasets; 8,825 [32.8%] deaths; Table 2:11); individual causes of death assigned without confirmed HIV status (7/22 [31.8%] datasets;

7,248 [27.0%] deaths; Table 2:12); population-level estimates of causes of death in individuals with confirmed HIV status (3/22 [13.6%] datasets; 2,344 [8.7%] deaths; Table 2:13); and populations of individuals with confirmed HIV status, without attempts to estimate causes of death by means other than VA (9/22 [40.9%] datasets; 8,473 [31.5%] deaths; Table 2:14). Several studies used different VA methods to compare VA and reference causes of death: a total 41 comparisons were conducted in the 30 studies included; PCVA was used most often (15/41 [36.6%]) times), followed by InterVA software (10/41 [24.4%] times).

Table 2:10. Description of studies included, datasets analysed, and adult deaths with VA, stratified by location, population, reference standard, and analysis methods (N = 30 studies; N = 22 reference datasets; N = 26,890 deaths)

Characteristic	Studies*, n (%) (N = 30)	Reference standard datasets*, n (%) (N = 22)	Adults included in analysis, n (%) (N = 26,890)
Region			
Sub-Saharan Africa	27 (90.0)	20 (90.9)	16,411 (61.0)
East Asia & The Pacific	11 (36.7)	3 (13.6)	5,918 (22.0)
South Asia	8 (26.7)	1 (4.5)	2,973 (11.1)
Latin America & The Caribbean	8 (26.7)	1 (4.5)	1,588 (5.9)
Income category			
Low	24 (80.)	17 (77.3)	13,828 (51.4)
Lower middle	11 (36.7)	3 (13.6)	4,610 (17.1)
Upper middle	14 (46.7)	6 (27.3)	8,452 (31.4)
Recruited at			
Hospital	16 (53.3)	7 (31.8)	13,927 (51.8)
HDSS site	6 (20.0)	7 (31.8)	8,026 (29.8)
Population-based cohort	7 (23.3)	7 (31.8)	3,025 (11.2)
Not specified	1 (3.3)	1 (4.5)	191 (0.7)
Died at			
Hospital only	17 (56.7)	8 (36.4)	13,968 (51.9)
Hospital and community	13 (43.3)	14 (63.6)	12,922 (48.1)
Reference standard used			
Individual CoD with confirmed HIV status	11 (36.7)	3 (13.6)	8,825 (32.8)
Individual CoD without confirmed HIV status	8 (26.7)	7 (31.8)	7,248 (27.0)
Population CoD with confirmed HIV status	3 (10.0)	3 (13.6)	2,344 (8.7)

Characteristic	Studies*, n (%) (N = 30)	Reference standard datasets*, n (%) (N = 22)	Adults included in analysis, n (%) (N = 26,890)
HIV status only	8 (26.7)	9 (40.9)	8,473 (31.5)

*Studies and datasets may have spanned more than one county and evaluated/been evaluated by more than one VA method. Section row totals may therefore be higher than 30 or 22, respectively, and percentages may total more than 100.

CCVA: computer-coded VA; CoD: cause of death; HDSS: health and demographic surveillance system; PCVA: physician-certified verbal autopsy; VA: verbal autopsy

2.3.3.2.1 Definitions used

All studies, except one [290], combined HIV-associated TB with other HIV-associated causes, per ICD guidelines. The most systematic and structured reference dataset, with published criteria for each diagnostic category, is the Population Health Metrics Research Consortium (PHMRC) dataset (Table 2:11), discussed in more detail below: for an individual to be assigned a 'Level 1' diagnosis of 'AIDS', they were required to be confirmed HIV-positive and have a diagnosis of an AIDS-defining condition. There was also potential for misclassification of causes of death in some studies. For example, in the study by Tensou et al. [288] (Table 2:11), some decedents with absent HIV data were diagnosed with pulmonary or disseminated TB (ICD-10 codes A16 or A19) and counted as 'AIDS' deaths, whereas others were not; the authors do not specify the criteria used to classify individuals one way or the other and, as HIV status was not confirmed, data from these individuals were not included in this review.

In the eight studies that assigned individual causes of death without HIV data (Table 2:12), there was considerable heterogeneity in the methods used to assign causes of death, from structured review by multiple physicians in at least two studies [293,296], to one that simply described the process as 'expert review' [294]. Three studies took a population approach to assigning causes of death (Table 2:13): Lopman et al., in two studies [298,300], counted as 'AIDS' all deaths among HIV-positive individuals that were not from trauma or maternal causes; and Kanjala et al. [299] used HIV prevalence data from the entire HDSS site at which the study was conducted to calculate an HIV-associated mortality rate, which they then applied to the individuals who had died.

2.3.3.3 Studies that compared VA-assigned causes of death to individually-assigned reference causes of death where most or all individuals assigned an HIV-associated cause were confirmed HIV-positive

Eleven studies, evaluating three datasets, were included that assigned individual causes of death to adults with confirmed HIV status (Table 2:11). The largest dataset, used in eight studies, which included 16 evaluations of VA

methods, is the PHMRC gold standard dataset, the compilation of which has been reported in detail [121]. In brief, VAs, using the PHMRC VA instrument, and clinical information were collected for over 12,000 hospital deaths at six sites in India, Mexico, the Philippines, and Tanzania. Clinical data were reviewed by physicians and each individual assigned one of 53 mutually exclusive reference causes of death using pre-defined criteria. Reference causes of death were also graded (as Level 1, 2A, 2B, or 3) based on the quality of the evidence available. HIV and TB deaths were initially divided into three groups: 'AIDS', 'AIDS with TB', and 'Pulmonary TB'. For an individual to be assigned a Level 1 'AIDS' cause of death, s/he was required to have a positive HIV test and an AIDS-defining illness (other than TB); for a Level 1 'AIDS with TB' cause of death, a positive HIV test and a positive culture for MTB; and for a Level 1 'Pulmonary TB' cause of death, a history consistent with pulmonary TB, a negative HIV test, and either a positive smear for acid-fast bacilli (AFB) or positive culture for MTB. As discussed in Chapter 1.2.3.1, these categories are consistent with ICD-10, where codes for TB (A15–A19) specifically exclude 'HIV disease resulting in tuberculosis' [76].

Of the 7,839 adults included, 353 (4.4%) were assigned an 'AIDS' cause of death (345/353 [97.7%] Level 1); 148 (1.9%) an 'AIDS with TB' cause of death (all Level 1); and 275 (3.5%) a 'Pulmonary TB' cause of death (196/275 [71.3%] Level 1). For all comparisons with VA, however, the authors combined some of the 53 cause of death categories to give a final list of 34 causes; among these were the 'AIDS' and 'AIDS with TB' categories, which, when combined, left 501 individuals with a broad 'AIDS' cause of death (493/501 [98.4%] Level 1).

The PHMRC dataset has been made publicly available [310], but comparisons with VA have been conducted mainly by IHME, the team partially responsible for compiling the dataset. In 2011, the IHME group published a series of articles comparing causes of death assigned by various VA methods with the PHMRC dataset. Established methods, such as PCVA [311] and InterVA [284], and more experimental methods, such as Tariff [120,285], Random Forests [287], and Simplified Symptom Pattern [286], were evaluated. In 2014, the team published a further comparison between all methods, using updated versions of Tariff and InterVA software [283], and reported that all CCVA methods, apart from InterVA-4, performed better than PCVA across all causes and ages. However, when narrowed to adult 'AIDS' and 'TB' deaths only, none of the CCVA methods performed better than PCVA in assigning individual or population an HIV/AIDS cause of death (PCVA chance-corrected concordance 67.5% and chance-corrected CSMF accuracy 96.6%) and only InterVA-4 performed better in assigning a TB cause of death (chance-corrected concordance 53.2% vs. 48.5% and chance-corrected CSMF accuracy 97.4% vs. 96.4% for InterVA-4 vs. PCVA).

Further evaluations of some of the CCVA methods, as well as more recent software that uses probabilistic methods (InSilicoVA), were conducted by McCormick et al. [123], who found that the simplified symptom pattern method was the most sensitive in assigning AIDS deaths (60.1%) and InSilicoVA most sensitive for TB (52.7%). InSilicoVA, however, was very insensitive for AIDS deaths (16.7%). The Murray 2014 and McCormick publications, both of which used the same reference dataset and very similar VA interpretation methods to assign causes of death, have some notable discrepancies in results: although most of the differences are minor, the estimates of specificity of InterVA-4 in assigning an HIV/AIDS cause of death vary quite dramatically; Murray et al. estimate specificity at 19.5% and McCormick et al. at 46.7% [123,283].

Two other reference datasets included individually-assigned causes of death in adults with confirmed HIV status. Data in the first [290], published in 1998, were collected in Ethiopia, Ghana, and Tanzania and involved 796 individuals, 72 (9.0%) of whom were confirmed HIV-positive. Deaths related to HIV and/or TB were categorised as 'AIDS', 'AIDS with TB', or 'TB', though specific criteria were not described for each category. In the primary analysis, sensitivity and specificity of PCVA were 58% and 94% for 'AIDS' deaths (n = 55 deaths); 8% and 99% for 'TB + AIDS' deaths (n = 37 deaths); and 59% and 96% for 'TB' deaths (n = 56 deaths). When all TB and AIDS deaths were combined (n = 148 deaths), the sensitivity and specificity of PCVA were 76% (95% CI 68–82) and 94% (95% CI 92–96), respectively.

The third study in this category is also the smallest [288]. Conducted in Ethiopia in 2003, it involved 193 adults recruited in hospital, who died either in hospital or in the community; 101 (52.3%) were confirmed HIV positive and 108 (64.7%) were assigned a reference 'AIDS' cause of death. InterVA was 81% sensitive and 80% specific, with a chance-corrected CSMF accuracy of 74.8% in assigning deaths due to 'AIDS' (estimate derived from data presented). It should be noted that the procedures for assigning references causes of death in this study were not robust: reviewers used clinical data (available for only 38% of decedents) as well as the admission or discharge diagnosis to assign the cause of death. A high proportion of individuals died outside of hospital and there is no indication as to the time between hospitalisation and death; it is therefore not clear how these diagnoses relate to the final cause of death.

A total 8,825 adult deaths were included in the three datasets described in Table 2:11, at least 666 (7.5%) were confirmed HIV-positive, and 701 (7.9%) were thought to have died from HIV/AIDS. All 'AIDS' definitions included HIV-associated TB, per ICD-10. Crude estimates of median sensitivity, specificity, chance-corrected concordance, and chance-corrected CSMF accuracy of all VA methods in assigning an HIV/AIDS cause of death compared with these data

were 57.1% (95% CI 47–67; n = 13 comparisons), 96.9% (95% CI 94–98; n = 13), 54.0% (95% CI 51–57; n = 6), and 92.6% (95% CI 86.2–94; n = 15), respectively (Figure 2:4). PCVA performed best overall: median estimates of the above measures were 71.4% (95% CI 67–76; n = 2 comparisons), 95.7% (95% CI 94–97; n = 2), 67.5% (n = 1) and 96.6% (95% CI 75–97; n = 3), respectively. Median sensitivities of InterVA and Tariff in assigning an HIV/AIDS cause, when compared with these three datasets, were lower, at 46.7% (95% CI 20–81; n = 3) and 48.3% (95% CI 44–53; n = 2), respectively, though median chance-corrected CSMF accuracies were comparable, at 85.8% (95% CI 75–90; n = 3) and 92.3% (95% CI 92–93; n = 2), respectively.

Table 2:11. Studies in adults in LMIC that compared VA-assigned causes of death with individually-assigned reference causes of death with data on HIV status for most/all individuals, listed by year of first death (n = 11 studies; n = 8,825 deaths; n = 666 confirmed HIV-positive)

First author, year published	Dates of death	Country /ies	Adults with VA, N (n, [%] confirmed HIV+)	Reference standard		VA interpretation method	Verbal autopsy CoD										
				Data source(s)	AIDS, n (%)		'AIDS' or HIV-associated					TB					
				Process	TB, n (%)		n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*	n (%)	Sens. % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*	
Mc-Cormick, 2016 [123]	2007 – 2010	India, Mexico, Philippines, Tanzania	7,836 (≥493 [6.3])	Hospital files, lab results, death certs, autopsies	501 (6.4)	IVA-4	NS	46.7	96.7				NS	35.3	97.6		
						Tariff	NS	44.1	98.8				NS	47.8	97.4		
						SSP	NS	60.1	96.3				NS	48.8	96.9		
						ISVA	NS	16.7	99.5				NS	52.7	95.8		
Serina, 2015 [120]			PHMRC dataset			Tariff 2.0	NS			51.0 (50.5–51.8)		NS			43.5 (43.1–44.3)		
Murray, 2014 [283]			PHMRC dataset			PCVA	418 (5.3)	66.7	97.3		96.9	169 (1.5)	48.7	97.7		96.2	
						IVA-4	118 (1.5)	19.5	96.9		85.8	187 (2.4)	55.2	95.8		96.8	
						Tariff	295 (3.8)	52.5	97.8		92.4	147 (1.9)	45.2	98.1		95.4	
						SSP	300 (4.8)	57.1	98.5		92.6	148 (1.9)	48.4	98.1		95.4	
						RF	302 (3.9)	55.3	97.9		97.3	143 (1.8)	46.9	98.2		95.3	
						KL	268 (3.4)				91.4	116 (1.5)				94.3	

First author, year published	Dates of death	Country /ies	Adults with VA, N (n, [%] confirmed HIV+)	Reference standard		VA interpretation method	Verbal autopsy CoD												
				Data source(s)	AIDS, n (%)		'AIDS' or HIV-associated					TB							
				Process	TB, n (%)		n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*	n (%)	Sens. % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*			
Lozano, 2011† [311]			PHMRC dataset			PCVA	410 (5.2)				67.5 (66.6–68.6)	96.6		175 (2.2)				48.5 (47.5–49.8)	96.4
Lozano, 2011† [284]			PHMRC dataset			IVA-3.2	243 (3.1)				39.0 (38.7–39.4)	90.4		204 (2.6)				53.2 (52.8–53.5)	97.4
James, 2011† [285]			PHMRC dataset			Tariff	305 (3.9)				52.4 (51.6–53.3)	92.7		162 (2.1)				46.4 (44.9–47.4)	95.9
Murray, 2011† [286]			PHMRC dataset			SSP	328 (4.2)				55.6 (54.9–56.4)	93.6		163 (2.1)				46.9 (46.0–47.8)	96.0
Flaxman, 2011† [287]			PHMRC dataset			RF	333 (4.3)				56.5 (55.7–57.1)	93.7		159 (2.0)				46.2 (45.1–47.0)	95.8
Tensou, 2010† [288]	2003	Ethiopia	193 (101 [52.3])	Hospital dx + patient card	108‡ (64.7)	IVA	103‡ (61.7)	81§ (73–88)	80§ (67–89)			74.8§		NS					
		Recruited in hosp.; died in/out of hosp.		AIDS vs. non-AIDS vs. unknown	NS														
Quigley, 1999 ^{ll} [289]	1993 – 1995	Ethiopia, Ghana, Tanzania	796 (72 [9.0])	Hospital files, death certificates	92 (11.6)	Data algorithm	78 ^{ll} (20.2)	65	90			94.0		NS					

First author, year published	Dates of death	Country /ies	Adults with VA, N (n, [%] confirmed HIV+)	Reference standard		VA interpretation method	Verbal autopsy CoD													
				Data source(s)	AIDS, n (%)		'AIDS' or HIV-associated					TB								
				Process	TB, n (%)		n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*	n (%)	Sens. % (95% CI)	Spec., % (95% CI)	CCC, % (95% CI)	CCCSMFa*				
				Two physicians; pre-defined, graded criteria	56 (7.0)															
Chandra-mohan, 1998[†] [290]			Per Quigley et al., 1999			PCVA	88 (11.1)	76** (68–82)	94** (92–96)		98.5	63 (7.9)	59 (45–72)	96 (94–97)						97.4
						Expert algorithm	115 (14.4)	68** (60–76)	89** (86–91)		91.1	53 (6.7)	35 (20–53)	95 (93–96)						98.9

*When calculation possible, all chance-corrected CSMF accuracies derived for two causes of death (AIDS vs. not AIDS; TB vs not TB) using formulae described by Murray et al. [259] and Flaxman et al. [312]

†Included in Leitao et al. 2014 systematic review [109]

‡AIDS deaths as proportion of those confirmed 'AIDS' or 'not AIDS' (n = 167)

§Sensitivity, specificity, and chance-corrected CSMF accuracy calculated across all 193 deaths

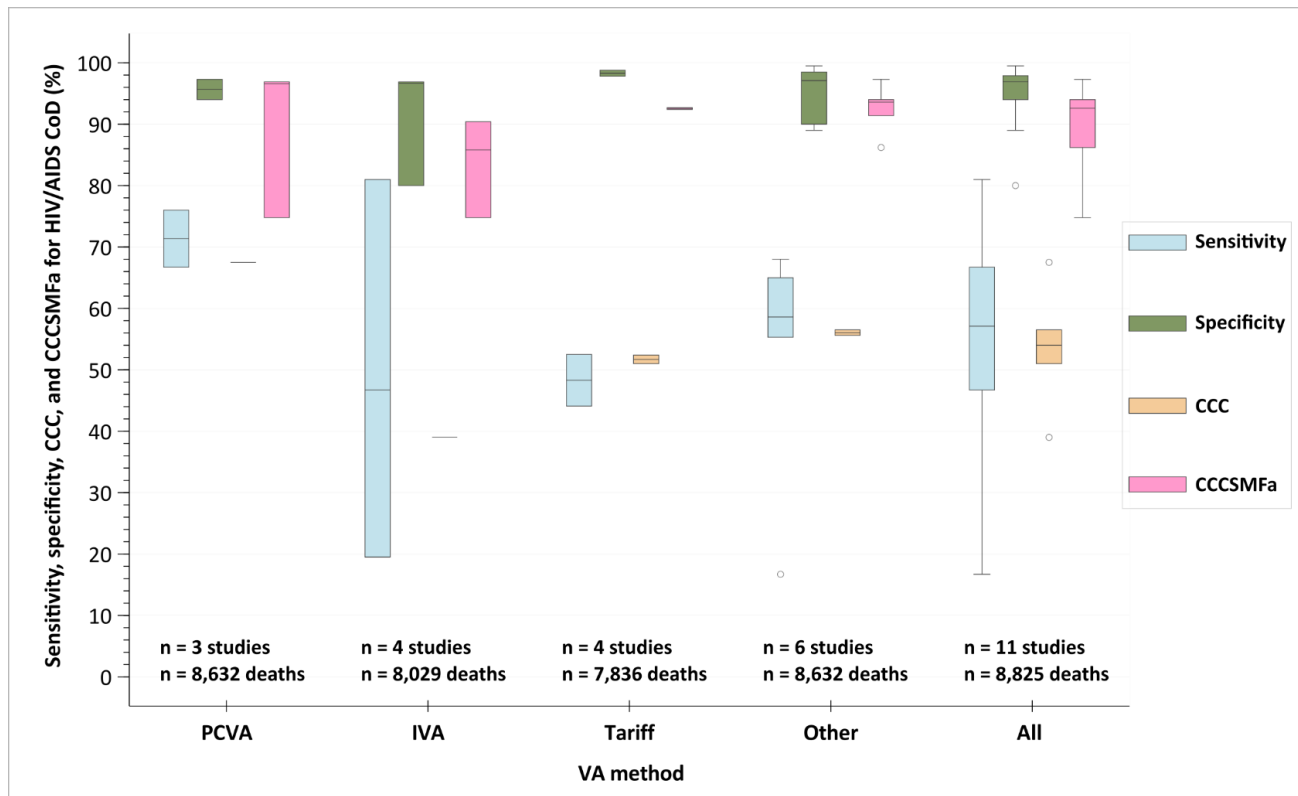
||All AIDS and TB deaths grouped together for analysis; measures of accuracy using only test dataset; n = 386 (71 [18.4%] with AIDS and/or TB)

¶'AIDS' deaths include 'AIDS + TB' (reference 37/796 [4.6%]; PCVA 11/796 [1.4%], sensitivity 8% [95% CI 2–21], specificity 99% [95% CI 98–100]; algorithm 53/796 [6.7%], sensitivity 35% [95% CI 20–53], specificity 95% [95% CI 93–96])

**As reported for any AIDS or TB cause of death

AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CCCSMFa: chance-corrected CSMF accuracy; certs: certificates; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; dx: diagnosis; HIV: human immunodeficiency virus; HIV+: HIV-positive; ISVA: InSilicoVA; IVA: InterVA; KL: King-Lu; LMIC: low- and middle-income countries; N/A: not applicable; NS: not specified; PCVA: physician-certified verbal autopsy; RF: Random Forests; sens.: sensitivity; spec: specificity; SSP: Simplified Symptom Pattern; TB: tuberculosis; VA: verbal autopsy; WHO: World Health Organization

Figure 2:4. Box plots illustrating crude summary measures of agreement between VA-assigned and reference HIV/AIDS causes of death from studies where most or all individuals assigned an HIV-associated cause of death were confirmed HIV-positive (n = 11 studies; n =666 confirmed HIV-positive)



Measures of agreement for individual deaths (sensitivity, specificity, and chance-corrected concordance) and at a population-level (chance-corrected CSMF accuracy) for VA methods in assigning an HIV/AIDS cause of death (per individual study definition), stratified by type of VA interpretation method used. Central horizontal line represents median; boxes represent interquartile range (IQR); and whiskers represent largest and smallest values within 1.5 IQR of the upper and lower quartiles, respectively

AIDS: acquired immune deficiency syndrome CCC: chance-corrected concordance; CCCSMFa: chance-corrected cause-specific mortality fraction accuracy; CoD: cause of death; CSMF: cause-specific mortality fraction; Ind: individual; PCVA: physician-certified verbal autopsy; Pop: population; VA: verbal autopsy

2.3.3.4 Studies that compared VA-assigned causes of death to individually-assigned reference causes of death in individuals without confirmed HIV status

Eight studies were included in this analysis, consisting of seven reference datasets and 7,248 deaths (Table 2:12). Six (75%) studies were conducted in sub-Saharan Africa (n = 2,590 deaths) and the remaining two (25%) in South-East Asia (China and Thailand, respectively; n = 4,658 deaths). All deaths occurred in hospitals. The study conducted in China, by Yang et al. [294], reported only estimates of deaths due to TB, not HIV, so was not included in estimates of mortality due to HIV/AIDS, leaving 5,148 deaths for the primary analysis. No study tried to differentiate between HIV and HIV-

associated TB, classifying all HIV-associated TB deaths as 'HIV/AIDS'. All studies, except one, used PCVA to interpret VA data.

Proportions of overall deaths attributed to HIV/AIDS varied widely, from 4.5% in South Africa (n = 102 [301]) to 33% in Tanzania (n = 1,912 [296]). Of a total 5,148 adult deaths included, at least 931 (18.1%) were assigned an HIV/AIDS cause of death. Crude summary estimates of VA sensitivity and specificity in assigning an HIV/AIDS cause of death were 69% (95% CI 63–82; n = 8) and 84% (95% CI 79–91; n = 8), respectively (Figure 2:5 shows crude estimates from studies included in Tables 2:12 and 2:13). Crude overall chance-corrected concordance was 36% (95% CI 33–39; n = 5) and chance-corrected CSMF accuracy 85% (95% CI 64–92; n = 7).

Table 2:12. Studies in adults in LMIC that compared VA-assigned causes of death with individually-assigned reference causes of death without confirmed HIV status; listed by year of first death (n = 8 studies; n = 7,248 deaths)

First author, year published	Dates of death	Country/ies	Adults with VA, N (confirmed HIV+, n [%])	Reference standard		VA interpretation method	Verbal autopsy CoD									
				Basis of CoD assignment	AIDS, n (%)		AIDS or HIV-associated					TB				
		Population		Data source/s	TB, n (%)		n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*	n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*
				Process												
Bauni, 2011 [291]	2007–2010	Kenya	145 (NS)	Individual	33 (22.7)	IVA	38 (26.2)	70 (5–84)	87 (79–92)	39.3†	87.9	18 (12.4)	83 (36–100)	91 (85–95)	66.7†	76.5
		HDSS; hosp. deaths		Clinical & lab data		PCVA	36 (24.8)	88 (72–97)	94 (88–98)	75.8†	92.7	11 (7.6)	100 (54–100)	96 (92–99)	100†	90.2
				Best judgement of physician	6 (4.1)											
Misganaw, 2012 [292]	2007–2010	Ethiopia	335 (NS)	Individual	69 (21)	PCVA	97 (29)	68 (57–79)	78 (72–83)	0.36†	71.4	77 (23)	63 (49–76)	84 (79–88)	25.0†	72.5
		Hosp. deaths		Hospital records												
				Hospital clerks blinded to VA	48 (14)											
Polprasert, 2010‡ [293]	2005	Thailand	2,558 (NS)	Individual	191 (7.5)	PCVA	123 (4.8)	60.7 (53–68)	99.7 (99–99)†	21.5†	92.2	46 (1.8)	32.3 (17–51)	98.6 (98–99)†	–35.5	98.4
		Hosp. deaths		Medical & lab records												
				Multiple reviewers; graded evidence [279]	31 (1.2)											
Yang, 2006‡ [294]	2002	China	2,100 (NS)	Individual	NS	PCVA	NS	NS	NS	NS	NS	39 (1.9)	62.2 (48–77)	99.3	24.4†	99.2
		Hosp. deaths		Medical records												
				'Expert review'	45 (2.1)											
Murray, 2007 [295]				Per Yang et al., 2006		SP	NS					NS				88§

First author, year published	Dates of death	Country/ies	Adults with VA, N (confirmed HIV+, n [%])	Reference standard		VA interpretation method	Verbal autopsy CoD										
				Basis of CoD assignment	AIDS, n (%)		AIDS or HIV-associated					TB					
Population				Data source/s	TB, n (%)		n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*	n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*	
				Process													
Setel, 2006† [296]	2000–2003	Tanzania	1,912 (NS)	Individual	634 (33.2)	PCVA	563 (29.4)	60 (55–65)	86 (84–88)	84.9	188 (9.8)	51 (43–58)	94 (93–95)			96.1	
		Facility or home deaths		Two physicians; graded based on evidence quality	163 (8.5)												
Hosegood, 2004 [297]	2000	South Africa	109 (NS)	Individual	NS	PCVA	NS	80	82		NS						
		Hosp. deaths		Hospital files	NS												
Kahn, 2000 [301]	1992–1995	South Africa	89 (NS)	Individual	4 (4.5)§§	PCVA	NS				NS	92	99				
		HDSS; hospital deaths		Hospital records	NS												
				One reviewer, blinded to VA	NS												

*When calculation possible, chance-corrected CSMF accuracies derived for two causes of death (AIDS vs. not AIDS; TB vs not TB) using formulae described by Murray et al. [259] and Flaxman et al. [312]

†Calculated from data provided; chance-corrected concordance derived using formula described by Murray et al. [259]

‡Included in Leitao et al. 2014 systematic review [109]

§Reports only ‘concordance’ of 88% for tuberculosis

||Includes all deaths in individuals >5 years of age; HIV-associated deaths include HIV-associated TB

¶AIDS-specific mortality fractions shown for n = 102 deaths used as ‘test’ dataset

**Number confirmed HIV-positive only reported for entire cohort (n = 1573), not specifically for those with VA (n = 1171)

††AIDS-specific mortality fractions shown for n = 555 deaths used as ‘test’ dataset

‡‡Range, based on variations to the algorithm

§§Reference CoD: ~23% ‘infectious/parasitic’ causes

AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CCCSMFa: chance-corrected CSMF accuracy; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; CSMFa: CSMF accuracy; HDSS: health and demographic surveillance system; HIV: human immunodeficiency virus; HIV+: HIV-positive; IVA: InterVA; KL: King-Lu; LMIC: low- and middle-income countries; N/A: not applicable; NS: not specified; PCVA: physician-certified verbal autopsy; popn: population; RF: Random Forests; sens.: sensitivity; spec.: specificity; SSP: Simplified Symptom Pattern; TB: tuberculosis; VA: verbal autopsy; WHO: World Health Organization

2.3.3.5 Studies that compared VA-assigned causes of death to population estimates of HIV-associated among individuals with confirmed HIV status

Three studies were included that used a reference standard derived from population estimates of HIV-associated mortality in individuals with known HIV status; these studies included three separate datasets reporting 2,065 deaths from Tanzania and Zimbabwe (Table 2:13). All data were from longitudinal community studies conducted within HDSS sites; investigators had access to HIV test data for all decedents described, but did not attempt to assign individual cause of death. In the two studies by Lopman et al., analysing deaths that occurred from 1998–2003 [300] and 1994–2005 [298], all deaths among HIV-positive adults from causes that were not traumatic or maternal were considered ‘AIDS’ deaths. These studies, unsurprisingly, report relatively high proportions of ‘AIDS’ deaths (65–76%) in populations with high HIV prevalence.

The third study, conducted in Tanzania [299], used HIV prevalence among individuals in the entire HDSS site (6% in 1994–1995, 8.3% in 2000–2001, and 6% in 2011), the estimated age-specific mortality rate due to HIV in the studied population, and the overall mortality rate in the population to estimate the proportion of HIV-attributable deaths among those that died. Among 1,573 (339 [21.6%] confirmed HIV-positive) deaths, 1,171 individuals also had VA data available. The authors estimated that around 20% of deaths were HIV-associated, though the estimate generated by InterVA-4 was higher, at 30.8%. The authors also attempted to assess mortality trends in relation to ART coverage; because of the absence of individual data, however, ART ‘availability’ was used as a proxy for ART use. They report an overall reduction in HIV-associated mortality among males aged 15–44 from over 40% in the pre-ART periods to under 40% after ART was widely available, but no similar changes among females. In addition, proportions of mortality attributable to HIV among HIV-positive individuals did not undergo any substantial change after ART became widely available, staying at around 90%.

Table 2:13. Studies in adults in LMIC that compared VA-assigned causes of death to population estimates of HIV-associated mortality among individuals with confirmed HIV status (n = 3 studies; n = 2,344 deaths; ≥631 confirmed HIV-positive)

First author, year published	Dates of death	Country/ies	Adults with VA, N (confirmed HIV+, n [%])	Reference standard		VA interpretation method	Verbal autopsy CoD													
				CoD basis	AIDS, n (%)		AIDS or HIV-associated					TB								
							Data source/s	TB, n (%)	n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*	n (%)	Sens., % (95% CI)	Spec., % (95% CI)	CCC, %	CCCS MFa*		
Lopman, 2006 [298]	1998–2003	Zimbabwe	381 (292 [77])	Population	77 (75.5) [†]	Algorithm	57 (55.8) [†]	66 (56–77)	76 (59–93)	32.5	29.4	NS								
		Population study, confirmed HIV status		NS	All HIV+ assigned 'AIDS' CoD unless trauma/maternal															
Kanjala, 2014 [299]	1994–2010	Tanzania	1,171 (339 [21.6]) [‡]	Population	(20.3)	IVA-4	361 (30.8)				64.1	NS								
		Population-based cohort		NS	Mortality estimated based on HIV prevalence															
Lopman, 2010 [300]	1994–2005	Tanzania, Zimbabwe	792 (NS)	Population	363 (65.4) [§]	Algorithm	NS	54–83	62–80			NS								
		Population study; confirmed HIV status		NS	All HIV+ assigned 'AIDS' CoD unless trauma/maternal															

*When calculation possible, all chance-corrected CSMF accuracies derived for two causes of death (AIDS vs. not AIDS; TB vs not TB) using formulae described by Murray et al. [259] and Flaxman et al. [312]

[†]AIDS-specific mortality fraction shown for n = 102 deaths used as 'test' dataset (n = 81 HIV-positive)

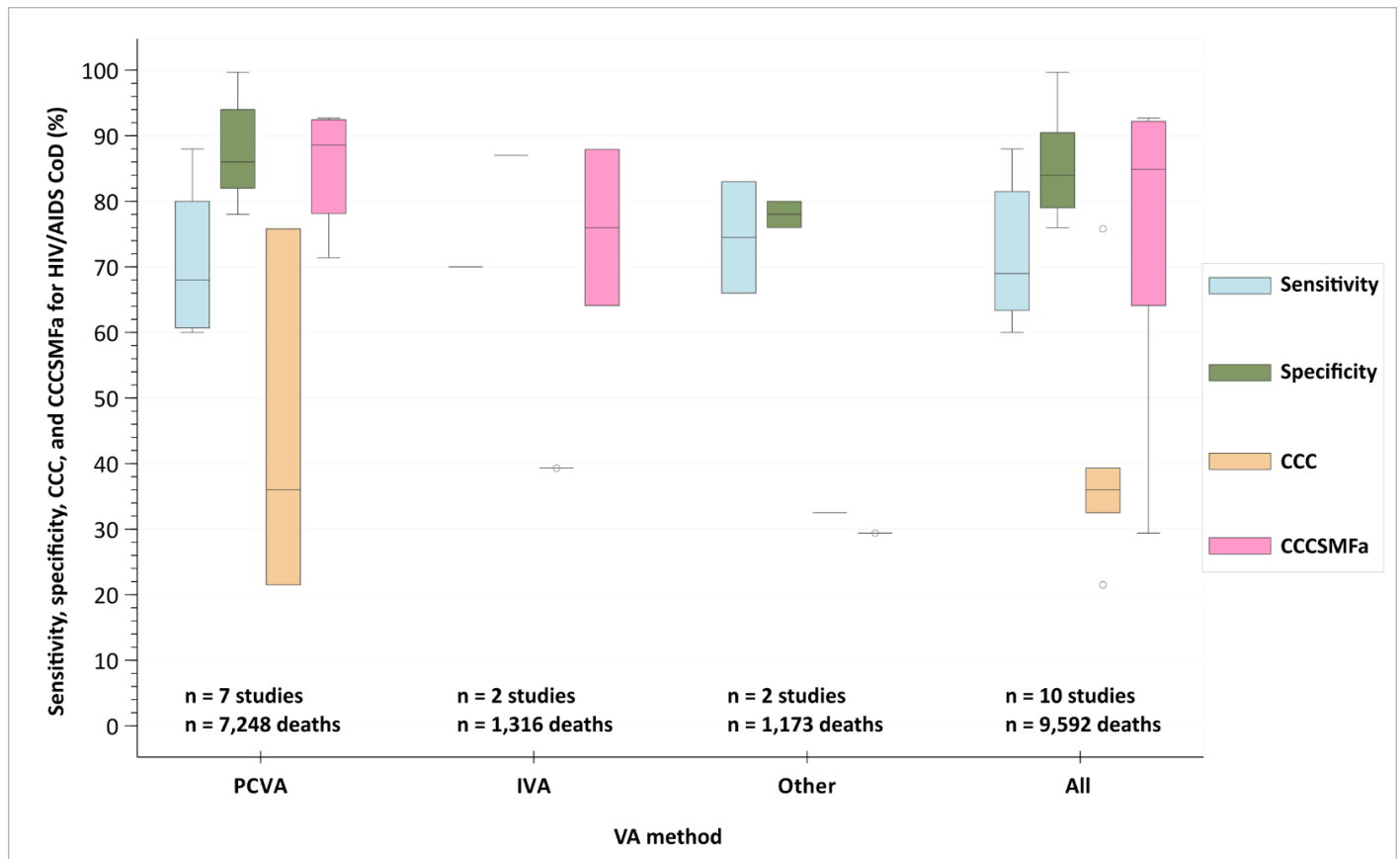
[‡]Number confirmed HIV-positive only reported for entire cohort (n = 1573), not specifically for those with VA (n = 1171)

§AIDS-specific mortality fraction shown for n = 555 deaths used as 'test' dataset

||Range, based on variations to the algorithm, i.e., variations in weight given to answers of specific questions (weight loss, jaundice, etc.) and age groups

AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CCCSMFa: chance-corrected CSMF accuracy; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; CSMFa: CSMF accuracy; HDSS: health and demographic surveillance system; HIV: human immunodeficiency virus; HIV+: HIV-positive; IVA: InterVA; KL: King-Lu; LMIC: low- and middle-income countries; N/A: not applicable; NS: not specified; PCVA: physician-certified verbal autopsy; popn: population; RF: Random Forests; sens.: sensitivity; spec.: specificity; SSP: Simplified Symptom Pattern; TB: tuberculosis; VA: verbal autopsy; WHO: World Health Organization

Figure 2:5. Box plots illustrating crude summary measures of agreement between VA-assigned and reference HIV/AIDS causes of death in studies that used, as reference, individually-assigned causes of death without confirmed HIV status or population-assigned causes of death with confirmed HIV status (n = 10 studies; n = 9,592 deaths)



Measures of agreement for individual deaths (sensitivity, specificity, and chance-corrected concordance) and at a population-level (chance-corrected CSMF accuracy) for VA methods in assigning HIV/AIDS CoD (per individual study definition), stratified by type of VA interpretation method used. The central horizontal line represents the median value; boxes represent the interquartile range (IQR); and whiskers represent the largest and smallest values within 1.5 IQR of the upper and lower quartiles, respectively.

AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CCCSMFa: chance-corrected cause-specific mortality fraction accuracy; CoD: cause of death; CSMF: cause-specific mortality fraction; IQR: interquartile range; PCVA: physician-certified verbal autopsy; VA: verbal autopsy

2.3.3.6 Studies conducted in adults with confirmed HIV status that assessed the specificity of VA methods in assigning HIV-associated causes of death and/or the performance of VA questions in assigning HIV status

The final group of studies is those that compared VA findings to a reference standard composed only from confirmed HIV status and did not attempt to assign reference causes of death. As HIV-positive individuals can die from a range of causes, including non-HIV-associated, these studies estimated only the specificity of VA methods in assigning an individual an HIV-associated cause of death and the sensitivity and specificity of individual questions in the VA instrument.

Eight studies were included in this group, including one PhD thesis [306]; 8,473 (4,819 [56.9%] confirmed HIV-positive) deaths from six countries, all in sub-Saharan Africa, were reported (Table 2:14). Some 7,792 (92.0%) deaths were in individuals from HDSS sites, with the remainder (n = 681 [8.0%]) in individuals from population-based cohort studies conducted in Uganda [302,309], Guinea-Bassau [305], and Tanzania [307]. All studies, except one, evaluated the specificity of VA methods in assigning HIV-associated deaths; two studies [303,308] evaluated the sensitivity and specificity of the VA HIV question (written as “Was there any diagnosis of HIV/AIDS?” in the WHO 2012 instrument [104]) in assigning HIV status. In the 10 comparisons conducted, PCVA was used five times to interpret VA data, InterVA four times, and a WHO checklist once. In two studies [304,306], more than one VA method was used to analyse deaths from the same dataset, meaning the effective number of deaths used to evaluate the specificity of VA methods was 8,307 (4,900 [59.0%] confirmed HIV-positive).

The specificity of VA methods in assigning an HIV-associated cause of death varied widely, from 60.2% (95% CI 53–67 [306]) to 100% (95% CI 83.2–100 [305]), depending on the VA interpretation method used. One study, conducted in Malawi [304], used PCVA and InterVA-4 and found both methods to be highly specific (PCVA 99.4%; InterVA-4 90.7%; n = 417 deaths). Reviewing physicians and InterVA software in this study, however, were not blinded to decedents’ HIV status. These data (n = 417 deaths, evaluated twice) were therefore excluded from the summary estimates described below, leaving 7,473 (4390 [58.7%] confirmed HIV-positive) deaths. A total 432/3,083 (14.0%) confirmed HIV-negative individuals were assigned an HIV/AIDS cause of death, giving an overall specificity of 86.0% (95% CI 84.7–87.2). Stratified by VA method, specificity was 90.4% (95% CI 87.3–92.9; n = 762 deaths) for PCVA and 85.1% (95% CI 83.6–86.4; n = 6,533 deaths) for InterVA (Figure 2:6). The WHO checklist used in one study [307] was 88.6% (95% CI 81–94) specific (n = 178 deaths).

Two studies, examining 5,871 deaths occurring in five sub-Saharan countries between 1990 and 2014, assessed the sensitivity and specificity of the VA HIV question in assigning HIV status [303,308] (Table 2:14). The VA HIV question was answered ‘Yes’ for 1,242/3,272 (38.0%) HIV-positive individuals and 144/2,599 (5.6%) HIV-negative individuals, giving an overall sensitivity of 38.0% (95% CI 36.3–39.6) and specificity of 94.5% (95% CI 93.5–95.3). Only one study [303] assessed the sensitivity and specificity of a VA question asking about use of ART, reporting a sensitivity and specificity of 92.1% and 46.4%, respectively (n = 154 deaths).

Table 2:14. Studies that compared VA (VA-assigned cause of death or specific VA questions) to HIV serostatus data without estimation of reference causes of death; listed by year of first death (n = 8 studies; n = 8,473 decedents; n = 4,819 confirmed HIV-positive)

First author, year published	Dates of deaths	Country/ies Population	Adults with VA and confirmed HIV status, N (HIV+, n [%])	Verbal autopsy Interpretation method	AIDS CoD assigned		Specificity, % (95% CI)*	VA HIV question				
					Overall, n (%/all)	In HIV-negative, n (%/HIV-)		Answered Yes			Sens., % (95% CI)*	Spec., % (95% CI)*
							All, n (%/all)	In HIV+, n (%/HIV+)	In HIV-, n (%/HIV-)			
Mayanja, 2011† [302]	2006–2008	Uganda Population-based cohort	264 (59 [22.3])	PCVA	68 (25.8)	20 (9.8)	90.2 (85.3–93.9)	NS				
McLean, 2016 [303]	2003–2014	Malawi HDSS site	842 (279 [33.1])	N/A	NS			246 (29.2)	232 (83.2)	14 (2.5)	82.3 (78.2–87.4)	97.5 (95.9–98.6)
Glynn, 2014‡ [304]	2002–2012	Malawi HDSS site	417 (255 [61.2])	PCVA	204 (48.9)	1 (0.6)	99.4 (96.6–100)	NS				
				IVA-4	136 (32.6)	15 (9.3)	90.7 (85.2–94.7)	NS				
Cooper, 2010 [305]	1999–2004	Guinea-Bassau Population-based cohort	84 (64 [76.2])	PCVA	23 (27.3)§	0	100 (83.2–100)	NS				
Grollman, 2014 [306]	1994–2011	Tanzania	259 (105 [40.5]) 	PCVA	137 (52.9)	18 (11.7)¶	88.3 (82.2–92.9)	NS				
		HDSS site	541 (250 [46.2]) 	IVA	203 (37.5)	56 (19.2)¶	80.8 (75.7–85.1)	NS				
	1998–2011	Zimbabwe HDSS site	963 (777 [80.7])	IVA	574 (74.5)	74 (29.8)¶	60.2 (52.8–67.3)	NS				
Todd, 1997 [307]	1991–1993	Tanzania Population-based rural cohort	178 (64 [36.0])	WHO / simplified checklist	40 (22.5)	13 (11.4)	88.6 (81.3–93.8)	NS				

First author, year published	Dates of deaths	Country/ies	Adults with VA and confirmed HIV status, N (HIV+, n [%])	Verbal autopsy interpretation method	AIDS CoD assigned		Specificity, % (95% CI)*	VA HIV question				
					Overall, n (%/all)	In HIV-negative, n (%/HIV-)		Answered Yes			Sens., % (95% CI)*	Spec., % (95% CI)*
							All, n (%/all)	In HIV+, n (%/HIV+)	In HIV-, n (%/HIV-)			
Byass, 2013 [308]	1990–2011	Malawi, South Africa, Tanzania, Uganda, & Zimbabwe HDSS sites	5,029 (2,993 [59.5])	IVA-4	1,436 (28.6)	245 (12.0)**	88.0 (86.5–89.3)**	1,140 (22.7)	1,010 (33.7)	130 (6.4)	33.7 (32.1–35.5)	93.6 (92.5–94.6)
Kamali, 1996 [309]	1990–1993	Uganda Population-based cohort	155 (78 [50.3])	PCVA	73 (47.1)	6 (7.8)	92.2 (83.8–97.1)	NS				

*Derived after excluding individuals with unknown HIV status

†Study discusses ‘HIV status’, but this was actually HIV-associated cause of death

‡VA cause of death estimates for ‘AIDS’ and ‘TB/AIDS’ combined; specificity derived based on 417 deaths with confirmed status; VA methods not blinded to HIV status

§Study reported a high proportion (35/84 [41.7%]) of indeterminate causes of death; 25/35 (71.4%) of these individuals were HIV-positive

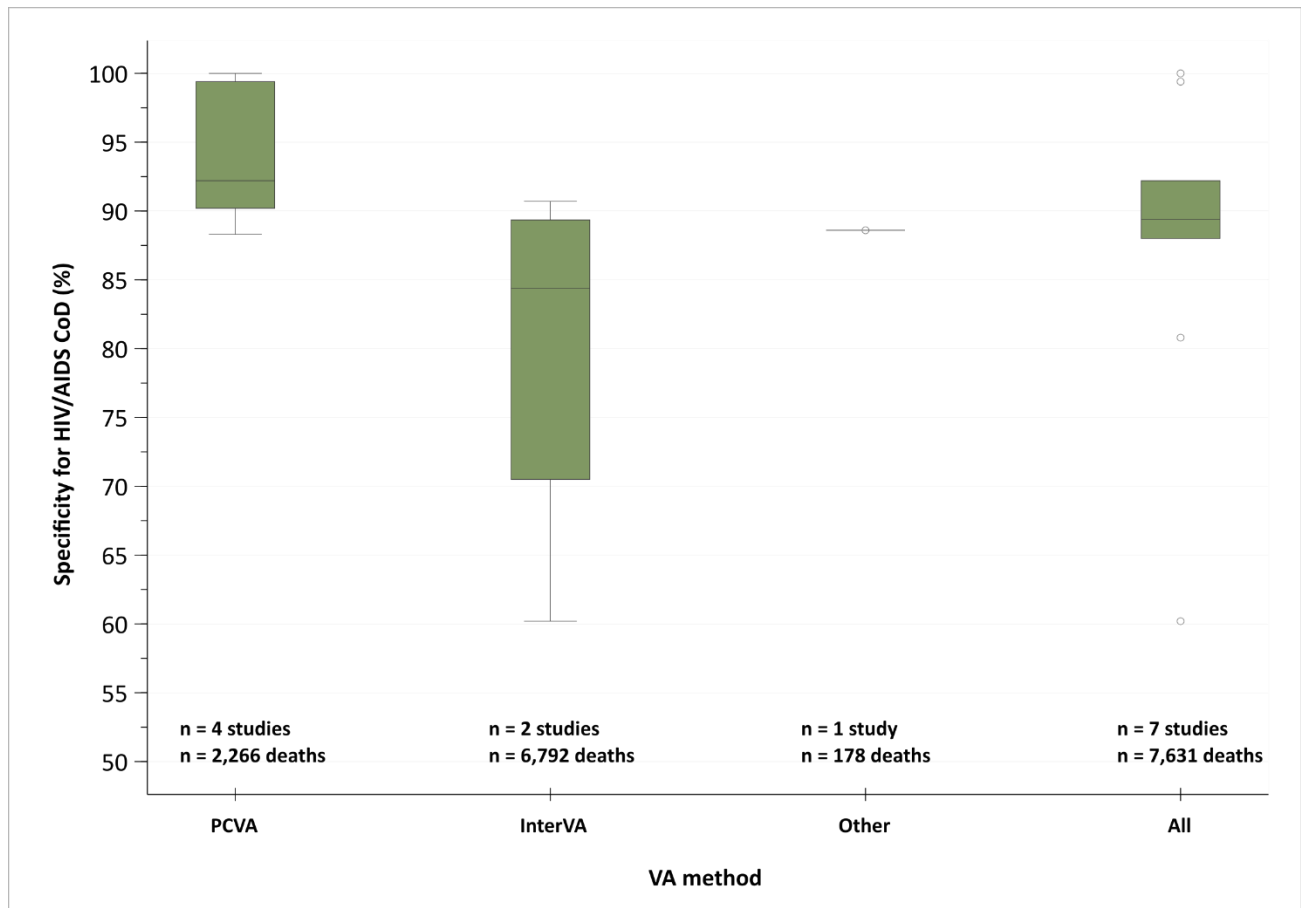
||Total n = 541 deaths at Tanzania site, but only n = 259 assessed by PCVA; all 541 assessed by InterVA-4

¶Individuals considered HIV-negative if negative test ≤5 years prior to death

**The study reports specificity of 90.1, but the authors did not include as HIV-negative the 130 individuals who tested HIV-negative but were reported HIV-positive by VA, 43% (n ≈ 56) of whom were assigned an HIV/AIDS cause of death, bringing the total number of HIV-negative individuals assigned an HIV/AIDS cause of death to 245

AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CCCSMFa: chance-corrected CSMF accuracy; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; CSMFa: CSMF accuracy; HDSS: health and demographic surveillance system; HIV: human immunodeficiency virus; HIV+: HIV-positive; IVA: InterVA; KL: King-Lu; LMIC: low- and middle-income countries; N/A: not applicable; NS: not specified; PCVA: physician-certified verbal autopsy; popn: population; RF: Random Forests; sens.: sensitivity; spec.: specificity; SSP: Simplified Symptom Pattern; TB: tuberculosis; VA: verbal autopsy; WHO: World Health Organization

Figure 2:6. Box plots illustrating crude summary measures of specificity of VA methods in assigning an HIV/AIDS cause of death (n = 7 studies; n = 7,631 deaths)



The central horizontal line represents the median value; boxes represent the interquartile range (IQR); and whiskers represent largest and smallest values within 1.5 IQR of the upper and lower quartiles, respectively.

AIDS: acquired immune deficiency syndrome; CoD: cause of death; PCVA: physician-certified verbal autopsy

2.3.4. Discussion

2.3.4.1 Summary of findings

The literature describing the validation of VA for HIV-associated deaths is extensive and diverse. Reference standards vary widely in the data and methods used in their development, with a general absence of standardisation between and within studies. ICD-10 coding rules were widely used, but were sometimes applied incorrectly; for example, in the grouping together of all HIV-associated and TB deaths (regardless of HIV status). PCVA has been evaluated more than other methods and was overall the most consistent and accurate. Despite the number of validation studies, however, there does not as yet appear to be an automated method that is sensitive, specific, and consistent in assigning HIV-associated causes of death.

2.3.4.2 Reference standards

The number of automated methods developed and tested has greatly increased in recent years, though the newest methods, such as Naïve Bayes [313], open-source Random Forests, and open-source Tariff [108] have only been tested, so far, using PCVA-assigned causes of death as a reference standard. There remains considerable heterogeneity between reference standards used for HIV and TB causes of death in these studies; this is a major obstacle to isolating the best method for use in high HIV prevalence settings. In addition, very few individuals had data available from pathological autopsy, other than a few cases in the PHMRC [121] dataset and in the study by Polprasert et al. (Thailand) [293].

The development of the PHMRC gold standard dataset [121] was a major step in establishing global VA standards [314], but, in addition to the issues discussed above, it does have some important limitations when used to estimate HIV-associated mortality. Data were collected from four countries, only one (Tanzania) with relatively high HIV prevalence (4.2% [95% CI 4.2–5.3] in 2015) [129]. As a result, the HIV-associated mortality fraction among the overall population is low, at 6.4%. The most recent global burden of disease study estimated that, in 2015, in countries with very high HIV prevalence, such as South Africa and Zimbabwe, 53.7% (95% CI 49–59) and 59.0% (95% CI 32–75), respectively, of mortality among individuals aged 15–49 years was due to HIV/AIDS [315]. Despite the precautions taken by various teams developing VA methods (i.e., repeated random sampling of the PHMRC dataset to generate CSMFs of varying distributions [283]), it is unlikely that test CSMFs generated will have reflected the extremely high HIV/AIDS-associated mortality fractions seen in populations such as these. Therefore, despite the rigour with which the dataset was constructed, the low proportion of HIV-associated deaths included means that it is of limited use in evaluating a VA method's accuracy in estimating HIV-associated mortality. Estimates of HIV-associated mortality generated by VA interpretation methods that have, in the published literature, been compared only with reference causes of death from the PHMRC dataset (Tariff, Tariff 2.0, simplified symptom pattern, random forests, and InSilicoVA) should be interpreted with caution.

2.3.4.3 HIV-associated TB

Only one study, conducted in 1998, attempted to differentiate between HIV-associated TB and other HIV-associated causes [290]. Although the team that compiled the PHMRC dataset did initially include this distinction, the two categories have been combined for all comparisons to VA to date. Several other studies, conducted in areas of high HIV

prevalence, combined all TB and HIV/AIDS deaths, which is not in accordance with ICD-10 and makes it difficult to assess VA specificity [270,271,304,316]

2.3.4.4 Previous reviews

Published in 2014, a review by Leitao et al. [109] compared causes of death assigned by CCVA methods to both clinical and PCVA reference standard causes of death. The review included 19 studies, conducted between 1992 and 2012 and reported on over 116,000 adult and child deaths. Due to the marked heterogeneity of the studies included, the authors did not attempt meta-analysis, describing instead mean sensitivities and specificities, with associated ranges, of different VA methods in assigning causes of death. As such, the estimates of mean VA sensitivity and specificity for deaths due to HIV/AIDS are of limited use, at 59% (range 0–61) and 90% (range 0–96), respectively (n = 3 studies). Estimates for deaths due to TB are more precise (mean sensitivity 39% [range 18–62] and mean specificity 97% [range 93–99]; n = 3 studies). The authors report that no single method performed better overall; this is also true for deaths due to HIV/AIDS (median chance-corrected concordance 0.58–0.64 for all of PCVA, InterVA, Tariff, and SSP) and TB (median chance-corrected concordance 0.46–0.49 for the four methods).

Of the 19 studies included in the Leitao 2014 systematic review, only 10 were included in this thesis: nine for comparison of causes of death [279,284–288,294,296,311], and one for comparison based on HIV status alone [302]. Five studies were excluded because they used PCVA-assigned (n = 4 [271,317–319]) or InterVA-assigned (n = 1 [119]) causes of death as the reference standard, leading to circular comparisons, and two because they used only partially verified death certificates as reference standard [320,321]. A further two studies were excluded because they reported only on child deaths (n = 1 [322]) or described only overall CSMFs for the population studied, which did not allow for separation of mortality due to HIV/AIDS or TB (n = 1 [323]).

2.3.4.5 Potential sources of bias

2.3.4.5.1 Publication and selection bias

This review did not use formal systematic review methodology; only one electronic database was searched, which may have led to potentially important publications being excluded. However, attempts were made to mitigate this, through inspection of the reference sections of the 2014 systematic review discussed above [109] and other included publications, as well as the inclusion of known relevant articles. The grey literature was also not searched, which will have biased selection towards data published in peer-reviewed journals. Articles were included only if they were

written in English, or had a detailed abstract in English, which may have led to the exclusion of important data from non-English-speaking countries.

Most individuals included in studies in this review died in hospitals, as seen in pathological autopsy studies (see Chapter 2.2). At least 53% of deaths were in hospitals, though the true figure is almost certainly much higher, as most studies conducted at HDSS sites did not specify the proportions of deaths occurring in hospitals vs. the community. This proportion is unlikely to reflect overall mortality patterns in resource-limited settings, although this is not verifiable, as reliable CRVS data are not available for many LMIC, particularly in sub-Saharan Africa.

Individuals who die out of hospitals are less likely to have access to facilities, are likely to fall into different socio-economic categories, and may be exposed to different risk factors than those in urban areas or with access to healthcare. Unfortunately, there are few reliable data on mortality patterns in rural populations, other than those collected through verbal autopsy. In sub-Saharan Africa, only ~38% of the population live in urban areas, compared with 80% in the Americas and 71% in Europe. Health services, too, are less accessible in these countries, with few clinics and fewer hospitals per 100,000 population [12]. South Africa, for example, has a population of 54.5 million; in 2013, 64% of the population lived in urban areas and there were 0.53 district or rural hospitals per 100,000 population [12,324]. South Africa also has some of the best CRVS data of countries in sub-Saharan Africa; Statistics South Africa estimated that at least 22% of deaths in 2015 occurred at home, with 6% occurring outside of facilities, and a further 22% in 'unknown' locations [144]. These proportions are likely to be much larger in countries with larger rural populations, poorer infrastructure, and reduced access to care; for example, Malawi (population of 17.2 million, only 15% of whom lived in urban areas in 2013; 0.45 health posts per 100,000 population in 2013; and HIV prevalence was 10.0% in 2014) and Zimbabwe (population of 15.6 million, 34% lived in urban areas in 2013; <0.01 health posts per 100,000 population in 2013; and HIV prevalence was 16.7% in 2014) [12,324]. The assumption is, therefore, that a sizeable proportion of deaths occur in the community, but few data are available to corroborate this. As VAs are used primarily in areas without adequate CRVS data, it follows that they should be tested in populations in whom they are going to be used; at present, the over-representation of individuals dying in hospitals may not allow for accurate estimations of CSMFs in the target populations.

The majority of individuals included in this review had confirmed HIV status or were assigned individual causes of death with or without HIV status. This may have led to the exclusion of groups with high proportions of HIV-associated deaths

but without HIV status data or sufficient data with which to estimate cause of death, which may have inflated or deflated the summary estimates of sensitivity or specificity. Additionally, all individuals included in this review had VA data available, which itself may be a source of bias. Individuals without families or carers, or whose family did not agree to be interviewed, may be at higher risk of certain causes of death and are not represented in these studies. Finally, a few studies selected specifically for HIV-positive individuals, which will have affected the constituent CSMFs and summary estimates of CSMF accuracy.

2.3.4.5.2 Bias inherent in measures of agreement

For individual-level agreement, the use of a purpose-developed metric, chance-corrected concordance, will have mitigated some of the issues with older measures such as Cohen's kappa, which is known to vary based on the CSMF distribution of the evaluated dataset [259]. Similarly, for estimation of population-level agreement, the use of chance-corrected CSMF accuracy, as opposed to CSMF accuracy or Lin's concordance correlation coefficient, corrects for random guessing and allows for fairer comparisons between studies and populations [312].

2.3.4.5.3 Bias inherent in verbal autopsy

VA is, by definition, a 'blunt instrument', involving a second-hand account of events collected by a non-technical interviewer. The way VAs are conducted, however, can bias the way data are collected and the causes of death assigned. A concern frequently cited is that of recall bias on the part of the respondent, where it is thought that a longer interval between death and the interview will lead to changes in information reported, with respondents perhaps able to remember some symptoms better than others [325,326]. WHO recommends a maximum 12 months between death and interview [104]; most studies included in this review were comfortably within this, with a few exceptions. Recent evidence, however, suggests that this may actually have little effect on the CSMFs assigned from data collected. Two studies, one using the WHO 2012 VA instrument (n = 10,822) [327], and another using the PHMRC VA instrument (n = 1,394) [328], found only minor changes in CSMFs or CSMF accuracy between VAs conducted 0–5 and 0–3 months, and 6–12 and 4–11 months after death, respectively.

Further sources of heterogeneity and possible systematic bias within VA studies include the amount and nature of training and support provided to lay interviewers; the beliefs and attitudes of the interviewers and respondents, which will relate to societal beliefs and norms (e.g., stigma or perceived stigma that may reduce the likelihood of specific

diagnoses, such as HIV, being reported); and the questionnaires used for interview, which were not standardised prior to 2007.

2.3.4.6 Gaps in the literature and remaining questions

Fundamentally, there remains a need for better quality reference standards and greater standardisation among VA validation studies. A major focus of this process should be in the development of large, shared, reference standard datasets that, ideally, use high quality diagnostic data and include findings from pathological autopsy. The PHMRC dataset shows that the process can be conducted with a degree of rigour, although it is itself problematic with respect to HIV and TB-related deaths, as discussed above. Shared reference datasets are therefore also needed that are more representative of mortality patterns in areas with high proportions of deaths due to HIV and TB, including representation of individuals who receive care and die in the community. The development and use of guidelines for assigning causes of death, particularly in HIV-positive individuals, similar to the Coding Causes of Death in HIV (CoDe) project [329], would make the validation process easier and allow for clearer comparisons between different VA validation studies and between research and routine data.

Another important aspect of standardisation is the way in which agreement, or VA performance, is measured. The IHME recommendations for metrics are very useful [330] and have been adopted by more recent studies [108,109,313]. In addition, the development of reporting guidelines for VA validation studies, such as those currently recommended for randomised controlled trials [331] and systematic reviews [332] would allow for greater reproducibility of results across studies.

Finally, there is a need for studies conducted in areas of high HIV and TB prevalence to separate deaths due to HIV-associated TB from deaths due to other HIV-associated causes. Although this may be very difficult in practice, both in the development of reference datasets and in the interpretation of VA data, the potential benefits of having better data on mortality due to HIV-associated TB, as discussed in Chapter 1, outweigh the time and cost risks involved in the exercise. More accurate estimates of mortality due to HIV-associated TB are essential if we are to adequately track progress towards the mortality reduction goals set by governing bodies [35,36]. Pathological autopsy, in research or routine practice, can only realistically be used for a small proportion of deaths. Despite indications that VA may not be the ideal method with which to estimate this mortality fraction, it is, at present, and in the absence of robust and

validated CRVS data, the most feasible and cost-effective method available [333,334]. The use of VA to measure mortality due to HIV-associated TB should not be dismissed when its ability to do so has not really been evaluated.

2.3.5. Summary and conclusions

The literature around the validation of VA in areas of high HIV prevalence is diverse. There is considerable heterogeneity in the quality of reference standards used for comparison, the methods used to interpret VA, and the metrics by which VA performance is measured. At present, PCVA remains the most accurate and consistent method in assigning HIV-associated causes of death in areas of high HIV prevalence. Very few attempts have been made to distinguish between HIV-associated TB and other HIV-associated causes of death; ICD-10 coding makes this difficult, although there is also variability in how ICD rules are interpreted. There is a need for higher quality reference standards that, ideally, include data from pathological autopsy, and greater standardisation of VA procedures; this will be made easier through the development of shared reference standard datasets which are representative of mortality patterns in different settings. Future studies should also attempt to differentiate between HIV-associated TB and other HIV-associated causes.

Chapter 3. Research paper 1: Directly estimated causes of death among HIV-positive adults in low- and middle-income countries. A systematic review and meta- analysis.

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3.1. Cover page

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Research paper cover sheet

Section A: Student details

Student	Aaron Karat
Principal supervisor	Alison Grant
Thesis title	An autopsy study exploring the spectrum of disease in individuals with advanced HIV in primary care clinics in South Africa

If the research paper has previously been published, please complete Section B.
 If not, please go to Section C.

Section B: Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion.	
Have you retained copyright for the work?*	Was the work subject to academic peer-review?

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

Section C: Prepared for publication, but not yet published

Where is the work intended to be published?	Lancet HIV
Please list the paper's authors in the intended authorship order:	Aaron S Karat, Katherine L Fielding, Salome Charalambous, Asha Daya, Kathleen Kahn, Sebastian B Lucas, Daniel Chandramohan, Alison D Grant
Stage of publication	Not yet submitted

Section D: Multi-authored work

For multi-authored work, give full details of your role in the research include in the paper and in the preparation of the paper (attach a further sheet if necessary).	The candidate conceived the idea; conducted the literature review, data extraction, and analysis; and wrote the paper.
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Student signature:

Date: 24 May 2017

Supervisor signature:

Date: 25 May 2017

3.2. Manuscript

Authors

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3.2.1. Abstract

3.2.1.1 Background

To track progress towards targets set by governing bodies for reductions in HIV- and tuberculosis (TB)-related mortality, it is important to know how many HIV-positive people die and from which causes. In studies of HIV-positive adults dying in low- and middle-income countries (LMIC) in whom causes of death (CoD) were directly estimated (from autopsy or clinical records), this systematic review and meta-analysis aimed to estimate the proportions assigned HIV-associated and TB CoD and to estimate these same proportions in individuals initiated on antiretroviral therapy (ART).

3.2.1.2 Methods

Three electronic databases were searched; studies were included that used autopsy or other clinical data to assign CoD in confirmed HIV-positive adults and excluded that required an additional diagnosis for participant inclusion, used non-clinical data (without validation) to assign CoD, or were published in a language other than English without an English abstract. The primary outcome was the proportion of decedents assigned HIV-associated CoD (defined as AIDS, other

infection, or ART toxicity). Pooled proportions were generated using random-effects meta-analysis; relationships between CoD proportions and other variables were assessed using meta-regression.

3.2.1.3 Results

A total 3,818 unique records were identified from electronic databases and other sources; 3,701 were excluded based on titles and/or abstracts; 117 full text articles were reviewed, 56 (47.9%) of which were included in the meta-analysis, reporting on 10,291 deaths in HIV-positive adults, 4,009 of whom had initiated ART. Heterogeneity between studies was very high ($I^2 > 94\%$; $p < 0.01$). The pooled proportion of decedents assigned HIV-associated CoD was 79% (95% confidence interval [CI] 74–83) and, among studies reporting TB as a specific CoD, the pooled proportion assigned a TB CoD was 29% (95% CI 24–34; $n = 47$ studies). TB accounted for 39% of all HIV-associated CoD. Studies with higher proportions of decedents who initiated ART assigned fewer HIV-associated CoD (assigned to 86%, 76%, and 70% if $< 33\%$, 33%–65%, or $\geq 66\%$ of decedents initiated, respectively; $p = 0.01$) but no changes were seen in proportions of TB CoD assigned (to 28%, 24%, and 26%, respectively; $p = 0.52$). Among decedents who initiated ART, a pooled 72% (95% CI 66–79) were assigned an HIV-associated CoD and 26% (95% CI 18–34) a TB cause of death. In general, studies that used autopsy data assigned higher proportions of HIV-associated and TB CoD than those that did not (all HIV-associated 87% vs. 73%, $p = 0.02$; TB 37% vs. 24%, $p = 0.04$).

3.2.1.4 Conclusions

HIV-associated causes, specifically TB, accounted for a high proportion of deaths in studies in LMIC, including in adults who initiated ART. More recent studies and those that included higher proportions of individuals on ART had fewer HIV-associated CoD assigned, but there was no reduction seen in proportions of TB CoD assigned. To track progress towards global targets for reducing HIV and TB mortality, standardised methods are needed to assign CoD in HIV-positive people who die in LMIC that are feasible for use at a programmatic level, allow for the quantification of all deaths in HIV-positive individuals, and correspond to the coding systems used to collate national, regional, and global estimates.

3.2.2. Introduction

An estimated 1.2 million individuals died in 2015 due to HIV-associated causes [1], but accurate estimates of causes of death in HIV-positive individuals dying in low- and middle-income countries (LMIC) remain elusive, with cause-specific mortality estimates generated indirectly through mathematical modelling [2]. Often, this is because many countries with high HIV prevalence do not have functional civil registration and vital statistics (CRVS) systems and information on causes of death must be obtained from other sources: between 2009 and 2013 over 80% of countries in the African region had incomplete or no CRVS death data available [3–5].

Complete diagnostic (pathological) autopsy is the gold standard in assigning causes of death. Even when pathological autopsy data are available, however, the process of cause of death assignment is often not precise [6,7]. Other than pathological autopsy studies, nearly all of which have been conducted among small numbers of individuals admitted to hospital, the next best direct estimates of cause-specific mortality in these populations are obtained from individual studies of in-hospital deaths, sub-studies of deaths after recruitment to a cohort study, or retrospective reviews of medical records with post hoc assignment of cause of death. The types and quality of data available to these studies are extremely variable, as are the methods used to assign causes of death. To remedy this, in 2004, the Coding Causes of Death in HIV (CoDe) project aimed to develop a standardised, validated method for assigning causes of death in HIV-positive individuals [8,9]. In this aim the project was relatively successful, but two major obstacles to the wider uptake of the method have not yet been overcome: (1) the relative complexity and time requirements of the protocol mean that it is not feasible for use outside of research settings, making it difficult to compare estimates obtained through research and those obtained through routine data collection; and (2) the causes of death assigned do not readily translate into International Statistical Classification of Diseases and Related Health Problems (ICD) codes, which further increases the difficulties in relating results to routinely collected data. In addition, the CoDe list of causes of death refers only to 'AIDS' or 'Other infections' and does not specifically mention tuberculosis (TB), the impact of which is already difficult to estimate.

TB is the most important cause of death in HIV-positive individuals, causing an estimated 390,000 deaths in 2015 [10]. In individuals with advanced HIV disease, however, TB disease is difficult to diagnose: a systematic review of pathological autopsy studies among HIV-positive adults in LMIC reported that almost half of those with evidence of active TB at autopsy had not been diagnosed before death [11]. The under-recognition of TB in people living with HIV

(PLHIV) suggests that we may underestimate the true burden of TB mortality in these populations. This is compounded by the way ICD-10 classifies HIV deaths [12], where all HIV-associated deaths, whether due to TB or other cause, are counted together under five broad 'HIV' codes (B20–B24) [13,14]. This, in addition to the absence of a system that records the death of an HIV-positive individual from a non-HIV-associated cause, makes it difficult to differentiate between those dying *from* HIV and those dying *with* HIV, a distinction that is likely to become increasingly important to make.

The global rollout of antiretroviral therapy (ART) has dramatically reduced the risk of mortality for many PLHIV [15,16], though those in lower-income settings are still at high risk of death early on treatment [17–19]. PLHIV on ART have greater life expectancies and reduced vulnerability to diseases associated with immunosuppression; changes in cause of death patterns among these individuals are inevitable [7,20] and the way that HIV-associated deaths are defined may need to change to mirror the evolving epidemic [21]. As seen in high-income countries, it is likely that causes of death in HIV-positive individuals will align more closely with those seen in the HIV-negative population, with non-communicable diseases such as diabetes mellitus, hypertension, and cardiovascular disease becoming increasingly prominent [22–24]. Systems currently used to collate cause of death statistics, however, are designed to estimate only mortality due to HIV and do not provide estimates of all-cause mortality among HIV-positive individuals [21]. As we seek to track progress towards reductions in absolute numbers of HIV-associated deaths, as set out by the Joint United Nations Committee on HIV and AIDS (UNAIDS) [25], it will be critical to distinguish between individuals dying from HIV and with HIV. To allow for estimation of trends and to measure the impact of interventions, however, it is important first to establish a baseline as regards the HIV-associated mortality fraction among HIV-positive individuals.

The aims of this systematic review were to assess, among studies that directly estimated causes of death in HIV-positive adults in LMIC (through pathological autopsy, other post-mortem examination, or review of clinical data), (1) the proportions of deaths attributed to HIV-associated causes and, specifically, HIV-associated TB; and (2) to estimate these same proportions among individuals who died after initiating ART.

3.2.3. Methods

3.2.3.1 Search strategy

MEDLINE®, EMBASE, and Web of Science searched for variations of the following terms, combined by AND parameters:

HIV, cause of death, and low- and middle-income countries (LMIC, as defined by the World Bank and the Cochrane

group [26,27]). Articles published on or before 31 October 2016 were included. Full details of the electronic search strategy are provided in Supplementary table 3:1. Additional titles were obtained from the references of reviewed articles, including a 2015 systematic review of autopsy studies in sub-Saharan Africa [11] and three other recent reviews [23,28,29].

3.2.3.2 Screening and exclusion criteria

After removal of duplicates, titles and abstracts were screened independently by two reviewers (ASK and AD). Studies were excluded that were conducted in high-income countries only; involved only pregnant women and/or children; included only individuals with a certain risk factor, such as mine-workers, or diagnosis, such as TB; or were written in a language other than English without an English abstract. Full-text articles were reviewed, where available; studies were excluded if they had recruited only individuals with an HIV-associated or 'AIDS' cause of death, if they involved only estimates through indirect methods (such as verbal autopsy) without clinical correlation, or if they described only population-level causes of death.

3.2.3.3 Data extraction

Data were extracted from each manuscript using a standardised case report form; data collected included population size, study design, recruitment criteria, site of recruitment, site of death, numbers on ART and/or TB treatment, clinical data available, methods used to assign causes of death, and causes of death assigned. Causes of death counted as 'HIV-associated' were AIDS (per the 1993 revised Center for Disease Control [CDC] definition [30]), other infections thought to have caused death, and adverse effects of ART thought to have caused death. This was implemented regardless of how individual studies had classified AIDS-associated or HIV-associated causes of death (for example, if studies classified deaths due to ART toxicity as 'non-AIDS', these were re-classified as 'HIV-associated' for the purposes of this review). For other, more specific causes of death (e.g., TB), numbers of deaths were reported per the study publication.

3.2.3.4 Assessment of quality, bias, and methods

The quality of studies, including risk of bias, was assessed using the National Heart, Lung, and Blood Institute (NHLBI) quality assessment tool for observation cohort and cross-sectional studies (Supplementary table 3:2 [31]). Based on the 14 variables included, each study was assigned a rating of 'Good', 'Fair', or 'Poor', in accordance with guidance provided. In addition, a separate assessment of methodological rigour was carried out using a purpose-built scale

(Supplementary table 3:3). Measures assessed were: the types of data available per participant per study; the numbers, roles, and independence of individuals assigning causes of death; the structure of the overall assignment process; the methods used to resolve discrepancies; and the standard of the case definitions used to assign each cause of death. Each element was scored individually and the scores aggregated to give each study a total score between zero and six.

3.2.3.5 Data management and statistical analyses

Manuscripts were organised using Mendeley software; data were entered into an EpiData (The EpiData Association, Odense, Denmark) database and managed in a Microsoft® Excel spreadsheet. Pooled estimates of HIV-associated mortality and mortality due to HIV-associated TB with 95% confidence intervals (CI) were generated using a random-effects model, incorporating the Freeman-Tukey double arcsine transformation of proportions, with the percentage of total variation due to heterogeneity (I^2) measured between studies [32]. Estimates were further stratified by geographic region, economic group, calendar period (three periods, based on ART availability in LMIC: pre-2005 [very limited availability]; 2005–2010 [increasing availability]; and 2011–2015 [widespread availability]), proportions of decedents initiated on ART, and study quality, with heterogeneity measured between groups. Forest plots were used to graphically represent mortality fractions in individual studies and pooled estimates. Meta-regression was used to examine for relationships between mortality fractions and other key variables, including the year of data collection, the proportion of individuals in each study initiated on ART, the data & methods score assigned to each study, and the use of pathological autopsy data to assign causes of death.

Further analysis was conducted to compare, where reported, causes of death assigned to individuals who had initiated ART with causes assigned with those who had not. Pooled proportions of HIV-associated and TB causes of death assigned were generated using random-effects meta-analysis, as previously, although stratification and meta-regression were conducted based only on geographic location, income group, and years of data collection, as quality and methods scores were assigned at study level. All analyses were carried out using Stata v14 (StataCorp, College Station, TX, USA); figures were edited and formatted using Inkscape™ software (<https://inkscape.org>).

3.2.3.6 Ethical considerations

No participants or individual data were involved in this study; ethical approvals were not required.

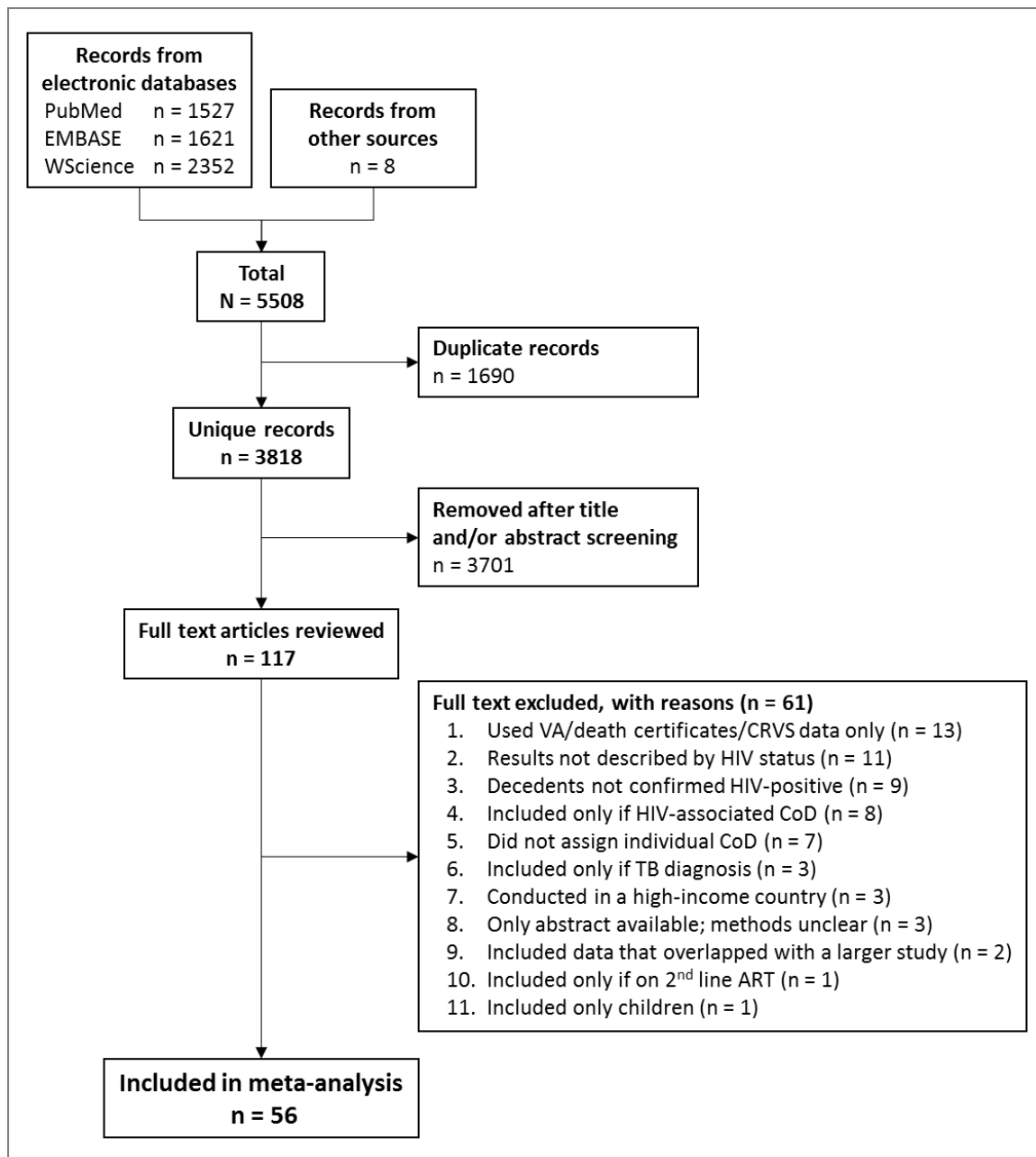
3.2.4. Results

3.2.4.1 Search results

A total 3,818 unique records were identified from electronic databases and other sources; 3,701 were excluded after review of title and abstracts; 117 full text articles were reviewed, 56 (47.9%) of which were included in the meta-analysis (Figure 3:1). Details of individual studies are described in Table 3:1 and summarised in Table 3:2. Of the 56 studies included, 32 (57.1%) were conducted in sub-Saharan Africa, eight (14.3%) in South Asia, seven (12.5%) in Latin America and the Caribbean, and four (7.1%) in East Asia and the Pacific. Studies were divided into three periods based on the last year of data collection: 15 (26.8%) studies completed data collection prior to 2005; 30 (53.6%) between 2005 and 2010; and 11 (19.6%) between 2011 and 2015. At least 34/56 (60.7%) studies reported causes of death in individuals who had initiated ART (11 studies did not specify): 1%– 66% of decedents initiated ART in 14/34 (41%) studies; 100% of decedents initiated ART in 17/34 (50%) studies. Only one study completed before 2005 included adults who had initiated ART.

Some 105,992 individuals were studied, 57,001 (53.8%) of them HIV-positive; 35,127 (61.6%) of these individuals had initiated ART. There were 11,791 deaths among HIV-positive adults, 4,128 [35.0%] of whom had initiated ART; attempts were made to assign individual causes of death to 10,291 (87.3%) HIV-positive individuals, of whom 4,009 (40.0%) had initiated ART.

Figure 3:1. PRISMA flow diagram showing records found, screened, reviewed, and included in analysis



ART: antiretroviral therapy; CoD: cause of death; CRVS: civil registration and vital statistics; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; TB: tuberculosis; VA: verbal autopsy; WScience: Web of Science

Table 3:1. Studies that directly estimated causes of death in HIV-positive individuals in LMIC: population, selection criteria, study quality, overall methods score*, and proportion of decedents on ART for each study; listed by period of data collection and geographic area (n = 56 studies)

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
Pre-2005									
[33] LAm + Car 01 (1993–2000)	Brazil	UM	R	Hospital in-patients 'submitted to autopsy'	Good	100	73.9	2.7	NS
[34] LAm + Car 02 (1996–2003)	Brazil	UM	R	'AIDS' patients undergoing autopsy	Poor	100	0	1.0	NS
[35] LAm + Car 03 (1999–2004)	Peru	UM	R	In-patient deaths, HIV+ or CDC AIDS; unclear CoD preferred	Good	100	100	2.0	0
[36] SAsia 01 (1991–2003)	India	LM	R	Hospital in-patients	Poor	100	100	2.5	0
[37] SAsia 02 (2000–2003)	India	LM	P	Consecutive HIV+ admissions to hospital; report only on in-patient deaths	Poor	0	100	2.0	NS
[38] SAsia 03 (2002–2003)	India	LM	R	HIV+ admitted to medical wards of a large public hospital; report only on in-patient deaths	Fair	0	100	2.0	NS
[39] SSAfr 01 (1986–1987)	Rwanda	L	P	Consecutive sample of HIV+ and HIV- women of childbearing age	Good	0	NS	2.3	0
[40] SSAfr 02 (1988–1989)	Cote d'Ivoire	LM	P	Consecutive cadavers admitted to two mortuaries; deaths in hosp. or community (forensic deaths)	Good	100	0	3.0	0
[41] SSAfr 03 (1989)	Cote d'Ivoire	LM	P	Consecutive deaths on pulmonary ward	Poor	100	100	2.0	0
[42] SSAfr 04 (1991)	Cote d'Ivoire	LM	P	Consecutive in-patient & community deaths brought into hospital	Good	100	88.8	3.9	0
[43] SSAfr 05 (1990–1996)	Uganda	L	P	Deaths after enrolment to population-based cohort	Good	0	0	3.5	0

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
[44] SSAfr 06 (1996–1997)	Kenya	LM	P	Consecutive in-patient deaths on medical wards; excluded patients referred for specialist care	Good	100	100	4.5	0
[45] SSAfr 07 (1997–1998)	Botswana	UM	P	Medical in-patient deaths, including DOA; favoured those without diagnosis/unexpected death, with pulmonary>gastro symptoms, or possible PCP; excluded forensic cases and DOA with trauma	Good	100	100	4.0	0
[46] SSAfr 08 (2001)	Congo, Rep.	LM	P	Bodies of all individuals >14 years registered at morgue	Poor	0	91.4	2.4	0
[47] SSAfr 09 (1998–2002)	Senegal	L	P	>15 years, ART-naïve, CD4 <350 cells/μL, VL >30,000 copies/ml, enrolled in observational cohort; consecutive starting ART in ISAARV	Good	0	64.5	4.2	100
2005–2010									
[48] EAsia + Pac 01 (2003–2007)	TAHOD+	LM	P	Enrolled to observational cohort, started ART and had at least one f/up visit; community & hospital deaths	Good	0	NS	4.3	100
[49] EAsia + Pac 02 (2007–2010)	Vietnam	LM	P	Deaths after enrolment to RCT to assess the effect of peer support on tx failure & drug resistance; ≥18 years, ART-naïve, willing to receive adherence support at home, eligible for ART	Fair	0	76.7	0.8	100
[50] Eur + CAsia 01 (2003–2008)	Russian Federation	UM	R	HIV-positive adults who died in hospital in the Smolensk region	Poor	100	0	1.0	NS
[51] Eur + CAsia 02 (1996–2009)	Turkey	UM	R	Unclear	Poor	0	0	0.0	NS
[52] LAm + Car 04 (1997–2006)	Brazil	UM	P	≥16 years, at least one f/up visit after enrolment to cohort study	Good	0	100	5.0	67.7
[53] LAm + Car 05 (1986–2009)	Brazil	UM	P	≥18 years, enrolled into IPEC cohort with minimum f/up of 60 days	Poor	0	NS	4.3	34.4

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
[54] MEast + N Afr 01 (1999–2009)	Morocco	LM	R	Adults attending ID unit of hospital, started ART and died in the ID unit; excluded if on ART for <1 month	Fair	0	100	2.5	100
[55] SAsia 04 (1992–2005)	India	LM	R	HIV+ deaths after admissions to hospital; report only on in-patient deaths	Poor	0	100	2.0	3.4
[56] SAsia 05 (1988–2007)	India	LM	P	Medical in-patient deaths among consecutive admissions to medical wards; excluded if admitted to neurology or neurosurgery wards	Fair	100	100	2.0	0.8
[57] SAsia 06 (1996–2008)	India	LM	P	≥18 years, ART-naïve prior to initiation; report only on in-patient deaths	Fair	0	100	3.0	100
[58] SAsia 07 (2000–2008)	India	LM	P	Adults initiating ART who died in hospital after enrolment to prospective cohort; report only on in-patient deaths	Fair	0	100	1.5	100
[59] SAsia 08 (2009–2010)	India	LM	R	Deaths among consecutive ART-naïve patients, aged ≥15 years, initiating first-line ART at tertiary hospital; excluded pregnant women	Poor	0	100	1.0	100
[60] SSAfr 10 (1996–2005)	Togo	L	R	In-patient deaths on neurology ward	Fair	0	100	1.0	0
[61] SSAfr 11 (2002–2005)	South Africa	UM	P	Adults (≥18 years) dying after referral for ART; excluded if previously initiated ART at other clinic	Good	8.8	100	2.6	54.4
[62] SSAfr 12 (2003–2005)	Uganda & Zimbabwe	L	P	Deaths among adults with CD4 <200 cells/μL and no previous ART enrolled to large pragmatic trial of ART monitoring (DART); excluded if death >12 months after enrolment	Good	0	NS	2.3	100
[63] SSAfr 13 (2004–2005)	Uganda	L	P	Adults living within 20 km of Kampala dying after recruitment to a cohort study; included if eligible for ART and willing to f/up for two years	Good	0	NS	1.3	100
[64] SSAfr 14 (2000–2007)	Cameroon	LM	R	In-patient deaths, HIV-positive and HIV-negative	Fair	0	100	1.5	27.4

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
[65] SSAfr 15 (2005–2007)	South Africa	UM	R	Initiated on ART and died in hospital	Good	0	74.2	4.2	100
[66] SSAfr 16 (2007)	Ghana	LM	R	Consecutive HIV-positive deaths within fevers unit in teaching hospital	Fair	61.1	100	5.6	14.5
[67] SSAfr 17 (2000–2008)	South Africa	UM	P + R	Medical in-patient deaths among HIV-positive adults	Poor	100	100	4.5	NS
[68] SSAfr 18 (2003–2008)	Burkina Faso	L	R	Death among adults ≥15 years who started ART for first time ≥6 months before start of study; excluded if started ART at different facility or before January 2003	Good	0	60.9	4.6	100
[69] SSAfr 19 (2004–2009)	South Africa	UM	R	Deaths (at home or in hospital) among adults (≥18 years) who initiated ART at hospital ART clinic	Good	0	75.4	3.8	100
[70] SSAfr 20 (2006–2009)	Nigeria	LM	R	Died ≥6 months after enrolment in ART programme; died while on home-based or in-patient care	Poor	0	49.5	2.0	100
[71] SSAfr 21 (2006–2009)	Nigeria	LM	R	Deaths among those aged >13 years, non-pregnant, and admitted to medical wards; included only those with 'complete clinical details'	Good	0	100	4.0	83.3
[72] SSAfr 22 (2009)	Uganda	L	P	Consecutive deaths on ID/gastro ward; excluded if no next-of-kin available	Fair	100	100	5.5	28.6
[73] SSAfr 23 (2009)	South Africa	UM	P	Consecutive deaths on medical wards among adults receiving/eligible for ART; excluded if pregnant or had a history of defaulting/restarting ART	Good	100	100	6.0	69.2
[74] SSAfr 24 (2004–2010)	South Africa	UM	R	ART-naïve adults (≥18 years) with CD4 ≤50 cells/μL attending a clinic for screening	Fair	0	34.9	0.9	100
[75] SSAfr 25 (2010)	Togo	L	P	Adults and children dying after admission to 16 hospitals; excluded if died at home or in ambulatory care	Poor	0	100	1.0	51.8
[76] SSAfr 26 (2010)	Mozambique	L	R	In-patient deaths among HIV-positive individuals undergoing autopsy	Good	100	100	2.0	NS

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
[77] SSAfr 27 (2010)	Cote d'Ivoire, Burkina Faso, Benin, Mali, & Senegal	LM	P	Newly hospitalised HIV-positive adults; death after admission	Fair	0	100	5.0	42.5
2011–2016									
[78] EAsia + Pac 03 (2009–2012)	China	UM	P	In-patient deaths among HIV-positive adults; deaths within 6 months of admission	Good	0	NS	0.3	34.3
[79] EAsia + Pac 04 (2007–2014)	Vietnam	LM	P	Adults in first year of ART attending HIV clinics in two large hospitals in Hanoi; excluded if never on ART or on ART for >1 year	Fair	0	0	1.5	100
[80] Eur + CAsia 03 (1989–2012)	Georgia	UM	R	HIV+ adults registered in Georgia HIV/AIDS database; died between 1989 and 2012	Good	0	NS	4.3	NS
[81] LAm + Car 06 (2000–2011)	Brazil	UM	P	Adults (≥18 years) in longitudinal database receiving HIV care with a minimum 60 days follow up; excluded if heavy cocaine use, IVDU, unknown route of HIV acquisition, or no CD4 count during f/up	Fair	0	NS	4.3	NS
[82] LAm + Car 07 (2010–2013)	Mexico	UM	R	Death in one of three hospitals; excluded if records not available	Fair	0	100	3.5	18.8
[83] MEast + NAfr 02 (2000–2014)	Tunisia	LM	R	Adults (≥15 years) followed up by hospital ID department	Good	0	NS	2.3	68.5
[84] SSAfr 28 (2002–2011)	Congo, Rep.	LM	R	Adults (>15 years) dying during follow up at outpatient ART centre	Poor	0	NS	0.3	100
[85] SSAfr 29 (2002–2012)	Uganda	L	R	Deaths among adults (≥18 years) receiving care at community HIV facility in Kampala; excluded those with unknown date of death	Good	0	NS	3.9	38.4

Calendar period, region, period of data collection	Country/ies	Income group	Study design	Population and selection	NHLBI grade	Data available from		Overall methods score	Proportion on ART (%)
						Autopsy (%)	Hospital (%)		
[86] SSAfr 30 (2012)	Kenya	LM	P	Deaths among patients receiving ART at a hospital	Poor	100	100	6.0	100
[87] SSAfr 31 (2013)	Uganda	L	P	Consecutive HIV+ adult deaths on medical wards + patients with autopsy requests from non-medical wards; excluded postpartum & traumatic deaths	Fair	100	0	4.5	61.5
[88] SSAfr 32 (2013–2015)	Mozambique	L	P	In-patient deaths with autopsy requested by clinician; excluded traumatic and maternal deaths	Good	100	100	6.0	NS

*Based on assessment of data available to reviewers, reviewers and process of assigning causes of death, and criteria defined for each cause (Supplementary table 3:3)

†TAHOD lower middle-income countries: Cambodia, India, Indonesia, Philippines, and Vietnam; TAHOD upper middle-income countries: China, Indonesia, Malaysia, and Thailand

ART: antiretroviral therapy; CDC: Center for Disease Control; CoD: cause of death; DART: Development of Antiretroviral Therapy in Africa; DOA: dead on arrival; EAsia + Pac: East Asia & the Pacific; Eur + CAsia: Europe & Central Asia; f/up: follow-up; gastro: gastroenterology; HIV+: HIV-positive; ID: infectious diseases; IPEC: Evandro Chagas Institute of Clinical Research (Brazil); ISAARV: Senegalese ART drug access initiative; IVDU: intravenous drug user; km: kilometres; L: low; Lam + Car: Latin America & the Caribbean; LM: lower middle; MEast + NAfr: Middle East & North Africa; NHLBI: National Heart, Lung, and Blood Institute; NS: not specified; P: prospective; R: retrospective; RCT: randomised controlled trial; SAsia: South Asia; SSAfr: sub-Saharan Africa; TAHOD: TREAT Asia HIV Observational Database; tx: treatment; UM: upper middle

Table 3:2. Characteristics of studies included in analysis (n = 56 studies; n = 10,291 HIV-positive adult deaths; n = 4,009 deaths after initiation of ART)

Characteristic	Studies, n (%) (N = 56)	Included HIV-positive adults with CoD assigned	
		Overall, n (%) (N = 10,291)	Initiated ART, n (row %)
Region*			
Sub-Saharan Africa	32 (57.1)	6,572 (63.9)	3,045 (46.3)
South Asia	8 (14.3)	791 (7.7)	164 (20.7)
Latin America & the Caribbean	7 (12.5)	1,562 (15.2)	476 (30.5)
East Asia & the Pacific	4 (7.1)	374 (3.6)	232 (62.0)
Europe & Central Asia	3 (5.4)	847 (8.2)	0
Middle East & North Africa	2 (3.6)	145 (1.4)	128 (88.3)
Income category*			
Low	13 (23.2)	3,065 (29.8)	1,685 (55.0)
Lower middle	25 (44.6)	3,812 (37.0)	1,254 (32.9)
Upper middle	18 (32.1)	3,414 (33.2)	1,106 (32.4)
Calendar period†			
Pre-2005	15 (26.8)	2,118 (20.6)	93 (4.4)
2005–2010	30 (53.6)	5,249 (51.0)	2,916 (55.6)
2011–2015	11 (19.6)	2,924 (28.4)	1,036 (35.4)
Site of recruitment			
Hospital only	32 (57.1)	4,339 (42.2)	1,117 (25.7)
Community only	14 (25.0)	4,250 (41.3)	2,136 (50.3)
Hospital + community	9 (16.1)	1,666 (16.2)	756 (45.4)
Site of death			
Hospital only	27 (48.2)	3,080 (29.9)	927 (30.1)
Hospital + community	24 (42.9)	6,849 (66.6)	3,040 (44.4)
NHLBI quality rating			
Good	25 (44.6)	5,258 (51.1)	2,288 (43.5)
Fair	16 (28.6)	2,091 (20.3)	695 (33.2)
Poor	15 (26.8)	2,942 (28.6)	1026 (34.9)
Data & methods			
Data available			
Pathological autopsy‡	20 (35.7)	2,442 (23.7)	420 (17.2)
Hospital records‡	49 (87.5)	9,627 (93.5)	3,994 (41.5)
Assignment process			
At least two reviewers	36 (64.3)	7,519 (73.1)	3,399 (45.2)
Structured process	33 (58.9)	7,541 (73.3)	3,136 (41.6)
System for resolving disagreements	25 (44.6)	6,123 (59.5)	3,179 (51.9)
Criteria for individual CoD	34 (60.7)	7,601 (73.9)	2,894 (38.1)
Data & methods score§, median (IQR)	2.5 (1.7–4.3)	2.5 (1.7–4.3)	2.5 (2.0–4.0)

*Per World Bank classification [27]

†Based on last year of data collection

‡All participants from each study did not necessarily have these data available; numbers shown are maximum possible with data available

§Based on data available and methods used to assign causes of death (Supplementary table 3)

ART: antiretroviral therapy; CoD: cause of death; IQR: interquartile range; NHLBI: National Heart, Lung, and Blood Institute

3.2.4.2 Proportions assigned HIV-associated causes of death

The pooled proportion of HIV-positive adult decedents assigned any HIV-associated cause of death was 79% (95% CI 74–83; Tables 3:3 and 3:4; Figure 3:2). There was a high degree of heterogeneity between studies, (I^2 96.8%, $p < 0.01$), with proportions of decedents assigned HIV-associated causes ranging from 30% to 100%. There were fewer differences between studies based on geographical regions ($p = 0.79$, based on meta-regression); income groups ($p = 0.85$); and study quality, based on NHLBI grade ($p = 0.25$). The 11 studies conducted from 2011–2015 assigned a slightly lower proportion of HIV-associated causes of death than those conducted in earlier periods (70% in 2011–2015 vs. 77% in 2005–2010 [$n = 30$ studies] vs. 87% pre-2005 [$n = 15$ studies]; $p = 0.14$; Figure 3:2). Similarly, studies where larger proportions of decedents initiated ART assigned lower proportions of HIV-associated causes of death (85% if <33% on ART [$n = 11$ studies] vs. 76% if 33–65% on ART [$n = 7$ studies] vs. 71% if >66% initiated on ART [$n = 21$ studies]; $p = 0.02$; Figure 3:3).

Meta-regression of the proportion of individuals on ART per study against middle year of data collection showed that proportions on ART were markedly higher in studies conducted more recently ($p < 0.001$; Supplementary figure 3:1). The 20 studies that used autopsy data assigned more HIV-associated causes of death than the 36 studies that did not (87% vs 73%; $p = 0.02$; Figure 3:4). Heterogeneity was high within groups ($I^2 > 93%$; $p < 0.01$) but was variable between groups (p 0.53–1.0 between regions, income groups, and NHLBI grades; $p = 0.06$ and $p = 0.01$ between calendar periods and proportions on ART, respectively).

3.2.4.3 Proportions assigned TB causes of death

Of the 56 studies included, 47 (83.9%) reported the proportion of individuals assigned HIV-associated TB as a cause of death: 29/47 (61.7%) studies were conducted in sub-Saharan Africa, seven (14.9%) in South Asia, and four (8.5%) in Latin America; 12 (25.5%), 22 (46.8%), and 13 (27.7%) studies were conducted in low-, lower middle-, and upper middle-income countries, respectively; 21 (44.7%), 14 (30.0%), and 12 (25.5%) studies were assigned a grade of ‘good’,

'fair', and 'poor', respectively, using the NHLBI grading tool; 18 (38.3%) and 40 (85.1%) studies used autopsy data and hospital data, respectively to assign causes of death; and the overall median methods score was 2.5 (IQR 1.5–4.0).

Among the 7,990 HIV-positive adult decedents included in studies where TB causes of death were reported, a pooled 29% (95% CI 24–34; Table 3:4) were assigned a TB cause of death. Heterogeneity between studies was high (I^2 96.0%; $p < 0.01$); proportions ranged from 0% to 76%. Meta-regression revealed differences between groups based on geographical region (higher in South Asia [51%] than sub-Saharan Africa [25%] or Latin America [22%]; $p = 0.01$; Figure 3:5) and income group (highest in lower-middle income countries [36%]; $p < 0.01$). In contrast to overall HIV-associated causes of death, no difference was seen in proportions of TB causes assigned based on calendar period of data collection ($p = 0.37$) or the proportions of decedents initiated on ART ($p = 0.61$; Figure 3:3). In the 18 studies where autopsy data were used, a pooled 37% (95% CI 30–45) of decedents were assigned a TB cause of death, compared with a pooled 24% (95% CI 18–30) of decedents in the 29 studies without autopsy data ($p = 0.04$).

When the analysis was restricted to studies conducted in sub-Saharan Africa ($n = 29$ studies, $n = 5,722$ deaths), differences were observed between income groups (15% low vs. 32% lower middle vs. 33% upper middle; $p = 0.05$; Table 3:4) and based on use of autopsy data (34% with autopsy data vs. 19% without; $p = 0.07$). As in the overall analysis, the proportion of individuals initiated on ART in each study appeared to have no effect on the proportions of TB causes of death assigned, despite a decline in the proportions of overall HIV-associated causes of death assigned (meta-regression against proportion on ART: any HIV-associated cause, $p = 0.02$; TB cause of death in all decedents, $p = 0.85$; Figure 3:3).

3.2.4.3.1 TB as a proportion of HIV-associated causes of death

Among decedents assigned an HIV-associated cause of death ($n = 5,688$ in the 47 studies where TB was reported), a pooled 39% (95% CI 33–46) were assigned a TB cause of death (heterogeneity 95.6%; $p < 0.01$; Table 3:4). This was much higher in studies conducted in South Asia (72%) compared with sub-Saharan Africa (34%), Latin America (24%), and other regions (38%; $p < 0.01$). Differences were also seen between income groups (lower middle 51% vs. upper middle 38% vs. low 21%; $p < 0.01$) but not in relation to the proportion of decedents initiated on ART in each study ($p = 0.98$).

Table 3:3. Summary of findings of studies that directly estimated causes of death in HIV-positive adults dying in low- and middle-income countries, listed by calendar period of data collection (n = 56 studies; n = 10,291 HIV-positive adult decedents)

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/ μ L), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD [†] (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD [†] (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)
Pre-2005														
[33] LAm+Car 01 (1993–2000)	Brazil	1,478	25	Mean 34.8 (range 19–68)	NS	92 (6.2)	92 (100)	86 (93.5)	22 (23.9)	NS				
[34] LAm+Car 02 (1996–2003)	Brazil	129	25.6	32 (range 13–64)	NS	129 (100)	129 (100)	107 (82.9)	36 (27.9)	NS				
[35] LAm+Car 03 (1999–2004)	Peru	281	37.5	32 (range 19–62)	NS	16 (5.7)	16 (100)	16 (100)	5 (31.3)	0	-	-	-	-
[36] SAsia 01 (1991–2003)	India	60	20	Mean 32.1 (range 19–55)	NS	60 (100)	60 (100)	35 (58.3)	35 (58.3)	0	-	-	-	-
[37] SAsia 02 (2000–2003)	India	135	17	Mean 34 (\pm 10)	Mean 121 (\pm 205)	21 (15.6)	21 (100)	21 (100)	16 (76.2)	NS				
[38] SAsia 03 (2002–2003)	India	655	25.3	Mean 35.2 (\pm 9)	66 (range 4–446)	172 (26.3)	172 (100)	112 (65.1)	91 (52.9)	NS				
[39] SSAfr 01 (1986–1987)	Rwanda	3,702	100	Mean 28	NS	39 (1.1)	39 (100)	38 (97.4)	4 (10.3)	0	-	-	-	-
[40] SSAfr 02 (1988–1989)	C. d'Iv.	698	25.9	Range 15–?	NS	266 (38.1)	266 (100)	130 (48.9)	32 (12.0)	0	-	-	-	-
[41] SSAfr 03 (1989)	C. d'Iv.	473	NS	NS	NS	NS	53	47 (88.7)	22 (41.5)	0	-	-	-	-

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/ μ L), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD [†] (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD [†] (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)
[42] SSAfr 04 (1991)	C. d'Iv.	6,752	24.1	34	80 (range 1–166)	1080 (16.0)	294 (27.2)	271 (92.2)	92 (31.3)	0	-	-	-	-
[43] SSAfr 05 (1990–1996)	Uganda	440	48.7	Range 22–80	NS	63 (14.3)	63 (100)	52 (82.5)	3 (4.8)	0	-	-	-	-
[44] SSAfr 06 (1996–1997)	Kenya	1,804	53.3	33 (29–42)	55 (10–230)	155 (8.6)	75 (48.4)	74 (98.7)	35 (46.7)	0	-	-	-	-
[45] SSAfr 07 (1997–1998)	Botswana	5,055	46.2	35 (range 14–87)	NS	565 (11.2)	104 (18.4)	86 (82.7)	38 (36.5)	NS				
[46] SSAfr 08 (2001)	Congo, Rep.	1,309	48.7	NS	NS	641 (49.0)	641 (100)	586 (91.4)		0	-	-	-	-
[47] SSAfr 09 (1998–2002)	Senegal	404	54.7	37 (31–43)	128 (54–217)	93 (23.0)	93 (100)	77 (82.8)	17 (18.3)	93 (23.0)	NS¶	93 (100)	77 (82.8)	17 (18.3)
2005–2010														
[48] EAsia+Pac 01 (2003–2007)	TAHOD‡	1,557	21.4	39 (32–46)	66 (23–218)	56 (3.6)	56 (100)	22 (39.3)		56 (3.6)	NS	56 (100)	22 (39.3)	
[49] EAsia+Pac 02 (2007–2010)	Vietnam	640	10	34.2 (29–38)	41 (17–104)	60 (9.4)	60 (100)	49 (81.7)	24 (40.0)	60 (9.4)	79 (29–185)	60 (100)	49 (81.7)	24 (40.0)
[50] Eur+CAsia 01 (2003–2008)	Russian Fed.	32	15.6	NS	NS	32 (100)	32 (100)	32 (100)	24 (75.0)	32 (100)	NS	NS		
[51] Eur+CAsia 02 (1996–2009)	Turkey	128	18	Mean 38.5 (range 23–67)	Mean 454 (range 10–1480)	36 (28.1)	36 (100)	25 (69.4)	7 (19.4)	NS				

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/ μ L), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD [†] (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD [†] (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)
[52] LAm+Car 04 (1997–2006)	Brazil	1,538	50.2	39 (24–53)	250	226 (14.7)	226 (100)	130 (57.5)		153 (9.9)	NS	153 (100)	75 (49.0)	
[53] LAm+Car 05 (1986–2009)	Brazil	3,530	26.4	35	265 (90–430)	868 (24.6)	868 (100)	639 (73.6)		299 (8.5)	NS	299 (100)	181 (60.5)	
[54] MEast+NAfr 01 (1999–2009)	Morocco	1,243	49.5	Mean 36 (range 24–56)	Mean 96 (range 1–626)	91 (7.3)	91 (100)	86 (94.5)	35 (38.5)	91 (7.3)	270	91 (100)	86 (94.5)	35 (38.5)
[55] SAsia 04 (1992–2005)	India	2,050	24.1	43 (range 16–69)	NS	145 (7.1)	145 (100)	90 (62.1)	66 (45.5)	5 (0.2)	NS	5 (100)	NS	NS
[56] SAsia 05 (1988–2007)	India	236	22.9	Range 18–?	NS	236 (100)	236 (100)	223 (94.5)	129 (54.7)	2 (0.8)	NS	2 (100)	NS	NS
[57] SAsia 06 (1996–2008)	India	4,848	24.6	34 (29–39)	58 (31–67)	155 (3.2)	69 (44.5)	56 (81.2)	20 (29.0)	155 (3.2)	150	69 (44.5)	56 (81.2)	20 (29.0)
[58] SAsia 07 (2000–2008)	India	822	18.8	NS	80	56 (6.8)	32 (57.1)	23 (71.9)		56 (6.8)	NS	32 (57.1)	23 (71.9)	
[59] SAsia 08 (2009–2010)	India	1,182	23.2	35 (–)	NS	56 (4.7)	56 (100)	45 (80.4)	27 (48.2)	56 (4.7)	73	56 (100)	45 (80.4)	27 (48.2)
[60] SSAfr 10 (1996–2005)	Togo	5,347	46.9	Mean 38.9 (\pm 13.4)	NS	147 (2.7)	147 (100)	104 (70.7)	0	NS				
[61] SSAfr 11 (2002–2005)	S. Africa	712	74	33 (29–38)	94	68 (9.6)	68 (100)	53 (77.9)	10 (14.7)	37 (5.2)	NS§	37 (100)	28 (75.7)	5 (13.5)
[62] SSAfr 12 (2003–2005)	Uganda & Zimbwe	3,316	65	36 (31–42)	86 (31–139)	179 (5.4)	179 (100)	124 (69.3)	14 (7.8)	179 (5.4)	NS§	179 (100)	124 (69.3)	14 (7.8)

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/ μ L), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD ⁺ (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD ⁺ (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)
[63] SSAfr 13 (2004–2005)	Uganda	559	NS	38 (33–44)	98 (21–163)	99 (17.7)	99 (100)	83 (83.8)	13 (13.1)	99 (17.7)	NS§	99 (100)	83 (83.8)	13 (13.1)
[64] SSAfr 14 (2000–2007)	Came-roon	362	51.9	Mean 40.2 (\pm 11.6)	NS	281 (77.6)	281 (100)	226 (80.4)	96 (34.2)	77 (21.3)	30 (13–60)	77 (100)	NS	NS
[65] SSAfr 15 (2005–2007)	S. Africa	1,353	67	37 (31–45)	93 (37–148)	124 (9.2)	124 (100)	93 (75.0)	47 (37.9)	124 (9.2)	57 (28–169)	124 (100)	93 (75.0)	47 (37.9)
[66] SSAfr 16 (2007)	Ghana	716	52	39.7 (\pm 9)	NS	221 (30.9)	221 (100)	215 (97.3)	69 (31.2)	32 (4.5)	NS	32 (100)	32 (100)	
[67] SSAfr 17 (2000–2008)	South Africa	86	NS	Range 18–70	NS	86 (100)	86 (100)	80 (93.0)		NS				
[68] SSAfr 18 (2003–2008)	B. Faso	5,608	70	35 (30–41)	NS	690 (12.3)	690 (100)	341 (49.4)	67 (9.7)	690 (12.3)	82 (30–270)	690 (100)	341 (49.4)	67 (9.7)
[69] SSAfr 19 (2004–2009)	S. Africa	2,943	40.1	36 (range 19–69)	26 (range 1–479)	305 (10.4)	305 (100)	208 (68.2)	55 (18.)	305 (10.4)	NS§	305 (100)	208 (68.2)	55 (18.0)
[70] SSAfr 20 (2006–2009)	Nigeria	110	46.5	39.5 (range 14–64)	75 (range 6–1003)	110 (100)	101 (91.8)	64 (63.4)	64 (63.4)	110 (100)	NS	101 (91.8)	64 (63.4)	64 (63.4)
[71] SSAfr 21 (2006–2009)	Nigeria	3,464	47.3	36 (30–45)	136 (56–199)	66 (1.9)	66 (100)	58 (87.9)	27 (40.9)	19 (0.5)	91 (62–1095)	19 (100)	16 (84.2)	7 (36.8)
[72] SSAfr 22 (2009)	Uganda	290	51.4	Mean 38	Mean 50 (IQR 14–87)	NS	35	32 (91.4)	13 (37.1)	NS		10	10 (100)	5 (50.0)
[73] SSAfr 23 (2009)	S. Africa	39	48.7	36 (32–40)	50 (27–154)	39 (100)	39 (100)	38 (97.4)	26 (66.7)	27 (69.2)	32 (16–50)**	27 (100)	25 (92.6)	25 (92.6)

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/μL), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD ⁺ (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD ⁺ (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)
[74] SSAfr 24 (2004–2010)	S. Africa	395	66.1	34.4 (29–40)	22 (8–37)	63 (15.9)	63 (100)	43 (68.3)	23 (36.5)	63 (15.9)	NS§	63 (100)	43 (68.3)	23 (36.5)
[75] SSAfr 25 (2010)	Togo	24,054	55.7	Mean 32.2	Mean 181	309 (1.3)	309 (100)	113 (36.6)	12 (3.9)	160 (0.7)	NS	160 (100)	55 (34.4)	6 (3.8)
[76] SSAfr 26 (2010)	Mozbque	742	NS	NS	NS	214 (28.8)	214 (100)	113 (52.8)	64 (29.9)	NS				
[77] SSAfr 27 (2010)	C. d'Iv., B. Faso, Benin, Mali, & Senegal	823	58	40 (33–48)	75 (25–177)	315 (38.3)	315 (100)	280 (88.9)	113 (35.9)	134 (16.3)	NS	134 (100)	NS	NS
2011–2016														
[78] EAsia+Pac 03 (2009–2012)	China	1,112	20.2	Mean 43 (± 11.7)	31 (11–104)	216 (19.4)	216 (100)	154 (71.3)		74 (6.7)	NS	74 (100)	51 (68.9)	
[79] EAsia+Pac 04 (2007–2014)	Vietnam	1,197	37.3	32 (18–73)	110 (range 1–693)	42 (3.5)	42 (100)	20 (47.6)	7 (16.7)	42 (3.5)	402	42 (100)	20 (47.6)	7 (16.7)
[80] Eur+CAsia 03 (1989–2012)	Georgia	3,554	26.5	36 (30–42)	238 (106–408)	779 (21.9)	779 (100)	426 (54.7)	166 (21.3)	NS				
[81] LAm+Car 06 (2000–2011)	Brazil	2,224	36.7	35.6 (29–43)	189 (72–299)	103 (4.6)	103 (100)	64 (62.1)		NS				
[82] LAm+Car 07 (2010–2013)	Mexico	145	10.3	38 (range 15–70)	47 (range 2–662)	145 (100)	128 (88.3)	118 (92.2)	15 (11.7)	61 (42.1)	NS	24 (39.3)	24 (100)	0

Reference, calendar period, region, study number, years data collected	Country /ies	Enrolled, N	Fe-male (%)	Age, median (IQR), mean (SD), or range	CD4 count (cells/ μ L), median (IQR), mean (SD), or range	All HIV-positive adults (n = 10,291)*				Adults who initiated ART (n = 4,009)				
						All deaths, n (%/enrolled)	Assd. CoD [†] (%/all deaths)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/CoD)	Deaths on ART, n (%/enrolled)	Time on ART (days), median (IQR)	Assd. CoD [†] (%/deaths on ART)	Assd. HIV-assoc. CoD (%/assd. CoD)	Assd. TB CoD (%/assd. CoD)
[83] MEast+NAfr 02 (2000–2014)	Tunisia	260	35.2	Mean 44 (\pm 11)	NS	54 (20.8)	54 (100)	38 (70.4)	2 (3.7)	37 (14.2)	NS	37 (100)	NS	NS
[84] SSAfr 28 (2002–2011)	Congo, Rep.	152	48.7	37 (16–71)	170 (1–772)	152 (100)	152 (100)	45 (29.6)	7 (4.6)	152 (100)	NS	152 (100)	45 (29.6)	7 (4.6)
[85] SSAfr 29 (2002–2012)	Uganda	4,784	58.1	36 (30–42)	90 (22–237)	1,249 (26.1)	1,028 (82.3)	848 (82.5)	347 (33.8)	395 (8.3)	NS	395 (100)	312 (79.0)	103 (26.1)
[86] SSAfr 30 (2012)	Kenya	253	60.1	40 (34–47)	87 (33–209)	253 (100)	253 (100)	160 (63.2)	80 (31.6)	253 (100)	330 (60–930)	253 (100)	160 (63.2)	80 (31.6)
[87] SSAfr 31 (2013)	Uganda	99	57.3	35 (29–40)	47 (17–165)	96 (97.0)	96 (100)	87 (90.6)	42 (43.8)	NS				
[88] SSAfr 32 (2013–2015)	Mozbque	112	49.1	37 (range 16–76)	NS	73 (65.2)	73 (100)	67 (91.8)		NS				

*Includes the 4,009 individuals who initiated ART

[†]Includes those assigned ‘unknown’ or ‘indeterminate’ cause of death

[‡]TAHOD lower middle-income countries: Cambodia, India, Indonesia, Philippines, and Vietnam; TAHOD upper middle-income countries: China, Indonesia, Malaysia, and Thailand

[§]At least 50% of deaths in those who initiated ART occurred within 3 months of initiation

^{||}At least 50% of deaths in those who initiated ART occurred within 6 months of initiation (3-month mortality not reported)

[¶]At least 50% of deaths in those who initiated ART occurred within 12 months of initiation

**Median time listed for ‘Early-ART’ group, died <90 days post-initiation (n = 15); for ‘Late-ART’ group (>90 days post-initiation; n = 10), median time on ART 326 (IQR 148–531) days

Assd.: assigned; assoc.: associated; ART: antiretroviral therapy; B. Faso: Burkina Faso; C. d’Iv.: Côte d’Ivoire; CoD: cause of death; EAsia + Pac: East Asia & the Pacific; Eur + CAsia:

Europe & Central Asia; IQR: interquartile range; L: low; Lam + Car: Latin America & the Caribbean; LM: lower middle; MEast + NAfr: Middle East & North Africa; Mozbque:

Mozambique; NS: not specified; Rep.: republic; Russian Fed.: Russian Federation; S. Africa: South Africa; SAsia: South Asia; SD: standard deviation; SSAfr: sub-Saharan Africa;

TAHOD: TREAT Asia HIV Observational Database; TB: tuberculosis; UM: upper middle; Zimbwe: Zimbabwe

Table 3:4. Pooled prevalence of decedents assigned any HIV-associated and TB causes of death in studies of HIV-positive adults dying in low- and middle-income countries (n = 56 studies) and restricted to studies conducted in sub-Saharan Africa (n = 32 studies)

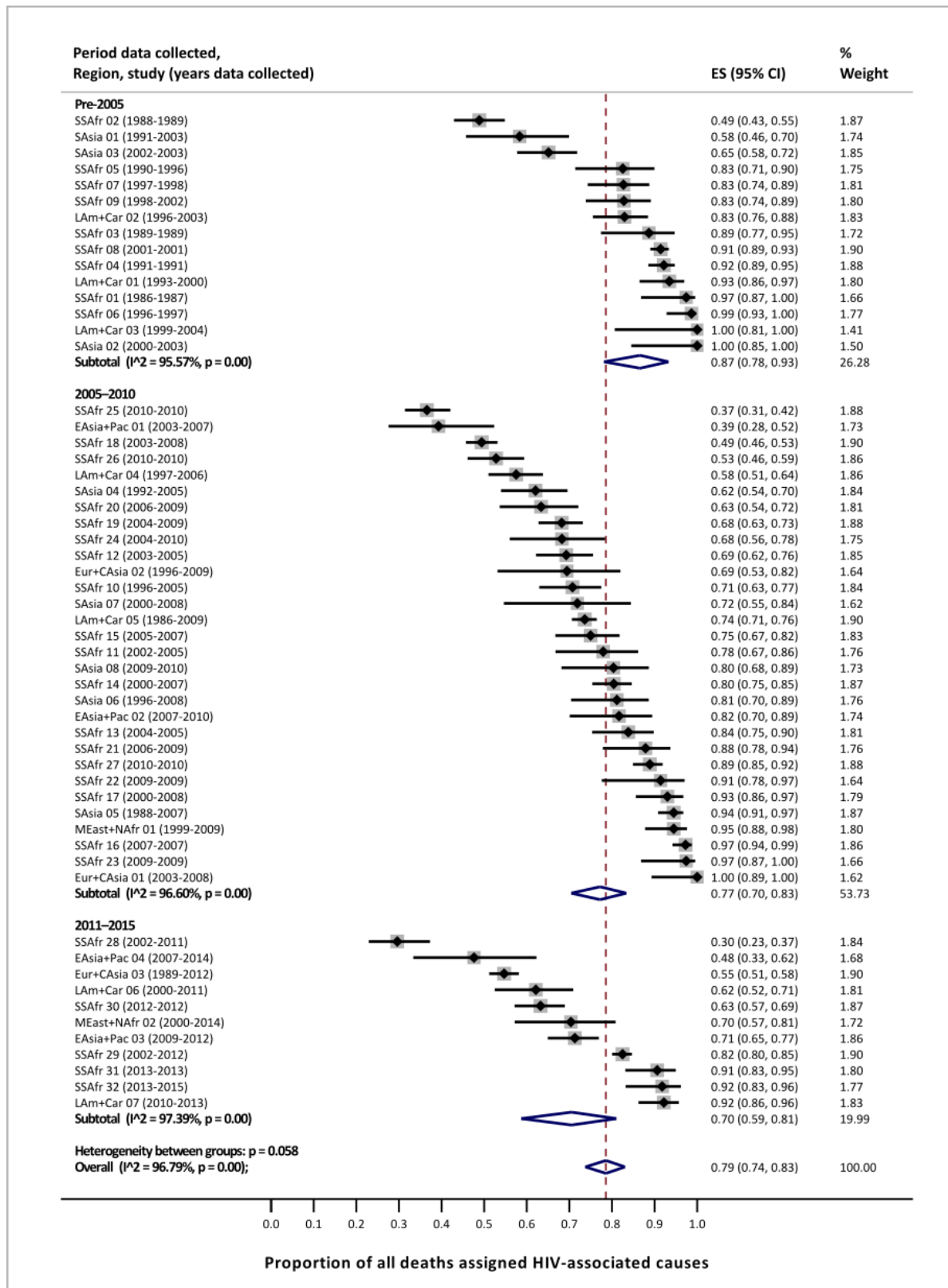
Group & sub-group	All regions					Sub-Saharan Africa only				
	Proportion of decedents assigned any HIV-associated CoD (N = 56 studies)		Pooled proportion of decedents assigned TB CoD (N = 47 studies)			Proportion of decedents assigned any HIV-associated CoD (n = 32 studies)		Proportion of decedents assigned TB CoD (n = 29 studies)		
	Among all HIV-positive adults (N = 10,290 deaths)	Among all HIV-positive adults (n = 7,990 deaths)	Among all HIV-positive adults (n = 7,990 deaths)	Among adults with HIV-associated CoD (n = 5,688 deaths)	Among adults with HIV-associated CoD (n = 5,688 deaths)	Among all HIV-positive adults (n = 6,572 deaths)	Among all HIV-positive adults (n = 5,772 deaths)	Among all HIV-positive adults (n = 5,772 deaths)	Among adults with HIV-associated CoD (n = 4,103 deaths)	Among adults with HIV-associated CoD (n = 4,103 deaths)
Studies, PP, n (%)	PP, % (95% CI) p*	Studies, PP, n (%)	PP, % (95% CI) p*	PP, % (95% CI) p*	Studies, PP, n (%)	PP, % (95% CI) p*	Studies, PP, n (%)	PP, % (95% CI) p*	PP, % (95% CI) p*	
Overall	56	79 (74–83) -	47	29 (24–34) -	39 (33–46) -	32	79 (73–85) -	29	25 (19–31) -	34 (27–42) -
Region										
Sub-Saharan Africa	32	79 (73–85)	29	25 (19–31)	34 (27–42)	-	-	-	-	-
South Asia	8	79 (65–90) 0.79	7	51 (43–58) 0.01	72 (56–85) <0.01	-	-	-	-	-
Latin America & The Caribbean	7	82 (71–90)	4	22 (13–32)	24 (14–37)	-	-	-	-	-
Other	9	73 (59–85)	7	28 (16–42)	38 (25–51)	-	-	-	-	-
Country income group										
Low	13	77 (66–86)	12	15 (7–25)	21 (11–32)	13	77 (66–86)	12	15 (7–25)	21 (11–32)
Lower middle	25	78 (70–85) 0.85	22	36 (29–44) <0.01	51 (41–61) <0.01	12	81 (68–90) 0.77	11	32 (23–42) 0.05	45 (33–57) 0.05
Upper middle	18	80 (74–86)	13	30 (23–38)	38 (29–47)	7	81 (72–89)	6	33 (21–47)	43 (29–57)
Calendar period										
Pre-2005	15	87 (78–93)	14	32 (22–42)	42 (28–56)	9	87 (76–95)	8	24 (14–35)	28 (19–39)
2005–2010	30	77 (70–83) 0.14†	25	31 (23–39) 0.37†	41 (31–51) 0.46†	18	77 (67–85) 0.38	17	25 (17–35) 0.25	36 (24–48) 0.10
2011–2015	11	70 (59–81)	8	19 (12–28)	30 (21–40)	5	74 (53–90)	4	26 (13–43)	40 (29–50)
Proportion on ART**										

Group & sub-group	All regions					Sub-Saharan Africa only										
	Proportion of decedents assigned any HIV-associated CoD (N = 56 studies)		Pooled proportion of decedents assigned TB CoD (N = 47 studies)			Proportion of decedents assigned any HIV-associated CoD (n = 32 studies)		Proportion of decedents assigned TB CoD (n = 29 studies)								
	Among all HIV-positive adults (N = 10,290 deaths)	Among all HIV-positive adults (n = 7,990 deaths)	Among adults with HIV-associated CoD (n = 5,688 deaths)	Among all HIV-positive adults (n = 6,572 deaths)	Among all HIV-positive adults (n = 5,772 deaths)	Among adults with HIV-associated CoD (n = 4,103 deaths)	Studies, PP, n (%)	PP, % (95% CI)	p*	Studies, PP, n (%)	PP, % (95% CI)	p*				
<33%	18	86 (79–92)	17	28 (19–39)	37 (25–49)	13	87 (79–94)	12	24 (15–35)	29 (18–40)						
33–65%	7	76 (63–86)	0.01‡	5	24 (10–42)	0.52‡	31 (20–44)	0.98‡	5	77 (56–93)	0.02	5	24 (10–42)	0.85	31 (20–44)	0.47
>66%	20	70 (62–77)		17	26 (18–35)		39 (26–51)		11	69 (59–78)		11	25 (15–36)		40 (24–57)	
Study quality																
Good	25	78 (71–84)		21	22 (17–28)		29 (24–36)		18	81 (72–88)		17	24 (17–31)		31 (24–38)	
Fair	16	82 (75–88)	0.25	14	31 (21–42)	0.09	39 (27–52)	0.02	7	85 (76–93)	0.17	7	28 (14–44)	0.74	33 (18–50)	0.52
Poor	15	75 (64–85)		12	39 (23–55)		59 (38–78)		7	69 (45–88)		5	25 (6–50)		47 (13–83)	
Data availability																
Autopsy data	20	87 (79–94)	0.02	18	37 (30–45)	0.04	47 (38–55)	0.17	14	86 (75–94)	0.15	12	34 (27–41)	0.07	42 (35–49)	0.13
No autopsy data	36	73 (67–79)		29	24 (18–30)		34 (26–43)		18	74 (65–82)		17	19 (12–28)		29 (19–40)	
Hospital data	49	79 (74–83)	0.82	40	29 (24–35)	0.63	40 (33–47)	0.61	29	80 (72–86)	0.72	26	26 (19–33)	0.52	35 (27–43)	0.51
No hospital data	7	78 (60–92)		7	26 (13–42)		34 (20–50)		3	76 (44–97)		3	18 (3–41)		24 (6–49)	
Methods score																
<2.0	15	71 (60–81)		13	26 (13–40)		39 (20–59)		7	62 (44–79)		7	17 (4–37)		32 (7–64)	
2.0–3.9	22	82 (75–88)	0.13	21	28 (21–35)	0.51	38 (28–48)	0.95	11	79 (69–87)	<0.01	10	19 (13–27)	0.02	27 (18–36)	0.16
≥4.0	19	80 (72–87)		13	34 (26–42)		41 (23–48)		14	87 (76–95)		12	35 (25–45)		42 (34–50)	

*Based on meta-regression | †Meta-regression conducted using last year of data collection | ‡Meta-regression conducted using proportion on ART (continuous)

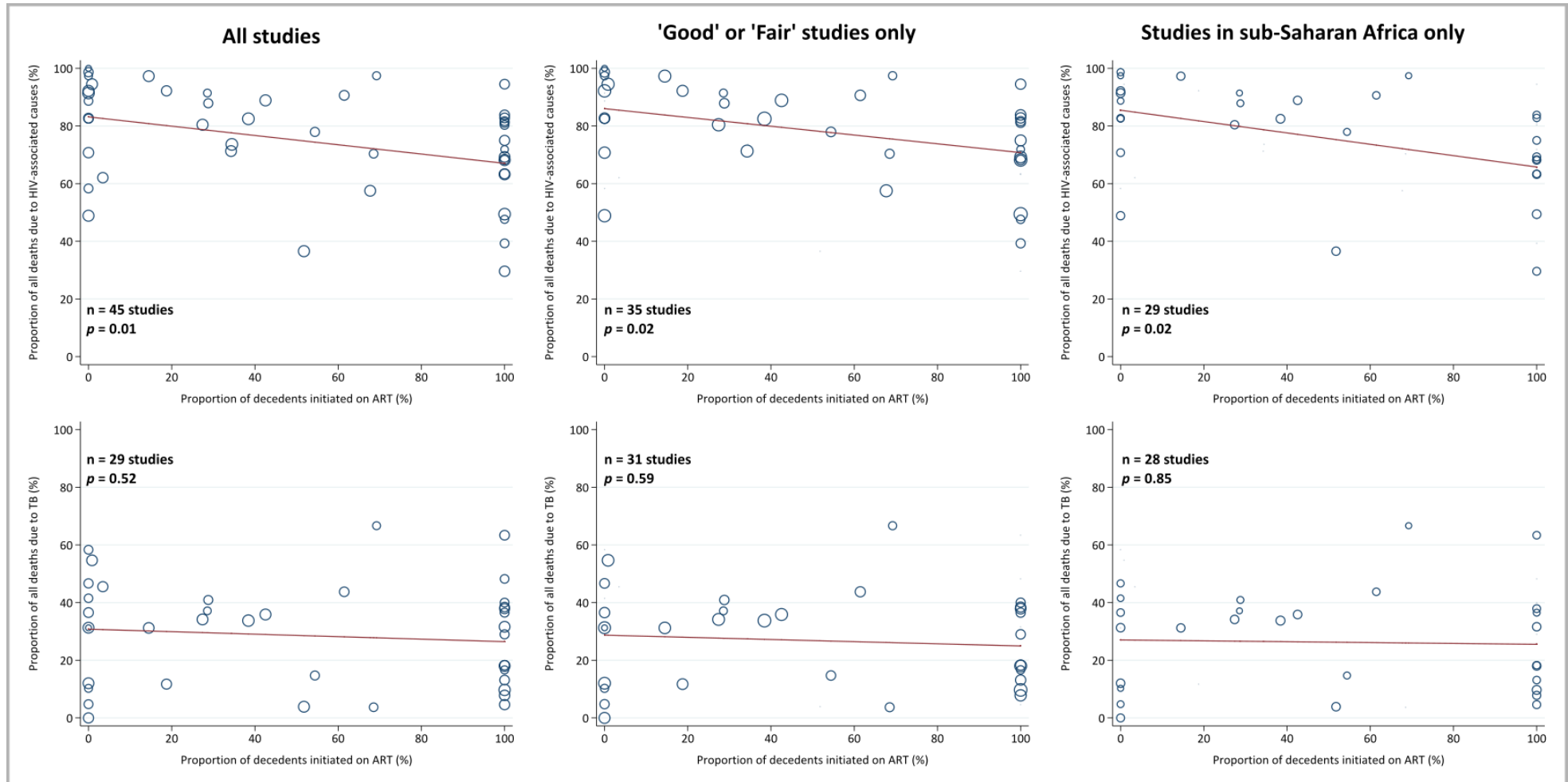
ART: antiretroviral therapy; CI: confidence interval; CoD: cause(s) of death; PP: pooled proportion; TB: tuberculosis

Figure 3:2. Forest plot showing the proportions of decedents assigned any HIV-associated cause of death in studies conducted in low- and middle-income countries, stratified by calendar period of data collection (n = 56 studies)



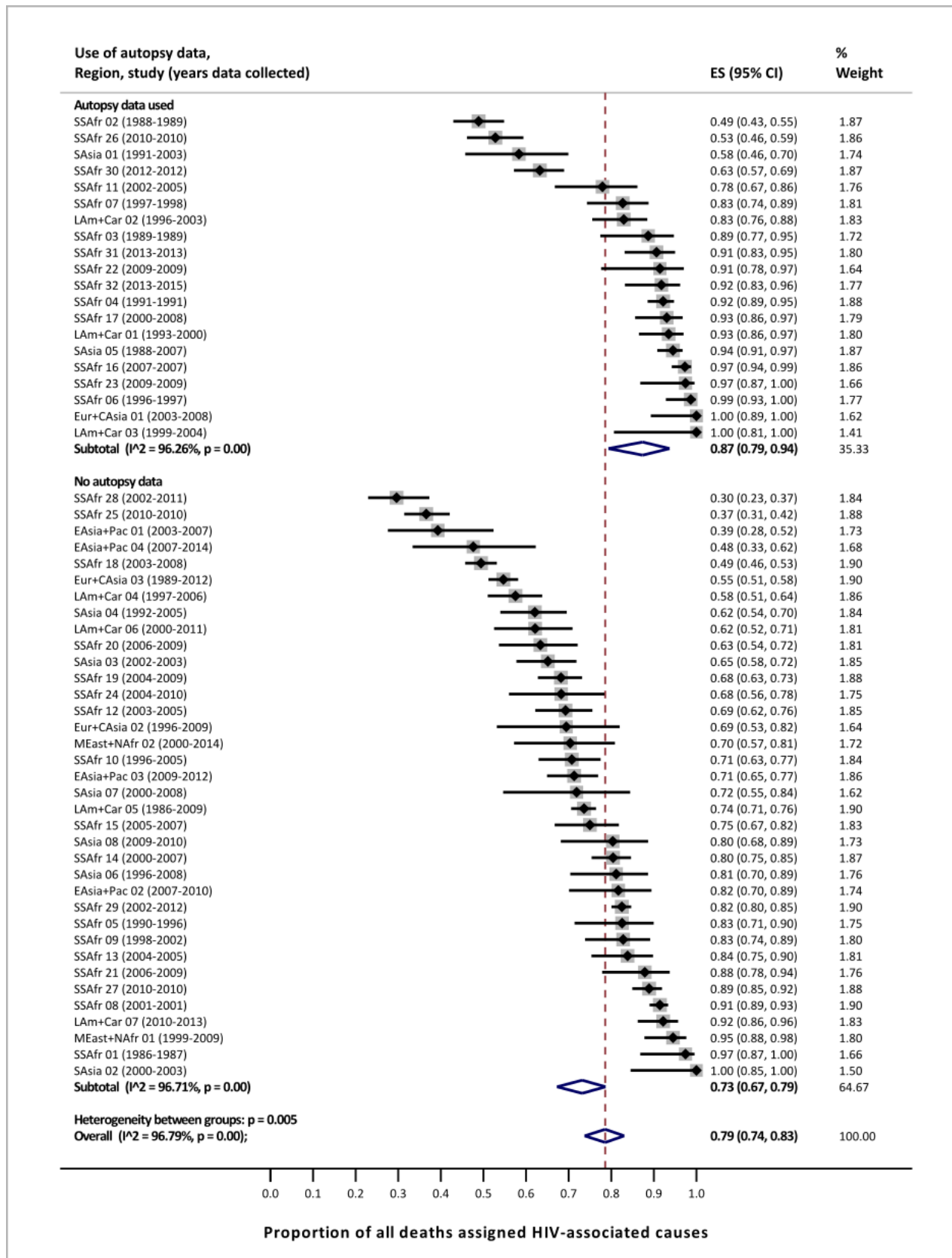
Car: Caribbean; CI: confidence interval; EAsia: East Asia; ES: estimated proportion/prevalence; Lam: Latin America; MEast: Middle East; NAfr: North Africa; Pac: Pacific; SAsia: South Asia; SSAfr: Sub-Saharan Africa

Figure 3:3. Bubble plots showing relationships, based on meta-regression, between proportions of decedents initiated on ART per study (x axis) and proportions of decedents assigned any HIV-associated cause of death (y axis, top row) and proportions of decedents assigned a TB cause of death (y axis, bottom row)



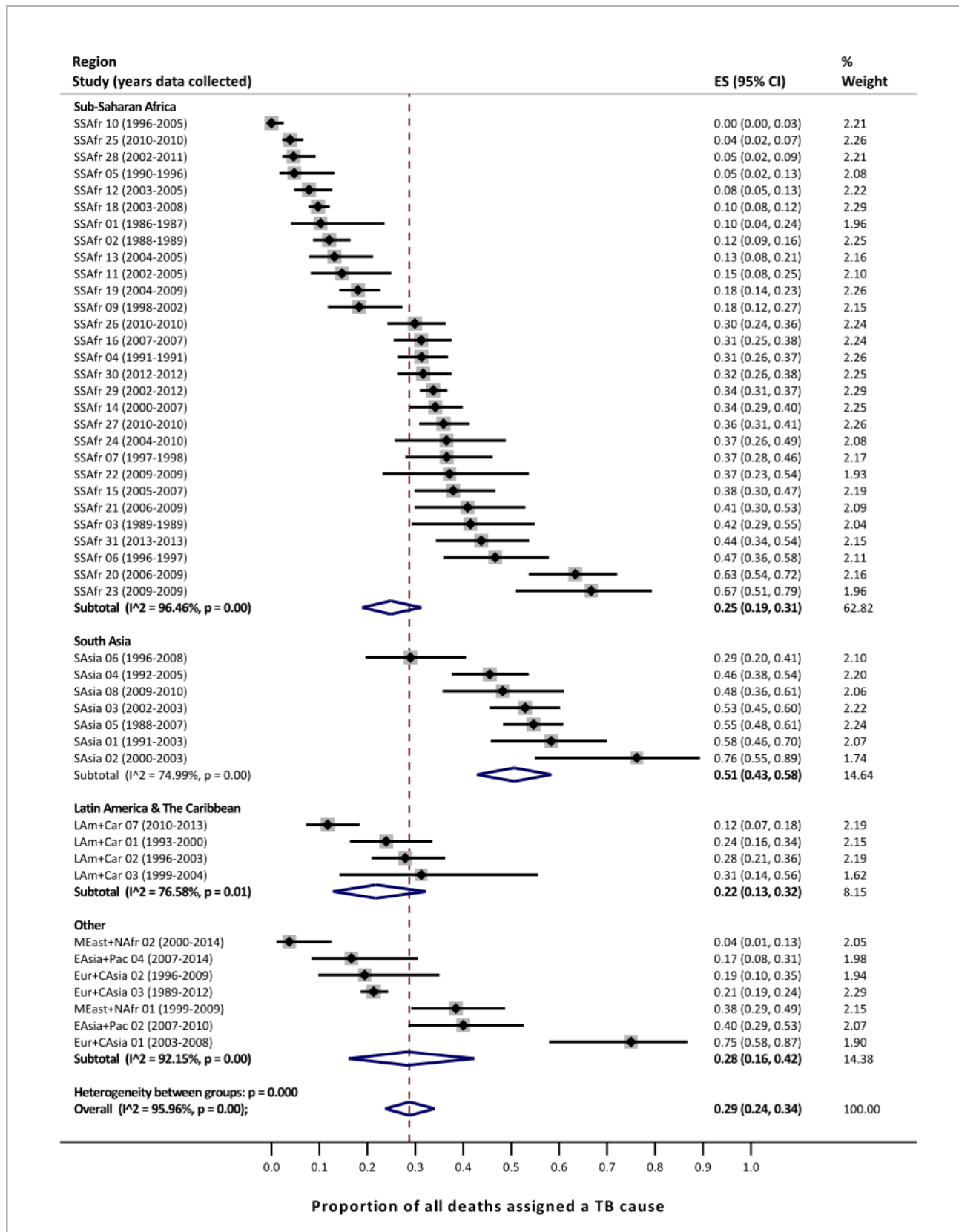
ART: antiretroviral therapy; CoD: cause of death; TB: tuberculosis

Figure 3:4. Forest plot showing the proportions of decedents assigned any HIV-associated cause of death, stratified by use of autopsy data (n = 56 studies)



Car: Caribbean; CI: confidence interval; EAsia: East Asia; ES: estimated proportion/prevalence; Lam: Latin America; MEast: Middle East; NAFr: North Africa; Pac: Pacific; SAsia: South Asia; SSAfr: Sub-Saharan Africa

Figure 3:5. Forest plot showing the proportions of decedents assigned a tuberculosis cause of death, stratified by geographic region (n = 47 studies)



Car: Caribbean; CI: confidence interval; EAsia: East Asia; ES: estimated proportion/prevalence; Lam: Latin America; MEast: Middle East; NAfr: North Africa; Pac: Pacific; SAsia: South Asia; SSAfr: Sub-Saharan Africa

3.2.4.4 Sub-analysis: causes of death in individuals who initiated ART vs. individuals who did not

In the 28 studies describing causes of death in those who had initiated ART ($n = 3,695$ deaths), 17 (60.7%) included only individuals on ART; proportions of decedents on ART in the remaining 11 studies ranged from 15% to 69%. The majority of studies were conducted in sub-Saharan Africa (17/28 [60.7%]); 21 (75.0%) studies had completed data collection between 2005 and 2010; and 11 (39.2%) studies only recruited individuals after admission to hospital, compared with 11 (39.2%) that recruited from the community, and six (21.4%) that recruited from both sites. Five (17.9%) studies used data from pathological autopsies to assign causes of death.

The pooled proportions of decedents assigned HIV-associated and TB causes of death were 84% (95% CI 78–90) and 22% (95% CI 13–33) in decedents who had not initiated ART ($n = 23$ studies, $n = 3,968$ deaths) and 72% (95% CI 66–79) and 26% (95% CI 18–34), respectively, in those who had (Table 3:5). Heterogeneity between studies was high in both groups (I^2 95.71 and 96.79, $p < 0.01$). There were very slight differences between proportions of causes of death assigned based on geographic or income group in either those who did not initiate ART or those who did (p 0.1–0.95 for HIV-associated and TB causes of death in both groups). Fewer HIV-associated and TB causes were assigned to individuals who initiated ART in studies that completed data collection after 2010 (67% any HIV associated, 14% TB) compared with earlier periods (73–83% any HIV associated; 18–31% TB), but this may have been due to chance ($p = 0.94$ and $p = 0.41$ for any HIV-associated and TB causes of death, respectively); this relationship was not seen in individuals who did not initiate ART.

Similar patterns were seen when the analysis was restricted to only studies that included either 0% or 100% of decedents who initiated ART ($n = 11$ studies, $n = 1,758$ deaths if 0% initiated ART; $n = 17$ studies, $n = 2,465$ deaths if 100% initiated ART). Studies with 0% ART initiation assigned 85% (95% CI 74–94) of decedents an HIV-associated cause, compared with 69% (95% CI 68–83) assigned in studies where all decedents initiated ART ($p = 0.05$). Almost no difference was seen, however, in the proportions of TB causes of death assigned (24% vs 26% in all decedents, 33% vs. 39% in decedents with HIV-associated causes of death, 0% ART vs. 100% ART, respectively; $p = 0.99$ and $p = 0.64$).

3.2.4.4.1 Duration of ART

Only 12 studies reported the median time from ART initiation to death, which ranged from 30 days ($n = 77$; Cameroon [64]) to over one year ($n = 42$; Vietnam [79]). The largest study that reported time on ART was a retrospective analysis of patient records across 13 districts of Burkina Faso [68], which included 690 decedents who initiated ART and died a

median 82 (IQR 30–270) days after initiation. A number of studies did not report time on ART, but instead reported the numbers of individuals who died after specific intervals of time. Seven studies [49,57,61–63,69,74], two of which also reported median time on ART, reported that at least 50% of the deaths among individuals who had initiated ART had occurred in the three months after initiation; a further three [58,78,82] reported that 54%–68% of deaths among those initiating ART had taken place in the six months after initiation (Table 3:3). Only three studies [58,63,79], conducted in Uganda, India, and Vietnam, presented causes of death stratified by duration of ART (n = 99, n = 32, and n = 42 on ART, respectively). Although all three studies found that overall mortality decreased with time on ART, the proportions of HIV-associated causes of death assigned were similar, regardless of duration of ART (Table 3:6).

Table 3:5. Pooled prevalence of deaths assigned any HIV-associated cause or TB cause in HIV-positive adult decedents who initiated ART (n = 28 studies) and in those who did not (n = 23 studies)

Group & sub-group	In HIV-positive adults who initiated ART (n = 28 studies), the proportion of decedents assigned						In HIV-positive adults who did not initiate ART (n = 23 studies), the proportion of decedents assigned					
	Any HIV-associated cause of death (n = 3,695 deaths)			A TB cause of death (n = 3,049 deaths)			Any HIV-associated cause of death (n = 3,968 deaths)			A TB cause of death (n = 2,120 deaths)		
	Studies, n (%)	PP, % (95% CI)	p*	Studies, n (%)	PP, % (95% CI)	p*	Studies, n (%)	PP, % (95% CI)	p*	Studies, n (%)	PP, % (95% CI)	p*
Overall	28	72 (66–79)	-	22	26 (18–34)	-	23	84 (78–90)	-	17	22 (13–33)	-
Region												
Sub-Saharan Africa	17	72 (63–81)		16	25 (17–35)		16	85 (76–92)		14	20 (11–31)	
South Asia	3	79 (72–85)		2	37 (29–46)		2	89 (86–93)		2	16 (9–23)	
Latin America & The Caribbean	3	73 (50–91)	0.83	1	0 (0–14)	0.61	4	86 (77–93)	0.95	1	58 (46–70)	0.21
Other	5	69 (45–88)		3	32 (19–46)		1	73 (65–79)		0	-	
Country income group†												
Low	7	72 (56–85)		7	14 (7–22)		6	79 (61–92)		6	11 (0–33)	
Lower middle	12	72 (58–84)	0.95	9	33 (20–48)	0.10	9	87 (76–95)	0.54	6	37 (23–53)	0.15
Upper middle	9	73 (64–81)		6	30 (13–51)		8	83 (78–88)		5	21 (10–34)	
Calendar period												
Pre-2005	1	83 (74–89)		1	18 (12–27)		10	87 (75–95)		9	28 (17–41)	
2005–2010	21	73 (66–81)	0.94‡	16	31 (21–42)	0.41‡	10	82 (70–92)	0.87‡	6	13 (1–31)	0.81‡
2011–2015	6	67 (48–84)		5	14 (5–27)		3	83 (74–91)		2	35 (31–38)	

*Based on meta-regression

†Based on World Bank classification [27]

‡Meta-regression conducted using middle year of data collection

ART: antiretroviral therapy; CI: confidence interval; CoD: cause of death; PP: pooled proportion; TB: tuberculosis

Table 3:6. Causes of death assigned to individuals who initiated ART in studies that reported causes by duration on ART (n = 3 studies; n = 173 deaths)

Reference, region, period of data collection	Country	Deaths after ART initiation, n	Causes of death by time on ART
[58] SAsia 07 (2000-2008)	India	32	15/18 (83%) deaths in the first 6 months were assigned HIV-associated CoD vs. 8/14 (57%) deaths >6 months post-initiation.
[63] SSAfr 13 (2004-2005)	Uganda	99	72/80 (90%) deaths in the first 12 months were assigned HIV-associated CoD, vs. 8/15 (53%) in months 13–24, and 3/4 (75%) in months 25–36.
[79] EAsia+Pac 04 (2007-2014)	Vietnam	42	Report ‘no significant differences’ in survival time between the 19 individuals who were assigned ‘AIDS-related’ CoD (median 1.05 years from ART initiation to death) and the 14 individuals assigned a ‘non-AIDS’ CoD (median 1.61 years from ART initiation to death).

AIDS: acquired immune deficiency syndrome; ART: antiretroviral therapy; CoD: cause(s) of death; EAsia+Pac: East Asia and the Pacific; SAsia: South Asia; SSAfr: sub-Saharan Africa

3.2.5. Discussion

Over 10,000 deaths among HIV-positive adults, occurring between 1986 and 2015, were included in this review: a pooled 79% of decedents were assigned an HIV-associated cause of death; almost 40% of these were thought to be due to HIV-associated TB (29% of all deaths). Proportions of decedents assigned HIV-associated causes of death declined over the 30 years analysed, a reduction very likely driven by the higher numbers of individuals initiated on ART in more recent studies. No differences were seen in the proportions of deaths attributed to TB, either by date of study or by the proportion of decedents in each study who initiated ART. The same patterns were evident when the analysis was restricted only to ‘fair’ or ‘good’ studies (per the NHLBI scale) and only to studies conducted in sub-Saharan Africa. Causes of death were reported in 3,900 individuals who died before and over 4,000 who died after initiating ART; HIV-associated causes of death were assigned to 84% and 71% and TB causes of death assigned to 22% and 25% of decedents in each group, respectively.

A recent systematic review of ‘non-AIDS’ deaths in individuals on ART by Farahani et al. [23] estimated that 18.5% (95% CI 14–24) and 28.1% (95% CI 16–42) of deaths in sub-Saharan Africa and other LMIC, respectively, were due to non-AIDS causes [23] (i.e., 76–86% and 58–84% of deaths, respectively, were due to AIDS). However, this review counted deaths due to ART toxicity and deaths from non-AIDS infections as ‘non-AIDS’, suggesting that it may have underestimated overall HIV-associated mortality. Additionally, the review included only studies in which all, or at least

66% of participants were on ART, did not include any pathological autopsy studies, and included at least two studies that used only death certificates to report causes of death [89,90].

Other reviews examining causes of death in PLHIV include a 2015 study of causes of hospital admission in PLHIV by Ford et al. [29], which reported that around 80% of in-hospital mortality was due to HIV-associated causes (57% [95% CI 46–68] AIDS and 23% [95% CI 17–30] bacterial infections). Although these estimates are similar to those generated by our analysis, they do include deaths in high-income countries; the proportions of decedents assigned HIV-associated causes of death would likely be higher if the analysis was restricted to LMIC only. The Ford review also did not differentiate by the methods used to assign causes of death; the inclusion of mortality estimates generated by death certification and/or verbal autopsy will likely have led to reduced accuracy in the causes assigned.

3.2.5.1 TB and the potential effects of ART

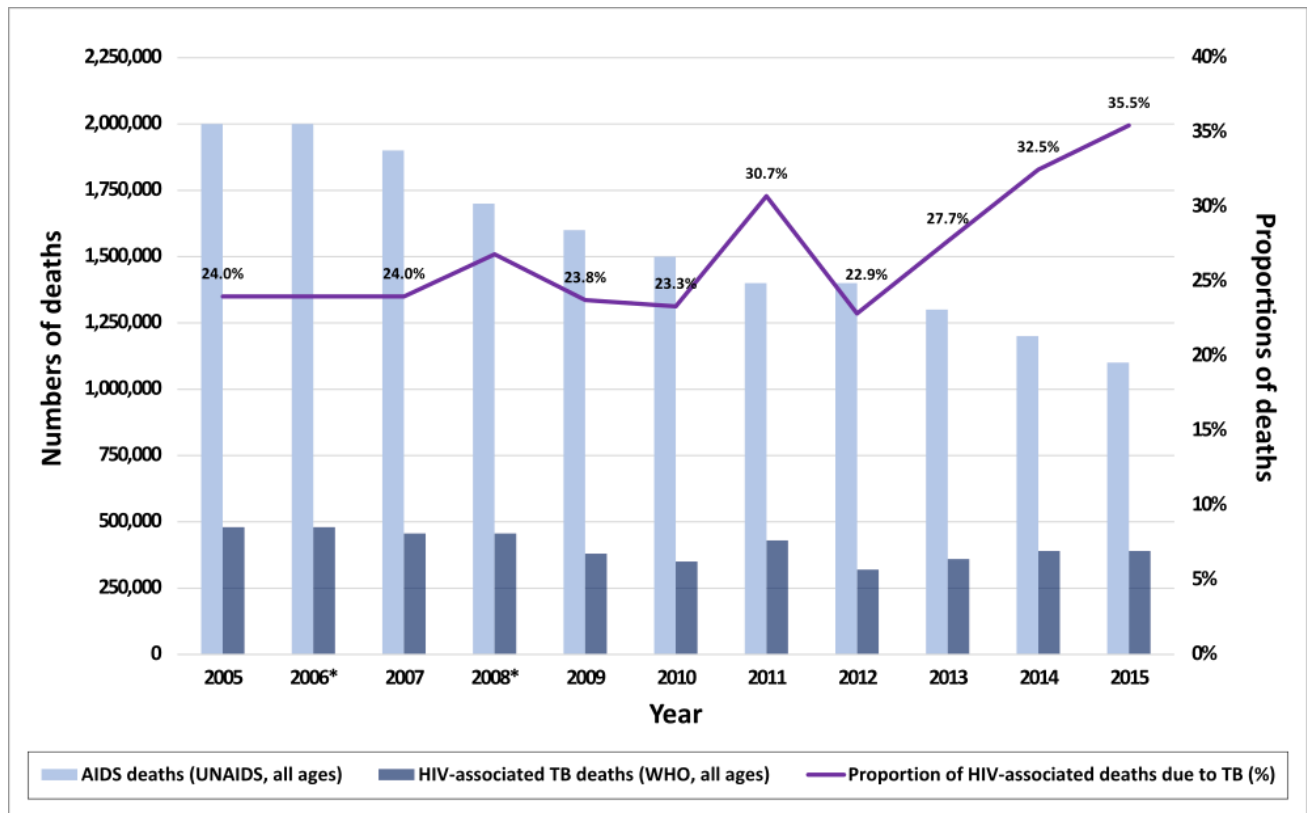
ART has consistently been shown to reduce, substantially, all-cause mortality [16] and the incidence of TB in PLHIV [91,92]. The pattern observed in our review, of lower proportions of HIV-associated causes of death assigned in studies that included higher proportions of decedents initiating ART, is consistent with this, but the apparent absence of a similar reduction in TB deaths is surprising. As the leading cause of death in PLHIV in LMIC, one would expect a reduction in overall mortality to also result in a reduction in TB mortality. This may be partially explained by the under-recognition, in older studies, of TB as a cause of death, due to limitations in diagnostics. However, older studies were also more likely to use pathological autopsy data to assign causes of death and were therefore more likely to detect TB that was not diagnosed prior to death. The two pathological autopsy studies that included individuals who had initiated ART both assigned a high proportion of decedents a TB cause of death (Wong et al. [South Africa [73]], 93% overall and 100% of HIV-associated; Siika et al. [Kenya [86]], 32% overall and 50% of HIV-associated).

A crude analysis of global estimates of mortality due to AIDS and HIV-associated TB shows a similar trend to that described above. Based on estimates from UNAIDS, numbers of deaths due to all AIDS causes have dropped from ~2 million in 2005 to ~1.2 million in 2015, an absolute reduction of 40% [93]. Per WHO figures, however, deaths attributable to HIV-associated TB have gone from ~480,000 in 2005 to ~320,000 in 2012 and ~390,000 in 2015 [10,94], a reduction of approximately 90,000 deaths per year over 10 years. There are currently no estimates of all-cause mortality among HIV-positive individuals; we are therefore unable to compare these figures with the estimates of TB causes as a proportion of all causes of death generated by our analysis. TB deaths as a proportion of all AIDS deaths,

however, appear to have increased steadily over the last few years, from 22.9% in 2012 to 35.5% in 2015 (Figure 3:6), which is to be expected, given the much steeper decline in overall AIDS deaths. However, even the highest estimate from these data is below the overall pooled proportion generated by our analysis (39% overall; 38% if initiated ART) and much lower than the estimates from pathological autopsy studies (pooled 47%), including the two studies that used autopsy data to assign causes of death in individuals on ART. The 2015 systematic review of pathological autopsy studies by Gupta et al. [11] also reported that a pooled 37.2% (95% CI 26–49) of ‘HIV/AIDS-related deaths’ were due to TB, based on findings from 10 studies.

In summary, our findings suggest that although ART may be reducing overall mortality due to HIV-associated causes, it may be having less of an impact on mortality due to HIV-associated TB. Our analysis also suggests, with the caveats mentioned above, that the numbers published by WHO and UNAIDS may be underestimates of the true mortality attributable to HIV-associated TB. It should be noted that estimates from mortality studies, and autopsy studies in particular, are vulnerable to several possible sources of bias, and that the margins of error for global estimates are very large, because of well-documented issues with methods used to estimate and code causes of death [2,3,13]. Caution, therefore, should be exercised in interpreting any comparisons of global surveillance data to research findings.

Figure 3:6. Crude estimate of the proportions of AIDS deaths due to HIV-associated TB per year (line), based on estimates of absolute numbers (vertical bars) published by UNAIDS [93] and WHO, 2005–2015 [10,94]



*Estimates of number of deaths due to HIV-associated TB not published for 2006 or 2008

AIDS: acquired immune deficiency syndrome; TB: tuberculosis; UNAIDS: Joint United Nations Programme on HIV/AIDS; WHO: World Health Organization

3.2.5.2 Methods for assigning causes of death

There is wide variation in the methods and data used to assign causes of death, which makes it difficult to compare estimates across different studies. This is illustrated by the high degree of heterogeneity seen in studies included in this review, despite the exclusion of studies that did not describe the methods used to assign causes of death or that used death certificates without validation (which have consistently been shown to correlate poorly with clinically-assigned causes of death [95,96]).

As discussed above, previous attempts have been made at creating a standardised method for assigning causes of death in PLHIV [8]; for any such system to succeed, however, it must be simple enough for routine use in resource-limited settings. Although using such a system to certify all deaths is unlikely to be feasible, it may be possible to use it to validate a sample of death certificates in a structured manner, particularly in countries without robust CRVS systems. This would also allow for ready comparison between research findings and routinely collected data. Crucially, any such

system would need to be compatible with the standard classification system for all deaths, in this case ICD-10, to allow for comparison with national, regional, and global estimates of disease and mortality.

3.2.5.3 Distinguishing between deaths *from* HIV and deaths *with* HIV

In addition to changes needed to ICD codes to better distinguish deaths from HIV-associated TB and other HIV-associated causes, it would be ideal to be able to track all deaths in HIV-positive individuals, from any cause, to allow for the estimation of deaths with HIV and from HIV. This could be done through the addition, to the standard death certification process, of a short list of yes/no questions regarding key comorbidities, including HIV, TB, and other non-communicable and mental health conditions. This may also allow for recording of non-biomedical factors, including socio-economic, education, and residential or migration status, all of which may improve the quality of cause-specific mortality data and could provide valuable insights into disease processes and risk profiles.

3.2.5.4 Limitations and strengths

This study had several limitations. The high heterogeneity of sample sizes, methods used, and populations studied reduce the generalisability of our findings. A few studies reported using the CoDe system, but some did not follow the full protocol and only used the cause classifications recommended by CoDe. As previously discussed, the disconnect between the CoDe and ICD-10 classification systems does not facilitate easy comparison between studies. Included studies were also predominantly conducted in sub-Saharan Africa, with North Africa, Central Asia, and East Asia poorly represented.

Pathological autopsy studies provided the best estimates of causes of death but have only been conducted among a small number of individuals who had initiated ART. There were also no reliable measures or real estimates of adherence to ART. Individuals who were poorly adherent during the study period may have been more susceptible to diseases (and causes of death) associated with immunosuppression. In addition, individuals whose adherence to ART during the study was good, but who had a history of poor adherence, will have been at increased risk of resistance to first-line therapy, which may not have been detected without viral load monitoring. This shortcoming therefore allowed for analysis based only on individuals who had 'initiated' ART, rather than those 'receiving' ART.

This study also had strengths. It represents the first attempt to systematically review the literature around studies that have directly estimated causes of death in HIV-positive adults in LMIC, providing a baseline reference for future

mortality studies and estimates made through less direct means, such as verbal autopsy. This study also provides additional evidence of the important contribution of TB to HIV-associated mortality, including in individuals who had initiated ART.

3.2.6. Conclusions

HIV-associated causes accounted for the majority of mortality among HIV-positive adults in LMIC, including in those who had initiated ART. TB accounted for 30% of overall mortality and 40% of HIV-associated mortality; these proportions were similar in individuals who had initiated ART. Studies that used pathological autopsy data assigned higher proportions of HIV-associated causes of death, suggesting that non-autopsy studies likely underestimate the true HIV-associated mortality fraction. Heterogeneity between studies was very high; a standardised approach to the direct estimation of causes of death in clinical settings that aligns with global classification systems (i.e., ICD-10) would be useful in more closely monitoring HIV-associated mortality and for validating death certificates and instruments such as verbal autopsy. A way for key diagnoses (including, but not limited to HIV and TB) to be recorded as part of an individual's death notification, regardless of cause of death, would improve the quality of mortality surveillance data and allow for long-term, large-scale monitoring of the effects of multiple, concurrent epidemics on population health.

3.2.7. References

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3.3. Supplementary material

Supplementary table 3:1. Details of search strategies used and records found in three electronic databases

PubMed	Embase®	Web of Science™
#1 HIV [MeSH] OR human immunodef* OR human immune def* OR HIV OR AIDS OR HIV/AIDS OR acquired immune def* OR acquired immunodef*	#1 TITLE-ABS-KEY (("human immunodef*") OR ("human immune def*") OR ("HIV") OR ("AIDS") OR ("HIV/AIDS")) OR ("acquired immune def*") OR ("acquired immunodef*")	#1 TI (title) and TS (topic) fields searched for: ((human immunodef*) OR (human immune def*) OR (HIV) OR (AIDS) OR (HIV/AIDS) OR (acquired immune def*) OR (acquired immunodef*)
#2 Cause of death [Mesh] OR cause of death OR cause-specific mortality OR CSMF	#2 TITLE-ABS-KEY (("cause* of death") OR ("cause-specific mortality") OR ("CSMF"))	#2 TI (title) and TS (topic) fields searched for: ((cause of death) OR (cause-specific mortality) OR (CSMF))
#3 Filters for low- and middle-income countries as described by the Cochrane Group, 2012 [26], updated as per the World Bank, 2016 [27]	#3 Filters for low- and middle-income countries as described by the Cochrane Group 2012 [26], updated as per the World Bank, 2016 [27]	#3 CU (country), TI (title) and TS (topic) fields searched for list of low- and middle-income countries as per the World Bank, 2016 [27]
	#4 (LIMIT-TO(DOCTYPE,"ar") OR LIMIT-TO(DOCTYPE,"re") OR LIMIT-TO(DOCTYPE,"cp"))	#4 CU (country), TI (title) and TS (topic) fields searched for: ((low-income countr*) OR (middle-income countr*) OR (low- and middle-income countr*) OR (developing countr*))
	#5 (LIMIT-TO(SUBJAREA,"MEDI") OR LIMIT-TO(SUBJAREA,"IMMU") OR LIMIT-TO(SUBJAREA,"MULT"))	
	#6 (LIMIT-TO(SRCTYPE,"j") OR LIMIT-TO(SRCTYPE,"p"))	
Final search #1 AND #2 AND #3	Final search #1 AND #2 AND #3 AND #4 AND #5 AND #6	Final search #1 AND #2 AND (#3 OR #4)

= 1527 records

= 1621 records

= 2352 records

Supplementary table 3:2. National Heart, Lung, and Blood Institute Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Criteria	Yes	No	Other (CD, NR, NA)
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?			
Quality Rating (Good, Fair, or Poor; see guidance [31])			
Rater #1 initials:			
Rater #2 initials:			
Additional Comments (If POOR, please state why):			

CD: cannot determine; NA: not applicable; NR: not reported

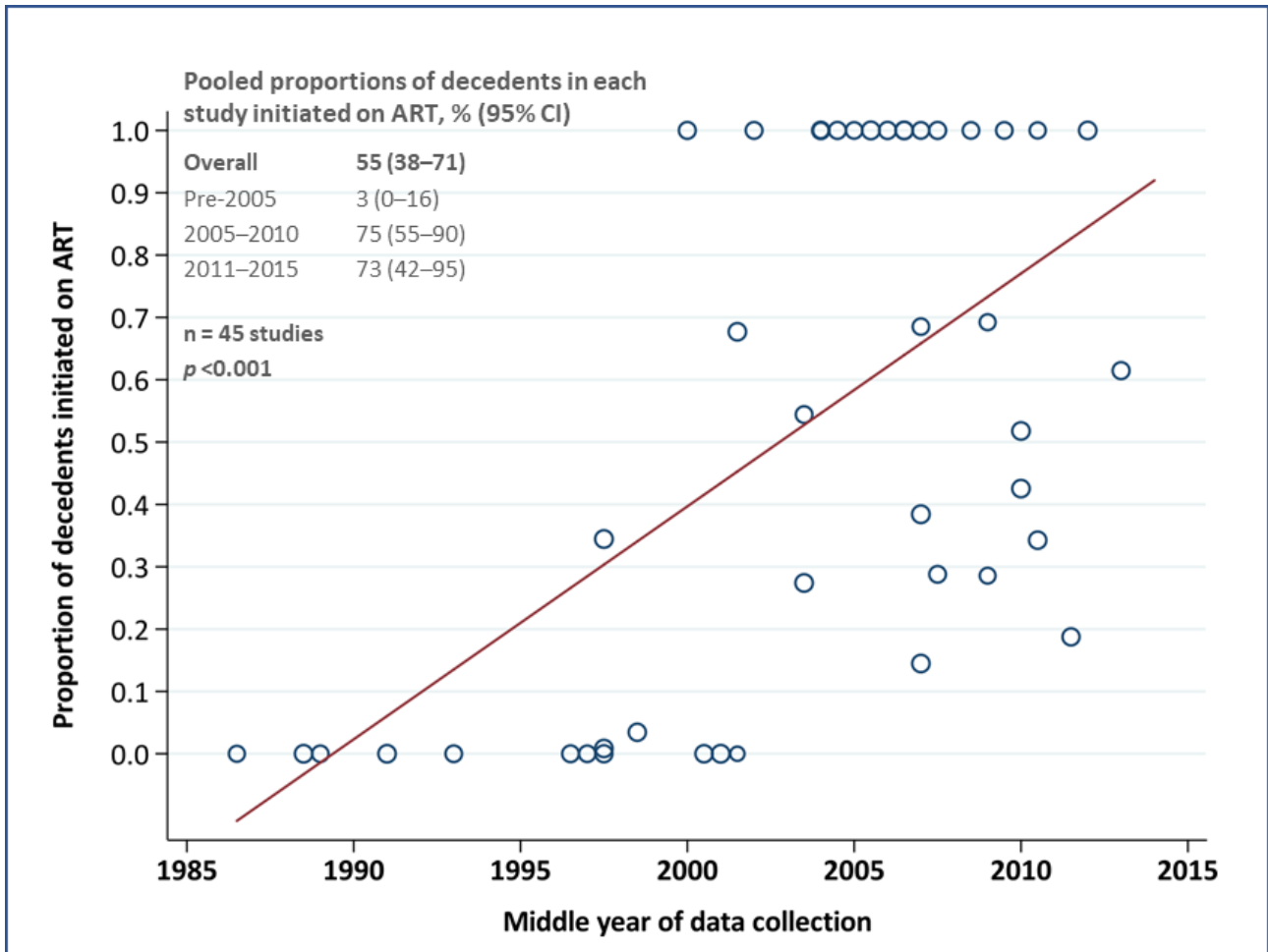
Supplementary table 3:3. Criteria used to grade study methods

Category	Score			Proportion or category score (0–1)
	0	0.5	1.0	
Proportion of deaths with CoD assigned using autopsy data*				
Proportion of deaths with CoD assigned using hospital data*				
Reviewers (number, role)	Only one, non-pathologist reviewer OR Not specified	At least two reviewers OR One pathologist reviewer	At least two independent reviewers OR Multidisciplinary panel	
Description of CoD assignment process	None	Partial description, or allusion to methods	Detailed description of standardised process OR Used CoDe protocol	
System for resolving disagreements	None	Attempt at independent review & validation, but not well implemented	Structured review process OR Used CoDe protocol	
CoD criteria	None	Categories described, but criteria not well defined	Described in detail with qualifying criteria for each OR Used CoDe CoD list	
			Total score (0–6)	

*Proportion (range 0–1) used as proxy score for these two criteria

CoD: cause of death; CoDe: Coding Causes of Death in HIV [8]; NS: not specified

Supplementary figure 3:1. Bubble plot showing results of meta-regression between the proportion of decedents per study who initiated ART and the middle year of data collection for that study (n = 45 studies)



ART: antiretroviral therapy; CI: confidence interval

Chapter 4. Research paper 2:

Autopsy prevalence of tuberculosis and other potentially treatable infections among adults with advanced HIV enrolled in out-patient care in South Africa

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4.1. Cover sheet

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Research paper cover sheet

Section A: Student details

Student	Aaron Karat
Principal supervisor	Alison Grant
Thesis title	An autopsy study exploring the spectrum of disease in individuals with advanced HIV in primary care clinics in South Africa

If the research paper has previously been published, please complete Section B.
If not, please go to Section C.

Section B: Paper already published

Where was the work published?	PLoS One		
When was the work published?	October 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion.	N/A		
Have you retained copyright for the work?*	Yes	Was the work subject to academic peer-review?	Yes


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Section C: Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	

Section D: Multi-authored work

For multi-authored work, give full details of your role in the research include in the paper and in the preparation of the paper (attach a further sheet if necessary). **The candidate collected the data, conducted all analyses, and wrote the paper.**

Student signature:  Date: 24 May 2017

Supervisor signature:  Date: 25 May 2017

4.2. Manuscript



RESEARCH ARTICLE

Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa

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Abstract

Background

Early mortality among HIV-positive adults starting antiretroviral therapy (ART) remains high in resource-limited settings, with tuberculosis (TB) the leading cause of death. However, current methods to estimate TB-related deaths are inadequate and most autopsy studies do not adequately represent those attending primary health clinics (PHCs). This study aimed to determine the autopsy prevalence of TB and other infections in adults enrolled at South African PHCs in the context of a pragmatic trial of empiric TB treatment ("TB Fast Track").

Methods and Findings

Adults with CD4 \leq 150 cells/ μ L, not on ART or TB treatment, were enrolled to TB Fast Track and followed up for at least six months. Minimally invasive autopsy (MIA) was conducted as soon as possible after death. Lungs, liver, and spleen were biopsied; blood,

Competing Interests: The authors have declared that no competing interests exist.

CSF, and urine aspirated; and bronchoalveolar lavage fluid obtained. Samples underwent mycobacterial, bacterial, and fungal culture; molecular testing (including Xpert® MTB/RIF); and histological examination. 34 MIAs were conducted: 18 (53%) decedents were female; median age was 39 (interquartile range 33–44) years; 25 (74%) deaths occurred in hospitals; median time from death to MIA was five (IQR 3–6) days. 16/34 (47%) had evidence of TB (14/16 [88%] with extrapulmonary disease; 6/16 [38%] not started on treatment ante-mortem); 23 (68%) had clinically important bacterial infections; four (12%) cryptococcal disease; three (9%) non-tuberculous mycobacterial disease; and two (6%) *Pneumocystis* pneumonia. Twenty decedents (59%) had evidence of two or more concurrent infections; 9/16 (56%) individuals with TB had evidence of bacterial disease and two (13%) cryptococcal disease.

Conclusions

TB, followed by bacterial infections, were the leading findings at autopsy among adults with advanced HIV enrolled from primary care clinics. To reduce mortality, strategies are needed to identify and direct those at highest risk into a structured pathway that includes expedited investigation and/or treatment of TB and other infections.

Background

Mortality among HIV-positive adults prior to starting and in the first year of antiretroviral therapy (ART) remains high, particularly among those with advanced disease in resource-constrained settings [1–3]. Pathological autopsy studies involving HIV-positive individuals have consistently found tuberculosis (TB) to be the leading cause of death and the overall prevalence of active TB to be extremely high, much of it undiagnosed ante-mortem [4–7]. The World Health Organization (WHO) aims to reduce TB deaths by 95% by 2035 [8], but questions remain around current estimates of TB deaths and the methods used to obtain them [9]. Death certification in many parts of sub-Saharan Africa remains sub-optimal [10,11] and the shortcomings of verbal autopsy, a structured interview with the family or carers of the deceased, in classifying HIV-related deaths are well documented [12–14]. Pathological autopsy studies provide the most accurate estimates, but almost all have only included individuals recruited after admission to hospital and do not necessarily represent the broader population who receive the majority of their care from primary health clinics and may die outside of hospitals [15]. Robust data on the prevalence of TB in deceased HIV-positive individuals and more accurate cause of death estimates are essential in measuring progress towards the reduction and eventual elimination of TB-related deaths and in the design of interventions to reduce mortality in this population [16].

Full pathological autopsy with visualisation and sampling of all organs remains the gold standard for assigning cause of death [17,18], but it is expensive, time consuming, and not well accepted by families, who are often required to provide consent [19]. There is growing evidence that minimally invasive autopsy (MIA) can provide useful information relevant to cause of death, particularly with regards to TB and other infectious diseases [20,21]; one study, involving 96 HIV-positive adults in Uganda, compared histology from MIA to that from full autopsy and found that MIA was 71% sensitive and 100% specific in detecting TB [18]. Adding culture and/or other bacteriological modalities to MIA would likely improve its sensitivity in this regard. The acceptance of MIA among recently bereaved families is considerably higher than

for full autopsy [5,22]; it is safer, cheaper, and procedures can be conducted outside of hospitals, in private mortuaries or other locations [23]. This study aimed to determine the autopsy prevalence of TB and other treatable infections in adults with advanced HIV disease recruited in public sector primary health care facilities who died after enrolment to a pragmatic trial of empiric TB treatment (“TB Fast Track”) [24].

Methods

Study population

The TB Fast Track study, described in detail elsewhere [24], was a cluster-randomised trial of targeted empirical TB treatment enrolling adults with $CD4 \leq 150$ cells/ μ L, not on TB treatment or ART at the point of enrolment, attending one of twenty-four public sector primary health clinics in three provinces of South Africa. At intervention sites, individuals were assessed for their probability of active TB disease using an experimental algorithm that included the results of point-of-care tests. Those considered ‘high’ probability were started on TB treatment immediately, followed by ART within two weeks; those considered ‘medium’ probability were further investigated; and those considered ‘low’ probability initiated ART immediately. Participants at control sites followed standard clinic procedures. All participants were followed up for at least six months. If the study team became aware of a participant’s death before the funeral had taken place, the family’s permission was sought to undertake MIA. Efforts were made to conduct autopsies as soon as possible after death, but no limits were placed on the time that could elapse between death and autopsy.

Autopsy procedures

Four organs were targeted for tissue biopsy: liver, spleen, and right and left lungs. Where possible, ultrasound was used to check for liver lesions and pleural effusions and to locate the spleen. Any lesions or effusions found were targeted during sampling. The skin overlying all sampling sites was cleaned using 70% isopropyl alcohol and 10% povidone iodine. Using 18 gauge core-biopsy needles, a minimum of five samples, each approximately 2cm in length, were taken from each site for each of bacterial and fungal microscopy and culture; mycobacterial culture; and histology. Cerebrospinal fluid (CSF) was extracted by insertion of a 22 gauge spinal needle inferior to the occipital base in the direction of the eyes to access the cerebellomedullary cistern. Flocked swabs were inserted into the oro- and naso-pharyngeal cavities and then immersed in PrimeStore media (Longhorn Vaccines and Diagnostics, Bethesda, MD, USA). Blood was aspirated from the right subclavian or internal jugular vein and a urinary catheter was used to collect residual urine. Modified bronchoalveolar lavage (BAL) was conducted by instilling 80–120ml of 0.9% saline through a horizontal incision of the cricothyroid membrane using a 16 gauge nasogastric tube inserted into the trachea in the direction of the lungs. Enlarged lymph nodes and skin lesions in areas other than the face were sampled by needle biopsy or scalpel excision, respectively.

As far as possible, MIA was conducted by a single, clinically-trained operator. If the primary operator was unavailable, limited MIA, without ultrasound or any attempt to obtain blood, urine, or BAL, was conducted by a non-clinical operator.

Laboratory procedures

All tissue samples, as well as CSF, urine, and BAL were cultured for mycobacteria using Mycobacterial Growth Indicator Tube (MGIT) culture (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Culture-positive samples underwent microscopy with Ziehl-Neelsen

(ZN) staining and polymerase chain reaction (PCR) testing for speciation and drug resistance (Hain Lifescience GmbH, Germany). Xpert[®] MTB/RIF (Cepheid, Sunnyvale, CA, USA) and microscopy of immunofluorescence-stained slides for *Pneumocystis jirovecii* were performed on BAL samples. Tissue samples, as well as CSF, blood, and BAL, underwent microscopy, Gram staining, and aerobic culture for bacteria and fungi. Real-time PCR assays were used to detect *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* in CSF and blood [25,26]; and *Legionella* spp., *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* [27], and *Bordetella* spp. [28] in naso-/oro-pharyngeal and BAL specimens. Naso-/oro-pharyngeal and BAL specimens were further tested by multiplex real-time reverse transcription PCR for parainfluenza viruses 1–3, adenovirus, enterovirus, human metapneumovirus, respiratory syncytial virus, rhinovirus, influenza A virus, and influenza B virus [29]. CSF was tested for the presence of parvovirus B19, cytomegalovirus (CMV), Epstein Barr virus (EBV), herpes simplex virus 1–2, human herpes viruses 6 and 7, and parechovirus (FTD Neuro 9 assay, Fast-track diagnostics Ltd., Malta). Urine was tested for *Legionella pneumophila* serogroup 1 and *S. pneumoniae* antigen (BinaxNOW, Alere, Waltham, MA, USA). Histological examination was carried out on all tissue samples, using ZN and Grocott stains for the identification of mycobacteria and fungi, respectively. Other stains and PCR procedures were performed as necessary, based on pathologist assessment.

Ethics

Separate approvals for the autopsy sub-study were obtained from the Research Ethics Committees of the London School of Hygiene & Tropical Medicine and The University of the Witwatersrand. Beginning in August 2013, participants in TB Fast Track were asked to give written informed consent for MIA if they should die during the parent study. Declining permission to take part in the autopsy sub-study did not affect their participation in TB Fast Track or their routine health care. If a participant who had given their written consent died during follow-up, verbal agreement from the next of kin was obtained to proceed with MIA. For participants who were enrolled to TB Fast Track prior to August 2013 and died during follow-up, formal written consent to undertake MIA was sought from the next of kin.

Data interpretation and statistical analysis

Data were entered into EpiData v3.1 (The EpiData Association, Odense, Denmark) and analysed using Stata v14 (StataCorp, College Station, TX, USA). Baseline demographics were compared using chi-square or Kruskal-Wallis tests, as appropriate. Analysis was conducted in order to estimate the autopsy prevalence of identified pathogens only; no inference was made as to possible cause(s) of death, which will be reported elsewhere. Microscopy and culture results were interpreted by experienced microbiologists who reviewed the data and designated, for each decedent, which organisms were considered 'artefact' and which 'pathogenic'. For all decedents, *Enterococcus* spp., *Enterobacter* spp., *E. coli*, *Proteus* spp., *Bacillus* spp., coagulase negative staphylococci, and *Viridans streptococci* were considered artefact. Additionally, Gram-negative organisms isolated only from the spleen of a decedent were considered artefact due to the high likelihood of contamination from abdominal viscera.

Results

Consent, demographics, and samples obtained

From December 2012 to December 2014, a total of 3022 individuals were enrolled into the TB Fast Track study: 626 (20.7%) were enrolled prior to autopsy sub-study initiation and were not

asked to consent at the point of enrolment. Of the remaining 2396 individuals, 1675 (69.9%) consented to autopsy at enrolment. A total of 364 TB Fast Track participants died after enrolment to the study, 285 (78%) within six months. Seventy-five of 364 (21%) deaths occurred prior to sub-study initiation and 229 (63%) were ascertained after burial, leaving 60 (16%) individuals for whom death was ascertained in time for MIA. Of these 60, 14 (23%) had already declined to participate and for three (5%) no family member could be contacted. Between October 2013 and June 2015, 43 families were approached in person for permission to conduct an autopsy: 36 (84%) gave their consent. Two autopsies did not proceed due to refusal by the mortuary; 34 autopsies were therefore conducted.

Decedents included in the MIA study are described in Table 1. Just over half the decedents (18/34 [53%]) were female; median age was 39 (interquartile range [IQR] 33–44) years; all were Black African; 32 (94%) were South African, with one (3%) from each of Lesotho and Mozambique; 13 (38%) individuals had completed South African grade 12 (high school) or equivalent; 17 (50%) were in some form of employment at the point of enrolment; and, of the 24 individuals able to estimate their household income at enrolment, 15 (63%) reported it to be less than R2000 (~USD150) per month. The majority of individuals died in hospitals (25/34 [74%]), the remainder died in the community; median time from enrolment to death was 60 (IQR 21–175) days; and 28 (82%) individuals died within six months of enrolment. The median CD4 count at enrolment was 34 (IQR 17–66) cells/ μ L. Twenty (59%) decedents were started on TB treatment a median 60 (IQR 26–175) days prior to death: 15/20 (75%) were enrolled to the intervention arm of the TB Fast Track study and 12 (80%) of these were started on TB treatment based on the study algorithm. Twenty-five (74%) decedents were initiated on ART a median 74 (IQR 30–126) days before death; 28 (82%) started co-trimoxazole prophylaxis at least one week before death; and one (3%) isoniazid preventative therapy at least one week before death. A comparison of baseline characteristics between individuals who had an MIA conducted ($n = 34$) and those who died within six months of enrolment but did not have an MIA ($n = 259$) showed only one important difference, in the proportion who started TB treatment between enrolment and death (59% in MIA group and 31% in group with no MIA; $p = 0.001$).

The primary operator conducted 31/34 (91%) MIA; median time from death to MIA was five (IQR 3–6) days; and 33/34 (97%) MIA were conducted within 10 days of death. Measured by histological examination, overall success rates in obtaining samples varied by site: 33/34 (97%) attempted lung biopsies were successful, compared to 30/34 (88%) liver and 21/34 (62%) splenic biopsies; 30/31 (97%), and 30/32 (94%) attempts to obtain BAL and CSF, respectively, were successful (S1 Table).

Tuberculosis

Evidence of TB disease was found in 16/34 (47%) decedents (Table 2), of whom 12 (75%) had evidence on histology (Fig 1); 11 (69%) on culture; five (31%) on testing with Xpert[®] MTB/RIF; and eight (50%) on histology plus bacteriology. Almost all (14/16 [88%]) decedents with evidence of TB had extrapulmonary disease; 3/14 (21%) had no evidence of pulmonary disease. BAL specimens provided the highest yield for culture (seven positive), followed by CSF and splenic tissue (five each), and lung and liver tissue (four each; S1 Table). Twelve decedents had histological evidence of TB: four (33%) in all of lungs, liver, and spleen; two (17%) in lungs and liver only; two (17%) in liver and spleen only; and two (17%) in lungs alone. A total of 164 samples underwent mycobacterial culture: 37 (23%) were positive and 13 (8%) could not be interpreted due to bacterial contamination. Of the 16 individuals with autopsy evidence of TB, 10 (63%) were started on TB treatment a median 56 (IQR 40–173) days before death; 14 (88%)

Table 1. Baseline characteristics of deceased TB Fast Track participants: MIA conducted (n = 34) vs no MIA (n = 259).

Characteristics	MIA conducted (n = 34) n (%) or median (IQR)	No MIA conducted (n = 259) n (%) or median (IQR)	p value (Chi ² or Kruskal-Wallis)
Demographics and past medical history			
Female	18 (53)	133 (51)	0.86
Age (years)	39 (33–44)	39 (34–46)	0.57
CD4 count at enrolment (cells/μL)	34 (17–66)	45 (21–88)	0.21
Black African	34 (100)	257 (99)	0.61
South African	32 (94)	242 (93)	0.88
Enrolled in a peri-urban area	23 (68)	187 (72)	0.58
Completed grade 12	13 (38)	82 (32)	0.44
Employed at time of enrolment	17 (50)	106 (41)	0.31
Household income of ≤R2000 per month*	15 (63) (n = 24)	90 (47) (n = 190)	0.16
Previously diagnosed with chronic illness**	5 (15)	34 (13)	0.80
Smoking history	7 (21) (n = 33)	51 (20) (n = 257)	0.85
Previous treatment for TB	6 (18)	27 (10)	0.21
Symptoms at enrolment			
Cough	14 (41)	127 (49)	0.39
Night sweats	11 (32)	85 (33)	0.96
Fever	11 (32)	73 (28)	0.61
Weight loss	28 (88) (n = 32)	205 (84) (n = 244)	0.61
Assessment at enrolment			
Body-mass index (kg/m ²)	20.2 (18.7–23.2)	20.2 (17.7–23.6)	0.48
Positive urine LAM (point of care) †	10 (29)	74 (29)	0.92
Between enrolment and death			
Started TB treatment	20 (59)	79 (31)	0.001
Started ART	25 (74)	153 (59)	0.10
Time from enrolment to death (days)	60 (21–175)	60 (31–113)	0.72

*Ten decedents in the MIA group and 69 in the no MIA group did not know their average household monthly income.

**Chronic illness included hypertension, diabetes, asthma, epilepsy, cancer, and cardiac, renal, and chronic lung disease.

†Samples that elicited one or more visible lines on the LAM test strip were considered positive.

ART: antiretroviral therapy; IQR: interquartile range; LAM: lipoarabinomannan; MIA: minimally-invasive autopsy; TB: tuberculosis

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received ART, initiated a median 65 (IQR 30–126) days before death; and nine (56%) were initiated on both TB treatment and ART. Six (38%) individuals with autopsy evidence of TB were not started on TB treatment antemortem, five of whom were enrolled to the control arm of TB Fast Track.

Infections with non-tuberculous mycobacteria and other bacteria

Of the 34 decedents, three (9%) had evidence of disease caused by non-tuberculous mycobacteria (NTM): two grew *Mycobacterium avium* and one grew *Mycobacterium intracellulare* from multiple sites. Excluding mycobacteria and organisms considered artefact, 23/34 (68%) decedents had culture evidence of pathogenic bacterial infections: 14/23 (61%) grew *Klebsiella* spp. from non-splenic samples; four (17%) grew *S. aureus*; two (9%) grew *Pseudomonas* spp.; and *S. pneumoniae*, *Salmonella* sp., *Nocardia* sp., and *Haemophilus* sp. were cultured from one (4%)

Table 2. Combined histopathological and microbiological findings; participants with evidence of each at autopsy; and demographics for select groups (n = 34).

Pathogen	Decedents with autopsy evidence of		Age at death (years) Median (IQR or range*)	CD4 count at enrolment (cells/ μ L) Median (IQR or range*)	Time from enrolment to death (days) Median (IQR or range*)
Major disease category	Major disease category n (%/34)	Disease sub-category n (row %)			
Disease sub-category					
All decedents	-	-	39 (33–44)	34 (17–66)	60 (21–175)
Mycobacteria					
Tuberculosis	16 (47)	-	38 (33–43)	44 (21–60)	63 (40–152)
Pulmonary only		2 (13)			
Extrapulmonary only		3 (19)			
Pulmonary & extrapulmonary		11 (69)			
Rifampicin-resistant		2 (13)			
Non-tuberculous mycobacteria	3 (9)		45 (27–56)	2 (2–7)	33 (21–324)
Disseminated		3 (100)			
Bacteria†					
Any bacterial infection	23 (68)		40 (34–45)	33 (10–75)	57 (21–184)
Pneumonia	11 (32)		41 (38–54)	33 (17–36)	33 (14–285)
Gram-negative		6			
Gram-positive		5			
Organism unclear		1			
Bacteria in CSF	4 (12)		40 (31–54)	106 (66–132)	82 (8–285)
<i>S. pneumoniae</i>		3			
<i>S. aureus</i>		1			
<i>Salmonella</i> sp.		1			
Bacteraemia	3 (9)		33 (31–36)	106 (29–132)	100 (8–184)
<i>H. influenzae</i>		1			
<i>K. pneumoniae</i>		1			
<i>Salmonella</i> sp.		1			
<i>S. aureus</i>		1			
<i>S. pneumoniae</i>		1			
Fungi & viruses‡					
Pneumonitis	4 (12)		38 (29–44)	86 (17–106)	67 (14–304)
Cryptococcal disease	4 (12)		40 (29–46)	8 (1–56)	53 (10–373)
Disseminated		2 (50)			
Pneumocystis pneumonia	2 (6)		40 (39–41)	41 (3–78)	133 (84–182)
CMV disease	2 (6)		33 (33–34)	20 (10–29)	106 (28–184)
Disseminated		2 (100)			
Other/non-infectious					
Hepatic	18 (53)		40 (34–45)	29 (17–56)	91 (33–184)
Steatosis		12 (67)			
Non-specific portal triaditis		6 (33)			
Chronic hepatitis? viral		4 (22)			
Acute hepatitis		3 (28)			

(Continued)

Table 2. (Continued)

Pathogen	Decedents with autopsy evidence of		Age at death (years) Median (IQR or range*)	CD4 count at enrolment (cells/μL) Median (IQR or range*)	Time from enrolment to death (days) Median (IQR or range*)
	Major disease category	Disease sub-category n (row %)			
	Major disease category n (%/34)	Disease sub-category n (row %)			
Renal	6 (18)		42 (23–62)	19 (1–107)	49 (9–324)
Acute tubular necrosis		3 (50)			
Interstitial nephritis		5 (83)			

*IQR shown if $n \geq 10$; range shown if $n < 10$

†Some decedents had more than one pathogenic organism identified

‡Positive viral PCR was not considered sufficient evidence of disease

CMV: cytomegalovirus; CSF: cerebrospinal fluid; IQR: interquartile range; MIA: minimally-invasive autopsy

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decedent each. Pathogenic bacteria were most frequently isolated from BAL samples (18 positive cultures) and lung tissue (12 positive; S1 Table). Of the 33 decedents with lung tissue available for histological examination, 11 (33%) had microscopic evidence of bacterial pneumonia (Table 2), nine (82%) of whom also grew pathogenic bacteria from BAL and/or lung tissue, most frequently *Klebsiella* spp.. Of the 30 decedents with CSF samples, two (7%) were culture positive, one growing *Salmonella* sp. and the other *S. aureus* and *S. pneumoniae*.

Bacterial PCR was conducted on 13 CSF samples and 10 blood samples: *S. pneumoniae* was detected in 3/13 (23%) CSF samples and 1/10 (10%) blood sample; and *H. influenzae* was detected in 1/10 (10%) blood sample. PCR of 11 BAL specimens and 13 naso-/oropharyngeal swabs for atypical pneumonia-causing pathogens and *Bordetella* spp. and testing of seven urine samples for *Legionella pneumophila* and pneumococcal antigen yielded no positive results (S1 Table).

Cryptococcal disease

Evidence of cryptococcal disease was found in 4/34 (12%) decedents with enrolment CD4 counts ranging from 1–56 cells/μL and time from enrolment to death 10–373 days. Three (75%) decedents had histological evidence of cryptococcal disease (Fig 1), two in more than one organ, and *Cryptococcus neoformans* was grown from two (50%) decedents, one from lungs and the other from liver and CSF. Two decedents were treated for cryptococcosis prior to death, receiving 800mg oral fluconazole for four days and 800mg intravenous fluconazole for one day, respectively (decedents 02 and 05; S2 Table).

Other fungal and viral infections

Two of 34 (6%) decedents had evidence of cytomegalovirus (CMV) disease on histological examination, both with disseminated disease, and a further two (6%) had evidence of *Pneumocystis pneumonia* (PCP; Table 2; Fig 1). Viral PCR assays were conducted on specimens from 14/34 (41%) decedents (S1 Table). Of the 13 with BAL specimens and/or naso-/oropharyngeal swabs available for viral testing, rhinovirus was detected four (31%) times and influenza virus and respiratory syncytial virus detected once each (8%). CSF samples from 11 decedents were available for viral testing, EBV was detected eight times (73%), CMV seven times (64%); parvovirus B19 twice (18%); and influenza A virus, human-Herpes virus 7, and varicella zoster virus once each (9%).

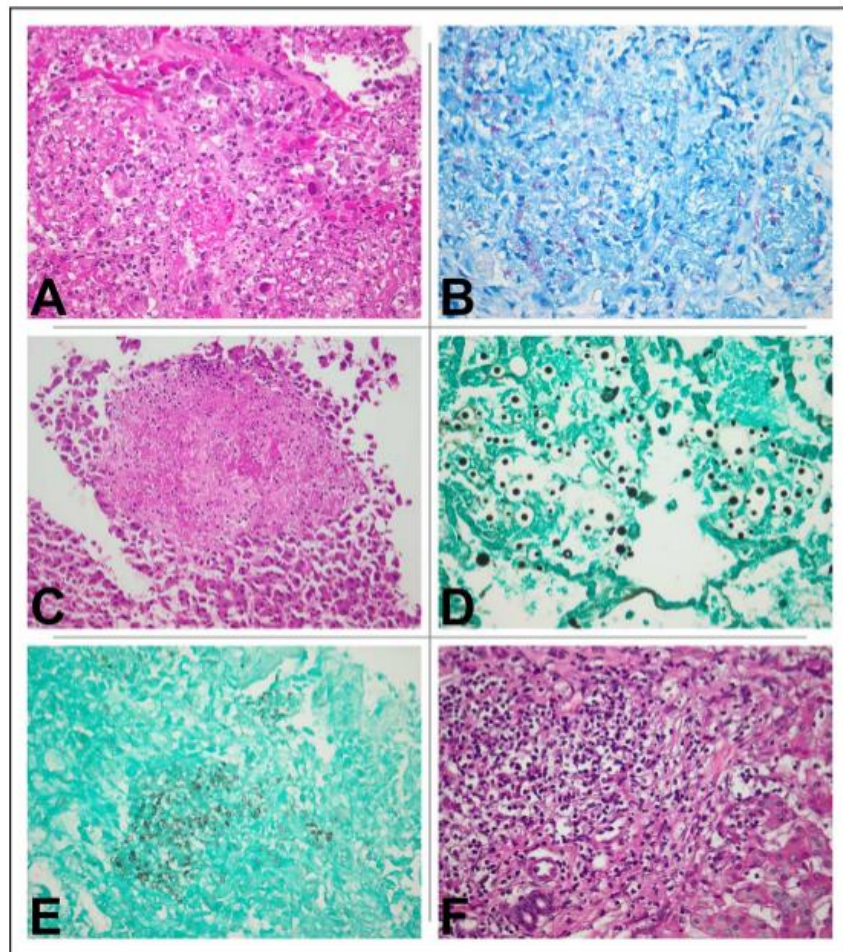


Fig 1. Six examples of histological changes observed in MIA tissue samples (n = 4). Panels (A), (B), and (C) demonstrate mycobacterial and CMV disease in the lung and liver of decedent 08: (A) necrotising, intra-alveolar granuloma with superadded CMV nuclear inclusions (lung; H&E x40); (B) numerous acid-fast bacilli (lung; ZN x40); and (C) mycobacterial granuloma (liver; H&E x20). Panel (D) demonstrates polymorphous cryptococcal yeasts in alveolar spaces (decedent 19; lung; Grocott's methenamine silver x40). Panel (E) illustrates the characteristic small, crescentic, yeast-like fungi of *Pneumocystis jirovecii* pneumonia (decedent 11; lung; Grocott's methenamine silver x40). Panel (F) shows active hepatitis B virus infection (decedent 34; liver; H&E x40). CMV: cytomegalovirus; H&E: haematoxylin and eosin; MIA: minimally invasive autopsy; ZN: Ziehl-Neelsen

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Decedents with multiple pathogens identified

Twenty (59%) decedents had either histological or culture evidence of at least two infections present at the same or different sites (Fig 2). Of the 16 decedents with evidence of TB, nine (56%) had evidence of bacterial disease at one or more sites; two (13%) had evidence of cryptococcal disease; and one (6%) each had evidence of CMV disease (Fig 1), PCP, and disease due

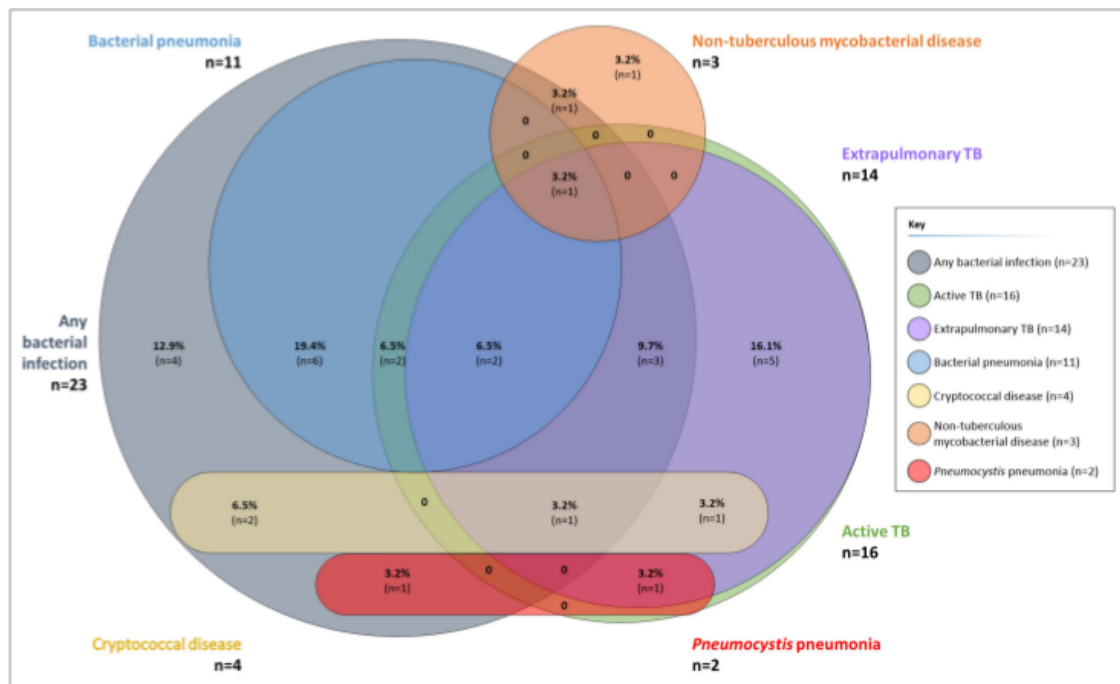


Fig 2. Venn diagram illustrating overlap between diagnoses made at autopsy: any active TB; extrapulmonary TB; any bacterial infection; bacterial pneumonia; disease due to non-tuberculous mycobacteria; cryptococcal disease; and *Pneumocystis pneumonia* (n = 31*).
* Figure only includes decedents with autopsy evidence of disease due to specified pathogens

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to NTM. Of the 23 decedents with evidence of bacterial infections, three (13%) also had evidence of cryptococcal disease; two (9%) disease due to CMV; two (9%) disease due to non-tuberculous mycobacteria; one (4%) due to PCP; and four (17%) due to diseases caused by other viruses.

Additional findings

Liver tissue from 30 decedents under went histological examination, 18 (60%) showed evidence of inflammation: 5/18 (28%) had acute or chronic hepatitis, four of which were thought likely to be viral in origin (Fig 1); 12/18 (67%) had evidence of steatosis, one with non-alcoholic steato-hepatitis; and 6/18 (33%) had evidence of non-specific portal triaditis. Renal samples were also collected incidentally (nine from the right kidney, thirteen from the left): 6/13 (46%) decedents showed evidence of interstitial nephritis (n = 5) and/or acute tubular necrosis (n = 3) in one or both kidneys (Table 2).

Discussion

To our knowledge this is the first study that has followed up a prospective cohort of HIV-positive outpatients conducting minimally invasive autopsies on those who died. This study is also

unusual in that it includes a number of deaths that occurred outside hospitals, thereby including individuals not well represented in previous autopsy studies of HIV-positive individuals.

Disease prevalence and overlap

We conducted MIA on as many TB Fast Track decedents as possible, all of whom were not taking ART or TB treatment at the time of enrolment. Those who had MIA and those who did not were largely similar with regard to characteristics at enrolment and during follow up, although those with MIA were more likely to have been treated for TB between enrolment and death. This, together with the likelihood that MIA will miss some cases that may be identified by full autopsy [18], suggests that the prevalence of TB at death among the entire population of decedents in TB Fast Track may have been even higher than the almost 50% found in this sample. Nearly 90% of those with evidence of TB had disease in at least one extrapulmonary site and 38% were not on TB treatment at the time of death. These findings, in line with data published by Omar et al. [5], suggest that TB is as frequent a pathogen among those attending primary care clinics as it is among the in-patients previously investigated in autopsy studies. Our findings are consistent with most autopsy studies done in sub-Saharan Africa over the last 20 years [4–6]. A recent systematic review [6] found the pooled prevalence of TB at autopsy in HIV-positive adults to be 39.7%, of which 87.9% was extrapulmonary and/or disseminated and 45.8% was not diagnosed before death. The review included four studies conducted during the ART era and, though overall ART coverage was not included, TB prevalence in these four studies was equal to or greater than in studies conducted prior to ART rollout, suggesting that individuals are initiating ART too late or are not linked to treatment at all. Studies conducted in clinics in South Africa have previously highlighted the large burden of undiagnosed TB among the ambulatory HIV-positive population [30,31], but investigation for TB is largely based on reporting respiratory symptoms, using sputum-based diagnostic tests [32,33]. A recent study conducted in Cape Town found that, of 139 HIV-positive individuals diagnosed with TB during admission to hospital (median CD4 count 80 cells/ μ L; 35% on ART for a median 1.3 years), 115 (83%) had at least one non-respiratory sample that was positive on testing with Xpert[®] MTB/RIF, or culture positive for *M. tuberculosis* [34]. Non-respiratory samples included blood, urine, and CSF. WHO recommends Xpert[®] MTB/RIF for use on extrapulmonary samples [35]. However, there are formidable practical and financial challenges in establishing alternative diagnostic algorithms and care pathways in primary care in resource-limited settings, in particular, the lack of a sensitive, cost-effective, point-of-care diagnostic test, and the difficulties in obtaining suitable specimens samples in individuals who cannot produce sputum. Regardless, the data suggest that these are obstacles that must be overcome in order to achieve any meaningful reduction in TB mortality.

A striking finding in our study was the high proportion of decedents with evidence of two or more infections at autopsy, sometimes at different sites, or concurrent at the same site. These are similar to results from a study conducted in 2009 among 39 HIV-positive ART-eligible adults in a hospital in South Africa [36], which attributed a third of deaths to bacterial infections, with several decedents showing evidence of multiple infections. Another study, involving full autopsy on adults (≥ 16 years) in a tertiary hospital in Zambia, 101 of whom were HIV-positive, found that 39/101 (39%) had autopsy evidence of 'pyogenic pneumonia', with 21/39 (54%) also showing evidence of active TB disease [4]. Among decedents in our study, most bacterial infections were due to common pathogens, such as *Klebsiella* spp., *Salmonella* spp., *H. influenzae*, and *S. aureus*. More needs to be done, through increased awareness among clinicians and in the design of clinical guidelines, to target and treat other opportunistic infections in those with advanced disease, even those already receiving prophylaxis, TB treatment,

or ART. Longer term, initiating ART at higher CD4 counts will help reduce the likelihood of progression to this stage of advanced disease; recent guidelines advocating ART in all people living with HIV are an important step towards making this a reality [37].

Interpretation of autopsy findings

M. tuberculosis can survive for years after the death of an infected individual and after preservation in formalin [38,39]. In our cohort, seven (64%) of the 11 individuals with *M. tuberculosis* culture-positive samples had received TB treatment for a median 122 (IQR 48–176) days. The interpretation of culture results from autopsy specimens is not straightforward and may be complicated further when the procedure is conducted many days after death, potentially providing greater opportunity for tissue autolysis and translocation of organisms to compartments they did not occupy in life [40–42]. However, there is evidence to show that the time from death to autopsy does not have a major effect on false-positive bacterial results if the body is kept refrigerated [43,44]. One study, conducted among 507 infants with sudden and unexpected deaths, even suggests that a longer interval may allow for fewer false-positive results [45]; and there are reports of pathogens recovered from bodies in states of advanced decomposition [46,47]. In our study, samples from all sites, except CSF, grew some organisms that were likely artefact (S1 Table); data from each decedent were reviewed individually by a microbiologist to decide on likely contaminants. This further highlights the challenges faced in assigning causes of death in these individuals, in whom many pathogens may cause disease at one time. Results of any microbiological tests, including mycobacterial, must be reviewed with clinical history and, ideally, histology in order to more accurately estimate prevalence of active disease and/or cause of death. The development of standardised guidelines to this end is suggested.

MIA in surveillance

The consistent under-diagnosis of TB ante mortem [6] suggests that MIA may have a role in routine surveillance to estimate TB prevalence at death more accurately. If MIA was to be simplified for this purpose, our data suggest that respiratory samples (BAL and lung tissue) would provide the highest yield for TB diagnosis. Combining BAL Xpert® MTB/RIF with BAL mycobacterial culture would have detected nine of our TB cases; adding mycobacterial culture of lung tissue would add a further two; and histological examination of lung tissue a further three; accounting for 88% of the TB seen in our decedents. The role of Xpert® MTB/RIF as part of the autopsy process is not established: a recent study testing post mortem liver, lung, and brain tissue found that it detected TB in 7/8 (87.5%) cases, although the gold standard used was another PCR technique, rather than liquid culture [21]. In our study, only 5/14 (35.7%) decedents with BAL specimens and evidence of TB had a positive Xpert® MTB/RIF, though this test was done only on BAL specimens, whereas culture and histology were conducted on specimens from many other anatomical sites, many of them extrapulmonary. There were three decedents whose BAL specimens were Xpert® MTB/RIF negative, but culture positive for MTB (decedents 2, 4, and 33; S2 Table). More work is needed to establish the optimum use of this and other molecular techniques in post mortem specimens.

This study has limitations: autopsy coverage of deaths occurring among participants in TB Fast Track was low, at around 10%, but those included appear largely representative of all those who died. Although MIA provides less information than complete autopsy, particularly with regards to non-infectious diagnoses, its greater acceptability to families and participants is a major advantage; and it was not possible for one operator to conduct all 34 MIAs, leading to some variability in tissue sampling. Strengths of the study include being nested within a trial that recruited a widely representative sample of adults with advanced HIV disease at high risk

of active TB; mycobacterial culture and histology being conducted on all decedents; and samples from different anatomical sites being individually tested, which may provide guidance for future MIA-based surveillance programmes.

Conclusions

The autopsy prevalence of tuberculosis among adults with advanced HIV disease who die before initiating ART or early on ART is high and is likely a major contributor to mortality. The large proportion of individuals with autopsy evidence of potentially treatable bacterial and/or fungal infections, often in addition to TB, should be considered when assessing these high-risk patients in primary or secondary care. Evidence-based interventions are urgently needed that prioritise timely and thorough investigation for those with advanced HIV disease; allow for empiric treatment when indicated; and promote early initiation of ART.

Supporting Information

S1 Table. Samples targeted, success rates, and yield from culture and molecular tests for each specimen (n = 34).
(DOCX)

S2 Table. Autopsy findings for each decedent: histological, microbiological, DNA, and immunological evidence of tuberculosis, bacterial disease, and other diseases, listed by time from death to MIA (n = 34).
(DOCX)

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4.4. Material provided as supplementary online appendices

Supplementary table 4:1. Samples targeted, success rates, and yield from culture and molecular tests for each specimen (n=34)

Site/sample		ALL n (%)	BAL n (%)	Blood n (%)	CSF n (%)	Liver n (%)	Lungs n (%)	NP/OP n (%)	Spleen n (%)	Urine n (%)	
Decedents where attempts made		-	31	13	32	34	34	13	34	12	
Successful attempts (based on histology for tissue)		-	30 (97)	10 (77)	30 (94)	30 (88)	33 (97)	13 (100)	21 (62)	7 (58)	
MYCOBACTERIA	Tested	164	30	-	28	33	32	-	34	7	
	Mycobacterial culture										
	Positive (MTB)	26 (16)	7 (23)		5 (18)	4 (12)	5 (16)		5 (15)	0	
	Positive (NTM)	11 (7)	1 (3)		1 (4)	3 (9)	2 (6)		3 (9)	1 (14)	
	Negative	113 (69)	16 (53)		20 (71)	24 (73)	23 (72)		25 (74)	5 (71)	
	Contaminated	14 (9)	6 (20)		2 (7)	2 (7)	2 (6)		1 (3)	1 (14)	
	Xpert® MTB/RIF	Tested	29	29	-	-	-	-	-	-	-
	Positive	5 (17)	5 (17)								
	BACTERIA	Tested	131	29	7	14	16	33	-	32	-
		Bacterial culture†									
Positive		37 (28)	18 (62)	2 (29)	2 (14)	1 (6)	12 (36)		2 (6)		
Negative		45 (34)	2 (7)	2 (29)	12 (86)	9 (56)	11 (33)		9 (28)		
Contaminated		49 (37)	9 (31)	3 (43)	0	6 (38)	10 (30)		21 (66)		
Multiplex PCR (meningitis)		Tested	23	-	10	13	-	-	-	-	-
<i>H. influenzae</i>		1 (4)		1 (10)	0						
<i>S. pneumoniae</i>		3 (13)		1 (10)	2 (23)						
<i>N. meningitidis</i>		0		0	0						
Multiplex PCR (atypical pneumonias)		Tested	24	11	-	-	-	-	13	-	-
<i>M. pneumoniae</i>	0	0						0			
<i>C. pneumoniae</i>	0	0						0			
<i>Legionella spp.</i>	0	0						0			
Bordetella spp.	Tested	24	11					13			
Positive	0	0						0			
Urinary antigen	Tested	7	-	-	-	-	-	-	-	7	
<i>S. pneumoniae</i>	0									0	
<i>L. pneumophila</i>	0									0	
Fungal culture	Tested	131	29	7	14	16	33	-	32	-	

Site/sample		ALL n (%)	BAL n (%)	Blood n (%)	CSF n (%)	Liver n (%)	Lungs n (%)	NP/OP n (%)	Spleen n (%)	Urine n (%)
Cryptococcal antigen	Positive	28 (21)	7 (24)	0	1 (7)	2 (13)	13 (40)		5 (16)	
	Tested	33	-	7	26	-	-	-	-	-
Pneumocystis IFA	Positive	2 (6)		0	2 (8)					
	Tested	26	26	-	-	-	-	-	-	-
VIRUSES	Tested	24	11	-	-	-	-	13	-	-
	Para-influenza viruses 1-3	0	0					0		
	Adenovirus	0	0					0		
	Enterovirus	0	0					0		
	Human metapneumovirus	0	0					0		
	Respiratory syncytial virus	1 (4)	0					1 (8)		
	Rhinovirus	8 (33)	4 (36)					4 (31)		
	Influenza A virus	1 (4)	0					1 (8)		
	Influenza B virus	0	0					0		
	Tested	11	-	-	11	-	-	-	-	-
Multiplex PCR (neurological)	Cytomegalovirus	7 (64)			7 (64)					
	Epstein-Barr virus	8 (73)			8 (73)					
	Herpes simplex virus 1 & 2	0			0					
	Human herpes virus 6	0			0					
	Human herpes virus 7	1 (9)			1 (9)					
	Parechoviruses	0			0					
	Parvovirus B19	2 (18)			2 (18)					
	Varicella zoster virus	1 (9)			1 (9)					

†A non-splenic sample growing only *Enterococcus* spp., and/or *Enterobacter* spp., and/or *E. coli*, and/or *Proteus* spp., and/or *Bacillus* spp., and/or coagulase negative staphylococci, and/or *Viridans streptococci* was considered contaminated, as was a splenic sample growing only one of the above, or growing only Gram-negative organisms in the absence of the growth of the same organism from another site.

BAL: bronchoalveolar lavage; CSF: cerebrospinal fluid; IFA: indirect fluorescent antibody; MTB: *M. tuberculosis*; NP/OP: naso-/oro-pharyngeal swabs; NTM: non-tuberculous mycobacteria; PCR: polymerase-chain reaction

Supplementary table 4.2. Autopsy findings for each participant: histological, microbiological, DNA, and immunological evidence of tuberculosis, bacterial disease, and other diseases, listed by time from death to MIA (n=34)

ID*	Time in days				Evidence of tuberculosis				Evidence of NTM and/or other bacterial disease		Other histological, microbiological, DNA, and immunological findings and site(s)
	From enrolment to death	From death to MIA	On TB Tx	On ART	Histological evidence and site(s)	Positive culture and site(s)	Xpert® MTB/RIF positive (BAL only)	If MTB found, INH/RIF sensitive?	Histological evidence and site(s)	Organism(s) grown or detected by PCR and site(s)	
07	48	1	48	-	-	CSF, LIV, SPL	-	✓	-	<i>Klebsiella</i> spp. (BAL)	CrAg positive (CSF)
30	1	2	-	-	-	-	-	-	LL, RL	<i>H. parainfluenzae</i> (lungs)	-
06	21	2	-	15	-	-	-	-	RL	<i>Pseudomonas</i> spp. (BAL, lungs)	Histology: Extensive autolysis (LK, RK)
15	34	2	-	30	-	-	-	-	-	-	Histology: Pneumonitis, ?viral + organising hyaline membrane disease (LL, RL)
31	63	2	-	56	LIV, LL, RL, SPL	-	✓	RIF resistant (BAL)	-	-	Histology: Extramedullary haematopoiesis (RL; LL) PCR: EBV (CSF); PVB19 (CSF)
08	28	3	-	-	LIV, LL, RL	BAL, LIV, lungs	✓	✓	RL	<i>K. pneumoniae</i> (BAL; lungs)	Histology: Florid superadded CMV infection (LIV; LL; RL)
24	33	3	33	13	LIV, SPL	-	-	-	LL, RL	<i>M. avium</i> (LIV, SPL)	PCR: CMV (CSF); EBV (CSF)
09	38	3	-	29	LL, LK	-	-	-	-	<i>K. pneumoniae</i> (BAL; lungs)	Histology: Acute tubular necrosis with underlying acute suppurative pyelonephritis (RK; LK)
10	41	3	48	32	RL	-	-	-	RL	<i>Klebsiella</i> spp. (BAL)	Histology: Acute tubular injury (RK; LK)
18	49	3	-	50	LIV, LL, RL, SPL	BAL, LIV, lungs, SPL	✓	RIF resistant (BAL)	-	-	-
33	182	3	182	148	-	BAL, CSF	-	✓	-	-	Histology: Severe PCP (LL; RL) PCR: <i>H. influenzae</i> (BLD); Rhinovirus (BAL; NP/OP)
12	9	4	9	-	-	-	-	-	RL	-	-
02	10	4	-	10	-	BAL, CSF	-	✓	-	-	Histology: Disseminated cryptococcosis (LIV; LL; RL)

ID*	Time in days				Evidence of tuberculosis				Evidence of NTM and/or other bacterial disease		Other histological, microbiological, DNA, and immunological findings and site(s)
	From enrolment to death	From death to MIA	On TB Tx	On ART	Histological evidence and site(s)	Positive culture and site(s)	Xpert® MTB/RIF positive (BAL only)	If MTB found, INH/RIF sensitive?	Histological evidence and site(s)	Organism(s) grown or detected by PCR and site(s)	
											Culture: <i>C. neoformans</i> (lungs) CrAg positive (CSF)
34	184	4	195	74	-	-	-	-		<i>K. pneumoniae</i> (BAL, BLD, lungs)	Histology: Disseminated CMV infection (LIV; LL; RL); Chronic active hepatitis B infection (LIV)
29	359	4	40	88	LL, RL	-	-	-	LL, RL	<i>Nocardia</i> sp. (lungs); <i>K. pneumoniae</i> (BAL; lungs)	PCR: CMV (CSF); Rhinovirus (BAL; NP/OP)
13	14	5	14	-	-	-	-	-	RL	<i>P. aeruginosa</i> (BAL); <i>K pneumoniae</i> (BAL)	Histology: Interstitial pneumonitis, ?viral (LL)
20	62	5	-	28	LIV, LL, RL, SPL	BAL, CSF, LIV, lungs, SPL	✓	✓	-	<i>S. pneumoniae</i> 15A/F (CSF); <i>Klebsiella</i> spp. (BAL)	PCR: Respiratory syncytial virus (NP/OP)
32	100	5	-	100	-	-	-	-	Soft tissue	<i>Salmonella</i> spp. (BAL, BLD, CSF, LIV, lungs, SPL) <i>S. aureus</i> (BAL, BLD) <i>H. influenzae</i> (BLD)	Histology: Pneumonitis, ?viral (LL; RL) PCR: CMV (CSF); EBV (CSF); PVB19 (CSF)
19	373	5	329	90	-	-	-	-	-	<i>S. aureus</i> (BAL); <i>K. pneumoniae</i> (BAL)	Histology: Disseminated cryptococcosis (LIV, LL, RL, SPL) Culture: <i>C. neoformans</i> (CSF, LIV) CrAg positive (CSF) PCR: Rhinovirus (BAL, NP/OP); CMV (CSF); EBV (CSF)
16	4	6	4	-	-	-	-	-	-	-	-
26	8	6	7	-	-	-	-	-	-	<i>S. pneumoniae</i> 19F (BLD; CSF)	PCR: EBV (CSF)
03	82	6	19	66	LIV, SPL	SPL	✓	✓	-	-	Histology: Significant steatosis (LIV)
14	128	6	63	126	LIV, RL	BAL, CSF	-	✓	LL	<i>K. pneumoniae</i> (BAL, lungs)	-
27	324	6	-	317	-	-	-	-	LIV	<i>M. intracellulare</i> (LIV; lungs; SPL)	Histology: Acute tubular necrosis (LK)

ID*	Time in days				Evidence of tuberculosis				Evidence of NTM and/or other bacterial disease		Other histological, microbiological, DNA, and immunological findings and site(s)
	From enrolment to death	From death to MIA	On TB Tx	On ART	Histological evidence and site(s)	Positive culture and site(s)	Xpert® MTB/RIF positive (BAL only)	If MTB found, INH/RIF sensitive?	Histological evidence and site(s)	Organism(s) grown or detected by PCR and site(s)	
										<i>K. pneumoniae</i> (lungs)	
28	450	6	71	422	LIV, LL, RL, SPL	Lungs	-	✓	-	-	PCR: EBV (CSF)
05	57	7	57	-	-	-	-	-	-	<i>K. pneumoniae</i> (lungs)	Histology: Severe cryptococcal pneumonia (LL, RL) Culture: <i>Aspergillus fumigatus</i> (lungs)
22	285	7	-	284	-	-	-	-	LL, RL	<i>S. aureus</i> (BAL; CSF; lungs; SPL); <i>S. pneumoniae</i> 9A/V, 1 (CSF)	PCR: Influenza A virus H32N (CSF, NP/OP); CMV (CSF) EBV (CSF); HHV-7 (CSF)
17	14	8	-	-	-	-	-	-	-	<i>S. aureus</i> (lungs); <i>P. aeruginosa</i> (BAL);	Histology: Profound non-alcoholic steato-hepatitis
23	21	8	-	21	-	-	-	-	LIV, LL, RL, SPL	<i>M. avium</i> (BAL; CSF; LIV; lungs; SPL; urine)	PCR: CMV (CSF); VZV (CSF)
04	122	8	120	108	LIV, LL, pleura, RL	BAL	-	✓	-	-	-
25†	122	8	126	46	-	-	-	-	-	-	Histology: mild autolytic changes (SPL)
21	304	8	304	276	-	-	-	-	LL	<i>Klebsiella</i> spp. (BAL, lungs)	Histology: Chronic hepatitis PCR: Rhinovirus (BAL, NP/OP); CMV (CSF); EBV (CSF)
01	175	9	173	153	-	SPL	-	✓	-	<i>Klebsiella</i> spp. (BAL)	Histology: Non-specific portal triaditis (LIV)
11	84	11	82	67	-	-	-	-	-	<i>K. pneumoniae</i> (BAL)	Histology: Severe bilateral PCP PCP immunofluorescence positive (BAL)

*ID denotes chronological order in which MIA was conducted (first October 2013, last June 2015)

†Samples obtained as part of complete autopsy – heavily contaminated

BAL: broncho-alveolar lavage; BLD: blood; CMV: Cytomegalovirus; CSF: cerebrospinal fluid; EBV: Epstein-Barr virus; HHV: Human Herpes virus; INH: Isoniazid; LIV: liver; LK: left kidney; LL: left lung; MIA: minimally-invasive autopsy; MTB: M. tuberculosis; NP/OP: nasopharyngeal/oropharyngeal swab; NTM: non-tuberculous mycobacteria; PCP: Pneumocystis pneumonia; PCR: polymerase chain reaction; PVB19: Parvovirus B19; RIF: Rifampicin; RK: right kidney; RL: right lung; SPL: spleen; TB: tuberculosis; Tx: treatment; VZV: Varicella zoster virus

Chapter 5. Research paper 3:

Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: Comparing verbal autopsy, minimally-invasive autopsy, and research data

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5.1. Cover sheet

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Research paper cover sheet

Section A: Student details

Student	Aaron Karat
Principal supervisor	Alison Grant
Thesis title	An autopsy study exploring the spectrum of disease in individuals with advanced HIV in primary care clinics in South Africa

If the research paper has previously been published, please complete Section B.
If not, please go to Section C.

Section B: Paper already published

Where was the work published?	PLOS One		
When was the work published?	March 2017		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion.	N/A		
Have you retained copyright for the work?*	Yes	Was the work subject to academic peer-review?	Yes

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Where is the work intended to be published?	
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Section D: Multi-authored work

For multi-authored work, give full details of your role in the research include in the paper and in the preparation of the paper (attach a further sheet if necessary).	The candidate coordinated data collection and management, conducted all analyses, and wrote the paper.
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Student signature: 

Date: 24 May 2017

Supervisor signature: 

Date: 25 May 2017

5.2. Manuscript



RESEARCH ARTICLE

Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: Comparing verbal autopsy, minimally-invasive autopsy, and research data

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Abstract

Background

The World Health Organization (WHO) aims to reduce tuberculosis (TB) deaths by 95% by 2035; tracking progress requires accurate measurement of TB mortality. International Classification of Diseases (ICD) codes do not differentiate between HIV-associated TB and HIV more generally. Verbal autopsy (VA) is used to estimate cause of death (CoD) patterns but has mostly been validated against a suboptimal gold standard for HIV and TB. This study, conducted among HIV-positive adults, aimed to estimate the accuracy of VA in ascertaining TB and HIV CoD when compared to a reference standard derived from a variety of clinical sources including, in some, minimally-invasive autopsy (MIA).

Methods and findings

Decedents were enrolled into a trial of empirical TB treatment or a cohort exploring diagnostic algorithms for TB in South Africa. The WHO 2012 instrument was used; VA CoD were

Competing interests: The authors have declared that no competing interests exist.

assigned using physician-certified VA (PCVA), InterVA-4, and SmartVA-Analyze. Reference CoD were assigned using MIA, research, and health facility data, as available. 259 VAs were completed: 147 (57%) decedents were female; median age was 39 (interquartile range [IQR] 33–47) years and CD4 count 51 (IQR 22–102) cells/ μ L. Compared to reference CoD that included MIA ($n = 34$), VA underestimated mortality due to HIV/AIDS (94% reference, 74% PCVA, 47% InterVA-4, and 41% SmartVA-Analyze; chance-corrected concordance [CCC] 0.71, 0.42, and 0.31, respectively) and HIV-associated TB (41% reference, 32% PCVA; CCC 0.23). For individual decedents, all VA methods agreed poorly with reference CoD that did not include MIA ($n = 259$; overall CCC 0.14, 0.06, and 0.15 for PCVA, InterVA-4, and SmartVA-Analyze); agreement was better at population level (cause-specific mortality fraction accuracy 0.78, 0.61, and 0.57, for the three methods, respectively).

Conclusions

Current VA methods underestimate mortality due to HIV-associated TB. ICD and VA methods need modifications that allow for more specific evaluation of HIV-related deaths and direct estimation of mortality due to HIV-associated TB.

Introduction

Data on causes of death (CoD) are essential in guiding public health strategy and research agendas. In South Africa in 2014, HIV prevalence was 18.9% among individuals aged 15–49 years [1] and there were an estimated 72,000 deaths due to HIV-associated tuberculosis (TB) [2]. However, methods to measure CoD, particularly HIV-associated TB, do not provide sufficiently accurate estimates [3]. Autopsy studies have typically investigated small numbers of deaths and mostly included individuals dying in hospitals [4–6]; death certification has consistently been shown to correspond poorly with retrospective clinical and autopsy diagnoses [7–9]; and verbal autopsy (VA), used to estimate CoD at a population level in many areas with poor civil registration systems, differentiates poorly between deaths due to HIV-associated TB and other HIV-associated causes [10–12]. These difficulties are compounded by the way deaths due to HIV-associated TB are coded by the International Classification of Diseases (ICD), which counts them as ‘HIV-related’ [13]. The World Health Organization (WHO) presents TB mortality data separately for HIV-negative and HIV-positive individuals. However, due to the coding issues described above, all estimates of mortality due to HIV-associated TB are generated indirectly through mathematical modelling that uses country-level data on TB incidence and case-fatality ratios [14,15]; WHO itself states the ‘urgent need’ for direct measurement of HIV-associated TB mortality [14]. An aim of the WHO ‘End TB’ strategy is to reduce TB deaths by 95% by 2035 [16].

Verbal autopsy is a structured interview with the family or carer of a deceased individual, carried out by a lay-interviewer. A standardised VA instrument has been available from WHO since 2007 [17] and is now used globally at health and socio-demographic surveillance system (HDSS) sites. In areas with poor civil registration systems, VA data are used to generate estimates of national and regional mortality [18] which may then be used to influence health policy. The last 10 years have seen the development of several automated methods (computer-coded VA [CCVA]) which, it is hoped, will eventually replace the expensive and time-consuming physician-certified VA (PCVA) [19,20]. Numerous studies have attempted to validate VA, but the vast majority compare VA CoD to clinical CoD derived from physician review of

hospital or clinic records, a gold standard of variable quality and consistency [21–24]. There have been few attempts to compare VA CoD to CoD derived from research-quality data or from pathological autopsy, which remains the highest standard for assigning CoD.

More accurate estimates of HIV-associated mortality are particularly needed in areas of high HIV prevalence, where civil registration systems are often weak [14]. This study, nested within two large studies of HIV-positive adults in South Africa ('TB Fast Track' [25] and 'XPHACTOR'), aimed to compare VA CoD to reference-standard CoD derived from clinical, research, and minimally-invasive autopsy (MIA) data.

Methods

Setting

HIV-positive adults (aged ≥ 18 years) were recruited to one of two studies of TB/HIV conducted in South Africa. The first, 'TB Fast Track', was a pragmatic trial of empirical TB treatment in ambulant HIV-positive adults enrolled in primary care with CD4 count ≤ 150 cells/ μL . Participants were eligible if not on antiretroviral therapy (ART) or TB treatment at the point of enrolment and were followed up for a minimum of six months [25]; MIA and VAs were conducted for as many decedents as possible. The second, 'XPHACTOR', was an interventional cohort study investigating the use of Xpert[®] MTB/RIF in a systematic sample of HIV-positive adults attending out-patient clinics for HIV care; participants were followed up for at least three months and VAs were conducted for as many decedents as possible, beginning about half-way through the study's follow-up.

Data collection

Verbal autopsy. All VA interviews were conducted by trained lay researchers at the home of the family/carers, or at another location of their choosing, one to twelve months after the death of the study participant, as recommended by WHO. Written informed consent for VA was obtained from respondents; all interviews were conducted using the WHO 2012 VA instrument, with additional questions around treatment for TB and HIV, health beliefs, and health service use added by the study team.

Clinical. Data used to inform reference-standard CoD were separated into three categories: those collected from routine clinic and/or hospital records by a trained lay researcher, research nurse, or physician using standardised paper forms, labelled *operational*; those collected by members of a clinical research team due to involvement in a parent study, including results of investigations retrieved from the national health laboratory service (NHLS) database, labelled *research*; and those collected through MIA, carried out on TB Fast Track decedents as soon as possible after death, labelled *autopsy* (S1 Fig). Detailed MIA methods and a description of the consent process have previously been described [26]. The procedure involved core biopsies of liver, spleen, and lungs; aspiration of cerebrospinal fluid (CSF), blood, and urine; sampling of naso- and oro-pharyngeal secretions; and broncho-alveolar lavage (BAL) by insertion of a nasogastric tube into the trachea directed toward the lungs through a cricothyroid incision. Laboratory testing of post-mortem specimens included liquid culture for mycobacteria; Xpert[®] MTB/RIF; microscopy and aerobic culture; molecular testing for a range of bacteria and viruses; and histological examination.

Data interpretation

Verbal autopsy. VA data included in the WHO 2012 VA instrument (i.e., excluding data from study-specific added questions around ART use, treatment for TB, health beliefs, or

health service use) were interpreted using both physician-certified verbal autopsy (PCVA) and computer-coded verbal autopsy (CCVA) methods. Using a PCVA method based on WHO recommendations, similar to that used at the Medical Research Council/Wits-Agincourt HDSS site, South Africa [21], two physicians, blinded to all other clinical information, independently reviewed all available VA data, including the free narratives, and separately assigned CoD using ICD-10 and study-defined codes (detailed below). Assigned CoD were compared and, where there were discrepancies in either immediate, underlying, or study-defined CoD, the cases were discussed by the two physicians, aiming for consensus. If a consensus could not be reached, the data were provided to a third physician who reviewed them independently. If the CoD assigned by physician 3 matched that assigned by physicians 1 or 2, it was considered the final CoD; if no consensus was reached after review by three physicians, the individual was assigned an 'indeterminate' CoD.

VA data were also processed by two CCVA methods. The first, InterVA-4 (www.interva.net), uses Bayesian probabilities to assign each decedent up to three CoD, each with an associated 'likelihood' expressed as a percentage probability; cause-specific mortality fractions (CSMFs) can be generated that combine all individual causes and likelihoods [27,28]. The model allows for user modification of two baseline variables: prevalence of malaria and HIV, each of which can be set to 'very low', 'low', or 'high'. The second method, SmartVA-Analyze (<http://www.healthdata.org/verbal-autopsy/tools>), uses the Tariff 2.0 system to assign each decedent one of 34 CoD [29,30]. SmartVA-Analyze also allows for the inclusion of data from the narrative section of the VA instrument and from healthcare records examined during the interview. Data were mapped from the WHO 2012 instrument to the 2014 framework for InterVA-4 and to the Population Health Metrics Research Consortium (PHMRC) full instrument for SmartVA-Analyze; free narrative and healthcare data were not provided to either software. Both CCVA methods assign CoD to lists of grouped ICD-10 codes [31,32]; these were further grouped into seven major categories for analysis (S1 Table).

Clinical. In order to meaningfully compare results with other VA validation studies, at least two sets of reference CoD were assigned to each participant. Operational data were used to generate level one (L1) CoD, comparable to the gold standard used in the majority of VA validation studies (S1 Fig). Both operational and research data were used to generate level two (L2) CoD for all decedents, representing a higher gold standard than would normally be available for comparison to VA. Finally, operational, research, and autopsy data were used to generate level three (L3) CoD for decedents for whom these data were available. All parties involved in the assignment of reference CoD were blinded to VA data, parent study arm, and any narratives around death obtained from family members as part of the research process, which were considered too similar to VA. L1 and L2 CoD were assigned using the same method as PCVA but involved different physicians; cases were processed in batches of 40–50. For each batch, all decedents were required to have finalised L1 CoD before the physicians were exposed to any higher level data.

L3 CoD were assigned in a different manner. Once all decedents with autopsy data had been assigned L1 and L2 CoD, a panel was convened to assign L3 CoD. The panel was made up of two infectious disease physicians, a microbiologist, and a pathologist, all of whom had extensive knowledge of local epidemiology; it was blinded to VA data, TB Fast Track study arm, and narratives from family members. The panel reviewed all available clinical data and attempted to reach consensus on CoD. In cases where full consensus could not be reached, consensus among three panel members was considered sufficient; if opinion was evenly split, the decedent was assigned an 'indeterminate' CoD.

Study-defined CoD

To differentiate HIV-associated TB from other HIV-associated causes, six broad study-defined CoD categories were constructed: TB in an HIV-positive individual; an HIV/AIDS-related cause, excluding TB; a cause unrelated to HIV in an HIV-positive individual; TB in an HIV-negative individual; a cause other than TB in an HIV-negative individual; and an indeterminate cause. As part of both PCVA and reference CoD processes, reviewers assigned each decedent ICD-10 and study-defined CoD, along with a probability of 'definite', 'probable', or 'possible'. For reference CoD only, probabilities were based on pre-defined criteria (S2 Table). CCVA outputs were not reclassified into study-defined categories as they do not allow HIV-associated TB to be distinguished from other HIV/AIDS-related CoD.

Data management and statistical analyses

VA quantitative data were entered directly into an online database (Mobenzi Technologies, Durban, South Africa) through a cell phone interface; narrative data were captured on paper. Data collected for reference CoD assignment were entered into EpiData v3.1 (The EpiData Association, Odense, Denmark) and data from the parent studies into a SQL database (Bytes Technology Group, Johannesburg, South Africa). InterVA-4.03 was used, with malaria prevalence set to 'Low' and HIV/AIDS to 'High'; InterVA-4 CSMFs were generated by dividing the sum of the likelihoods of each cause category by the sum of likelihoods for all causes [27]. SmartVA-Analyze v1.1.1 was used, with 'Malaria region', 'Health Care Experience', and 'Free text' options deselected; CSMFs, including deaths with 'Undetermined' cause, were calculated after outputs were grouped further (S1 Table). The Mortality Medical Data System (MMDS) 2011 software package [33] was used to generate a single 'underlying' CoD from ICD-10 codes assigned by PCVA and clinical panels; CSMFs were calculated using ACME/TRANSAX output. All analyses were conducted using Stata v14 (StataCorp, College Station, TX, USA).

Two forms of agreement were measured: between CoD assigned to individual decedents; and between the proportion of deaths assigned to each cause category across the study population. Cohen's kappa (K) was used to measure agreement between individual decedents and overall chance-corrected concordance (CCC) used for agreement between cause categories; 1 equated to perfect agreement and 0 to agreement no greater than chance. Lin's concordance correlation coefficient (ρ_c) and CSMF accuracy were calculated for population-level comparisons [34]. In line with previous uses of ρ_c , a value of less than 0.90 was considered 'poor' agreement [35].

Ethical considerations

Separate approvals were obtained for the parent studies and the sub-study from the human research ethics committees of the London School of Hygiene & Tropical Medicine and the University of the Witwatersrand. Beginning in August 2013, participants in TB Fast Track were asked to give written informed consent for MIA in the event of their death while undergoing follow-up as part of the parent study. If a participant who had given written consent died during follow-up, verbal agreement from the next of kin was obtained to proceed with MIA. For participants who were enrolled to TB Fast Track prior to August 2013 and died during follow-up, formal written consent to undertake MIA was sought from the next of kin. All VA respondents gave written informed consent for interview.

Table 1. Demographics for all decedents for whom a VA was conducted, stratified by parent study (n = 259).

Characteristic	All, n (%) or median (IQR)	TB Fast Track, n (%) or median (IQR)	XPHACTOR, n (%) or median (IQR)
N	259	212	47
Female	147 (57)	115 (54)	32 (68)
Age at death	39 (33–47)	39 (33–46)	43 (37–51)
CD4 count at enrolment* (cells/ μ L)	51 (22–102) (n = 257)	44 (19–88)	161 (42–335) (n = 45)
Black African	258 (99.6)	211 (99.5)	47 (100)
South African	248 (96)	203 (96)	45 (96)
Enrolled in a peri-urban area	203 (78)	156 (74)	47 (100)
Completed grade 12	85 (33)	69 (33)	16 (34)
Household income of \leq R2000 per month†	98 (49) (n = 202)	78 (49) (n = 159)	20 (47) (n = 43)
On ART at enrolment*	32 (12)	0	32 (68)
Previous treatment for TB	46 (18)	32 (15)	14 (30)
Time from enrolment* to death (days)	84 (39–184)	76 (33–168)	109 (70–344)
Time from death to VA (days)	146 (82–290)	142 (78–288)	170 (95–316)
Parent as VA respondent	75 (29)	61 (28)	14 (30)

* Refers to enrolment in the parent study (TB Fast Track or XPHACTOR)

† 202/259 (80%) individuals were able to estimate household income

ART: antiretroviral therapy; IQR: interquartile range; R: South African Rand; TB: tuberculosis; VA: verbal autopsy

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Results

Demographics

A total 3022 individuals were recruited to the TB Fast Track study between December 2012 and December 2014; 364 died after enrolment. XPHACTOR enrolled a total 3722 individuals between September 2012 and March 2014; 125 died after enrolment. Attempts were made to contact the families of all deceased individuals between August 2013 and October 2015: contact could not be made in 218/489 (44.6%) cases and respondents declined to participate in 12 (2.4%) cases; 212/364 (58.2%) TB Fast Track decedents and 47/125 (37.6%) XPHACTOR decedents had a VA completed; a total 259 VAs were conducted (S1 Fig).

Among the 259 decedents (Table 1), 147 (57%) were female; the median age was 39 (interquartile range [IQR] 33–47) years; the median CD4 count at enrolment into the parent study was 51 (IQR 22–102) cells/ μ L; 258 (99.6%) were black African; 248 (96%) were South African; and 203 (78%) were enrolled in a peri-urban area, as opposed to semi-rural. The median time from enrolment to death was 84 (IQR 39–184) days and from death to VA was 146 (IQR 82–290) days.

Data availability and consistency in physician assignment

Of the 212 TB Fast Track decedents, 196 (92%) had clinic files available; 122 (58%) had hospital files available; all had research data available; 207 (98%) had data from the NHLS database available; and 34 (26%) had MIA conducted, a median five (IQR 3–6) days after death. Of the 47 XPHACTOR decedents, all had clinic files available; none had hospital files available; all had research data available; and 33 (70%) had data from the NHLS database available (S1 Fig).

In assigning L1 CoD, physicians were in agreement on all three of immediate, underlying, and study-defined causes for 129/259 (49.8%) decedents; in assigning L2, they agreed 138/259 (53.3%) times. Physicians assigning VA CoD agreed on all three causes for 90/259 (34.7%) decedents. If agreement was measured based only on study-defined causes, it improved to

146/259 (56.4%), 162 (62.5%), and 141 (54.4%) times for L1, L2, and PCVA CoD, respectively. All disagreements were resolved through discussion; arbitrating physicians were not required.

Performance of VA against L3 reference standard

Thirty-four decedents underwent MIA, detailed results of which have previously been reported [26]: 18 (53%) were female; median age at death was 39 (IQR 33–44) years, and median CD4 count at enrolment to TB Fast Track was 34 (IQR 17–66) cells/μL. Using ICD-10 codes, 32/34 (94.1%) individuals were assigned an ‘HIV/AIDS-related’ reference CoD by the clinicopathological panel, compared to 25 (73.5%) assigned by PCVA, 47.1% assigned by InterVA-4, and 14 (41.2%) assigned by SmartVA-Analyze (Table 2). A ‘pulmonary TB’ CoD was assigned to 20.6% by InterVA-4 and to one (2.9%) decedent by SmartVA-Analyze; this was not assigned to any decedents by either PCVA or the clinical panel. All VA methods performed poorly when CoD assigned to individual decedents were compared to L3 (Figs 1 and 2): PCVA (K 0.13; CCC 0.22) performed better than the CCVA methods (K 0.05 and 0.03; overall CCC 0.01 and 0.16; for InterVA-4 and SmartVA-Analyze, respectively; Table 3). When compared at a population level, PCVA performed better (ρ_C 0.95; CSMF accuracy 0.79), but both CCVA methods were still sub-optimal (ρ_C 0.67 and 0.58; CSMF accuracy 0.49 and 0.43; for InterVA-4 and SmartVA-Analyze, respectively).

Using study-defined codes, the clinicopathological panel assigned 14/34 (41.2%) individuals a ‘TB in an HIV-positive individual’ CoD and 15 (44.1%) an ‘HIV/AIDS-related, excluding TB’ CoD (Table 2; S3 Table); PCVA assigned these CoD to 11 (32.4%) and 17 (50%) individuals, respectively. Agreement between PCVA and L3, at an individual level, was very poor (K 0.04; overall CCC 0.04; Table 3); agreement was better at a population level (ρ_C 0.91; CSMF accuracy 0.79).

Table 2. Numbers and resultant CSMFs generated for grouped ICD-10 and study-defined CoD categories in those with autopsy data available, as assigned by the clinicopathological panel, PCVA, InterVA-4, and SmartVA-Analyze (n = 34).

CoD category	L3 reference standard, n (%)	VA method		
		PCVA, n (%)	InterVA-4, % (CSMF)*	SmartVA-Analyze, n (%)
Grouped ICD-10				
HIV/AIDS	32 (94.1)	25 (73.5)	47.1	14 (41.2)
PTB	0	0	20.6	1 (2.9)
Other infectious	1 (2.9)	5 (14.7)	8.8	5 (14.7)
Malignancy	0	0	11.8	2 (5.9)
Other NCD	0	3 (8.8)	11.8	5 (14.7)
External/traumatic or pregnancy-related	1 (2.9)	1 (2.9)	0	0
Indeterminate	0	0	0	7 (20.6)
Study-defined				
TB in an HIV-positive individual	14 (41.2)	11 (32.4)	-	-
HIV-related cause other than TB	15 (44.1)	17 (50.0)	-	-
Other cause in an HIV-positive individual	1 (2.9)	3 (8.8)	-	-
TB in an HIV-negative individual	0	0	-	-
Other cause in an HIV-negative individual	0	3 (8.8)	-	-
Indeterminate cause	4 (11.8)	0	-	-

* InterVA-4 CSMFs generated from all assigned CoD and associated likelihoods
 AIDS: acquired immune deficiency syndrome; CoD: cause of death; CSMF: cause-specific mortality fraction; HIV: human immunodeficiency virus; ICD: International Classification of Diseases; L3: level three (operational, research, and autopsy data); NCD: non-communicable disease; PCVA: physician-certified verbal autopsy; PTB: pulmonary tuberculosis; TB: tuberculosis

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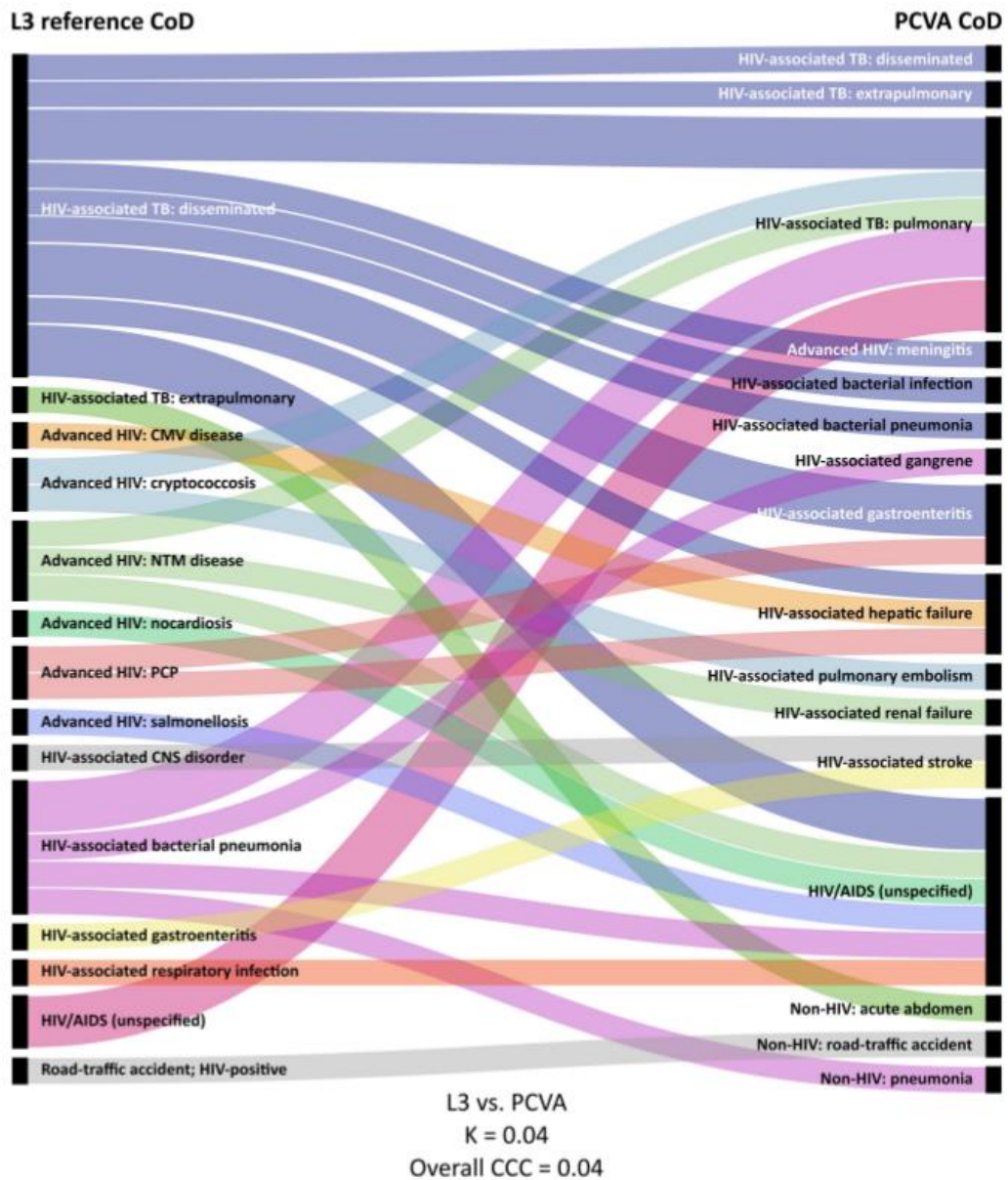


Fig 1. Alluvial * diagram showing CoD† as assigned by the clinicopathological panel (left) and PCVA (right); n = 34 [36]. *Each horizontal band represents one decedent. †Combined 'immediate' and 'underlying' ICD-10 CoD. ‡Comparison based on study-defined codes. AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CMV: cytomegalovirus; CNS: central nervous system; CoD: cause of death; HIV: human immunodeficiency virus; K: Cohen's kappa; L3: level three (using operational, research, and autopsy data); NCD: non-communicable diseases; NTM: non-tuberculous mycobacteria; PCP: *Pneumocystis pneumonia*; PCVA: physician-certified verbal autopsy; TB: tuberculosis.

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Fig 2. Alluvial* diagram showing CoD as assigned by clinicopathological panel† (centre), InterVA-4‡ (left) and SmartVA-Analyze (right); n = 34 [36]. *Each horizontal band represents one decedent. †Combined 'immediate' and 'underlying' ICD-10 CoD. ‡InterVA-4 CoD with highest associated likelihood. AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CMV: cytomegalovirus; CNS: central nervous system; CoD: cause of death; HIV: human immunodeficiency virus; K: Cohen's kappa; L3: level three (using operational, research, and autopsy data); NCD: non-communicable diseases; NTM: non-tuberculous mycobacteria; PCP: *Pneumocystis pneumonia*; PCVA: physician-certified verbal autopsy; TB: tuberculosis.

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Table 3. Summary of measures of performance of PCVA, InterVA-4, and SmartVA-Analyze in assigning CoD in ICD-10 or study-defined categories, compared to L3 (n = 34) and L2 (n = 259) reference standards.

CoD category and VA method	Compared to L3 reference standard (n = 34)				Compared to L2 reference standard (n = 259)			
	Individual measures [‡]		Population measures		Individual measures [‡]		Population measures	
	Cohen's kappa (95% CI)	Overall CCC	ρ_C (95% CI)	CSMF accuracy	Cohen's kappa (95% CI)	Overall CCC	ρ_C (95% CI)	CSMF accuracy
Grouped ICD-10*								
PCVA	0.13 (0–0.47)	0.22	0.95 (0.86–0.98)	0.79	0.06 (0–0.13)	0.14	0.93 (0.69–0.99)	0.78
InterVA-4	0.05 (0–0.08)	0.01	0.67 (0.38–0.84)	0.49	0.08 (0.03–0.11)	0.06	0.78 (0.44–0.93)	0.61
SmartVA-Analyze	0.03 (0–0.11)	0.16	0.58 (0.26–0.78)	0.43	0.05 (0.04–0.08)	0.15	0.52 (0.06–0.79)	0.57
Study-defined[†]								
PCVA	0.04 (0–0.20)	0.04	0.91 (0.51–0.99)	0.79	0.06 (0.04–0.08)	0.08	0.70 (0–0.95)	0.71

* Overall CCC for grouped ICD-10 calculated across seven possible CoD categories

† Overall CCC for study-defined calculated across six possible CoD categories

‡ For InterVA-4, measures of individual agreement calculated using assigned CoD with highest associated likelihood

CCC: chance-corrected concordance; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; ICD: International Classification of Diseases; L2: level two (operational and research data); L3: level three (operational, research, and autopsy data); PCVA: physician-certified verbal autopsy; ρ_C : Lin's concordance correlation coefficient

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Performance of VA against L2 reference standard

Using ICD-10 codes, an HIV/AIDS reference CoD was assigned to 183/259 (70.7%) individuals, to 206 (79.5%) by PCVA, to 48.1% by InterVA-4, and to 75 (29%) by SmartVA-Analyze (Table 4). One (0.4%), 14.3%, and five (1.9%) individuals were assigned a 'pulmonary TB' CoD by PCVA, InterVA-4, and SmartVA-Analyze, respectively. All VA methods performed poorly when individual agreement was measured: K 0.06, 0.08, and 0.05; overall CCC 0.14, 0.06, and 0.15, for PCVA, InterVA-4, and SmartVA-Analyze, respectively (Table 3). PCVA performed best at population level (ρ_C 0.93; CSMF accuracy 0.78), InterVA-4 second-best (ρ_C 0.78; CSMF accuracy 0.61), and SmartVA-Analyze least well (ρ_C 0.52; CSMF accuracy 0.57). Using study-defined codes, physicians assigned 69/259 (26.6%) individuals a reference CoD of 'TB in an HIV-positive individual' and 103 (39.8%) individuals an 'HIV/AIDS-related, excluding TB' CoD (Table 4). PCVA agreement with L2 was poor at an individual level (K 0.03; overall CCC 0.08) but better at population level (ρ_C 0.70; CSMF accuracy 0.71).

Cause-specific comparisons

Compared to L3 and L2, PCVA was more sensitive, but less specific, than both CCVA methods in assigning ICD-10 HIV/AIDS-related CoD (sensitivity against L3 75.0%, 50.0%, and 40.6%; sensitivity against L2 80.9%, 55.7%, and 31.7%; and specificity against L2 23.7%, 64.5% and 77.6% for PCVA, InterVA-4, and SmartVA-Analyze, respectively; Table 5). Compared to L2, PCVA was only 44.9% sensitive and 58.9% specific for a study-defined CoD of 'TB in an HIV-positive individual', with CCC measured at 0.42; sensitivity and CCC were lower when compared to L3, at 35.7% and 0.23, respectively.

Discussion

This study, conducted among HIV-positive adults in a setting of high TB prevalence, compared CoD assigned by VA to a robust gold standard, including MIA, and found the proportion of

Table 4. Numbers and resultant CSMFs generated for grouped ICD-10 and study-defined CoD categories, as assigned by L2 and L1 clinical panels, PCVA, InterVA-4, and SmartVA-Analyze (n = 259).

CoD category	Clinical panel		VA method		
	L2, n (%)	L1, n (%)	PCVA, n (%)	InterVA-4, % (CSMF)*	SmartVA-Analyze, n (%)
Grouped ICD-10					
HIV/AIDS	183 (70.7)	143 (55.2)	206 (79.5)	48.1	75 (29.0)
PTB	0	0	1 (0.4)	14.3	5 (1.9)
Other infectious	2 (0.8)	4 (1.5)	17 (6.6)	9.7	26 (10.0)
Malignancy	8 (3.1)	8 (3.1)	7 (2.7)	13.7	58 (22.4)
Other NCD	7 (2.7)	9 (3.5)	21 (8.1)	7.9	34 (13.1)
External/traumatic or pregnancy-related	3 (1.2)	3 (1.2)	7 (2.7)	1.2	1 (0.4)
Indeterminate	56 (21.7)	92 (35.5)	0	5.1	60 (23.2)
Study-defined					
TB in an HIV-positive individual	69 (26.6)	56 (21.6)	109 (42.1)	-	-
HIV-related cause other than TB	103 (39.8)	82 (31.7)	110 (42.5)	-	-
Other cause in an HIV-positive individual	18 (6.9)	16 (6.2)	20 (7.7)	-	-
TB in an HIV-negative individual	0	0	1 (0.4)	-	-
Other cause in an HIV-negative individual	0	0	19 (7.3)	-	-
Indeterminate cause	69 (26.6)	105 (40.5)	0	-	-

* InterVA-4 CSMFs generated from all assigned CoD and associated likelihoods

AIDS: acquired immune deficiency syndrome; CoD: cause of death; CSMF: cause-specific mortality fraction; HIV: human immunodeficiency virus; ICD: International Classification of Diseases; L1: level one (operational data only); L2: level two (operational and research data); NCD: non-communicable disease; PCVA: physician-certified verbal autopsy; PTB: pulmonary tuberculosis; TB: tuberculosis

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deaths attributable to TB was underestimated by methods that did not include data from pathological autopsy. Overall HIV-associated mortality was also underestimated by all VA methods when compared to the L3 (autopsy) reference standard, with poor agreement at an individual level; all methods performed better at a population level.

Table 5. Sensitivity, specificity, and chance-corrected concordance of PCVA, InterVA-4, and SmartVA-Analyze in the detection of specific CoD compared to L3 (n = 34) and L2 (n = 259) clinical reference standards.

CoD category and VA method	Compared to L3 (n = 34)			Compared to L2 (n = 259)		
	Sensitivity, % (95% CI)	Specificity, % (95% CI)	CCC	Sensitivity, % (95% CI)	Specificity, % (95% CI)	CCC
HIV/AIDS (ICD-defined)						
PCVA	75.0 (56.6–88.5)	50.0 (1.3–98.7)	0.71	80.9 (74.4–86.3)	23.7 (14.7–34.8)	0.78
InterVA-4*	50.0 (31.9–68.1)	100.0 (15.8–100.0)	0.42	55.7 (48.2–63.1)	64.5 (52.7–75.1)	0.48
SmartVA-Analyze	40.6 (23.7–59.4)	50.0 (1.3–98.7)	0.31	31.7 (25.0–39.0)	77.6 (66.6–86.4)	0.20
TB in an HIV-positive individual (study-defined)						
PCVA	35.7 (12.8–64.9)	70.0 (45.7–88.1)	0.23	44.9 (32.9–57.4)	58.9 (51.6–66.0)	0.42
HIV-related cause other than TB (study-defined)						
PCVA	53.3 (26.6–78.7)	52.6 (28.9–75.6)	0.44	26.2 (17.2–36.9)	77.1 (70.2–83.1)	0.36

* Measures of InterVA-4 sensitivity and specificity calculated using individuals assigned HIV/AIDS as 'most likely' CoD (n = 129 overall; n = 16 with L3 CoD) AIDS: acquired immune deficiency syndrome; CCC: chance-corrected concordance; CI: confidence interval; CoD: cause of death; HIV: human immunodeficiency virus; ICD: International Classification of Diseases; L2: level two cause of death (operational and research data); L3: level three cause of death (operational, research, and a autopsy data); PCVA: physician-certified verbal autopsy; TB: tuberculosis; VA: verbal autopsy

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HIV-associated TB

A recent systematic review of autopsy studies in HIV-positive individuals found that 46% of TB diagnosed at autopsy had not been diagnosed ante-mortem and that almost 90% of HIV-positive individuals with evidence of TB at autopsy had disseminated disease [4]. In our study, every individual with MIA data assigned a reference-standard CoD of HIV-associated TB had evidence of extrapulmonary and/or disseminated disease at post-mortem (S3 Table); 6/16 (38%) individuals with evidence of active TB at autopsy had not been started on TB treatment between enrolment and death. The under-diagnosis of TB in the absence of autopsy data suggests that the long-standing emphasis on respiratory symptoms and sputum-based investigation make it less likely for physicians to consider a TB diagnosis in patients who do not report a cough. This has important implications for the development of TB diagnostics, the design of guidelines, and in the training of clinicians operating in areas of high HIV prevalence. It may also mean that current CCVA algorithms used to generate VA CoD need recalibration to account for those with advanced HIV and extrapulmonary or disseminated TB, who may have few or no respiratory symptoms.

ICD-10 coding of HIV-deaths

ICD-10 coding does not differentiate HIV-associated TB death from other HIV-associated causes, therefore deaths due to HIV-associated TB are effectively 'hidden' within HIV-related deaths a whole. TB is the leading cause of death in HIV-positive individuals and being unable to directly measure mortality attributable to it is an enormous disadvantage. As the global ART rollout continues and the HIV epidemic evolves, it is no longer sufficient to talk simply about deaths due to HIV; a more nuanced approach to disease and mortality measurement is needed [10,37] and central to this approach must be modifications to the ICD system to allow for differentiation of HIV-associated TB deaths from other HIV-related deaths. The current draft of ICD-11, due for release in 2018 [38,39], allows for the separation of HIV disease by clinical stage and for the inclusion of certain co-morbidities, such as TB and malaria. A separate three-character code denoting HIV-associated TB would be a welcome addition to these developments.

Comparison to previous studies

To our knowledge, only one other study, conducted in Kenya, has attempted to compare VA CoD to CoD derived from pathological autopsy. In this study both PCVA and InterVA-4, when compared to MIA, overestimated mortality due to TB in an area of high HIV prevalence [11]. However, in addition to HIV-positive adults, this study included children and HIV-negative individuals and selected only individuals who reported respiratory symptoms. This may have led to the exclusion of those with extrapulmonary TB and no respiratory symptoms and may account for the relatively low prevalence of TB seen at pathological autopsy. Our findings suggest the opposite, that VA underestimates deaths due to TB, but we would nevertheless agree with the authors of the Kenyan study that, at present, VA is not a suitable tool for assigning individual CoD in areas of high HIV prevalence and would be cautious about its use in registering individual deaths [40–42].

A number of studies have attempted to use VA to estimate CSMFs in areas of high HIV prevalence using a variety of gold standards. Most studies grouped HIV-associated TB deaths with other HIV/AIDS deaths, as per ICD-10 [10,22,43–51], or did not clearly differentiate between 'TB' and 'HIV/AIDS' categories [52–56]. At least some of this inconsistency is due to the issues with ICD coding discussed above. For example, one of the largest studies, an analysis of 54,000 deaths from the International Network for the Demographic Evaluation of

Populations and Their Health (INDEPTH) HDSS sites in Africa and Asia, compared InterVA-4 to a PCVA gold standard; ρ_C was 0.831 overall and increased to 0.974 when HIV/AIDS and pulmonary TB CoD were combined for deaths from sub-Saharan Africa [57]. This high figure is misleading, however, as the two categories are intended to be mutually exclusive [58]; classifying HIV-associated TB deaths as 'pulmonary TB' will lead to the overall underestimation of HIV-associated deaths if current ICD rules are correctly applied. In our study, 35/41 (85.4%) individuals assigned a 'PTB' CoD by InterVA-4 were reported HIV-positive during the VA interview, but 32/35 (91%) did not have HIV/AIDS mentioned as a second or third CoD. Another important issue is that of extrapulmonary and disseminated disease: the WHO truncated CoD list classifies extrapulmonary and disseminated TB (ICD-10 codes A17–A19) under 'Other or unspecified infectious diseases' [59]; even if PTB and HIV/AIDS categories were combined for analysis purposes, the exclusion of these forms of TB would still result in the underestimation of TB-related deaths.

Only one previous study, conducted in 1998 across sites in Tanzania, Ethiopia, and Ghana, attempted to use VA to differentiate HIV-associated TB from other HIV-associated CoD, comparing CoD from PCVA and an early CCVA algorithm to CoD derived from hospital diagnoses [24]. Similar to our findings, both VA analysis methods showed low sensitivity and high specificity for 'TB + AIDS' diagnoses (respectively, 8% and 99% for PCVA and 35% and 95% for the CCVA algorithm), with PCVA detecting only 11/35 (31%) cases of 'TB + AIDS'. More recently, in the construction of the PHMRC gold standard dataset, CoD were initially classified as 'AIDS'; 'AIDS with TB'; or 'Pulmonary TB', with criteria explicitly stated for each [32]. The categories were consistent with ICD-10: inclusion in the 'Pulmonary TB' category required the individual to have tested HIV-negative. However, to be included in the 'AIDS with TB' category, an individual was required to have both a positive HIV test and a positive culture for *M. tuberculosis*, which likely led to the exclusion of individuals with disseminated TB and limited or no respiratory symptoms. To date, all comparisons of VA to the PHMRC dataset, including those conducted by the PHMRC team, have combined the 'AIDS' and 'AIDS with TB' categories, and have therefore not attempted to assess VA's ability to detect HIV-associated TB [19,20,30,60–64]. The PHMRC gold standard dataset nevertheless remains a valuable resource; we would suggest that any future validation exercises use the differentiated, 'AIDS with TB' and 'AIDS' categories, rather than the combined 'AIDS' category, for comparison to VA.

Moving forward

In the absence of robust, validated CRVS data, there are few alternatives to VA that are both feasible and cost-effective in generating estimates of cause-specific mortality in countries with high HIV and TB prevalence [65,66]. Although, in this study, VA methods performed poorly in assigning individual CoD, it should be noted that VA is primarily intended to generate population-level estimates [18], and that performance in this regard was better. However, when using study-defined codes, which were designed to allow for the differentiation of HIV-associated TB from other HIV-associated causes, the population-level accuracy of PCVA was still sub-optimal (ρ_C 0.70 and CSMF accuracy 0.71 compared to L2 standard [$n = 259$]; Table 3), confirming the difficulty of making this distinction.

The challenges of diagnosing HIV-associated TB disease are well documented [67,68] and, as found in the systematic review of autopsy studies [4], in the absence of new diagnostics it is likely that clinicians will continue to underdiagnose TB, which will have important implications for measuring progress towards the WHO targets described above [16]. Improvements are needed to TB surveillance methods, which, at present, consist mostly of enumerating

individuals already diagnosed and started on treatment [69–71]. MIA is a useful technique for estimating the prevalence of infectious diseases [72,73], is acceptable to a high proportion of families [26,74], and could be used periodically for surveillance at sentinel sites [75], allowing for more accurate evaluation of the impact of disease-focused interventions.

Population-level estimates of cause-specific mortality are extremely valuable and improving the accuracy of VA-generated estimates would be of benefit, regardless of whether or not VA is used to assign individual CoD. The continued development and sharing of gold standard datasets that include pathological autopsy data, better reflecting the high proportions of HIV-associated mortality seen in high-burden countries and including both hospital and community deaths in different populations, would allow for greater standardisation in future validation studies. The parallel development of a structured, standardised process for CoD assignment, similar to that described in the Coding Causes of Death in HIV (CoDe) project [76], but assigning CoD matched to ICD codes [77], would increase the value of this exercise.

Limitations and strengths

This study had limitations: the median time from death to VA was slightly longer than the ideal three months that some recommend, but was well within the maximum 12 months recommended by WHO and was therefore considered unlikely to have had a substantial effect on VA-generated estimates [78,79]; physicians who reviewed clinical and VA data were aware that most decedents were likely HIV-positive and had been enrolled into TB-focused studies, which may have led to greater assignment of HIV- and TB-related CoD; missing operational and research data may have affected consistency of the reference standard; pathological autopsy data were available for a small number of decedents; and, although the reference CoD assigned represent our best estimates using the data available, the true CoD may still differ. Questions on ART and TB treatment, added to the VA instrument by the study team, may have led to changes in how events were reported in the free narrative section; the answers to the questions themselves, however, were not provided to reviewing physicians or to either software. InterVA-4 and SmartVA-Analyze are designed for use with the WHO 2014 and PHMRC VA instruments, respectively, therefore using the WHO 2012 instrument may have resulted in some missing variables; healthcare and narrative data were not provided to SmartVA-Analyze, which may have affected its assignment of CoD. Individuals included in this analysis are likely representative of those with advanced HIV disease in resource-scarce settings, but may not necessarily represent the patterns of mortality seen in the wider community. This may have affected measures of agreement that are dependent on the composition of the gold standard CSMF and may, in turn, limit the generalisability of our findings. This study's strengths include: having recent, reliable information regarding CD4 count, ART status, and investigation and treatment of TB; using the same physicians to assign PCVA CoD for all decedents; using robust methods to assign reference CoD; comparing VA-assigned CoD to a reference standard that included MIA findings; comparing between CoD using a range of metrics, allowing for evaluation of different potential applications of VA; and classifying HIV-associated TB separately from other HIV-associated CoD, something made difficult by ICD-10 and generally neglected by previous VA studies.

Conclusions

Current VA methods underestimate mortality due to HIV-associated TB. At present, VA does not assign individual CoD in areas of high HIV prevalence with sufficient accuracy and, in part due to the limitations of ICD-10, does not distinguish between deaths due to HIV-associated TB and advanced HIV disease. More accurate methods are needed that allow for direct

estimation of deaths due to HIV-associated TB; unless TB mortality is more accurately measured, it will be extremely difficult to track progress towards the goals set by the post-2015 global strategy.

Supporting information

S1 Fig. Numbers of individuals enrolled to each of the parent studies, number of deaths, number of VAs conducted, data sources for clinical cause of death assignment, data availability by parent study, and which data contributed to different CoD levels.

(TIF)

S1 Table. Grouped ICD-10 CoD categories and corresponding WHO 2014 CoD codes.

(DOCX)

S2 Table. Criteria used by clinical panels to assign study-defined CoD and associated certainty.

(DOCX)

S3 Table. Causes of Death (CoD) for decedents with 'Autopsy' data: ICD-10 immediate and underlying CoD, as assigned by reviewers; grouped ICD-10 category; and study-specific categories as assigned by clinicopathological panel (L3), physician-certified VA, InterVA-4, and SmartVA-Analyze (n = 34).

(DOCX)

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5.4. Material provided as supplementary online appendices

Supplementary table 5:1. Grouped ICD-10 CoD categories and corresponding WHO 2014 CoD codes

Grouped ICD-10 category*	Corresponding WHO 2014 CoD codes [106]
HIV/AIDS-related	01.03 HIV/AIDS-related death
Pulmonary TB	01.09 Pulmonary tuberculosis
Other infections	01.01 Sepsis (non-obstetric)
	01.02 Acute resp. infection, incl. pneumonia
	01.04 Diarrhoeal illness
	01.05 Malaria
	01.06 Measles
	01.07 Meningitis and encephalitis
	01.08, 10.05 Tetanus
	01.10 Pertussis
	01.11 Haemorrhagic fever
	01.99 Other and unspecified infectious diseases
Non-HIV malignancy	02.01 Oral neoplasms
	02.02 Digestive neoplasms
	02.03 Respiratory neoplasms
	02.04 Breast neoplasms
	02.05, 02.06 Reproductive neoplasms M, F
	02.99 Other and unspecified neoplasms
Other non-communicable diseases	03.01 Severe anaemia
	03.02 Severe malnutrition
	03.03 Diabetes mellitus
	04.01 Acute cardiac disease
	04.02 Stroke
	04.03 Sickle cell with crisis
	04.99 Other and unspecified cardiac disease
	05.01 Chronic obstructive pulmonary disease
	05.02 Asthma
	06.01 Acute abdomen
	06.02 Liver cirrhosis
	07.01 Renal failure
	08.01 Epilepsy
	98 Other and unspecified NCD
External / Traumatic / Pregnancy	09.01 Ectopic pregnancy

Grouped ICD-10 category*	Corresponding WHO 2014 CoD codes [106]
	09.02 Abortion-related death
	09.03 Pregnancy-induced hypertension
	09.04 Obstetric haemorrhage
	09.05 Obstructed labour
	09.06 Pregnancy-related sepsis
	09.07 Anaemia of pregnancy
	09.08 Ruptured uterus
	09.99 Other and unspecified maternal CoD
	12.01 Road traffic accident
	12.02 Other transport accident
	12.03 Accidental fall
	12.04 Accidental drowning and submersion
	12.05 Accidental exposure to smoke, fire, & flame
	12.06 Contact with venomous plant/animal
	12.07 Accidental poisoning and noxious substances
	12.08 Intentional self-harm
	12.09 Assault
	12.10 Exposure to force of nature
	12.99 Other and unspecified external CoD
Indeterminate	Cause of death unknown

*Categories used for analysis purposes

AIDS: Acquired immune deficiency syndrome; CoD: cause of death; F: Female; HIV: Human immunodeficiency virus; ICD: International Classification of Diseases; M: Male; NCD: Non-communicable disease; TB: tuberculosis; WHO: World Health Organization

Supplementary table 5:2. Criteria used by clinical panels to assign study-defined CoD and associated certainty

Study-defined category	Certainty		
	'Definite'	'Probable'	'Possible'
Death due to TB in an HIV positive individual	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History and clinicopathological features consistent with active TB disease AND Microbiological evidence of TB (culture positive or Xpert® MTB/RIF positive)	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History suggestive of active TB disease AND Positive sputum smear, urine LAM or chest x-ray	History suggestive of HIV and active TB, but no clear evidence available
Death due to other HIV/AIDS-associated cause (excluding TB)	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History and clinicopathological findings consistent with an AIDS-defining or HIV-associated condition (other than TB), or a condition due to ART (only applicable if ART use documented) AND Microbiological/histological/cytological evidence for one of the above	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History consistent with an AIDS-defining or HIV-associated condition (other than TB), or a condition likely due to ART (only applicable if ART use suggested) AND Documentation of one of the above conditions by a senior clinician	History suggestive of an AIDS-defining or HIV-associated condition (other than TB) in a person thought to be HIV-positive or a condition thought to be due to ART
Death due to cause unrelated to HIV in an HIV-positive adult	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History and clinicopathological findings consistent with a condition unrelated to HIV	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History consistent with a condition unlikely to be related to HIV	Documented evidence of positive HIV point-of-care test or ELISA OR patient on ART AND History suggestive of a condition unlikely to be related to HIV
Death due to TB in an HIV-negative adult	Documented evidence of negative HIV point-of-care test or ELISA in the 90 days prior to death AND History consistent with active TB disease of a severity sufficient to cause death	Documented HIV-negative point-of-care test or ELISA over 90 days but less than 180 days prior to death) AND History suggestive of active TB disease of a severity sufficient to cause death	Patient self-reports HIV negative AND No other evidence of HIV disease or an HIV test

Study-defined category	Certainty		
	'Definite'	'Probable'	'Possible'
	AND Microbiological evidence of TB (culture positive, Xpert® MTB/RIF positive)	AND Positive sputum smear or urine LAM or chest x-ray	AND History suggestive of active TB disease (of a severity sufficient to cause death)
Death due to cause other than TB in an HIV-negative adult	Documented evidence of negative HIV point-of-care test or ELISA in the 90 days prior to death AND No evidence (history or investigations) of TB	Documented HIV-negative point-of-care test or ELISA over 90 days but less than 180 days prior to death) AND No evidence (history or investigations) of TB OR Possible evidence of TB disease (e.g.: suggestive symptoms) but clinical history not consistent	Patient self-reports HIV negative AND No other evidence of HIV disease or HIV test AND No evidence (history or investigations) of TB OR Possible evidence of TB infection/previous exposure but clinical history not consistent
Death due to indeterminate cause	Cause of death unclear		

AIDS: Acquired immune deficiency syndrome; ART: antiretroviral therapy; CoD: cause of death; ELISA: Enzyme-linked immunosorbent assay; HIV: human immunodeficiency virus; LAM: lipoarabinomannan; TB: tuberculosis

Supplementary table 5:3. CoD for decedents with 'Autopsy' data: ICD-10 immediate and underlying CoD, as assigned by reviewers; grouped ICD-10 category; and study-specific categories as assigned by clinicopathological panel (L3), PCVA, InterVA-4, and SmartVA-Analyze (n=34)

ID†	Level three CoD				Physician-certified VA CoD				InterVA-4 CoD	SmartVA-Analyze CoD
	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	Grouped ICD-10	
1	HIV disease		HIV/AIDS-related	Indeterminate	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV/AIDS-related	HIV/AIDS-related
2	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Pulmonary TB	HIV/AIDS-related
3	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV/AIDS-related	Other NCD
4	Cryptococcal disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV/AIDS-related	HIV/AIDS-related
5	Gastroenteritis	HIV disease	Other infectious	HIV/AIDS, excl. TB	Stroke	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Other NCD	Other infectious
6	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Bacterial meningitis	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Non-HIV malignancy	HIV/AIDS-related
7	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Bacterial infection	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	Other NCD
8	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV/AIDS-related	HIV/AIDS-related
9	Bacterial pneumonia	HIV disease	HIV/AIDS-related	TB in HIV-positive	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Pulmonary TB	HIV/AIDS-related
10	Salmonellosis	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	Other infectious	Non-HIV malignancy
11	PCP	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Gastroenteritis	HIV disease	Other infectious	HIV/AIDS, excl. TB	HIV/AIDS-related	HIV/AIDS-related
12	CNS disorder	HIV disease	HIV/AIDS-related	Indeterminate	Stroke	HIV disease	HIV/AIDS-related	Non-HIV in HIV-positive	Other NCD	Indeterminate
13	Bacterial pneumonia	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Pulmonary TB	HIV/AIDS-related

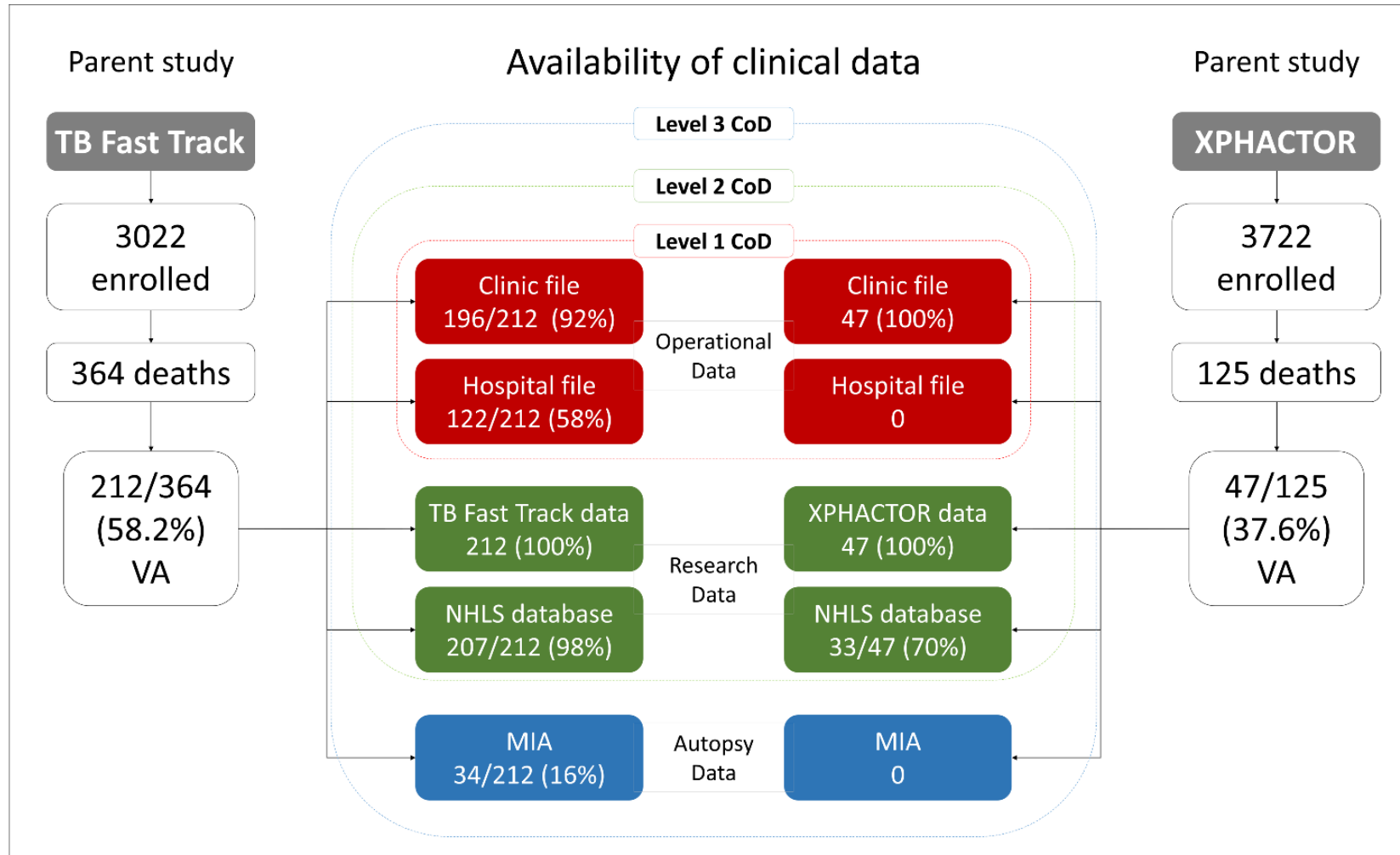
ID†	Level three CoD				Physician-certified VA CoD				InterVA-4 CoD	SmartVA-Analyze CoD
	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	Grouped ICD-10	
14	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Gastroenteritis	HIV disease	Other infectious	HIV/AIDS, excl. TB	Other infectious	Other infectious
15	LRTI	HIV disease	HIV/AIDS-related	Indeterminate	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	Other NCD
16	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Peptic ulcer	EPTB	Other NCD	TB in HIV-positive	Non-HIV malignancy	Non-HIV malignancy
17	Indeterminate	HIV disease	HIV/AIDS-related	Indeterminate	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Other NCD	HIV/AIDS-related
18	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	HIV/AIDS-related
19	Cryptococcal disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	PE	HIV disease	HIV/AIDS-related	Non-HIV in HIV-positive	Non-HIV malignancy	Indeterminate
20	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Liver disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	Indeterminate
21	Bacterial pneumonia	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Pneumonia	Type two diabetes	Other infectious	Non-TB in HIV-negative	Other infectious	Other NCD
22	Bacterial pneumonia	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Gas gangrene	HIV disease	HIV/AIDS-related	Non-HIV in HIV-positive	Non-HIV malignancy	Other NCD
23	NTM disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Chronic renal failure	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	Other infectious
24	NTM disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	PTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Pulmonary TB	Indeterminate
25	Transport accident		External/trauma	Non-HIV in HIV-positive	Injuries to spine and trunk	Transport accident	External/trauma	Non-TB in HIV-negative	Pulmonary TB	HIV/AIDS-related
26	EPTB	HIV disease	HIV/AIDS-related	TB in HIV-positive	GI obstruction		Other NCD	Non-TB in HIV-negative	Other NCD	Indeterminate
27	NTM disease	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	Other infectious

ID†	Level three CoD				Physician-certified VA CoD				InterVA-4 CoD	SmartVA-Analyze CoD
	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	'Immediate'	'Underlying'	Grouped ICD-10	Study-defined	Grouped ICD-10	
28	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	HIV/AIDS-related
29	Nocardiosis	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	HIV/AIDS-related
30	Pneumonia	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	HIV disease		HIV/AIDS-related	HIV/AIDS, excl. TB	HIV/AIDS-related	4. Indeterminate
31	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Gastroenteritis	HIV disease	Other infectious	HIV/AIDS, excl. TB	HIV/AIDS-related	Other infectious
32	Disseminated TB	HIV disease	HIV/AIDS-related	TB in HIV-positive	Bacterial pneumonia	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Pulmonary TB	4. Indeterminate
33	CMV	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Hepatic failure	PTB	Other NCD	TB in HIV-positive	HIV/AIDS-related	HIV/AIDS-related
34	PCP	HIV disease	HIV/AIDS-related	HIV/AIDS, excl. TB	Hepatic failure	PTB	Other NCD	HIV/AIDS, excl. TB	Pulmonary TB	PTB

†ID denotes chronological order in which participants died

AIDS: Acquired immune deficiency syndrome; CMV: cytomegalovirus; CNS: central nervous system; CoD: cause of death; excl.: excluding; EPTB: Extrapulmonary tuberculosis; GI: gastrointestinal; HIV: Human immunodeficiency virus; ICD: International Classification of Diseases; L3: level three reference cause of death ('operational', 'research', and 'autopsy data'); LRTI: lower respiratory tract infection; NCD: non-communicable disease; NTM: Non-tuberculous mycobacteria; PCP: *Pneumocystis pneumonia*; PCVA: physician-certified verbal autopsy; PE: pulmonary embolism; PTB: pulmonary tuberculosis; TB: tuberculosis; VA: verbal autopsy

Supplementary figure 5:1. Numbers of individuals enrolled to each of the parent studies, number of deaths, number of VAs conducted, data sources for clinical cause of death assignment, data availability by parent study, and which data contributed to different CoD levels



CoD: cause of death; MIA: minimally-invasive autopsy; NHLS: National Health Laboratory Services; TB: tuberculosis; VA: verbal autopsy

Chapter 6. Research paper 4:

Performance of verbal autopsy methods in estimating HIV-associated mortality among adults in South Africa

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6.1. Cover page

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Research paper cover sheet

Section A: Student details

Student	Aaron Karat
Principal supervisor	Alison Grant
Thesis title	An autopsy study exploring the spectrum of disease in individuals with advanced HIV in primary care clinics in South Africa

If the research paper has previously been published, please complete Section B.
If not, please go to Section C.

Section B: Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion.	
Have you retained copyright for the work?*	Was the work subject to academic peer-review?

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

Section C: Prepared for publication, but not yet published

Where is the work intended to be published?	Lancet HIV
Please list the paper's authors in the intended authorship order:	Aaron S Karat, Noriah Maraba, Mpho Tlali, Salome Charalambous, Violet N Chihota, Gavin J Churchyard, Katherine L Fielding, Yasmeen Hanifa, Suzanne Johnson, Kerrigan McCarthy, Kathleen Kahn, Daniel Chandramohan, Alison D Grant
Stage of publication	Not yet submitted

Section D: Multi-authored work

For multi-authored work, give full details of your role in the research include in the paper and in the preparation of the paper (attach a further sheet if necessary). **The candidate was involved in data collection and management, conducted all analyses, and wrote the paper.**

Student signature: 

Date: 24 May 2017

Supervisor signature: 

Date: 25 May 2017

6.2. Manuscript

Short title

Performance of VA to estimate HIV deaths in South Africa

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6.2.1. Abstract

6.2.1.1 Background

Causes of death (CoD) among HIV-positive adults are difficult to measure, and global estimates of HIV-associated mortality are partially derived through mathematical modelling. Antiretroviral therapy (ART) is changing mortality patterns; verbal autopsy (VA) is to be integrated into civil registration systems, but has not undergone robust validation for HIV-associated CoD. This study aimed to assess the sensitivity and specificity of VA questions in detecting HIV status and ART initiation, and to compare the HIV-associated mortality fractions assigned by the three leading VA interpretation methods.

6.2.1.2 Methods and findings

HIV-positive adults (≥ 18 years) in South Africa who died after enrolment to one of three studies of tuberculosis (TB) diagnosis and/or treatment, and HIV-negative adults who died in hospitals from non-traumatic and non-maternal causes were included. The 2012 World Health Organization VA instrument was used with questions added around ART; CoD were assigned by physician-certified (PCVA) and computer-coded (CCVA) methods (InterVA-4 and SmartVA-Analyze).

Data from 356 HIV-positive and 103 HIV-negative adults were analysed: sensitivity and specificity of VA questions were 84.3% (95% confidence interval [CI] 80–88) and 94.2% (95% CI 88–98) in assigning HIV status, and 91.0% (95% CI 86–95) and 53.2% (95% CI 43–64) in detecting ART initiation. Of 356 HIV-positive individuals, 283 (79.5%) were assigned an HIV-associated CoD by PCVA, 166 (46.6%; cause-specific mortality fraction [CSMF] 44.7%) by InterVA-4, and 80 (22.5%) by SmartVA-Analyze. Agreement between VA methods was poor at individual and population levels (Cohen's kappa 0.05 and 0.04 and CSMF accuracy 56% and 28% for PCVA vs. InterVA-4 and PCVA vs. SmartVA-Analyze, respectively). All VA interpretation methods showed high specificity in assigning HIV-associated CoD, at 96.1%, 89.3%, and 95.1% for PCVA, InterVA-4, and SmartVA-Analyze, respectively.

This study was limited by inability to estimate sensitivity of VA methods (because of the absence of a reference CoD); HIV-positive and HIV-negative groups were not age-matched, which may have led to overestimation of specificity; and narrative data were not provided to SmartVA-Analyze, which may have influenced its accuracy in assigning CoD.

6.2.1.3 Conclusions

VA interpretation methods gave very different estimates of HIV-associated mortality, and CCVA methods underestimated the HIV-associated mortality fraction. However, VA questions were sensitive and specific in detecting HIV status and sensitive in detecting ART initiation. To track progress towards international targets to reduce HIV-associated mortality, adjustments to automated VA methods and changes to classification systems are needed that allow for estimation of all CoD among HIV-positive individuals.

6.2.2. Introduction

An estimated 1.2 million individuals died due to HIV-associated causes in 2015; 75% of these deaths occurred in low- and middle-income countries (LMIC) [1]. The Joint United Nations Programme on HIV/AIDS (UNAIDS) aims to bring down the number of AIDS-related deaths to under 0.5 million by the year 2020 [2]. Mortality data form the bedrock of health research and policy agendas [3,4] but, at present, there are few direct estimates of overall or cause-specific mortality among HIV-positive individuals in LMIC. Figures from the Global Burden of Disease study are arrived at through complex mathematical models that use data from a variety of sources, including civil registration and vital statistics (CRVS) systems, seroprevalence surveys, and antenatal and antiretroviral therapy (ART) monitoring programmes [1,5–7]. Estimates published by UNAIDS are developed through similar methods [8]. The scarcity of reliable cause of death (CoD) data suggests it will be extremely difficult to track progress towards the ambitious goals set by UNAIDS.

The direct estimation of HIV-associated mortality is challenging. The disease remains associated with considerable stigma in many areas; this may lead to the diagnosis being omitted from death certificates, even when the certifying physician is aware of an individual's HIV status and considers the CoD to be HIV-related [9–11]. A number of studies have shown poor agreement between CoD listed on death certificates and those estimated through clinical or autopsy methods [12–15]. Even when death certificates are correctly completed, many countries do not have robust CRVS systems; between 2009 and 2013, over 80% of countries in the African region had incomplete or no CRVS death data available [16–19]. In these situations, estimates of cause-specific mortality are generated through verbal autopsy (VA), a structured interview with a relative or carer of a deceased individual [7], conducted at health and demographic surveillance system (HDSS) sites. VA data can be interpreted using several methods, all of which assign CoD per International Classification of Diseases (ICD) rules. For demographic purposes, most countries use only a single 'underlying' CoD for each decedent, thereby classifying all HIV-associated CoD under one of five ICD codes: B20, *HIV disease resulting in infectious and parasitic diseases*; B21, *HIV disease resulting in malignant neoplasms*; B22, *HIV disease resulting in other specified diseases*; B23, *HIV disease resulting in other conditions*; or B24, *Unspecified HIV disease* [20].

HIV-positive individuals who die from causes thought to be unrelated to HIV are likely to be increasingly important in monitoring the evolution of the epidemic. The global rollout of ART has already had an impact on all-cause mortality in

areas of high HIV prevalence [21–23] and cause-specific patterns are certain to change as more individuals receive treatment [24,25]. Recent guidelines advocating ART for all people living with HIV will likely accelerate this process [26], but current national and global systems do not allow for the estimation of all-cause mortality among HIV-positive individuals, estimates which are needed to distinguish deaths due to HIV-related immunosuppression from other CoD among HIV-positive people. The World Health Organization (WHO) standardised VA instrument does ask about HIV status but, until very recently, did not include questions about ART [27]. Although VA has been used extensively in areas of high HIV prevalence [28–30], it has not been validated against a robust gold standard for HIV- and tuberculosis (TB)-related deaths [31].

This article describes an analysis of data from a study partly nested within three large studies of HIV and TB in South Africa. The aims were to estimate the sensitivity and specificity of existing WHO VA questions in detecting HIV status and of additional questions in detecting ART initiation, compared to a reference standard of confirmed HIV status from clinical and research data; the proportions of deaths among HIV-positive adults attributed by VA methods to HIV-associated causes; and the specificity of VA interpretation methods in assigning HIV-associated CoD, compared to confirmed and VA-reported HIV status.

6.2.3. Methods

6.2.3.1 Parent studies

This study was nested within three large studies conducted in South Africa between 2012 and 2015. (1) ‘TB Fast Track’ was an open, cluster-randomised trial of empirical TB treatment in ambulatory adults with advanced HIV who were not on ART or TB treatment at the point of enrolment; it was conducted across 24 primary health care clinics (PHCs) in three provinces of South Africa and completed follow-up in June 2015 [32]. (2) ‘XPHACTOR’ was an interventional cohort study investigating the use of Xpert® MTB/RIF in a systematic sample of HIV-positive adults attending out-patient clinics for HIV care; participants were followed up for at least three months. (3) ‘XTEND’ was an evaluation of the impact on mortality of Xpert® MTB/RIF rollout; it enrolled adults, both HIV-positive and HIV-negative, from 40 PHCs across South Africa having sputum sent for TB investigation and followed them up for six months [33].

6.2.3.2 HIV-negative individuals

To estimate the specificity of the VA question “Was there any diagnosis of HIV/AIDS?” in detecting HIV status, VAs were conducted among confirmed HIV-negative adults who died in one of five TB Fast Track referral hospitals. Based on an

estimated sample of 329 VAs in HIV-positive individuals and a predicted VA specificity of 90% in assigning HIV-associated deaths (based on figures from a large study in five African countries [29]), a target was set of 121 VAs in HIV-negative adults (giving an HIV prevalence of 73%) to achieve 95% confidence intervals (CI) of width $\pm 5\%$ around a point estimate of specificity of 90%. Registers of in-patient deaths were reviewed at hospital mortuaries; all adults who died between June 2014 and October 2015 with a hospital-assigned CoD that was not explicitly HIV-associated, traumatic, or maternal were included for further review. Inclusion was initially restricted to individuals aged 18–55 years to be similar in age to HIV-positive decedents, but due to the low numbers of deaths among young individuals from non-HIV, non-traumatic, and non-maternal causes, this was expanded to 18–70 years. The name, sex, date of birth, date of death, and hospital identifiers of each included adult were recorded; hospital files and the National Health Laboratory Service (NHLS) online database were then searched for HIV test results. For those with a negative HIV test (rapid test or Enzyme Linked Immunosorbent Assay [ELISA]) in the one year prior to death and no evidence of a subsequent positive test, the date of negative HIV test and contact details of next-of-kin, where available, were recorded. The relatives of these individuals were contacted and a VA conducted per standard procedures; all decedents were assigned a unique identifier to ensure anonymity.

6.2.3.3 Data collection

VAs were conducted for as many individuals as possible who died after enrolment to one of the parent studies; trained lay-interviewers conducted the VA at the home of the family/carers or at another location of their choosing, one to twelve months after the death of the individual, as recommended by WHO [27]. All interviews were conducted using the WHO 2012 VA instrument [34], with questions around ART initiation, treatment for TB, and adherence to treatment added by the study team (Supplementary table 6:1). Questions around ART and TB treatment were asked only to respondents who had answered “Yes” to either the “Was there any diagnosis of HIV/AIDS?” or the “Was there any diagnosis of tuberculosis?” question, respectively. For example, questions around ART initiation were not asked if a respondent reported that the decedent in question was HIV-negative.

VA data were interpreted by both physician-certified verbal autopsy (PCVA) and computer-coded verbal autopsy (CCVA). The PCVA method was based on WHO recommendations, modified in line with changes made at the Medical Research Council/Wits University Agincourt HDSS site, South Africa [35]. Two physicians, blinded to all other clinical information, including information on ART obtained through VA, independently reviewed standard VA data and

separately assigned CoD using ICD-10 codes. Assigned CoD were compared and, where there were discrepancies, the cases were discussed by the two physicians, aiming for consensus. If a consensus could not be reached, a third, independent physician reviewed the data; if the CoD assigned by physician 3 matched that assigned by physicians 1 or 2, it was considered the final CoD. If no consensus was reached after review by three physicians, the individual was assigned an 'indeterminate' CoD.

6.2.3.4 Reference definitions

Clinical data to confirm HIV status, ART initiation, TB diagnosis, and TB treatment were obtained from clinic and hospital files, parent study databases, and the NHLS online database. Data were available for all decedents regarding HIV status and for all HIV-positive individuals regarding initiation of ART. An individual was considered HIV-positive if a positive test result had been recorded at any point prior to death and HIV-negative if a negative test result had been recorded in the one year prior to death without a subsequent positive result. An individual was considered to have initiated ART if this had been recorded in a clinic, hospital, or research file; estimation of adherence to treatment was not possible based on the data available. Self-report was not considered sufficient evidence of HIV status (positive or negative) or ART initiation. A death was considered HIV-associated if it was assigned an underlying CoD of one of ICD-10 codes B20–B24 by PCVA, an 'HIV/AIDS' CoD by InterVA-4, or an 'AIDS' CoD by SmartVA-Analyze; a death from any other cause was considered non-HIV-associated.

6.2.3.5 Data management and statistical analyses

Quantitative VA data were entered directly into an online database (Mobenzi Technologies, Durban, South Africa) through a cell phone interface; narrative data were captured on paper. Data collected from hospital files and the NHLS database were entered into an EpiData v3.1 database (The EpiData Association, Odense, Denmark) and data from the parent studies into SQL databases (Bytes Technology Group, Johannesburg, South Africa). VA data were mapped to the WHO 2014 instrument and fed into InterVA-4.03 (<http://www.interva.net/>), with the prevalence of malaria set to 'Low' and HIV/AIDS to 'High'. InterVA-4 cause-specific mortality fractions (CSMFs) were generated by dividing the sum of the likelihoods of each cause category by the sum of likelihoods for all causes [36]; estimations of specificity and individual agreement used the 'most likely' assigned cause for comparison. VA data were also mapped to the Population Health Metrics and Research Consortium (PHMRC) full instrument and fed into SmartVA-Analyze v1.1.1 (<http://www.healthdata.org/verbal-autopsy/tools>), with 'Malaria region', 'Health Care Experience', and 'Free text' options deselected. Narrative data were not provided to SmartVA-Analyze as they were captured on paper. The

Mortality Medical Data System (MMDS) 2011 software package

(ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/Software/mmds/2011) was used to generate a single 'underlying' CoD from ICD-10 codes assigned by PCVA. All analyses were conducted using Stata v14 (StataCorp, College Station, TX, USA).

Individuals with unknown HIV status were excluded from all analyses. For estimation of sensitivity and specificity, answers to VA questions of 'Do not know' were re-coded as 'No', to match the methods used by both InterVA-4 and SmartVA-Analyze [36–39]. The sensitivity and specificity of VA questions regarding HIV status and ART initiation were calculated with exact binomial 95% CI. The specificities of the three VA methods in assigning HIV-associated CoD were calculated compared to both confirmed HIV status, collected from clinical records, and VA-reported HIV status, to simulate situations in which clinical HIV data may not be available. Reference CoD were not available for all decedents and, because HIV-positive individuals may die from a range of causes, including those unrelated to HIV, sensitivity of the VA methods in assigning CoD could not be estimated. In line with previous recommendations [40,41], Cohen's kappa (K) and chance-corrected concordance (CCC) were used to measure individual agreement between VA methods and Lin's concordance correlation coefficient (ρ_c), CSMF accuracy, and chance-corrected CSMF (CCCSMF) accuracy used to measure population-level agreement based on two possible CoD: HIV-associated and non-HIV-associated (as defined above).

6.2.3.6 Ethical considerations

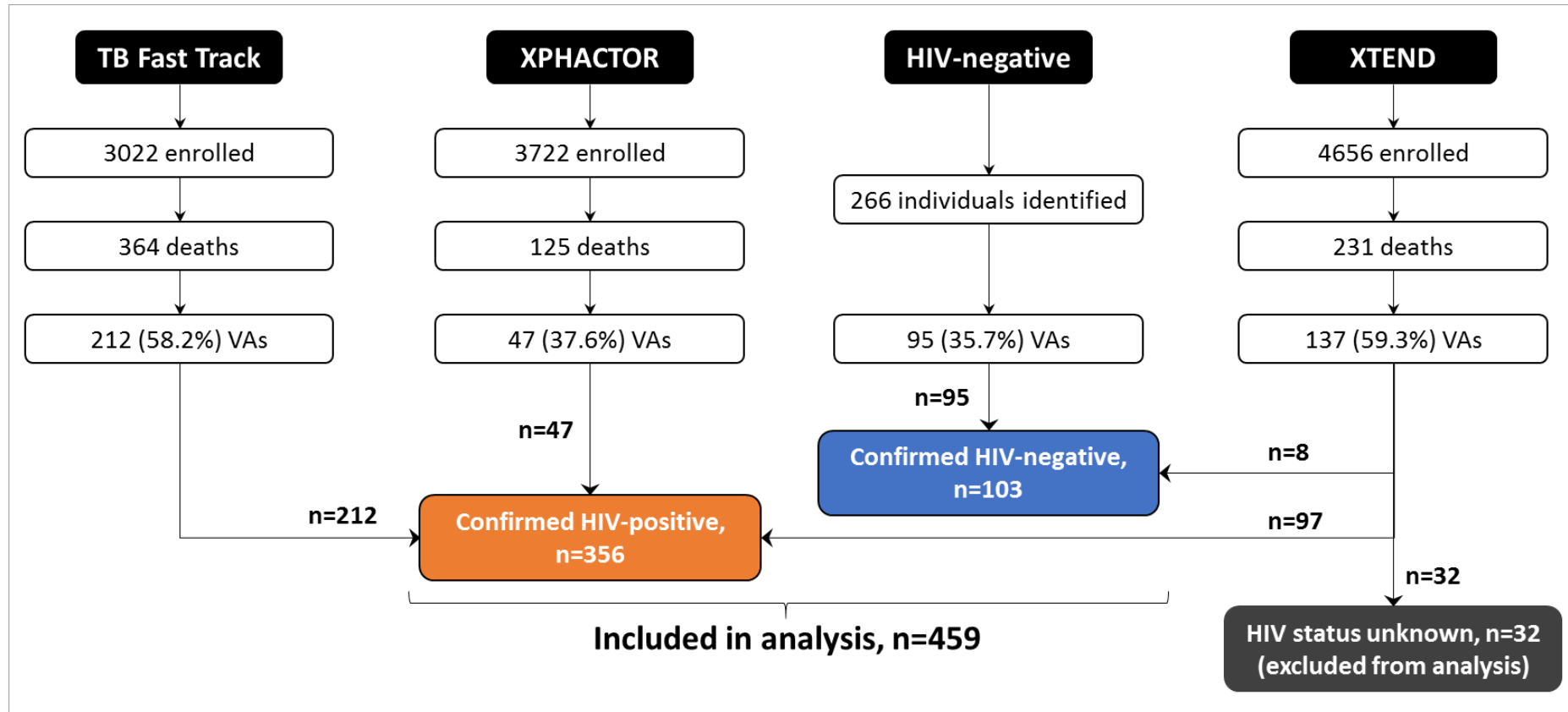
Separate approvals were obtained for this sub-study from the human research ethics committees of the London School of Hygiene & Tropical Medicine and the University of the Witwatersrand. All decedents were assigned numeric identities to ensure anonymity during collection of clinical data and all VA respondents gave written informed consent for interview.

6.2.4. Results

6.2.4.1 Demographics

VA data from 459 individuals were included, 356 (77.6%) HIV-positive and 103 (22.4%) HIV-negative (Figure 6:1; Table 6:1). Overall, 240 (52.3%) were female and the median age was 41.5 (interquartile range [IQR] 34–52) years. Further demographics are described in Table 6:1; a comparison between HIV-positive and HIV-negative individuals showed important differences only in median age (39.6 vs. 52.1 years; $p < 0.001$).

Figure 6:1. Flow diagram showing numbers enrolled into each of the parent studies*, subsequent deaths, numbers of verbal autopsies completed, and numbers of confirmed HIV-positive and HIV-negative individuals included in final analysis



*TB Fast Track and XPHACTOR enrolled only HIV-positive adults; XTEND enrolled HIV-positive and HIV-negative adults being investigated for TB

HIV: human immunodeficiency virus; TB: tuberculosis; VA: verbal autopsy

Table 6:1. Demographics for all decedents, stratified by confirmed HIV status (n=459)

Characteristic	All (n=459) n (%) or median (IQR)	Confirmed HIV-positive (n=356) n (%) or median (IQR)	Confirmed HIV-negative (n=103) n (%) or median (IQR)	p‡
Female	240 (52.3)	195 (54.8)	45 (43.7)	0.05
Age at death, years	41.5 (33.6–51.5)	39.6 (33.0–47.4)	52.2 (42.4–60.9)	<0.001
Black African	457 (99.6)	354 (99.4)	103 (100)	0.45
South African national	433 (94.3)	336 (94.4)	97 (94.2)	0.94
Enrolled or hospitalised in peri-urban area*	330 (71.9)	253 (71.1)	77 (74.8)	0.46
Initiated ART after enrolment†	117 (25.5)	117 (32.9)	NA	-
Time from				
Enrolment† to death, days	80.5 (35–161) (n=356)	80.5 (35–161) (n=356)	NA	-
HIV-negative test to death, days	14 (5–59) (n=103)	NA	14 (5–59)	-
Death to VA, days	218.5 (106–325)	235 (102–338)	174 (110–271)	0.04

*Site of enrolment for individuals enrolled to one of the three parent studies; site of hospitalisation for HIV-negative individuals recruited from hospitals

†Enrolment into parent study

‡Kruskal-Wallis or Chi-squared test, as appropriate

ART: antiretroviral therapy; HIV: human immunodeficiency virus; IQR: interquartile range; VA: verbal autopsy

6.2.4.2 Sensitivity and specificity of VA questions

The VA question, “Was there any diagnosis of HIV/AIDS?” correctly identified 300/356 (sensitivity 84.3% [95% CI 80.1–87.9]) HIV-positive individuals and incorrectly identified 6/103 (specificity 94.2% [95% CI 87.8–97.8]) HIV-negative individuals as HIV-positive (K 0.68; CCC 0.69; Table 6:2). The question “Did the deceased ever take ART?”, added to the WHO VA instrument, was asked to the 306 individuals who answered ‘Yes’ to the HIV diagnosis question: 193/212 (sensitivity 91.0% [95% CI 86.4–94.5]) individuals who had initiated ART were correctly reported as having done so, but the question was also answered ‘Yes’ for 44/94 (specificity 53.2% [95% CI 42.6–63.6]) individuals who had not initiated ART (K 0.48; CCC 0.82).

6.2.4.3 ART initiation dates

Dates of ART initiation were available from both VA interviews and clinical records for 125 individuals. The median difference between the dates obtained from the two sources was 29 (IQR 11–111) days; 90/125 (72.0%) and 108/125 (86.4%) ART initiation dates obtained from VA interviews were within 90 days and one year, respectively, of the dates in clinical records. Of the 237 individuals who were reported, at VA, to have been taking ART, 36 (15.1%) were reported not to have been taking it at death and 62 (26.2%) not to have been taking it every day.

Table 6:2. Sensitivity, specificity, and measures of agreement of VA questions regarding HIV status and ART initiation (n=459)

VA question	Answers			Sensitivity†, % (95% CI)	Specificity†, % (95% CI)	Overall agreement†‡, %	Cohen’s kappa† (95% CI)	CCC†
	Yes	No	DNK					
Was there any diagnosis of HIV/AIDS? (n=459)	306 (66.7)	111 (24.2)	42 (9.2)	84.3 (80.1–87.9)	94.2 (87.8–97.8)	86.5	0.68 (0.60–0.74)	0.69
Did s/he ever take ART? (n=306)*	237 (77.5)	50 (16.3)	19 (6.2)	91.0 (86.4–94.5)	53.2 (42.6–63.6)	81.4	0.48 (0.37–0.59)	0.82

*VA respondents were only asked about ART initiation if they answered ‘Yes’ to HIV question, therefore confirmed HIV-positive individuals who were not reported as such by the respondent were not included in this analysis. Of the 306 individuals reported as HIV-positive at VA, 212 (69.3%) had initiated ART and 94 (30.7%) had not.

†‘No’ and ‘Do not know’ answers combined for analysis.

‡Overall agreement is the proportion considered ‘positive’ or ‘negative’ by both clinical and VA methods (i.e., [true positives + true negatives]/total).

AIDS: acquired immune deficiency syndrome; ART: antiretroviral therapy; CCC: chance-corrected concordance; CI: confidence interval; DNK: do not know; TB: tuberculosis; VA: verbal autopsy

6.2.4.4 Performance of three VA interpretation methods

6.2.4.4.1 Estimating HIV-associated mortality among HIV-positive adults

Of the 356 HIV-positive adults in our study, 283 (79.5%) were assigned an HIV-associated CoD by PCVA, 166 (46.6%; CSMF 44.7% when all assigned CoD and associated likelihoods included) by InterVA-4, and 80 (22.5%) by SmartVA-Analyze (Table 6:3). These proportions were slightly higher among the 300 confirmed HIV-positive individuals who were also reported HIV-positive at VA (PCVA 255 [85%], InterVA-4 150 [50.0%; CSMF 47.6%], and SmartVA-Analyze 69 [23%]). Agreement between the three VA methods was uniformly poor, measured at both individual (CCC<0 for PCVA vs. InterVA-4 and PCVA vs. SmartVA-Analyze) and population levels (CSMF accuracy 56% for PCVA vs. InterVA-4 and 28% for PCVA vs. SmartVA-Analyze; Table 6:3; Figure 6:2).

6.2.4.4.2 Specificity in assigning HIV-associated CoD

Among all 459 individuals, PCVA assigned 287 (62.5%) an HIV-associated CoD, compared to 177 (38.6%; CSMF 37.1% when all assigned CoD and associated likelihoods included) by InterVA-4 and 85 (18.5%) by SmartVA-Analyze (Table 6:4). Compared to confirmed HIV status, PCVA had a specificity of 96.1% (95% CI 90.4–98.9); the specificities of InterVA-4 and SmartVA-Analyze were 89.3% (95% CI 81.7–94.5) and 95.1% (95% CI 89.0–98.4), respectively. All three methods had lower specificities when compared to VA-reported HIV status, at 81.0%, 83.0%, and 90.2% for PCVA, InterVA-4, and SmartVA-Analyze, respectively (Table 6:4).

Table 6:3. Number of confirmed HIV-positive individuals (N=356) assigned an HIV-associated CoD by three VA methods, stratified by VA-reported HIV status; and agreement between PCVA and CCVA methods

VA interpretation method	Number assigned an HIV-associated CoD				Agreement with PCVA†				
	Confirmed HIV-positive, n (CSMF; %/356)	VA-reported HIV status			Individual level		Population level		
		Positive, n (CSMF; %/300)	Negative, n (CSMF; %/23)	Do not know, n (CSMF; %/33)	K (95% CI)	CCC	ρ_c	CSMF accuracy (%)	CCCSMF accuracy (%)
PCVA	283 (79.5)	255 (85.0)	6 (26.1)	22 (66.7)	-	-	-	-	-
InterVA-4*	(44.7)	(47.6)‡	(28.0)‡	(29.7)‡	0.05 (0–0.13)§	-0.03§	-0.348	56.2	-18.9
SmartVA-Analyze	80 (22.5)	69 (23.0)	3 (13.0)	8 (24.2)	0.04 (0–0.09)	-0.52	-0.998	28.3	-94.8

*InterVA-4 CSMFs calculated from all assigned CoD with associated likelihoods

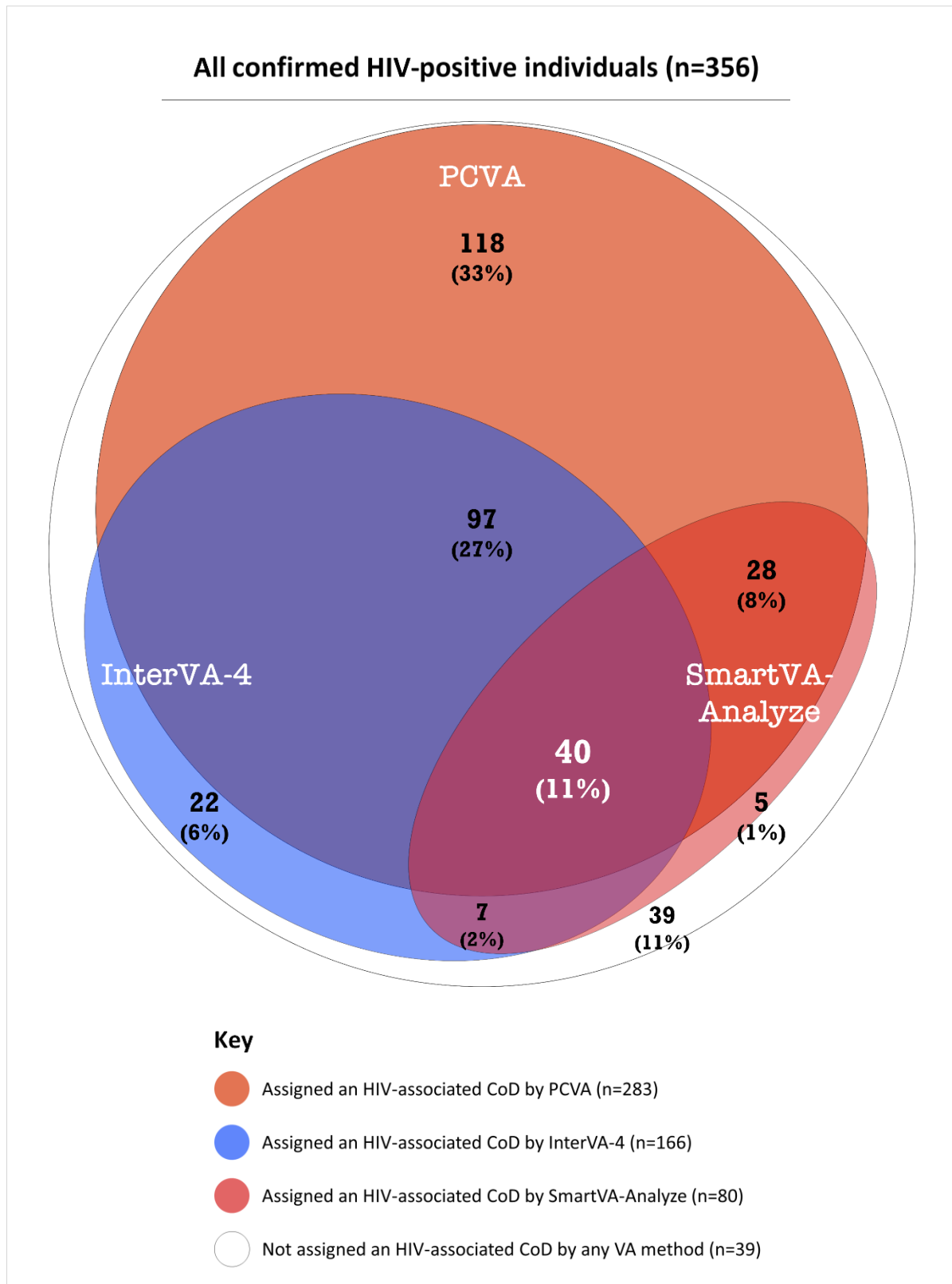
†Answers of 'Do not know' listed as 'Negative' for purposes of comparison

‡CSMF calculated separately per stratum

§Measures of individual agreement for InterVA-4 calculated using individuals assigned HIV/AIDS as 'most likely' CoD (n=166)

CCC: chance-corrected concordance; CCCSMF: chance-corrected cause-specific mortality fraction; CCVA: computer-coded verbal autopsy; CI: confidence interval; CoD: cause of death; CSMF: cause-specific mortality fraction; K: Cohen's kappa; PCVA: physician-certified verbal autopsy; ρ_c : Lin's concordance correlation coefficient; VA: verbal autopsy

Figure 6.2. Venn diagram illustrating the number of confirmed HIV-positive individuals assigned HIV-associated CoD by the three VA methods and overlap between methods (n, [%/356])



CoD: cause of death; PCVA: physician-certified verbal autopsy; VA: verbal autopsy

Table 6:4. Number assigned (n=459) and specificity of three VA methods in assigning HIV-associated causes of death, compared to confirmed (n=103) and VA-reported (n=153) HIV status

VA method	Number assigned an HIV-associated CoD, n (CSMF; %/459)	Specificity of VA method	
		Based on confirmed serostatus (95% CI)†	Based on VA-reported HIV status (95% CI)‡
PCVA	287 (62.5)	96.1 (90.4–98.9)	81.0 (73.9–86.9)
InterVA-4*	(37.1)	89.3 (81.7–94.5)§	83.0 (76.1–88.6)
SmartVA-Analyze	85 (18.5)	95.1 (89.0–98.4)	90.2 (84.3–94.4)

* InterVA-4 CSMFs calculated from all assigned CoD with associated likelihoods

† n=103 individuals confirmed HIV-negative

‡ n=153 individuals reported HIV-negative or with HIV status unknown

§ Individuals considered 'test positive' if HIV/AIDS assigned as most likely CoD (n=177)

AIDS: acquired immune deficiency syndrome; CI: confidence interval; CoD: cause of death; PCVA: physician-certified verbal autopsy; VA: verbal autopsy

6.2.5. Discussion

The VA question regarding HIV diagnosis showed moderate to high sensitivity and specificity in correctly identifying HIV status. Questions added by the research team regarding ART initiation were also sensitive and a high number of ART initiation dates obtained from VA were within three months of the confirmed date from clinical records. VA interpretation methods differed widely in their estimation of the HIV-associated mortality fraction among individuals confirmed HIV-positive; in particular, estimates by both CCVA methods were likely considerably lower than the true fraction. All VA interpretation methods showed high specificity in assigning HIV-associated CoD.

6.2.5.1 Detecting HIV prevalence and ART initiation

The moderate-to-high sensitivity and specificity of the HIV question in detecting HIV status seen here and in a study conducted in Malawi (sensitivity 83%, specificity 98%; n=842) [42] suggests that VA may be useful in generating estimates of HIV prevalence among deceased individuals. However, sensitivity was lower when tested in a larger, more diverse population: a study conducted across six sites in Uganda, Tanzania, Malawi, Zimbabwe, and South Africa between 1990 and 2011 focused primarily on estimating the specificity of InterVA-4 in diagnosing HIV-associated CoD [29]; crude estimates of sensitivity and specificity of the VA instrument in detecting HIV status can, however, be derived from the data presented (sensitivity 33.7% [95% CI 32.1–35.5], specificity 93.6% [95% CI 92.5–94.6]; n=4899).

Differences in sensitivity estimates may be attributable to increases in awareness, increases in availability of testing, and reductions in stigma over time, particularly as the multi-country study analysed deaths that occurred over a 20-

year period [29]. In the Malawian study, the proportion of respondents reporting knowledge of HIV status increased from 48% in 2003/04 (n=300) to 99% in 2013/14 (n=303) [42]. The consistent estimates of high specificity suggest that VA is unlikely to overestimate HIV prevalence; further evaluations of the VA question are needed, including re-analysis of existing raw VA data from HDSS sites, to better assess its sensitivity and suitability for assessing HIV prevalence in different contexts.

The only other study reported to have evaluated the sensitivity and specificity of VA questions in detecting ART use estimated them at 92% and 46%, respectively (Malawi, 2009–2014, n=154) [42]. The low specificity seen also in our study suggests a need to further refine questions around ART; HIV-positive individuals are often prescribed several different drugs and confusion among VA respondents is understandable. Inclusion of variants of these questions in future VA validation studies is recommended.

6.2.5.2 Estimating HIV-associated mortality

The high specificity of VA interpretation methods in assigning HIV-associated CoD seen in our study is consistent with previous evaluations. Several studies have described the specificity of PCVA in Uganda (92%, n=155) [43]; Tanzania (89%, n=168) [44]; and Malawi (99%, n=498) [30]. In the multi-country study referred to above [29], InterVA-4 assigned an 'HIV/AIDS' CoD with a specificity of 90.1% (95% CI 89–91; n=4629); a smaller evaluation of InterVA-4 in Malawi produced similar results (90.3% [n=498], derived from data presented) [30]; and specificities of 77% and 78% from two studies in Ethiopia were reported in a systematic review of VA accuracy among hospitalised patients [45]. No studies were found in the literature that assessed SmartVA-Analyze in this way.

The consistently high specificity of VA interpretation methods suggest that they are unlikely to overestimate HIV-associated mortality. Estimating the sensitivity of a VA method, however, is challenging even when high-quality data are available, given the inherent uncertainty involved in assigning CoD. Only one study, in South Africa, has compared VA to CoD derived from pathological autopsy in HIV-positive individuals, and found that VA methods likely underestimated HIV-associated CoD [46]. In part due to challenges with ICD coding, there are few direct (autopsy or clinical) estimates of the HIV-associated mortality fraction among HIV-positive adults. A recent systematic review, which included only studies of HIV-positive individuals entirely or mostly on ART (n=19 studies), estimated that 18.5% (95% CI 13–24) of deaths in HIV-positive individuals on ART in sub-Saharan countries were due to 'non-AIDS' causes (therefore 76–83% due to AIDS causes) [25]. This review, however, treated deaths due to ART toxicity as 'non-AIDS',

which may have led to an underestimation of overall HIV-associated mortality. In populations containing fewer individuals on ART, the HIV-associated mortality fraction is likely to be even higher, as seen in several pathological autopsy studies [47]. In our study, only PCVA estimated an HIV-associated mortality fraction close to the figure above (79.5%); estimates by both CCVA methods were much lower (44.7% and 22.5%). A high number of individuals in our study had not initiated ART; CCVA-generated estimates are therefore likely much lower than the true HIV-associated mortality fraction in this population.

6.2.5.3 CRVS integration

Plans are underway to integrate VA into CRVS systems [48–50], a proposal in part made feasible by the increased efficiency of CCVA methods. Data from this and other studies, however, suggest that the two most prominent CCVA methods are not, as yet, sensitive or consistent enough in assigning HIV-associated CoD in areas of high HIV prevalence [46,51]. Both methods likely allocate insufficient weight to the question regarding HIV diagnosis, which has now been shown to be sensitive and specific in Malawian and South African contexts. InterVA-4 assigns CoD based on a probabilistic algorithm, which weights particular questions per the recommendations of a panel of expert physicians, convened in 2006 [52]. In our study, physicians directly reviewing VA data gave the answer to the HIV question high importance, assigning an HIV-associated CoD to 85% of those reported HIV-positive and to 67% of cases where the respondent answered ‘Do not know’ (compared to 47% and 30% by InterVA-4 and 23% and 24% by SmartVA-Analyze [Table 6:4]). In part, this is likely because all ‘Do not know’ answers are converted to ‘No’ by both CCVA methods. Along with adjustments to weighting, an ability to differentiate between the two answers may be needed in the interpretation of key questions such as this.

SmartVA-Analyze is calibrated using CoD from the PHMRC gold standard dataset, which includes over 12,000 deaths from India, the Philippines, Mexico, and Tanzania [53,54]. Of 7,841 adult deaths, 443 (5.5%) were reported as HIV-positive during VA (227 [51%] in Tanzania) and 501 (6.4%) were assigned an ‘AIDS’ CoD by clinical methods. Even in Dar es Salaam, the site with the highest background HIV prevalence [55], the number of deaths among adults aged 15–49 years considered due to AIDS was 158/983 (16.1%); cause-specific estimates from the Global Burden of Disease study for the same period (2007–2010) suggest that HIV/AIDS accounted for 52–58% and 67–71% of deaths among individuals aged 15–49 years in Tanzania and South Africa, respectively [56]. Despite the random resampling of datasets conducted as part of the model’s training [53], it is unlikely that CSMFs will have been generated with HIV-

associated mortality fractions as high as seen in populations such as these, suggesting that recalibration may be needed for SmartVA-Analyze to be used more widely in areas of high HIV prevalence.

6.2.5.4 Next steps

The challenges involved in producing accurate estimates of HIV incidence, prevalence, and mortality are well documented [6,57]. Though changes in CSMFs may be estimated through measurement of mortality at a population level, the UNAIDS goal of less than 0.5 million HIV-related deaths by 2020 calls not for a reduction in the mortality fraction, but for an absolute reduction in events [2], which will require more accurate methods to identify individual HIV-associated deaths. Additionally, with patterns of HIV-associated mortality likely to undergo major change in the coming years, current definitions may not allow for all HIV-associated deaths to be counted [31]. The only reliable way to track progress towards this goal is if all-cause mortality among HIV-positive individuals is regularly estimated. Current classification systems require alterations that will allow for the identification of all deaths in HIV-positive individuals and better predict possible changes in mortality patterns through increased availability of ART. ICD-10 was adapted by WHO in 1994 and ICD-11 is not due for release until 2018 [58]. A system with greater flexibility, through shorter or partial revision cycles, would allow for more adaptability in the face of an evolving epidemic. In the short term, other methods may provide useful data in monitoring changes in mortality patterns: a recent study from Mozambique found good agreement between CoD assigned by minimally-invasive autopsy and complete autopsy [59]. These findings, and previous evaluations of this technique [60–62], suggest it may be useful in providing more accurate CoD data in LMIC as an adjunct to surveillance at sentinel sites.

6.2.5.5 Limitations and strengths

This study had limitations. The relatively low number of HIV-negative individuals reduced the accuracy of specificity estimates and, despite best efforts, the HIV-positive and HIV-negative groups were not age-matched. HIV is generally less likely to be considered as CoD in older individuals [63], though recent data from South Africa suggest this may no longer be appropriate [64], and the higher median age of those HIV-negative may have led to overestimation of the specificities of both the HIV diagnosis question in detecting HIV status and VA methods in assigning HIV-associated CoD. Estimates of the question's sensitivity, however, should not have been affected.

There were limitations also in the evaluation of VA interpretation methods. Physicians assigning CoD were aware that some decedents were likely to have been enrolled in studies of HIV/TB and this may have led to their assigning higher

numbers of HIV-associated CoD. The PHMRC VA instrument was not used for data collection and SmartVA-Analyze was not provided with narrative or health care experience data, which may have led to sub-optimal performance of the software, although the omission of these data will likely have had minimal effect, as the phrases 'HIV' or 'AIDS' are not classed by the software as words of interest [65]. Clinical reference CoD were not available for all decedents, meaning the sensitivity of VA methods in assigning HIV-associated CoD could not be assessed.

This study's strengths include having laboratory- or rapid test-confirmed HIV status for all those included in the analysis; the median time from HIV-negative test to death being two weeks, with 75% of HIV-negative individuals with a test result less than two months before death; and using the three leading VA interpretation methods to estimate the HIV-associated mortality fraction among HIV-positive individuals, something not previously done in VA studies.

6.2.6. Conclusions

VA interpretation methods gave very different estimates of HIV-associated mortality and CCVA methods underestimated the HIV-associated mortality fraction. However, VA questions were sensitive and specific in detecting HIV status and sensitive in detecting ART initiation. The addition of an HIV/TB module to the VA instrument, including questions on treatment dates and adherence, is suggested; future VA validation studies should be used to trial new or modified questions prior to inclusion in the standardised VA instrument. Modifications to classification systems are needed that allow for the inclusion of presumed non-HIV-associated deaths within estimates of mortality among HIV-positive individuals; closely aligned is a need for more accurate direct estimates of HIV-associated mortality to better track progress towards goals set by UNAIDS.

6.2.7. Acknowledgments

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6.4. Supplementary information

Supplementary table 6:1. Questions regarding antiretroviral therapy and treatment for TB added to the WHO 2012 VA instrument by the study team and the parent questions to which they were attached

From WHO 2012 VA instrument	Added by study team	
Parent question	Sub-question(s) (if parent question = 'Yes')	Sub-sub-question(s) (if sub-question = 'Yes')
Was there any diagnosis of Tuberculosis?	Did s/he ever take treatment for TB from a clinic/hospital?	When did s/he start TB treatment? (If treated more than once, record the most recent episode)
		Which clinic/health facility did s/he attend most recently (name)?
Was there any diagnosis of HIV/AIDS?	Did the deceased ever take Antiretroviral Therapy (ART)?	Location of clinic/health facility (nearest town)
		Were they still taking TB treatment at the time of death?
		If TB treatment was stopped early why was that?
		Was s/he taking medication every day in the way they s/he was supposed to?
		When did s/he start ART?
Was there any diagnosis of HIV/AIDS?	Did the deceased ever take Antiretroviral Therapy (ART)?	Which clinic/health facility did s/he attend most recently (name)?
		Location of clinic/health facility (nearest town)
		Were they still taking ART at the time of death?
		If ART was stopped, why was that?
		Was s/he taking the medication every day?

AIDS: acquired immune deficiency syndrome; ART: antiretroviral therapy; HIV: human immunodeficiency virus; TB: tuberculosis; VA: verbal autopsy; WHO: World Health Organization

Chapter 7. Summary, discussion, and conclusions

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7.1. Introduction

This thesis aimed to describe the spectrum of disease and causes of death in HIV-positive adults dying in South Africa. I endeavoured to do this through analysing novel data, collected from a population of mostly HIV-positive adults in South Africa, using MIA, VA, and abstraction from a variety of clinical sources, and through reviewing published reports of studies from LMIC describing tissue autopsy findings in HIV-positive adults, validation of VA for HIV- and TB-associated deaths, and directly estimated causes of death in HIV-positive adults. This chapter will provide a summary of the data presented in the thesis and relate the main findings to the existing literature; discuss some of the implications and potential applications of the work presented; examine the limitations of the methods used and some of the challenges faced in data collection and analysis; and present a summary set of recommendations and conclusions drawn from the findings described.

7.2. Discussion of findings and comparisons with literature

7.2.1. Prevalence of TB at autopsy is high among HIV-positive adults in LMIC

As detailed in Chapter 4, MIA was conducted in 34 HIV-positive adults who died after enrolment to the TB Fast Track study; evidence of active TB disease was found in 16 (47%) decedents, 14/16 (88%) individuals had extrapulmonary disease, and six (38%) had not been started on TB treatment between enrolment and death. Although the autopsy prevalence of TB in the Lesedi Kamoso study was similar to pooled estimates of prevalence from the 2015 systematic review by Gupta et al. [71] (discussed in more detail below and in Chapter 2.2.4.1.1) and more recent studies from Uganda [94] and Zambia [180], it is important to note that the individuals included in our study were enrolled in primary care clinics and followed-up prospectively, something not done before in studies in LMIC, and that around 25% died out of hospital. The majority of individuals included in previous autopsy studies were recruited and died in hospitals (see Chapter 2.2.3.2). Together with findings from a community-based autopsy study by Omar et al. [97] (which found high TB prevalence but did not report on the HIV status of decedents), and despite the relatively small sample size and potential lack of representativeness of the general HIV-positive population (see Chapters 4.2.5.3 and 7.4.1, below), our data suggest that TB is as important a cause of morbidity for individuals who receive most of their care in the community as it is for those treated mostly in secondary care.

The results from our field research are broadly supportive of the findings of my review of autopsy studies, presented in Chapter 2.2, which included data from over 5,000 autopsies in HIV-positive adults conducted between 1984 and 2013.

Evidence of active TB was found in around 37% (crude summary estimate) of the 2,999 autopsies that included the lungs, in around 18% of 971 autopsies that sampled only extra-pulmonary tissue, and in over 30% of 58 autopsies in studies that excluded individuals with an ante mortem diagnosis of TB. The heterogeneity of laboratory testing methods used, the differences in autopsy methods (complete vs. minimally-invasive approaches), and the estimation only of crude prevalence, without attempt at meta-analysis, all reduce the external validity of the summary results presented in this review. However, the inclusion of a range of studies with varying inclusion criteria also allowed for review of data that may not have been included by an attempt at more rigorous meta-analysis, as conducted by Gupta et al. in their systematic review of 2015 [71].

My review included 53 autopsy studies, 25 of which were also included the systematic review by Gupta et al., where they found similar (or higher) pooled prevalence of TB overall (40% [95% CI 32–47]) and in sub-Saharan Africa only (43% [95% CI 38–48]), as well as, among those with evidence of TB at autopsy, similar prevalence of extrapulmonary disease (88% [95% CI 82–97]) and proportions of individuals who were not diagnosed before death (46% [95% CI 33–59]). The criteria for inclusion in the Gupta review were much narrower than those I used in this thesis. As a result, the pooled summary estimates presented by the authors are likely more representative of the autopsy prevalence of TB among HIV-positive people in LMIC overall, particularly as the authors specifically excluded studies that focused on “sub-populations with potentially limited generalizability”, such as mine-workers or pregnant women, and those with a specific diagnosis, such as TB. The use of these narrow criteria, however, also means that estimates of autopsy prevalence of disease in these sub-populations were not included; comparing these data with more general estimates of prevalence would be useful, as strategies for prevention, diagnosis, and treatment are sometimes very specific to certain groups (e.g., mine-workers) and knowledge of differences in disease patterns may help to refine these approaches. In addition, the exclusion from the Gupta review of studies that included only individuals with a prior diagnosis of TB is a missed opportunity to examine the specificity of current TB diagnostic practices. A recurring theme in the debate around strategies to reduce mortality in individuals with advanced HIV is the value (or perceived lack thereof) of empiric TB treatment in this group [335,336] (for example, the hypothetical benefit of this approach was central to the design of the TB Fast Track study [148,155]). As is the case for HIV-related deaths, we do not, at present, have a reliable way to differentiate between people dying *from* TB and those dying *with* TB [337]; evaluation of the prevalence of TB disease among individuals who die after starting treatment for TB may be one way to help make this distinction.

My review of the literature does fill some of the gaps left by the Gupta review; the inclusion of a much wider range of studies means that data are available for some of the key groups discussed above. For example, the seminal work of Jill Murray and colleagues among mine-workers in South Africa is described [227] (TB was found in the cardiorespiratory organs of 21% of HIV-positive individuals), as is the study by Neil Martinson et al. [87], where autopsy evidence of TB was found in 79% of 47 individuals diagnosed with TB in a Johannesburg hospital. Importantly, my review included several studies which described findings only from extrapulmonary sites, something explicitly excluded by the Gupta review. As discussed in Chapter 2.2.4.1.1, the exclusion of these data from the systematic review may have led to the underestimation of overall TB prevalence at autopsy; these data can also be used to guide future efforts to diagnose TB disease in living patients.

7.2.2. TB is a leading cause of death in HIV-positive adults in LMIC

Data presented in this thesis, both from direct estimation of cause-specific mortality in HIV-positive people in South Africa using clinical, research, and autopsy data (Chapter 5), and from the systematic review of studies estimating cause-specific mortality in HIV-positive people dying in LMIC (Chapter 3), suggest that TB remains a leading cause of death in those who have access to ART and who initiate ART before death. As discussed in more detail below, this is likely to be largely because individuals start ART when their HIV disease is already at an advanced stage and the risk of death is extremely high.

Of the 259 HIV-positive adults assigned a 'Level 2' reference cause of death by clinical reviewers in the Lesedi Kamoso study (i.e., using clinical and research data, but not pathological data; Chapter 5.2.3.3.2), at least 69 (27%) were thought to have died from HIV-associated TB; 51/69 (74%) had initiated ART between enrolment and death (median 63 [IQR 30–125] days before death; $n = 46$). Among the 34 individuals with autopsy data available (also included in the 259 above), 14 (41%) were thought to have died from HIV-associated TB; 10/14 (72%) initiated ART after enrolment, a median 53 (IQR 30–108) days before death (Tables 5:3 and 5:4). Similarly, in the systematic review of studies that directly estimated causes of death in HIV-positive adults in LMIC, presented in Chapter 3, a pooled 29% (95% CI 24–34; random-effects meta-analysis; $n = 47$ studies; $n = 7,990$ deaths) of decedents were assigned a TB cause of death. This proportion did not change with the proportions of individuals initiated on ART per study (pooled 28% in 17 studies where <33% of participants initiated ART vs. 26% in 17 studies where >66% initiated ART; $p = 0.52$), though time

between ART initiation and death was reported by very few studies (Chapter 3.2.4.4.1). TB was considered the cause of death in a pooled 39% (95% CI 33–46) of individuals assigned an HIV-associated cause of death ($n = 5,688$).

There is evidence from large, population-based studies to support the assertion that ART is driving a reduction in overall mortality among HIV-positive people [264,272,338], and that it is starting to create a shift in cause-specific mortality patterns away from causes associated with immunosuppression, towards causes seen more often in the HIV-negative population [339,340]. This is also one of the main findings of Chapter 3, a systematic review of studies assigning causes of death at an individual level, where the proportion of deaths attributable to HIV-associated causes was seen to have an inverse relationship with the proportion of decedents in each study who had initiated ART (85% if <33% initiated ART, 75% if 33–65% initiated ART, and 71% if $\geq 66\%$ initiated ART; $p = 0.02$). However, despite good evidence to support the use of ART to reduce TB incidence among HIV-positive people overall [341] and reduce mortality among HIV-positive people receiving treatment for TB [342], changes such as those described above for all HIV-associated causes of death do not, as yet, appear to be taking place for overall mortality due to HIV-associated TB; estimated absolute numbers of deaths due to HIV-associated TB published by WHO have remained relatively static over the last 10 years (from $\sim 480,000$ in 2005 to $\sim 390,000$ in 2015; see Chapter 3.2.5.1).

Individuals with severe immunosuppression are at higher risk of death overall and particularly from death associated with HIV-associated TB [343–345]; the relatively small reduction in global HIV-associated TB-related mortality since the introduction of ART suggests that there remains a substantial proportion of the HIV-positive population who either do not have access to ART, or whose access to care is delayed to the point where ART alone is not enough to reduce sufficiently their risk of premature death. In the systematic review in Chapter 3, in the few included studies in that reported it, the median time from ART initiation to death was often less than 90 days, as was the median time on ART among individuals in the Lesedi Kamoso study who were assigned TB causes of death. Systematic reviews have consistently shown that overall mortality among HIV-positive individuals in LMIC, particularly those with low CD4 counts, is high in the first 3–6 months after initiating ART [23,25]. Encouraging all HIV-positive people to start ART, regardless of CD4 count or disease stage [346,347], will hopefully prevent individuals from ever becoming so severely immunosuppressed. Broader interventions to strengthen health systems, minimise barriers to care, and improve adherence to treatment [348,31,349,350] will also be critical in shaping a sustainable strategy to reduce HIV and TB mortality in the next decades.

7.2.3. Methods used to estimate causes of death in HIV-positive individuals are sub-optimal

Though several studies include estimation of cause-specific mortality and discussion around the potential implications of excessive mortality due to specific causes, particularly TB, in HIV-positive people, the process of estimating cause-specific mortality is less well discussed; methodological discussions appear to be reserved mostly for editorials or the appendices of statistical reports [351,72,84,85,11]. An issue that emerged several times in the development of this thesis is the high uncertainty around estimates of cause-specific mortality in HIV-positive individuals, most importantly around estimates of mortality due to HIV-associated TB (Chapters 1.2.2, 2.3.1, 3.2.2, 3.2.5.3, and 5.2.5.1). Some of this is due to the inherent difficulty in assigning causes of death in these individuals, particularly those with advanced immunosuppression, who may have several disease processes underway at one time. For example, among the 34 individuals in whom we conducted MIA as part of the Lesedi Kamoso study, 20 (59%) had culture and/or histological evidence of two or more infections at the same or different sites (Chapter 4.2.4.6 and Figure 4:2). In the autopsy studies reviewed in Chapter 2.2, high proportions of decedents had other infections: 19% had evidence of bacterial pneumonia, a further 10% evidence of other bacterial infections; 17% CMV disease; 16% toxoplasmosis; 10% *Pneumocystis pneumonia*; and 9% cryptococcal disease (crude summary estimates; Chapter 2.2.3.4 and Figure 2:2); any one of these conditions may lead to death in an individual with advanced HIV disease. Few studies conducted non-TB cultures or reported systematically on co-prevalent infections, but when this was done, evidence of more than one infection was often found (>25% of decedents in two studies that did not use non-TB cultures [94,195]; >40% in one study that did conduct aerobic and anaerobic cultures [184]; Chapter 2.2.4.1.2). When using study-defined codes to assign causes of death from data in the Lesedi Kamoso study, despite all decedents having been enrolled in large epidemiological studies and the extensive additional efforts undertaken to collect clinical and laboratory data, 69/259 (27%) individuals were still assigned an 'indeterminate' cause of death by clinical reviewers (Chapter 5.2.4.4). This was often because data were insufficient to make an assignment, or, more specifically, insufficient to distinguish between HIV-associated TB and other HIV-associated causes. As discussed further below, even when sufficient data are available to make this distinction, however, ICD-10 conventions make this process more complicated.

7.2.3.1 ICD-10 conventions are an obstacle to better estimates of mortality due to HIV-associated TB

The ICD-10 system includes HIV-associated TB in one of five, broad 'HIV' categories; 'TB' definitions explicitly exclude HIV-associated disease (see Chapters 1.2.3.1, 2.3.1, and 5.2.2). At a national level, most countries report only the three-character ICD-10 code, instead of the full, five-character code, of the single 'underlying' cause of death assigned

by automated systems such as MMDS and Iris (i.e., B20, “HIV disease resulting in infectious or parasitic diseases”, rather than B20.0, “HIV disease resulting in mycobacterial infection”). This does not allow governing bodies and groups producing estimates of national and global burdens of disease to differentiate between mortality due to HIV-associated TB and other HIV-associated infectious diseases and means that national and global estimates of mortality due to HIV-associated TB, even in many countries with functional civil registration systems, are generated through modelling of proxy measures, such as disease incidence and case-fatality rates, which are themselves often derived, in-part, from mathematical models [84].

I encountered these difficulties first-hand when conducting the work presented in this thesis. For example, in the systematic review of studies directly estimating causes of death in HIV-positive individuals in LMIC, presented in Chapter 3, nine of the 56 studies included did not describe the proportion of HIV-associated deaths that were TB-related. The Lesedi Kamoso study set out, as much as possible, to produce data comparable with previous research and with international systems used to measure mortality. Much of this involved ensuring that definitions and nomenclature were aligned with international norms, but we discovered that doing so would severely limit our ability to estimate mortality attributable to HIV-associated TB among decedents included in our study. We were, therefore, required to create additional, study-specific cause of death categories to allow us to estimate this mortality fraction (Chapter 5.2.3.4); attempts to assess VA performance in this regard both in the literature (Chapter 2.3.3.2.1) and among decedents in our study (Table 5:5) were also hampered by ICD-10 coding conventions.

7.2.3.2 VA does not differentiate well between HIV-associated TB and other HIV-associated causes of death

VA is used routinely at HDSS sites in areas of high HIV and TB prevalence and VA data are increasingly being used to supplement to other sources of mortality data, for example, as part of large-scale exercises estimating cause-specific mortality (e.g., the Global Burden of Disease study, conducted by IHME [352]), or to help strengthen CRVS systems in specific countries [353,115]. To assess the ability of the three leading VA interpretation methods to estimate cause-specific mortality among HIV-positive adults and, more specifically, mortality due to HIV-associated TB, I reviewed the literature around validation of VA for HIV- and TB-associated causes of death, and conducted a validation exercise as part of the Lesedi Kamoso study.

The review of the literature yielded 22 reference datasets that have been used to validate VA for HIV- and TB-associated causes of death; they include over 26,000 deaths that occurred between 1990 and 2012, mostly in sub-Saharan countries. There was considerable heterogeneity between the reference standards used to validate VA interpretation methods, from the fundamental approach taken in assigning causes of death (at an individual level vs. at a population level), to the quality of the data, and the rigour of the process of assigning causes of death. PCVA was the most accurate and precise method in assigning HIV-associated causes of death (sensitivity 65%–75%; specificity 85%–95%). In general, CCVA methods performed less well (sensitivity 25%–65%, specificity 80%–95%, chance-corrected concordance 35%–55%, and chance-corrected CSMF accuracy 70%–90%). Only one study [290] attempted to differentiate between HIV-associated TB and other HIV-associated causes of death; it was therefore not possible, from the literature, to evaluate the overall accuracy of VA in estimating mortality due to HIV-associated TB.

In the Lesedi Kamoso study, we compared VA-assigned causes of death with three ‘levels’ of reference standard, determined by the quality of data available. VA data were interpreted by PCVA, InterVA-4.03 and SmartVA-Analyze v1.1.1; clinical and PCVA reviewers were asked to assign causes of death using both ICD-10 and six purpose-designed codes, specifically to try to estimate mortality due to HIV-associated TB. In line with the findings of the literature review, PCVA performed best overall (CSMF accuracy 0.70–0.80 compared with level 2 or level 3 reference standards, using ICD-10 or study-defined codes; Chapter 5.2.5.4) and automated VA methods generally underestimated the proportion of mortality due to HIV/AIDS (sensitivity 30%–55%); this was the case whether or not autopsy data had been used to inform the reference standard. Agreement between VA methods and the reference standard was uniformly poor at individual level, but was slightly better at population level, particularly for PCVA (Table 5:3).

7.2.3.3 Automated VA methods underestimate overall HIV-associated mortality among HIV-positive people

In addition to the 259 HIV-positive adults analysed in Chapter 5, the analysis presented in Chapter 6 included a further 200 adult decedents (97 HIV-positive and 103 HIV-negative), all of whom had VAs completed. Reference causes of death were not available for all decedents; instead, this analysis focused on evaluating the sensitivity and specificity of the ‘HIV diagnosis’ question in the WHO 2012 VA instrument in assigning HIV status, and the specificity of VA methods in assigning HIV-associated causes of death, using laboratory- or rapid test-confirmed HIV status as a reference standard.

Among 459 individuals with confirmed HIV status ($n = 356$ HIV-positive; $n = 103$ HIV-negative), the HIV diagnosis question was sensitive (84% [95% CI 80–88]) and specific (94% [95% CI 88–98]) in assigning HIV status. Only one previous study, conducted in Malawi in 2016 [303], formally assessed the sensitivity and specificity of the VA question around diagnosis of HIV; results from that study were similar to ours, suggesting that algorithms used to interpret VA data should perhaps be recalibrated to give greater weight to the answer to this question. Among the 306 decedents who were reported HIV-positive by VA, a question added to the VA instrument, “Did the decedent ever take ART?”, was 91% sensitive and 53% specific in detecting ART use. Again, only the study from Malawi had previously assessed this question, finding it 92% sensitive and 46% specific [303]. These findings suggest that, after refinement to improve specificity, this question should be included in the standard VA instrument (which it has been, in the 2016 instrument [107]), and the answers included in the algorithms used to assign causes of death in areas of high HIV prevalence.

The process of empirically evaluating individual questions and, if needed, adjusting them before including them in the standardised instrument should, ideally, be adopted for future changes to VA instruments. This will allow for questions to be piloted in a range of contexts, and for their relative weight to be assessed when considering the makeup of diagnostic algorithms. It is not clear as to how much of this has been done for existing VA questions, but raw VA interviews, of which there are thousands at HDSS sites across Africa and Asia, could be used to test, retrospectively, particular questions of interest if a reference standard were available. This is not, of course, always as straightforward as is the case for a diagnosis of HIV, where the line between positive and negative is very well defined. However, if VA instruments and methods are to be refined to the point where they can reliably estimate causes of death in individual decedents, continuous validation, at as granular a level of detail as possible, may be needed.

In the second part of the analysis in Chapter 6, all three VA interpretation methods showed high specificity in assigning HIV-associated causes of death (PCVA 96%, InterVA-4 89%, and SmartVA-Analyze 95%). However, agreement between the three methods was poor at individual and population level (chance-corrected concordance <0 ; chance-corrected CSMF accuracy $<0\%$). CCVA methods, in particular, appeared to underestimate the HIV-associated mortality fraction: when the analysis was restricted to confirmed HIV-positive individuals only ($n = 356$), InterVA-4 and SmartVA-Analyze assigned 45% and 22% of decedents, respectively, an HIV-associated cause of death. The consequences of this for countries with high HIV prevalence are potentially very important. As discussed in Chapter 3 (a systematic review of studies directly estimating causes of death in HIV-positive adults) and in Chapter 7.2.2, above, HIV-associated causes

still account for a high proportion of overall mortality among HIV-positive people in LMIC. Classification systems do not, at present, allow for adjustments to be made to the definition of what does and does not constitute an ‘HIV-associated’ death, based on changes in disease and mortality patterns that may be observed as greater numbers of people are initiated on ART (see Chapter 7.3.3 for a more detailed discussion). The current draft of the ICD-11, due for release in 2018, appears not to address this issue either, with HIV-associated deaths now classified by ‘clinical stage’, rather than more objective measures [354], and the continued absence of a way to record all deaths among HIV-positive individuals. As mentioned above, VA is being integrated into CRVS systems in several countries [115]; the underestimation of HIV-associated mortality in countries with high HIV prevalence may have serious negative effects on funding streams and overall efforts to reduce morbidity and death.

7.3. Implications of this work

7.3.1. For clinical practice and policy

TB disease was not diagnosed ante mortem in 6/16 (37.5%) individuals with autopsy evidence of TB (Chapter 4), something also found in several previous autopsy studies [71]. This, in addition to the underestimation of HIV-associated TB mortality by physicians reviewing clinical data that did not include findings from pathological autopsy (Chapter 5), suggests that clinicians likely underestimate both the prevalence of TB disease and its contribution to mortality in HIV-positive adults. As discussed in Chapter 5.2.5.1, the historic focus on cough as the key to TB diagnosis (which may have been sufficient in the pre-HIV era) and reliance on sputum-based investigation, including Xpert® MTB/RIF, likely lead physicians not to consider or to discount prematurely a TB diagnosis in HIV-positive individuals without cough or with negative results from sputum [64].

The very high proportions of individuals with TB who had extrapulmonary and/or disseminated disease seen in our and previous autopsy studies suggests that alternative diagnostic strategies, including, but not limited to, non-pulmonary imaging and testing of extrapulmonary samples [355,356], should be employed in those at highest risk of disease. These are often individuals in whom routine, sputum-based diagnostic methods are less sensitive and for whom the consequences of a missed diagnosis very severe. Recent studies exploring the diagnostic value of testing non-pulmonary samples in HIV-positive individuals have shown encouraging results [357,245,358,359]. However, there remains a need for a cheap, sensitive, point-of-care test that allows for detection of TB disease, especially extrapulmonary disease, in these individuals [62].

Further challenges are illustrated by the findings at MIA, of multiple, concomitant infections in individuals with advanced HIV and by some of the difficulties faced by reviewing physicians in assigning causes of death, even when provided with extensive clinical data. These are complex patients, with many competing risks, and integrated strategies are needed if morbidity and mortality are to be successfully reduced. Supporting this is evidence from studies directly estimating causes of death in HIV (Chapter 3), which suggests that making ART available, alone, may not be enough to reduce HIV-associated TB mortality at the desired rate. Many of the individuals who die from HIV-associated TB are severely immunosuppressed; interventions are therefore needed that promote the early identification and treatment of these individuals before they reach these levels of immune compromise. In addition to better TB diagnostics and streamlined diagnostic pathways [360], systemic interventions are needed that reduce delays within the health system; minimise loss to follow-up at various points in the treatment cascade, and promote, measure, and reinforce adherence to ART [361]. A cohesive ‘package of care’ is needed for these patients, particularly at hospitals, but ideally even at community clinics. Encouragingly, in 2017, WHO published guidelines for the management of individuals with advanced HIV disease [362], which talk to the importance of “differentiated service delivery” in maximising the capacity of a health system to manage, optimally, patients who are most ill.

Underlying all these issues is a need to better understand the structural, socio-economic, and behavioural factors that have enormous impacts on clinical outcomes, but are too often unaccounted for in the development of biomedical interventions [363,364]. VA narratives, collected as part of the interviews, are a potential source of rich and diverse data and may provide some insight into the beliefs, practices, and actions that may contribute to adverse outcomes.

7.3.2. For HIV & TB surveillance

Many of the difficulties involved in diagnosing TB disease in life also affect estimates of TB-associated mortality. In the meta-analysis of directly estimated causes of death in HIV-positive adults, presented in Chapter 3, estimates of the TB-specific mortality fraction among HIV-positive individuals from studies that did not use autopsy data were lower than estimates from those that did (pooled prevalence 24% without autopsy data [n = 29 studies] vs. 37% with autopsy data [n = 18 studies]). Estimates of TB-specific mortality derived from death certificates or PCVA also rely on physician recognition of TB as a potential cause of death. As discussed above, this is guided by the presence of symptoms, particularly cough, and/or the results of sputum-based investigations, both of which are unreliable indicators of disease in individuals with advanced HIV.

This highlights a more fundamental problem regarding TB surveillance, that the majority of surveillance activities in high-burden settings generally focus on individuals in whom a TB diagnosis has been confirmed or TB treatment started [365–367]. WHO guidance states that a “major goal of TB surveillance is to provide an accurate measure of the number of new TB cases and related deaths” within the population in question [368]. These are, by definition, individuals who have engaged, at least briefly, with health services; as the guidelines go on to concede, for notification data to provide accurate estimates of TB incidence, the fraction of undiagnosed cases must be negligible, requiring not only a well-functioning health system and diagnostic infrastructure, but also good access to care for the majority of the population [368]. The lack of a sensitive test for HIV-associated TB, particularly extra-pulmonary TB, in individuals with advanced HIV disease and the documented gaps in health systems in the areas most affected by the HIV and TB epidemics [369,370] suggest that these criteria are often not met (see also Chapter 1).

Including estimates of undiagnosed disease in routine surveillance may be one way to provide more comprehensive data around TB control in high-burden settings. Additionally, as it requires acknowledgment of the burden of undiagnosed disease, this may allow for more realistic target-setting and resource allocation. To do this in practice would, of course, be challenging; the validation of estimation methods would be difficult and their implementation logistically complex. One approach may be to use MIA to estimate the prevalence of disease among individuals who die within the catchment areas of sentinel surveillance sites. The Lesedi Kamoso study has shown that this can be done in community mortuaries in urban and semi-rural settings in South Africa (Chapter 4) and several studies have shown the sensitivity of the procedure in estimating the prevalence of infectious diseases at death, particularly TB [94,99,95] (see also Chapter 4.2.2). The collection of viable samples, including for aerobic culture, in community settings, as well as high consent rates among families and TB Fast Track participants, add to the growing body of evidence [184,98] regarding the acceptability of MIA among families (and, for the first time, among a large group of adults with advanced HIV disease) and the feasibility of conducting community-based autopsies. The development of a standardised, cost-effective protocol for the autopsy procedure, laboratory testing, and interpretation of results that could be implemented at sentinel surveillance sites in resource-limited settings is an achievable goal, and would provide quality data to allow better monitoring of progress towards the global goals for reducing HIV- and TB-associated mortality.

7.3.3. For international disease classification systems

The problems with ICD-10 around classifying HIV-associated TB have been discussed at length above; the addition of a specific three-character code for HIV-associated TB would improve the accuracy of national and global estimates of TB mortality, particularly in countries where CRVS networks are already established. In the interim, studies using or evaluating any method(s) to assign causes of death in HIV-positive individuals, including VA, should, whenever possible, aim to separate HIV-associated TB from other HIV-associated causes.

Less well discussed in the literature is a second issue with ICD-10, that a death due to the adverse effects or toxicity of ART may not necessarily be counted as 'HIV-associated'. There are already real-world examples of the confusion this may create: the systematic review of 'non-AIDS' mortality by Farahani et al. [340] (discussed in detail in Chapter 3.2.5) considered deaths due to ART toxicity as 'non-AIDS', which is technically correct, but runs contrary to guidance from WHO, which states that ART toxicity should be included in estimates of HIV mortality [371]. This leads to the underestimation of overall HIV-associated mortality, which may have severe repercussions for the structures of national health systems and funding streams (see also Chapter 6.2.5.2). At present, we rely on observational studies or secondary analyses from trials for estimates of ART-associated mortality in LMIC and do not have reliable estimates of all-cause mortality among HIV-positive individuals. A more efficient system for monitoring mortality among HIV-positive individuals would be one that is integrated into our existing (and developing [114]) notification systems, allowing for consistent measurement across greater numbers of individuals in different contexts.

This is particularly relevant because of the widespread demographic changes being seen in disease and mortality patterns among HIV-positive people on ART [26]; individuals who initiate and continue on ART are living longer and are therefore exposed to age-related co-morbidities [372,373]. In addition, there are non-communicable disease epidemics emerging in many LMIC, including those most heavily affected by the HIV and TB co-epidemics [374]. Individuals may develop multiple co-morbidities and, as is the case with the HIV and TB epidemics, the presence of one disease may affect the susceptibility to, clinical course of, and/or severity of another. For example, there is already some evidence to suggest an interaction between TB and diabetes [375]. A final layer of complexity lies in the polypharmacy involved in treating individuals with multiple chronic comorbidities. There are still few data available regarding the longer-term effects (i.e., more than 25–30 years) of ART, or how second or third-line antiretroviral drugs may interact with anti-diabetic or anti-hypertensive agents, or the effects that these may have on disease and mortality patterns.

As discussed briefly in Chapter 3.2.5.3, a classification system that is too rigid, for example, one that requires the person assigning the cause of death to decide what is and is not an 'HIV-associated' death, limits our ability to evaluate disease patterns at population-level and leaves us vulnerable to unpredictable changes. Using the same example, a system that permitted the enumeration of all deaths among HIV-positive individuals, regardless of cause, would allow for the evaluation of demographic trends and the early identification of new 'HIV-associated' conditions that may have been excluded from previous definitions. There is also no reason such a system could not be extended to other key, chronic disease of interest (e.g., diabetes or hypertension). I would suggest that the ideal surveillance system is one that allows us to predict events of interest, or, at the least, react to them in time to minimise potentially adverse effects. A system that is too unwieldy to be adapted to changing patterns will eventually necessitate the development of workaround strategies (as has happened with the multiple steps currently needed to produce estimates of mortality due to HIV-associated TB), at which point it could be argued that it becomes part of the problem, instead of the solution.

7.4. Limitation and strengths

In addition to the limitations and strengths described in individual chapters and those discussed above, there are other, overarching, factors that may have affected the internal and external validity of the data presented in this thesis.

7.4.1. Selection bias

7.4.1.1 In enrolment to parent studies

The majority of decedents included in this study were enrolled to one of the three parent studies: TB Fast Track, XPHACTOR, or XTEND. All three studies were conducted in South African public clinics and, though these clinics are free at the point of care and situated within the community, will have included only individuals both willing or able to access these facilities and to give written informed consent for participation. This may have biased selection away from individuals who did not have the resources or support needed to get to health facilities, and those unable or unwilling to give consent. These individuals may be considered at higher risk of certain illnesses, particularly those related to severe poverty [376], and are possibly under-represented in our data. All three parent studies also excluded individuals who were on TB treatment at the point of enrolment and may therefore have selected for individuals who, if they had TB, presented atypically or were more difficult to diagnose for other reasons.

7.4.1.2 Representation among those deceased

The proportions of participants lost to follow-up were low in all the parent trials (retention in TB Fast Track 98.6% [155], XTEND 99.0% [159], and XPHACTOR 93.7% [Y. Hanifa, personal communication]), suggesting that there were relatively few individuals who died and were not accounted for. In TB Fast Track, in particular, extensive efforts were made to ascertain deaths, including the cross-checking of South African identification numbers with lists of death notifications held by the South African Medical Research Council and review of hospital databases for names and other identifiers of participants who could not be contacted. However, despite the fairly comprehensive enumeration of deaths, only those decedents with relatives or friends that could be contacted and were willing to give consent for MIA and/or VA were included. For MIA, this depended on the agreement of both the participant and their family. The need for family involvement may, again, have led to under-representation of individuals with poor or absent support networks or may have selected for individuals more receptive to healthcare-related interventions, all of which may have affected our estimates of autopsy prevalence of disease and mortality patterns.

7.4.2. Bias inherent in methods used

7.4.2.1 Verbal autopsy data collection

Our use of the WHO 2012 instrument may have led to systematic inconsistencies in the collection of VA data that made comparisons with other VA studies less valid. Additionally, despite our adhering to international recommendations around staff training, the way in which respondents were chosen, and the way in which specific questions were asked, there remained several opportunities for our procedures to vary from those conducted by other groups or in other contexts. A degree of variability in interpretation of questions between individual interviewers is inevitable, and this should not necessarily be considered a source of bias in comparing our results with other VA studies, as this is likely to occur in almost any context. Nevertheless, elements that may have had a systematic effect on the data collected include the language in which training was conducted (English), which may have affected the interpretation or understanding of particular questions for all the interviewers; the languages in which the interviews themselves were conducted (majority Tswana, Sepedi, and isiZulu) and how particular concepts were translated into (and back-translated from) those languages, which may differ from other studies done in South Africa and will certainly differ from studies done outside of South Africa; the 'type' of respondent interviewed (i.e., relation to the deceased), which may have been affected by the times at which research assistants were available to conduct interviews, and may have led to the exclusion of family members or carers who were bread-winners and were therefore at work during the day;

and the overall demographics of respondents (and research assistants), including socio-economic characteristics, education, health beliefs, and their own health status, which will have affected the way questions were understood or answered, or both, and which are likely to be different from respondents in other countries.

To estimate the specificity of the VA HIV diagnosis question and the specificity of VA methods in assigning HIV-associated causes of death (Chapter 6), we selected HIV-negative adults from the registers of hospital mortuaries who had died from causes that were not related to maternity or trauma. By definition, these were people who died in, or within reach of, a hospital, and were therefore not necessarily representative of the HIV-negative population more generally. Aside from the issue of generalisability of these results to other South African or sub-Saharan African settings, where a high proportion of deaths occur outside of hospitals, this raises a broader issue of how a hospital death may affect the information to which a family has access, and how this may affect the validity of data collected through VA. The families of individuals who were cared for or died in hospitals may be more likely to be familiar with the language used in the VA interview (which is written and interpreted by physicians) than the families of those who were cared for at home or in a more traditional setting. The development and validation of VA interpretation methods using data only from individuals who died in hospitals may therefore lead to overestimation of the overall accuracy of the VA method in question, particularly in the assignment of causes of death that depend heavily on the confirmation of a particular diagnosis, such as HIV. By including in this study only HIV-negative individuals who died in hospitals, our results may have been susceptible to a form of verification bias and may have led to our overestimating the sensitivity of the VA instrument in detecting HIV status.

7.4.2.2 Verbal autopsy data interpretation

Use of the WHO 2012 instrument, rather than instruments from 2014, 2016, or the PHMRC instrument may have affected translation into InterVA and SmartVA-Analyze input files and, by proxy, the causes of death assigned. As described in Chapters 5 and 6, PCVA reviewers were aware that decedents were likely to have been enrolled in a study of HIV and/or TB and that there was a high likelihood of a decedent being HIV-positive. Confirmation bias may therefore have led to the increased assignment of HIV- and TB-associated causes of death. Reviewers assigning reference causes of death may have been subject to the same confirmation bias, although the effect on the proportions of HIV- and TB-causes assigned will have varied between reviewers, because of individual differences in process and

differences in the data available to PCVA and clinical reviewers. This may have been further affected by our use of study-specific causes of death, which focused attention on HIV-associated TB and other HIV-associated causes.

7.4.2.3 Assigning reference-standard causes of death

There was also variability in the information available for individual decedents, even after attempts to separate different 'levels' of information (see Chapter 5.2.4.2): better information was available to reviewers assigning reference causes of death for those decedents who died in hospital (and had hospital data available). These individuals were therefore less likely to be assigned an 'indeterminate' cause of death. Finally, despite the precautions taken in assigning reference causes of death for levels 1 and 2 in batches of 40–50 decedents, there was still potential for learning among reviewing physicians. As individual physicians became accustomed to the data that were available at each level, and to the classifying tendencies of their reviewing colleague(s), they may have assigned causes of death based on subconscious expectations of data not yet available, or adjusted their approach to align their assignments more closely with their colleague. These elements were not measured or accounted for in the analysis.

7.4.3. Strengths

This study also had several novel elements and methodological strengths. The recruitment of all HIV-positive individuals from community-based clinics, which is more representative of people receiving HIV care in South Africa, provides much-needed data on disease and mortality patterns in this population. In addition to the study conducted by Omar et al. in the homes of patients in North West province [97], this study has shown that community-based MIA is feasible and acceptable in a variety of South African settings. This strengthens the case [90] for the broader use of MIA in resource-limited settings, for example, as an adjunct to routine surveillance activities at sentinel sites (Chapters 5 and 7.6.1). As previously discussed (Chapters 5, 6, and 7.2.3), this study is the first to systematically compare VA-assigned causes of death with a reference standard that incorporated pathological autopsy data. In addition, the process employed in this study for assigning reference causes of death was designed and implemented carefully and with consideration of transparency and reproducibility. Clear criteria were established for each major cause of death, along with standardised guidelines for reviewers to follow; the process was regulated, through the independence of reviewing physicians; and simple arbitration procedures were in place to resolve disagreements between reviewers. Although the precautions taken to reduce physician learning may not have eliminated it completely, they marked at

least a recognition of, and attempts to counteract, the threats posed to the internal validity of the process, something that unfortunately cannot be said for many previous VA validation studies (Chapter 2.3).

The use of study-defined causes of death (Chapter 5), in addition to ICD-10, allowed for illustration of some of the issues around estimating mortality due to HIV-associated TB, but also allowed for comparisons between our data, data from previous studies, and routine operational data. Finally, the systematic review and meta-analysis of studies that directly estimated causes of death in HIV-positive adults (Chapter 3) is the first of its kind, and has limitations, but still provides a useful baseline for future estimations of cause-specific mortality among HIV-positive adults dying in LMIC.

7.5. Reflective commentary

7.5.1. Personal reflection

I began this process in mid-2012, as an MSc student arriving in South Africa for the first time, with no prior experience of clinical research. Every day of the last five years has been one of learning: I have been fortunate to have been involved in almost all stages of the research process, from developing a protocol, to planning and executing data collection, designing and coordinating data management and analysis, writing up results, and making policy recommendations. I have also been extremely lucky to work with a diverse, multidisciplinary team, many of whom are leaders in their fields.

As part of the data collection for this study, obtaining consent for MIA from the families of participants, I spent time in mortuaries and with recently bereaved families, talking about the principles of research and the ideas behind the study. Having to explain, on a regular basis, why I believed in the study and why research, in general, was a positive thing, forced me to re-examine these issues for myself. Many individuals with whom I spoke reported having been treated badly at health facilities, or had been failed, repeatedly, by the structures that were put in place to support them. All had very recently lost a young family member to a disease for which treatment was safe and freely available. Having conversations about the value of research and, more specifically, of autopsies, in these intensely emotional situations, would have been impossible had I not been so sure of the importance of this work and its potential to have a positive impact on the lives of the people with whom I was speaking.

As the project moved into its analysis stages, I gained an appreciation of how the data we had worked so hard to collect fit into national and global systems, and started to develop a better understanding of some of the challenges involved

in compiling the global estimates of disease and mortality that I had previously taken for granted. Each MIA, VA, and set of hospital notes was a minor victory, and each was hard-fought, often involving considerable amounts of travel and negotiation with clinic, hospital, and mortuary staff. I did struggle, at times, to reconcile the complex, often heart-breaking, and deeply human stories that we were told, with the four or five characters assigned by VA software as the cause of death, or the crisp, clean lines of a statistical report that discussed thousands of deaths with apparent detachment. I have come to recognise, however, that the choice I thought was required, between the 'compassionate' clinician and the 'objective' researcher, is an artificial divide. They are, or should be, in my view, two parts of a synergistic whole. As I move into the next stages of my career, I hope to strike that balance; to focus with accuracy and precision on the areas of life, health, and death that may, at first, cause one to want to look away.

7.5.2. Practical lessons learnt

Although we were successful in conducting 34 MIAs as part of the Lesedi Kamoso study, our task would have been made much easier by better, and earlier, engagement with the communities in which we were working. Mostly, this would have involved detailed discussions, prior to starting any fieldwork, with mortuary owners and managers, local community and/or religious leaders, and representatives of health services, patient bodies, and law enforcement. Some of the difficulties we encountered were because of the size of the area we were covering: although the study was conducted across the three provinces mentioned, we also carried out two MIAs in the Free State, as the individual had travelled there before death. However, even taking this into account, better communication between ourselves and mortuary owners, in particular, would have avoided some difficult and sometimes confrontational interactions.

The process of assigning reference causes of death was enormously time-consuming and a much bigger undertaking than we initially thought. The data we had access to were sometimes quite complex, often qualitative (e.g., extracts from hospital notes), and had to be collected from various sources and manually organised in chronological order to form a coherent narrative. In addition, our decision to use at least two reviewers for each death and to assign causes of death based on different 'levels' of data meant that each decedent's data were reviewed on at least four occasions, sometimes more, depending on agreement between reviewers. Although our main analysis presented causes of death for only 259 individuals, this had involved well over 1,000 reviews. This process made clear to me the impossibility of trying to replicate this process in any setting other than research and the need, therefore, for a simple system that provides a balance between accuracy and feasibility. I hope to continue to explore this area in my future work.

7.6. Conclusions and recommendations

7.6.1. Summary of recommendations

As discussed above, the work presented in this thesis has implications across a wide range of disciplines, from everyday clinical practice to the way diseases are classified and counted. Table 7:1 provides an overview of key recommendations and refers the reader to other parts of the thesis for more detailed discussions of specific issues.

Table 7:1. Summary of key recommendations made in this thesis

Subject area	Key recommendation (s)	See also
1. Clinical practice and policy	a) Changes to guidelines in order to stress the high prevalence of EPTB in areas with high HIV prevalence and encourage more thorough investigation of high-risk individuals, particularly with, but even without, persistent symptoms; greater awareness of limitations of sputum-based investigation in these individuals.	Chapters 2.2, 4.2.5.1, and 7.3.1
	b) Encourage sampling and testing of non-pulmonary samples as part of TB diagnosis in high-risk individuals; use autopsy data to guide to anatomical sites that are most likely to yield a positive result.	
2. HIV & TB surveillance	a) Changes to the international classification of diseases that allow for the separation of HIV-associated TB and other HIV-associated causes	Chapters 2.3, 3, 5, 6, and 7.3.3
	b) Use of MIA at sentinel surveillance sites to monitor, periodically, the autopsy prevalence of TB in high-risk populations and to allow for direct estimation of mortality due to HIV-associated TB. This will be helped by the development of a standardised protocol, for use in high prevalence settings, that specifies sampling of the anatomical sites where TB is most likely to be found.	Chapters 2.2, 4, and 7.3.2
	c) Development of an HIV/TB module to be added to the VA instrument for use in high prevalence settings. This should include questions about treatment for TB, ART initiation, and adherence to therapy.	Chapters 5 and 6
3. Measuring cause-specific mortality	a) Development of a standardised system for assigning causes of death in HIV-positive people that is compatible with ICD coding	Chapters 3, 5, and 6
	b) Development of high-quality, representative, shared datasets for validation of VA methods.	Chapters 2.3, and 5
4. Future research	a) The inclusion of new or experimental questions in any future VA validation studies to allow for empirical assessment of accuracy prior to their inclusion in the standardised instrument.	Chapter 6
	b) An agreed format for the reporting of VA validation studies, with structured criteria and requirements for assigning reference causes of death and metrics for reporting agreement between VA-assigned and reference standard causes of death.	Chapters 2.3, 5, and 6

ART: antiretroviral therapy; HDSS: health and demographic surveillance system; MIA: minimally-invasive autopsy; TB: tuberculosis; VA: verbal autopsy

7.6.2. Conclusions

TB is highly prevalent among HIV-positive individuals dying in LMIC. It remains a serious clinical and public health concern and a leading cause of death in these individuals. Pathological autopsies provide the most accurate estimates of TB prevalence; estimates that are consistently higher than those made among living individuals, in part due to high prevalence of extrapulmonary disease and the absence of diagnostics that are sufficiently sensitive in individuals with advanced HIV. Methods used to enumerate deaths due to HIV-associated TB are limited by ICD conventions, which group together all HIV-associated causes of death. VAs, particularly when interpreted by automated methods, consistently underestimated HIV-associated mortality among HIV-positive individuals; because of ICD rules, VAs were also unable to estimate mortality due to HIV-associated TB, except when interpreted by physicians using a purpose-designed coding system.

The addition of MIA to other surveillance activities at sentinel sites would allow for regular direct estimation of the autopsy prevalence of TB and provide a real-world comparator for the modelled estimates of mortality due to HIV-associated TB currently used to track progress and determine policy. At a structural level, modifications to the ICD system to allow for the differentiation of HIV-associated TB from other HIV-associated causes would better enable surveillance and governing bodies to make this distinction, leading to more accurate and useful mortality data. VA, though not ideal for this purpose, is currently the only feasible method for estimating cause-specific mortality among large groups of decedents in areas with weak or absent CRVS systems. In regions of high HIV and TB prevalence, therefore, VA methods should be developed and trained using reference datasets that more accurately represent the mortality patterns seen in these areas, ideally with a distinction made between HIV-associated TB and other HIV-associated causes of death.

ART alone is not enough, at present, to reduce mortality at the rate needed to meet international targets. Meaningful reductions in mortality due to HIV-associated TB will therefore require more specific, TB-focused, interventions as well as broader, systemic measures that aim to strengthen health services, diagnostic capabilities, and reporting mechanisms. CRVS strengthening, with or without the integration of verbal autopsy, is a key part of that process and presents an opportunity to restructure the way that disease and cause-specific mortality are measured. A system is needed that can adapt to the needs of the health and research community and that is flexible in the face of evolving disease and mortality patterns.

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Chapter 8. Appendices

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8.1. Appendix 1: Consent forms for minimally-invasive autopsy and verbal autopsy (extracted from Lesedi Kamoso study protocol, version 5.1 [30 Jan 2015])

8.1.1. Participant information sheet: study participants, at enrolment to TB Fast Track/XPHACTOR

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

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Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

[Greeting], my name is [_____]. I am a researcher with [Aurum Institute/FPD]. We are doing a research study and we would like to invite you to take part. Research is the process to learn the answer to a question. This information sheet explains the study. You are free to decide if you want to take part or not. If you decide to take part, we will ask you to sign or make your mark on a consent form, or give a thumbprint. Signing or marking the form means that you agree to take part in the study. It also means that you are aware of your right not to take part, or to stop taking part at any time. If you decide not to take part, this will not affect your right to health care at this clinic.

Why are we doing this study? Tuberculosis (TB) is a very serious health problem in South Africa. TB can be cured, but occasionally can be fatal if treatment is not started quickly. You have already agreed to take part in the [as appropriate, TB Fast Track or XPHACTOR] study, which is trying to improve testing and treatment for TB. We hope that the care you receive will ensure that you stay well. However, some people in this study will be seriously ill. Even with treatment, it is likely that a small number will pass away. If we can find out why people pass away, we can design better treatments to keep other patients like them healthy. We will ask about 150 people in South Africa to take part in this study. The study is paid for by the Bill and Melinda Gates Foundation.

If you take part in this study, what will happen? If you agree to take part in this study, in the unlikely event you should pass away, we will take some samples from your internal organs with a needle. The samples will come from the lungs, liver, spleen, veins, bladder and fluid round the spine. The needle will make a small mark in the skin on the chest and abdomen. This will not affect how the body looks. We will also take a swab from the inside of the nose and some washings from the lungs by putting a small tube into the lungs from the base of the neck. We will make sure that taking the samples does not delay the funeral arrangements. If you stay well during the study, as we hope you will, this study will not apply to you and no samples will be taken.

It will not cost you any money to take part in this study. Equally, there is no payment to people who take part.

What are the risks and benefits of taking part in this study? There are no risks to taking part in this study. This study will not benefit you directly. It will help us to understand the causes of serious illness in people like you. We could then design treatments to improve care, and reduce the risk of serious illness in people like you in the future.

What happens if I do not agree to take part in this study? You do not have to take part in this study. If you do not take part, this will not affect the medical care that you receive at this clinic. You can decide to stop taking part in the study at any time, without giving a reason.

How will the information collected during this study be kept confidential? We will do this in the same way as we explained earlier for the [TB Fast Track / XPHACTOR] study.

Are there reasons why the study might be stopped early?

We will stop the study if:

the study is cancelled

there could be other reasons we don't know about yet.

You can stop taking part in the study at any time. If you want to stop taking part, just tell the study team at any time.

What if I have more questions I wish to ask about this study? If you have any questions about this study, please ask us now. If you have questions later you can ask study staff, or telephone Dr Charalambous on 010 590 1389. The committees giving ethical approval for this study are the Research Ethics Committees of the University of the Witwatersrand, South Africa and the London School of Hygiene & Tropical Medicine, UK. If you have any questions concerning your rights as a person taking part in a research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC) at 011 717 2301. This is an independent committee established to protect the rights of research participants.

We will give you a copy of this sheet which explains the study to take away with you.

If you would like a copy of a report on this study, please give us an email or postal address. The final results may not be available until 2 years or more from now.

(This information sheet will be available in the most common local languages in study clinics: e.g. Sesotho, isiXhosa, isiZulu, Setswana, as appropriate)

8.1.1.1 Consent form: study participants

Ppt ID: AUR- - -

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

Investigators:

London School of Hygiene & Tropical Medicine, UK: Prof. Alison Grant, Dr Katherine Fielding, Dr Aaron Karat, Dr Yasmeen Hanifa

Aurum Institute for Health Research: Dr Salome Charalambous, Dr Kerrigan McCarthy, Dr Mpho Tlali, Prof. Gavin Churchyard

Foundation for Professional Development: Ms Suzanne Johnson

National Health Laboratory Service and University of the Witwatersrand, Johannesburg: Dr Tanvier Omar

Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

I have read the information sheet about this study (or the information sheet about this study has been read to me) and I understand what will be required of me and what will happen if I take part in the study.

My questions concerning this study have been answered by:

_____	_____	_____
Research staff name (printed)	Signature	Date

I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management. I agree to take part in the study

_____	_____	_____
Study participant name (printed)	Signature/mark/thumbprint	Date

If the information sheet and consent form were translated or explained to the participant, enter the name of the translator here and their signature:

_____	_____	_____
Translator name (printed)	Signature/mark/thumbprint	Date

If the participant gave verbal consent, enter the name of the person who witnessed the consent here and their signature:

_____	_____	_____
Witness name (printed)	Signature/mark/thumbprint	Date

8.1.2. Information sheet for next of kin: consent for needle autopsy

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

Investigators:

London School of Hygiene & Tropical Medicine, UK: Prof. Alison Grant, Dr Katherine Fielding, Dr Aaron Karat, Dr Yasmine Hanifa

Aurum Institute for Health Research: Dr Salome Charalambous, Dr Kerrigan McCarthy, Dr Mpho Tlali, Prof. Gavin Churchyard

Foundation for Professional Development: Ms Suzanne Johnson

National Health Laboratory Service and University of the Witwatersrand, Johannesburg: Dr Tanvier Omar

Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

[Greeting], my name is [_____]. I am a researcher with [Aurum Institute/FPD]. We are very sorry to hear about the loss of [name of participant]. We offer our sincere condolences to you and the family. [Pause for response: researcher to respond appropriately to the family member's response.]

We would like to invite you to give permission for your relative to contribute to a research study. Research is the process to learn the answer to a question. This information sheet explains the study. You are free to decide if you will give permission for the study or not. If you agree, we will ask you to sign or make your mark on a consent form, or give a thumbprint. Signing or marking the form means that you agree for your relative to contribute to the study. It also means that you are aware of your right to say no. If you decide not to take part, this will not affect your right to health care.

Why are we doing this study? Tuberculosis, also called TB, is a very important health problem in South Africa. TB can be cured, but occasionally can be fatal. It is possible that TB played a part in the death of your relative, even if they were treated for it. There may be other treatable diseases that also played a part. If we can find out why people pass away, we may be able to design better treatments to keep other patients like them healthy. We aim to include about 150 people in South Africa in this study. The study is paid for by the Bill and Melinda Gates Foundation.

If you agree for your relative to contribute to this study, what will happen?

If you agree, we will take some samples from [name of participant]'s body with a needle. The samples will come from the lungs, liver, spleen, veins, bladder and fluid round the spine. The needle will make a small mark in the skin on the chest and abdomen. This will not affect how the body looks. We will also take a swab from the inside of the nose and some washings from the lungs by putting a small tube into the lungs from the base of the neck. We will make sure that taking the samples does not delay the funeral arrangements.

If you would like us to let you know what we find out from the samples, and you give us contact details, we can call you to say what we found. Because we are taking only small samples of tissue, we will not be able to examine the body organs in detail. This means that we will be able to say whether we found TB or other infections, and if we found cancer

cells. However we will not have information on all the diseases that may have been there. It would likely take about 3 months before we can give you these results.

If we find TB, we would recommend that you and others who lived with your relative are checked for TB, if this has not already been done. We can give you a letter of referral to your health centre to be checked for TB, if you would like that.

It will not cost you any money if your relative contributes to this study. Equally, there is no payment for taking part.

What are the risks and benefits of taking part in this study?

There are no risks to taking part in this study. This study will not benefit you directly. It will help us to understand the causes of serious illness in people like your relative. We could then design treatments to improve care, and reduce the risk of death, for people like your relative in the future.

What happens if I do not agree to this study? You do not have to agree for your relative to contribute to this study. If you do not agree, there will be no negative consequences.

How will the information collected during this study be kept confidential? All information collected during the course of this study will be kept securely and confidentially in a locked cupboard or filing cabinet: Prof Grant and Dr Charalambous are responsible for this. Your relative's name and contact details will only be available to a restricted group of study staff, and when we store this information on a computer, it will be protected by a password, and kept separate from other information. Information from the samples will be identified on forms and on computer files only by a study number, not your name or contact details. When the information is analysed, nobody will know who the people in the study are, and the information will remain private and confidential. Information that we collect as part of the study may be reviewed by the Ethics Committee, and independent monitors, to check that the study procedures were done correctly and the information is correct. Reports about the study and results that may be published in scientific journals will never include any information which allows your relative to be identified.

What if I have more questions I wish to ask about this study? If you have any questions about this study, please ask us now. If you have questions later you can ask study staff, or telephone Dr Charalambous on 010 590 1389. The committees giving ethical approval for this study are the Research Ethics Committees of the University of the Witwatersrand, South Africa and the London School of Hygiene & Tropical Medicine, UK. If you have any questions concerning your rights as a person taking part in a research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC) at 011 717 2301. This is an independent committee established to protect the rights of research participants.

We will give you a copy of this sheet which explains the study.

If you would like a copy of a report on this study, we can send one. We will need an email or postal address. The final results may not be available until 2 years from now.

(This information sheet will be available in the most common local languages: e.g. Sesotho, isiXhosa, isiZulu, Setswana, as appropriate)

8.1.2.1 Next of kin: consent for needle autopsy

Ppt ID: AUR- - -

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

Investigators:

London School of Hygiene & Tropical Medicine, UK: Prof. Alison Grant, Dr Katherine Fielding, Dr Aaron Karat, Dr Yasmeen Hanifa

Aurum Institute for Health Research: Dr Salome Charalambous, Dr Kerrigan McCarthy, Dr Mpho Tlali, Prof. Gavin Churchyard

Foundation for Professional Development: Ms Suzanne Johnson

National Health Laboratory Service and University of the Witwatersrand, Johannesburg: Dr Tanvier Omar

Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

I have read the information sheet about this study (or the information sheet about this study has been read to me) and I understand what will happen if I agree for my relative to contribute to the study.

My questions concerning this study have been answered by:

_____	_____	_____
Research staff name (printed)	Signature	Date

I understand that I may withdraw consent for this study at any time without giving a reason

I agree for my relative to contribute to the study

_____	_____	_____
Study participant name (printed)	Signature/mark/thumbprint	Date

If the information sheet and consent form were translated or explained to the participant, enter the name of the translator here and their signature:

_____	_____	_____
Translator name (printed)	Signature/mark/thumbprint	Date

If the participant gave verbal consent, enter the name of the person who witnessed the consent here and their signature:

_____	_____	_____
Witness name (printed)	Signature/mark/thumbprint	Date

8.1.3. Information sheet: next of kin consent for verbal autopsy

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

Investigators:

London School of Hygiene & Tropical Medicine, UK: Prof. Alison Grant, Dr Katherine Fielding, Dr Aaron Karat, Dr Yasmeen Hanifa

Aurum Institute for Health Research: Dr Salome Charalambous, Dr Kerrigan McCarthy, Dr Mpho Tlali, Prof. Gavin Churchyard

Foundation for Professional Development: Ms Suzanne Johnson

National Health Laboratory Service and University of the Witwatersrand, Johannesburg: Dr Tanvier Omar

Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

[Greeting], my name is [_____]. I am a researcher with [Aurum Institute/FPD]. We are very sorry to hear about the loss of [name of participant]. We offer our sincere condolences to you and the family. [Pause for response: researcher to respond appropriately to the family member's response.]

We are doing a research study and we would like to invite you to take part. Research is the process to learn the answer to a question. This information sheet explains the study. You are free to decide if you want to take part or not. If you decide to take part, we will ask you to sign or make your mark on a consent form, or give a thumbprint. Signing or marking the form means that you agree to take part in the study. It also means that you are aware of your right not to take part, or to stop taking part at any time. If you decide not to take part, this will not affect your right to health care.

Why are we doing this study? Tuberculosis, also called TB, is a very important health problem in South Africa. TB can be cured, but occasionally can be fatal. It is possible that TB played a part in the death of your relative, even if they were treated for it. There may have been other treatable diseases that played a part. If we can find out why people pass away, we may be able to design better treatments to keep other patients like them healthy. We will ask about 600 people in South Africa to take part in this study. The study is paid for by the Bill and Melinda Gates Foundation.

If you take part in this study, what will happen? If you agree to take part in this study, we will ask you general questions about your relative. This will include where s/he was born; the sort of work s/he did; questions about illness s/he had during his/her life. We will ask details about what happened in his/her final illness, including what treatment s/he received at clinics and hospitals. We would be very grateful if you would let us record the interview. If you give us permission we will record the interview on tape, so that we don't miss any of the things you say. We will also make notes on your answers during the interview and we may quote things that you say in future reports, although there will be no way to identify you (your name or other details will never appear). These questions will take about 45-60 minutes in total.

If you agree to take part in this study, we will give your ZAR100 in recognition of your time. [If you have had to spend money on travel to talk to us, we will give you ZAR20 to reimburse your costs.]

What are the risks and benefits of taking part in this study? Thinking about your relative's final illness may bring back the grief of losing your relative. You can stop the interview at any time, without giving a reason. There are no direct benefits to you of taking part in this study. This study will help us understand the causes of serious illness in people like your relative. We could then design treatments to improve care, and reduce the risk of death, for people like your relative in the future.

What happens if I do not agree to take part in this study? You do not have to take part in this study. You can stop taking part in the study at any time, without giving a reason.

How will the information collected during this study be kept confidential? All information collected during the course of this study will be kept securely and confidentially in a locked cupboard or filing cabinet: Prof Grant and Dr Charalambous are responsible for this. If you give permission, we will tape record the interview we have with you. The interview may be written down and translated into English. We may type the information into a computer database. The computer texts will be stored anonymously, which means that you will be identified by a study number only, not your name. Tapes will also be stored only with study numbers attached. When we have finished the study, we will destroy any links between the information you gave us and your name. If we publish the results of the study, we will keep interview data for at least two years after the study is finished for checking or further analysis. If we do not publish the results, we will keep interview data for up to 6 years for the same purposes. At this time it will not be possible to link the information you give us back to you.

Your relative's name and contact details will only be available to a restricted group of study staff, and when we store this information on a computer, it will be protected by a password, and kept separate from other information (such as information about their health) that you give us. The health information you give us will be identified on forms and on computer files only by a study number, not your relative's name or contact details. When the information is analysed, nobody will know who the people in the study are, and your information will remain private and confidential. Information that we collect as part of the study may be reviewed by the Ethics Committee and independent monitors, to check that the study procedures were done correctly and the information is correct. Reports about the study and results that may be published in scientific journals will never include any information which allows your relative to be identified.

What if I have more questions I wish to ask about this study? If you have any questions about this study, please ask us now. If you have questions later you can ask study staff, or telephone Dr Karat on 010 590 1360. The committees giving ethical approval for this study are the Research Ethics Committees of the University of the Witwatersrand, South Africa and the London School of Hygiene & Tropical Medicine, UK. If you have any questions concerning your rights as a person taking part in a research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC) at 011 717 2301. This is an independent committee established to protect the rights of research participants.

We will give you a copy of this sheet which explains the study.

If you would like a copy of a report on this study, please give us an email or postal address. The final results may not be available until 2 years or more from now.

(This information sheet will be available in the most common local languages: e.g. Sesotho, isiXhosa, isiZulu, Setswana, as appropriate)

8.1.3.1 Consent form: next of kin consent for verbal autopsy

Ppt ID: AUR-□□□-□□□□□-□

Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa

Investigators:

London School of Hygiene & Tropical Medicine, UK: Prof. Alison Grant, Dr Katherine Fielding, Dr Aaron Karat, Dr Yasmeen Hanifa

Aurum Institute for Health Research: Dr Salome Charalambous, Dr Kerrigan McCarthy, Dr Mpho Tlali, Prof. Gavin Churchyard

Foundation for Professional Development: Ms Suzanne Johnson

National Health Laboratory Service and University of the Witwatersrand, Johannesburg: Dr Tanvier Omar

Johns Hopkins University, USA: Dr Neil Martinson

University of the Witwatersrand: Prof. Kathleen Kahn

- I have read the information sheet about this study (or the information sheet about this study has been read to me) and I understand what will be required of me and what will happen if I take part in the study.
- My questions concerning this study have been answered by:

_____	_____	_____
Research staff name (printed)	Signature	Date

I understand that I may withdraw from this study at any time without giving a reason. I agree to take part in the study

_____	_____	_____
Study participant name (printed)	Signature/mark/thumbprint	Date

I agree for the interview to be tape-recorded

_____	_____	_____
Study participant name (printed)	Signature/mark/thumbprint	Date

If the information sheet and consent form were translated or explained to the participant, enter the name of the translator here and their signature:

_____	_____	_____
Translator name (printed)	Signature/mark/thumbprint	Date

If the participant gave verbal consent, enter the name of the person who witnessed the consent here and their signature:

_____	_____	_____
Witness name (printed)	Signature/mark/thumbprint	Date

8.2. Appendix 2: Summaries of three parent studies (adapted from the Lesedi Kamoso study protocol)

8.2.1. The TB Fast Track study

Research ethics committee reference numbers

University of the Witwatersrand: R14/49 M111177

London School of Hygiene & Tropical Medicine: 6099

Background: New TB diagnostics have potential to reduce mortality due to TB among people with HIV in developing countries. Urine lipoarabinomannan (LAM) antigen testing has high specificity and moderate sensitivity among HIV-positive individuals with advanced immunosuppression. This test is now available formulated for point-of-care use, at low cost, and could be used in primary care settings to identify TB among those at highest risk of both TB and of death among people with HIV, as could haemoglobin and body mass index.

Aims: To determine 6-month mortality among adults with HIV and CD4 ≤ 150 , presenting for ART, managed using a point-of-care technology-based algorithm to rapidly identify individuals at high risk of TB and ensure they start TB treatment, then ART; and to compare this to 6-month mortality among adults managed according to standard practice based on South African guidelines.

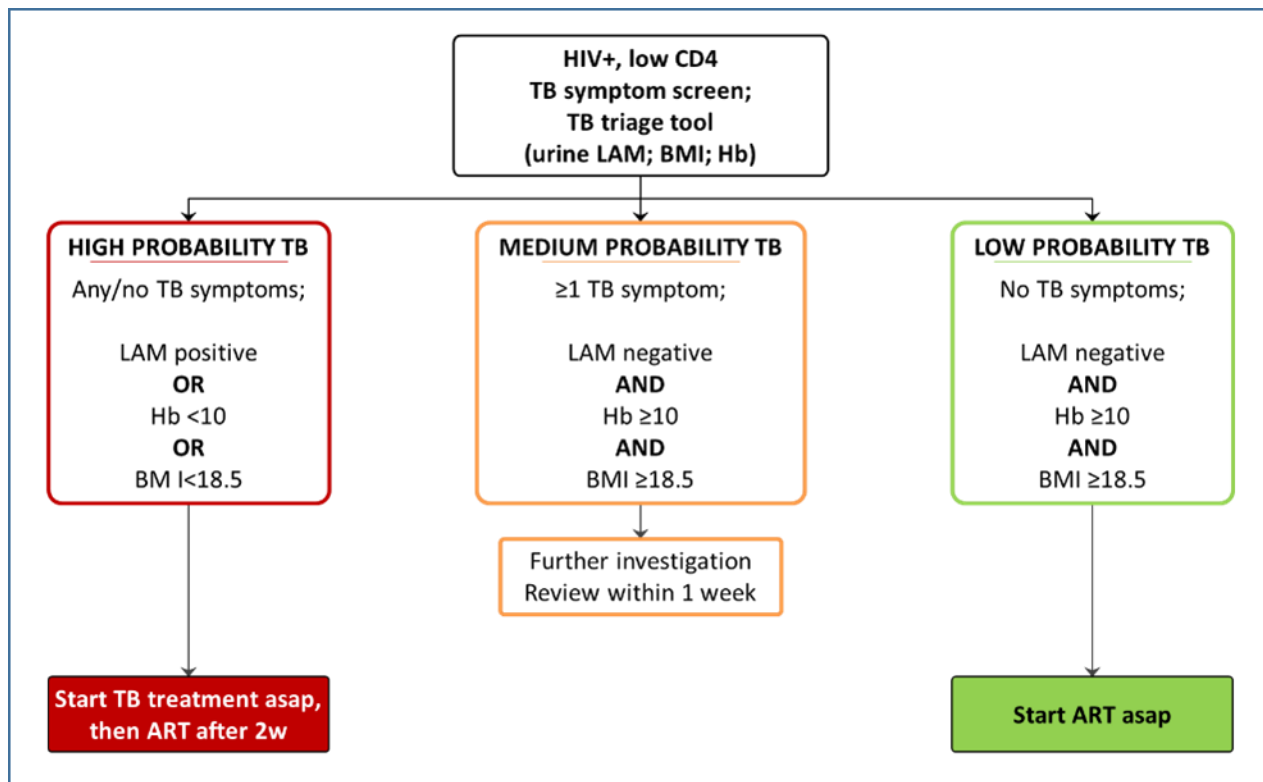
Methods: Open-label, pragmatic cluster-randomised trial, with primary health clinics as the unit of randomisation. Adults (at least 18 years) with CD4 ≤ 150 cells/ μl who are not yet taking ART and who have not had TB treatment in the last 3 months will be eligible. There are few exclusion criteria, such that study participants will be broadly representative of ambulatory patients with HIV and low CD4 counts.

In 12 intervention clinics, consenting adults will be assessed based on symptoms, urine LAM, haemoglobin concentration and body mass index. The study algorithm (Figure 1) will use the results of these assessments to classify individuals as high, medium or low probability of TB. Those with high probability will start TB treatment immediately, followed by ART after 2 weeks. Those with medium probability of TB will follow the South African guidelines (sputum smear, chest radiography and trial of antibiotics) and will be reviewed after one week, to be re-categorised as low or high risk of TB. Those categorised as low probability of TB will start ART as soon as possible.

In 12 control clinics, patients will be managed in the usual way, following South African national guidelines. All participants in both arms will be followed up to at least 6 months. The primary outcome is all-cause mortality at 6 months. We will also explore the cost-effectiveness of this management strategy.

Significance: If the study management strategy proves effective in reducing mortality, this algorithm could be implemented in resource-constrained settings and could substantially reduce early on-ART mortality.

Appendix figure 1. TB Fast Track study algorithm, used with the kind permission of Prof Alison Grant



ART: antiretroviral therapy; asap: as soon as possible; BMI: body mass index; Hb: Haemoglobin; HIV: Human immunodeficiency virus; LAM: lipoarabinomannan; TB: tuberculosis

8.2.2. The XPHACTOR study

Research ethics committee reference numbers

University of the Witwatersrand: M120343

London School of Hygiene & Tropical Medicine: 6165

Background: Intensified tuberculosis (TB) case finding is a key activity in HIV/TB programmes, and is recommended for people with HIV at every clinical encounter. Updated guidelines from WHO recommend TB screening using a new tool comprising any of current cough, fever, night sweats or weight loss (hereafter the "WHO screening tool") which was designed to maximise sensitivity and negative predictive value. However, the specificity, and consequently positive predictive value, of the WHO screening tool is low.

The new polymerase chain-based TB diagnostic test Xpert MTB/RIF (Cepheid) has higher sensitivity than sputum smear, but lower sensitivity than mycobacterial culture, particularly for sputum smear negative specimens, and is costly. Xpert MTB/RIF is recommended by WHO as the initial test for TB suspects who have HIV infection. The combination of new screening guidelines generating an expanded number of HIV-infected TB suspects requiring testing, plus a new test at higher cost, has major implications for health services in resource-constrained settings with a high burden of TB.

We hypothesise that an algorithm which prioritises immediate testing for individuals at highest risk of TB mortality (based on CD4 count and BMI) and/or transmitting TB (based on cough), while allowing deferral of investigation for those assigned lower priority, will reduce health service costs with minimal risk to patients. We propose to test such an algorithm among adults attending clinics in South Africa for HIV care.

The optimal management of symptomatic individuals whose first Xpert MTB/RIF test has a negative result is not yet clear. Current guidelines recommend that the “smear negative” algorithm is followed (sputum for mycobacterial culture, chest radiograph, a course of antibiotics then review). It has been proposed that it would be more cost-effective to undertake an immediate second Xpert MTB/RIF test, but there are no empiric data on which to base this decision.

Aims:

To evaluate an algorithm which identifies, among HIV-infected clinic attendees, those who are "high priority" for immediate investigation with Xpert MTB/RIF, and allows watchful waiting for those assessed as lower priority, comparing

- (i) outcomes and costs using the study algorithm vs.
- (ii) modelled outcomes and costs assuming testing according to the WHO-recommended strategy.

To compare two strategies for further investigation of adults with HIV who are suspected of having TB, but whose first Xpert MTB/RIF test is negative, comparing

- (i) outcomes and costs following the South African national guidelines for further investigation vs.
- (ii) modelled outcomes and costs assuming a second Xpert MTB/RIF test was performed immediately.

To determine diagnoses among HIV-infected clinic attendees who have symptoms suggesting TB, but who do not have a diagnosis of TB based on investigations performed before or at the 3-month visit.

To determine the "natural history" of TB symptoms among individuals without a final diagnosis of TB, in order to estimate the likely demand for repeat Xpert MTB/RIF testing among patients attending regularly for HIV care.

Methods:

Aim 1: interventional cohort study, in which we implement the study algorithm among a representative sample of adults attending three HIV clinics in South Africa. All participants will be assessed and the study algorithm used to assign each to either "high priority" for immediate testing with Xpert MTB/RIF, or "medium/low priority" for watchful waiting. Those assigned medium/low priority will be asked to give a sputum specimen to be stored for study purposes. All participants will be reviewed monthly for TB symptoms, with Xpert MTB/RIF testing initiated if, at the follow-up visit, they now fulfil criteria for "high priority" or have persistent “medium priority” features. All participants will be followed to three months, then tested for TB based on sputum and blood samples for mycobacterial culture. At the end of the

study we will compare outcomes and costs observed based on the study algorithm with modelled outcomes and costs assuming immediate testing with Xpert MTB/RIF based on the WHO recommended algorithm.

Aim 2: cohort study, using data from participants in study aim 1 who undergo Xpert MTB/RIF testing with a negative result, and have a CD4 count below 200 cells/ μ l. Study staff will facilitate further management according to South African guidelines; patients will be followed to determine outcomes, and costs estimated. In addition, at the point where the first Xpert MTB/RIF is negative, an additional sputum specimen will be collected for storage, and tested with Xpert MTB/RIF at the end of the study. Outcomes and costs observed following the South African guidelines will be compared to modelled outcomes and costs that would have been incurred if a second Xpert MTB/RIF test had been carried out immediately.

An additional group of participants will be recruited to strengthen aim 2: these are individuals not taking ART who have a CD4 count below 200 cells/ μ l, who may be identified following a recent HIV test, or as part of follow-up in pre-ART care. These individuals will not be recruited to follow the main XPHACTOR study algorithm since the risk of active TB is very high; thus, all will have sputum sent immediately for MTB/RIF, and none will have testing deferred. However, if their initial Xpert MTB/RIF test result is negative, they could contribute to aim 2. Subsequent follow up will be as for the main study.

Aim 3: at each of two of the study clinics, a subgroup of the first 100 individuals who remain symptomatic by the month 3 visit but whose investigations for TB are negative will be assessed by a research clinician, further investigations arranged, and final diagnosis assigned by month 6.

Aim 4: data from monthly visits of participants contributing to study aims 1 and 3 will be used to estimate the frequency of TB symptoms at repeated visits and thus estimate the demand for repeat testing in routine HIV care.

Significance

This study will identify an evidence-based algorithm, which is feasible to implement within HIV clinics, to guide the use of TB diagnostic investigations. It will address how the effectiveness and cost-effectiveness of a Xpert MTB/RIF based diagnostic algorithm are influenced by how many, and which, patients are selected to undergo testing. The results will complement our larger study of the effectiveness, cost-effectiveness and impact on TB control of Xpert MTB/RIF, and provide evidence to guide the rational use of Xpert MTB/RIF in national roll-out in South Africa and other settings where HIV and TB are both prevalent.

8.2.3. The XTEND study

Research ethics committee reference numbers

University of the Witwatersrand: R14/49 M110827

London School of Hygiene & Tropical Medicine: 6041

Background

South Africa is rolling out Xpert MTB/RIF, a new diagnostic test with improved sensitivity for detection of active tuberculosis combined with simultaneous detection of rifampicin resistance. The test has a greatly reduced turn-around time compared with mycobacterial culture, but cost per test substantially greater than the smear microscopy it is intended to replace. It is not yet clear whether this will improve patient-relevant outcomes, and how it will contribute to tuberculosis control (both drug susceptible and drug resistant) in South Africa and elsewhere.

Aim

To evaluate the effectiveness and cost effectiveness of Xpert MTB/RIF in the investigation of TB and TB drug resistance, and its impact on patient and programme outcomes and transmission at a population level.

Specific objectives

- To measure the effectiveness of Xpert MTB/RIF in improving patient and programme outcomes.
- To determine the likely population level impact on TB transmission of using Xpert MTB/RIF in the investigation of TB and TB drug resistance, using mathematical modelling
- To estimate the cost-effectiveness of Xpert MTB/RIF from a patient and health system perspective

Methods

Study design

A cluster randomised pragmatic trial (CRT) will be conducted, where 20 laboratories in South Africa, in high burden TB districts will be randomised to receive Xpert MTB/Rif technology early, and 20 to receive this technology later.

- Intervention laboratories will receive Xpert MTB/RIF early
 - TB suspects who attend facilities that use these laboratories will have one Xpert MTB/RIF instead of two smears at diagnosis
 - Patients that are sputum Xpert MTB/RIF positive will have a second sputum specimen collected for smear microscopy (with additional culture and DST for those detected with rifampicin resistant TB), in line with South African national guidelines
- Control laboratories will use standard of care diagnostic tests for TB, as per South African national guidelines, during the study, and then implement Xpert MTB/RIF:
 - two smears using fluorescent staining (Auramine O) will be done for all TB suspects,
 - Culture will be done if any of the following criteria are met:
 - Suspects are smear negative, and symptomatic, including those who are HIV positive;
 - high MDR-TB risk;
 - or a prior history of TB.

The NHLS is currently rolling out Xpert MTB/Rif in a phased way. The laboratories in high burden TB districts have all already received Xpert. The 20 laboratories in medium to high districts are all due to receive Xpert MTB/Rif as part of this study. In other words, this study is 'nested' within the national roll out of Xpert. The laboratories that are participating in this study were identified in early 2011 by the NDoH and role-players. For planning needs, the random allocation of laboratories in this study to receive Xpert diagnostic tests early (Dec/Jan 2011) or late (June 2012) was done earlier in the year.

Selection of PHCs for participation

Prior to initiation of the study, two community or primary health care facilities will be chosen for each participating laboratory. The sub-district and district health managers will select the clinics, based on the volume of TB suspects seen per quarter, the availability of space within the facility to accommodate two researchers, and other factors (including presence of other research projects at that facility, or other active NGO support for facilities that may interfere with the study).

Enrolment and follow-up of participants

At each PHC, TB suspects will be offered an opportunity to participate, until 120 suspects have been enrolled at each site. Informed consent will be taken according to approved protocols. Participants will be followed up for 6 months. Amongst the participants, some will develop TB disease and be started on TB treatment. These participants will be followed up for 6 months after the start of initiation of TB treatment. During the course of the study, research nurses will collect information on demographic characteristics, TB and HIV history, clinic usage, economic indicators, diagnostic tests done, and results, and outcome.

Evaluation and analysis

On completion of the study patient outcomes will be measured on TB suspects and TB patients attending clinics being serviced by these laboratories, and will include six month mortality amongst TB suspects as the primary outcome. We will determine whether the advent of Xpert MTB/RIF alters provider behaviour with respect to investigating TB suspects, and to estimate costs from the patients' perspective. Comprehensive economic costs (including costs to the health system) will also be measured, together with the parameters required for the modelling of population impact.

Significance of this study

These data will enable us to estimate effectiveness, cost and cost-effectiveness of implementation of Xpert MTB/RIF in the context of national roll-out. The data generated will populate mathematical and economic models which will explore the impact of roll-out of Xpert MTB/RIF on TB control, in South Africa and elsewhere. In addition, the data will allow us to model the effect of varying test algorithms on cost-effectiveness and future resource requirements; and will guide the development of further work to test how Xpert MTB/RIF can best be used within the health system to improve patient outcomes and TB control.

8.3. Appendix 3: Regulatory documents

8.3.1. Ethical approvals

8.3.1.1 London School of Hygiene & Tropical Medicine

London School of Hygiene & Tropical Medicine

Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk



Observational / Interventions Research Ethics Committee

Professor Alison Grant
Professor of International Health
Department of Clinical Research (CRD)
Infectious and Tropical Diseases (ITD)
LSHTM

18 March 2015

Dear Alison

Study Title: Lesedi Kamoso: Prevalence of TB and other treatable diseases at autopsy in South Africa (2)

LSHTM Ethics Ref: 9128

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Other	LK_LSHTMEthicsApplication_20130314	14/03/2013	n/a
Other	LK_LSHTMEthicsApplication_SupportingDocs_20130314	14/03/2013	1.0
Other	LK_Protocol_v2.0_20130607	07/06/2013	2.0
Other	LK_Protocol_v3.0_20130915_clean	15/09/2013	3.0
Other	LK_Protocol_v4.0_20140331_clean	31/03/2014	4.0
Other	LK_Protocol_v5.0_20140615_clean	15/06/2014	5.0
Other	LesediKamoso_Protocol_v5.1_20150130_changesmarked	30/01/2015	5.1
Other	LK_ExtraInfoBereaved_v1.1_20150130_changesmarked	30/01/2015	1.1
Other	LK_ExtraInfoPpts_v1.1_20150130_changesmarked	30/01/2015	1.1
Covering Letter	LK_LSHTMEthics_ResponseToQuery_20150310	10/03/2015	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: <http://leo.lshtm.ac.uk>

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

A handwritten signature in blue ink, appearing to be 'A Grant'.

8.3.1.2 University of the Witwatersrand

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Phillip Tobias Building, 2nd Floor Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265



24 February 2015

Dr Salome Charalambous

Aurum House, The Ridge
29 Queens Road
Parktown
2193

Sent by email to: scharalambous@auruminstitute.org

Dear Dr Charalambous

Re: Protocol Ref no: M130381

Protocol Title: Lesedi Kamoso: Prevalence of TB and Other Treatable Diseases at Autopsy in South Africa (A Study Nested Within the TB Fast Track, Xphactor and Xtend Studies).

Principal Investigator: Dr Salome Charalambous

Protocol Amendment

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved your amendments of stopping to conduct needle autopsies in Limpopo Province the on the above mentioned protocol, as detailed in your letter received on 06 February 2015.

The following documents were received:

- Amended Protocol v5.1 dd 20150205 (clean and with track changes).
- Information for Participants and Families- Extra Info Ppts v1.1 dd 20150130 (track changes).
- Information for Bereaved Families- Extra Info Bereaved- v1.1 dd 20150130 (track changes).

Thank you for keeping us informed and updated,

Yours Sincerely,

A handwritten signature in black ink, appearing to be 'M. Masingi', written over a dotted line.

Mr Langutani Masingi
Administrative Officer
Human Research Ethics Committee (Medical)



8.3.2. Cause of death notification letter to families (MIA only)



Lesedi Kamoso

Notification of likely cause of death

[DATE]

To the family of _____,

Thank you again for your help in allowing us to do this study. We are sure that the information we are collecting will be very useful in improving the way we treat tuberculosis (TB) in South Africa. You are a part of that improvement.

After reviewing the results of the needle-autopsy procedure conducted on _____, we think it is likely that your relative died due to _____ and advanced HIV disease.

Please do not hesitate to contact me if you have any questions or require any further information.

Kind regards,

Dr Aaron Karat
Research Fellow

Telephone: 010 590 1360
Mobile: 081 026 2001
Email: aaron.karat@lshtm.ac.uk

On behalf of the Principal Investigators:

Professor Alison Grant, London School of Hygiene and Tropical Medicine, United Kingdom
Dr Salome Charalambous, The Aurum Institute, Johannesburg, South Africa

**Please note that this letter is not a legal document
and does not serve as a replacement for the official death certificate**

8.3.3. Referral to clinical letter for families (MIA or VA)



NPC
Registration No. 1998/009355/08
041-083-NPO

Aurum House
The Ridge, 29 Queens Road
Parktown
Johannesburg
South Africa 2001
PostNet Suite300
Private Bag X30500
Houghton 2041
South Africa
Tel: +27 (0) 11 484 8844
Fax: +27 (0) 11 484 7841
Website: www.auruminstitute.org

TO ALL CLINICAL STAFF AT _____ CLINIC

Date: _____

Dear Doctor/Sister,

Please accept the referral of _____ for
TB screening +/- investigation (and screening for any other relevant illnesses). She/he has been in
close contact with a recently deceased individual who was at high risk of TB.

Please do not hesitate to contact me if you require any further information.

Kind regards,

Dr Aaron Karat
Research Fellow
aaron.karat@lshtm.ac.uk
010 590 1360; 081 026 2001

On behalf of Principal Investigators: Prof Alison Grant & Dr Salome Charalambous

STUDY NUMBER

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/ /



15. What was her/his highest level of schooling?

- | | |
|--------------------------------|---|
| 01 = No school/pre-school only | 06 = Matric with Technical Qualification or Diploma |
| 02 = Grade 1-3 | 07 = Bachelor's Degree |
| 03 = Grade 4-7 | 08 = Master's or Doctoral Degree |
| 04 = Grade 8-11 | |
| 05 = Grade 12 | 99 = Don't know |

16. Was s/he able to read and write? 1=Yes, 0=No, 9=Don't know

17. What was her/his employment status in the year prior to death?

- | | |
|-----------------------|--------------------------|
| 1 = Mainly employed | 5 = Pensioner |
| 2 = Mainly unemployed | 6 = Other, specify _____ |
| 3 = Home-maker | |
| 4 = Student | 9 = Don't know |

18. What was her/his occupation (what kind of work did s/he mainly do)? _____

19. What was her/his South African ID number?

Score out if not available or not South African

SECTION 3: Registration of the death

20. Do you have a death certificate or death registration form? 1=Yes, 0=No

**If NO, score out Q21-22 and go to Q23 (section 4) →
If YES, but the form is NOT AVAILABLE, score out Q21 and go to Q22**

21. Date of Death recorded on certificate/registration form: dd/mmm/yyyy / /

21a. Cause of death recorded on death certificate/registration form: _____

21b. Contributing causes: _____

21c. Underlying causes: _____

22. If you have a death form, but unavailable, where is it? _____

CONTINUE TO SECTION 4 →

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NARRATIVE:

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QA:

Data entry:

Date Entered: //

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24. What do you think was the matter with your loved one? _____

25. Did your loved one ever tell you what the matter with her/him was? Please explain: _____

26. Were there any difficulties that stopped her/him from seeking care at a clinic or hospital? _____

27. Cause of death declared by respondent/family (#1): _____

28. Cause of death declared by respondent/family (#2): _____

CONTINUE TO SECTION 5 →

Completed by: [] [] [] QA: [] [] [] Data entry: [] [] [] Date Entered: [] [] / [] [] [] / [] [] [] []

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SECTION 5: Context and history of previously known medical conditions

29. Was there any diagnosis of Tuberculosis?1=Yes, 0=No, 9=Don't know

If NO or DON'T KNOW, score out Q29a-29g and go to Q30

29a. Did s/he ever take treatment for TB from a clinic/hospital?1=Yes, 0=No, 9=Don't know

29b. When did s/he start TB treatment?dd/mmm/yyyy [] [] / [] [] [] / [] [] [] []
(If treated more than once, record the most recent episode; 97/997/9997=NA; 99/999/9999=Don't know)

29c. Which clinic/health facility did s/he attend most recently (name)? _____

29d. Location of clinic/health facility (nearest town): _____

29e. Were they still taking TB treatment at the time of death?1=Yes, 0=No, 7=NA, 9=Don't know

29f. If TB treatment was stopped early why was that?

- | | |
|--|---------------------------|
| 1 = Treatment side-effects (stopped him/herself) | 5 = Moved to a new area |
| 2 = Treatment side-effects (stopped by clinic) | 6 = Other, specify: _____ |
| 3 = Unable to reach the clinic | 7 = Not applicable |
| 4 = Felt s/he didn't need them any more | 9 = Don't know |

29g. Was s/he taking medication every day in the way they s/he was supposed to?
.....1=Yes, 0=No, 7=NA, 9=Don't know

30. Was there any diagnosis of HIV/AIDS?1=Yes, 0=No, 9=Don't know

If NO or DON'T KNOW, score out Q30a-30g and go to Q31

30a. Did the deceased ever take Antiretroviral Therapy (ART)?1=Yes, 0=No, 9=Don't know

30b. When did s/he start ART?dd/mmm/yyyy [] [] / [] [] [] / [] [] [] []
(If started more than once, record the most recent episode; 97/997/9997=NA; 99/999/9999=Don't know)

30c. Which clinic/health facility did s/he attend most recently (name)? _____

30d. Location of clinic/health facility (nearest town): _____

30e. Were they still taking ART at the time of death?1=Yes, 0=No, 7=NA, 9=Don't know

30f. If ART was stopped, why was that?

- | | |
|--|---------------------------|
| 1 = Treatment side-effects (stopped him/herself) | 5 = Moved to a new area |
| 2 = Treatment side-effects (stopped by clinic) | 6 = Other, specify: _____ |
| 3 = Unable to reach the clinic | 7 = Not applicable |
| 4 = Felt s/he didn't need them any more | 9 = Don't know |

30g. Was s/he taking the medication every day?1=Yes, 0=No, 7=NA, 9=Don't know

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31. Did s/he have a recent POSITIVE test for Malaria (within 1 week of death)? 1=Yes, 0=No, 9=Don't know
- 31a. Did s/he have a recent NEGATIVE test for Malaria? 1=Yes, 0=No, 7=NA, 9=Don't know
32. Was there any diagnosis of Measles (within 1 month of death)? 1=Yes, 0=No, 9=Don't know
33. Was there any diagnosis of High Blood Pressure? 1=Yes, 0=No, 9=Don't know
34. Was there any diagnosis of Heart Disease? 1=Yes, 0=No, 9=Don't know
35. Was there any diagnosis of Diabetes? 1=Yes, 0=No, 9=Don't know
36. Was there any diagnosis of Asthma? 1=Yes, 0=No, 9=Don't know
37. Was there any diagnosis of Epilepsy (fits)? 1=Yes, 0=No, 9=Don't know
38. Was there any diagnosis of Cancer? 1=Yes, 0=No, 9=Don't know
39. Was there any diagnosis of Chronic Obstructive Pulmonary Disease (COPD)? 1=Yes, 0=No, 9=Don't know
40. Was there any diagnosis of Dementia? 1=Yes, 0=No, 9=Don't know
41. Was there any diagnosis of Depression? 1=Yes, 0=No, 9=Don't know
42. Was there any diagnosis of other mental illness (schizophrenia, psychoses, bipolar disorder)?
..... 1=Yes, 0=No, 9=Don't know
43. Was there any diagnosis of Stroke? 1=Yes, 0=No, 9=Don't know
44. Was there any diagnosis of Sickle Cell disease/Sickle Cell anaemia? 1=Yes, 0=No, 9=Don't know
45. Was there any diagnosis of Kidney disease? 1=Yes, 0=No, 9=Don't know
46. Was there any diagnosis of Liver disease? 1=Yes, 0=No, 9=Don't know
47. Did s/he die during the summer? 1=Yes, 0=No, 9=Don't know
48. Did s/he die during the winter? 1=Yes, 0=No, 9=Don't know
49. For how long was s/he ill before s/he died (in weeks & days)?..... www:d □□□□:□□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:9=Don't know)
50. Did s/he die suddenly (illness of ≤1 day, or death unexpected)? 1=Yes, 0=No, 9=Don't know

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SECTION 6: History of injuries/accidents

51. Did s/he suffer from any injury or accident that led to her/his death?1=Yes, 0=No, 9=Don't know

If NO and the deceased was MALE (Q4=1), score out Q52-100 and go to Q101 (section 9) →→→

If NO and the deceased was FEMALE (Q4=2), score out Q52-66 and go to Q67 (section 7) →

52. Did s/he suffer from a road traffic accident?1=Yes, 0=No

If NO, score out Q52a-Q52f7a and go to Q53

52a. Was s/he injured as a pedestrian/walking?1=Yes, 0=No, 9=Don't know

52b. Was s/he injured as an occupant of a car vehicle?1=Yes, 0=No, 9=Don't know

52c. Was s/he injured as an occupant of a bus/heavy transport vehicle?1=Yes, 0=No, 9=Don't know

52d. Was s/he injured as a driver or passenger of a motorcycle?1=Yes, 0=No, 9=Don't know

52e. Was s/he injured as a pedal cyclist?1=Yes, 0=No, 9=Don't know

52f. Do you know anything about the counter-part that was hit during the road traffic accident?

.....1=Yes, 0=No

52f1. Was it a pedestrian?1=Yes, 0=No, 7=NA, 9=Don't know

52f2. Was it a stationary object?1=Yes, 0=No, 7=NA, 9=Don't know

52f3. Was it a car vehicle?1=Yes, 0=No, 7=NA, 9=Don't know

52f4. Was it a bus or heavy transport vehicle?1=Yes, 0=No, 7=NA, 9=Don't know

52f5. Was it a motorcycle?1=Yes, 0=No, 7=NA, 9=Don't know

52f6. Was it a pedal cycle?1=Yes, 0=No, 7=NA, 9=Don't know

52f7. Was it something else (not included in Q53f1-53f6)?1=Yes, 0=No, 7=NA, 9=Don't know

52f7a. Please specify (or enter NA): _____

53. Was s/he injured in a transport-related accident that did not occur on the road? .1=Yes, 0=No, 9=Don't know

54. Did s/he die as a result of a fall?1=Yes, 0=No, 9=Don't know

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55. Did s/he die of drowning? 1=Yes, 0=No, 9=Don't know
56. Did s/he suffer from burns? 1=Yes, 0=No, 9=Don't know
57. Did s/he suffer from any plant/animal/insect bite or sting that led to her/his death? 1=Yes, 0=No
- 57a. Was it a dog? 1=Yes, 0=No, 7=NA, 9=Don't know
- 57b. Was it a snake? 1=Yes, 0=No, 7=NA, 9=Don't know
- 57c. Was it an insect? 1=Yes, 0=No, 7=NA, 9=Don't know
- 57d. Was it something else? 1=Yes, 0=No, 7=NA, 9=Don't know
- 57d1. Please specify (or enter NA): _____
58. Was s/he injured by a force of nature? 1=Yes, 0=No, 9=Don't know
59. Was there any poisoning? 1=Yes, 0=No, 9=Don't know
60. Was s/he subject to violence or assault? 1=Yes, 0=No, 9=Don't know
61. Was the injury intentionally inflicted by someone else? 1=Yes, 0=No, 9=Don't know
62. Was s/he injured with a firearm? 1=Yes, 0=No, 9=Don't know
63. Was s/he injured from a stab, cut, or pierce? 1=Yes, 0=No, 9=Don't know
64. Was s/he injured by machinery? 1=Yes, 0=No, 9=Don't know
65. Was s/he struck by an animal or object? 1=Yes, 0=No, 9=Don't know
66. Do you think s/he committed suicide? 1=Yes, 0=No, 9=Don't know

If the deceased was MALE (Q4=1), score out Q67-100 and go to Q101 (Section 9) →→→

If the deceased was FEMALE (Q4=2), go to Q67 (Section 7) →

Completed by: □□□□

QA: □□□□

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SECTION 7: Symptoms and signs associated with illness of women

Instructions: Only ask the following questions if the deceased was FEMALE.

- 67. Did she have an ulcer or swelling in the breast?.....1=Yes, 0=No, 9=Don't know
- 68. Did she have excessive vaginal bleeding in between menstrual periods?1=Yes, 0=No, 9=Don't know
- 69. Did her vaginal bleeding stop naturally during menopause?1=Yes, 0=No, 7=NA, 9=Don't know
- 70. Did she have vaginal bleeding after menopause?1=Yes, 0=No,7=NA, 9=Don't know

SECTION 8: Symptoms and signs associated with pregnancy (see pg 20 for pregnancy timeline)

Instructions: Only ask the following questions if the deceased was FEMALE.

- 71. Was she pregnant or had she delivered within 6 weeks of her death?1=Yes, 0=No, 9=Don't know

If NO or DON'T KNOW, score out Q72-100 and go to Q101 (Section 9) →

- 72. Was she pregnant at the time of death?1=Yes, 0=No, 9=Don't know
- 73. Did she die within 6 weeks of giving birth?.....1=Yes, 0=No, 9=Don't know
- 74. Did she die within 6 weeks of a pregnancy that lasted less than 6 months?1=Yes, 0=No, 9=Don't know
- 75. Did she die within 24 hours after delivery?1=Yes, 0=No, 9=Don't know
- 76. Did she die during labour, but undelivered?.....1=Yes, 0=No, 9=Don't know
- 77. Was she breastfeeding at death?.....1=Yes, 0=No, 9=Don't know
- 78. How many births, including stillbirths did she have before this baby?99=Don't know
- 79. Did she have any previous C-section?.....1=Yes, 0=No, 9=Don't know
- 80. Did she die during or after a multiple pregnancy?.....1=Yes, 0=No, 9=Don't know
- 81. During pregnancy, did she suffer from high blood pressure?.....1=Yes, 0=No, 9=Don't know
- 82. Did she have foul smelling vaginal discharge during pregnancy or after delivery?..1=Yes, 0=No, 9=Don't know
- 83. During the last 3 months of pregnancy, did she suffer from convulsions?1=Yes, 0=No, 9=Don't know
- 84. During the last 3 months of pregnancy, did she suffer blurred vision?1=Yes, 0=No, 9=Don't know
- 85. Did she give birth to a live, healthy baby within 6 weeks of death?.....1=Yes, 0=No, 9=Don't know

Completed by: □□□□

QA: □□□□

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- 86. Was there any vaginal bleeding during pregnancy or after delivery? 1=Yes, 0=No, 9=Don't know
- 86a. Was there any vaginal bleeding during the **first 6 months** of pregnancy?.....
..... 1=Yes, 0=No, 7=NA, 9=Don't know
- 86b. Was there vaginal bleeding during the **last 3 months** of pregnancy but before labour started?
..... 1=Yes, 0=No, 7=NA, 9=Don't know
- 86c. Was there excessive vaginal bleeding **during labour**? 1=Yes, 0=No, 7=NA, 9=Don't know
- 86d. Was there excessive vaginal bleeding **after delivering the baby**? 1=Yes, 0=No, 7=NA, 9=Don't know
- 87. Was the placenta completely delivered?..... 1=Yes, 0=No, 9=Don't know
- 88. Did she deliver or try to deliver an abnormally positioned baby?..... 1=Yes, 0=No, 9=Don't know
- 89. Was she in labour for unusually long (more than 24 hours)?..... 1=Yes, 0=No, 9=Don't know
- 90. Did she attempt to terminate the pregnancy? 1=Yes, 0=No, 9=Don't know
- 91. Did she recently have a pregnancy that ended in an abortion (spontaneous or induced)?.....
..... 1=Yes, 0=No, 9=Don't know
- 92. Did she give birth in a health facility? 1=Yes, 0=No, 9=Don't know
- 93. Did she give birth at home? 1=Yes, 0=No, 9=Don't know
- 94. Did she give birth elsewhere, e.g. on the way to the facility? 1=Yes, 0=No, 9=Don't know
- 95. Was there a midwife, nurse or doctor present at the delivery?..... 1=Yes, 0=No, 9=Don't know
- 96. Did she have an operation to remove her uterus shortly before death? 1=Yes, 0=No, 9=Don't know
- 97. Did she have a normal vaginal delivery? 1=Yes, 0=No, 9=Don't know
- 98. Did she have an assisted delivery, with forceps/vacuum? 1=Yes, 0=No, 9=Don't know
- 99. Was it a delivery with caesarean section? 1=Yes, 0=No, 9=Don't know
- 100. Was the baby born more than one month early?..... 1=Yes, 0=No, 9=Don't know

Completed by: □□□□ QA: □□□□ Data entry: □□□□ Date Entered: □□/□□□□/□□□□

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Section 9: Symptoms noted during the final illness

101. Did s/he have a **fever**? 1=Yes, 0=No, 9=Don't know

101a. For how long did s/he have a fever? www:d □□□:□
 (If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)

102. Did s/he have **night sweats**? 1=Yes, 0=No, 9=Don't know

102a. For how long did s/he have night sweats? www:d □□□:□
 (If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)

103. Did s/he have a **cough**? 1=Yes, 0=No, 9=Don't know

103a. For how long did s/he have a cough? www:d □□□:□
 (If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)

103b. Was the cough productive with sputum? 1=Yes, 0=No, 7=NA, 9=Don't know

103c. Did s/he cough out blood? 1=Yes, 0=No, 7=NA, 9=Don't know

104. Did s/he have any **breathing problem**? 1=Yes, 0=No, 9=Don't know

104a. Did s/he have fast breathing? 1=Yes, 0=No, 7=NA, 9=Don't know

104a1. For how long did s/he have fast breathing? www:d □□□:□
 (If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)

104b. Did s/he have breathlessness? 1=Yes, 0=No, 7=NA, 9=Don't know

104b1. For how long did s/he have breathlessness? www:d □□□:□
 (If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)

104b2. Was s/he unable to carry out daily routine activities due to breathlessness?
 1=Yes, 0=No, 7=NA, 9=Don't know

104b3. Was s/he breathless while lying flat? 1=Yes, 0=No, 7=NA, 9=Don't know

104c. Did s/he have noisy breathing (wheezing)? 1=Yes, 0=No, 7=NA, 9=Don't know

[DEMONSTRATE IF NEEDED]

105. Did s/he have **severe chest pain**? 1=Yes, 0=No, 9=Don't know

106. Did s/he have **diarrhoea**? 1=Yes, 0=No, 9=Don't know

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- 106a. For how long did s/he have a diarrhoea?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
107. At any time in the final illness was there **blood in the stools**?1=Yes, 0=No, 9=Don't know
108. Did s/he **vomit**?1=Yes, 0=No, 9=Don't know
- 108a. Did s/he vomit 'coffee grounds' or bright red/blood?1=Yes, 0=No, 7=NA, 9=Don't know
109. Did s/he have any **abdominal problem**?1=Yes, 0=No, 9=Don't know
- 109a. Did s/he have severe abdominal pain?1=Yes, 0=No, 7=NA, 9=Don't know
- 109a1. For how long before death did s/he have severe abdominal pain?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
- 109b. Did s/he have a more than usual protruding abdomen?1=Yes, 0=No, 7=NA, 9=Don't know
- 109b1. For how long did s/he have a more than usual protruding abdomen?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
- 109c. Did s/he have any lump inside the abdomen?1=Yes, 0=No, 7=NA, 9=Don't know
- 109c1. For how long did s/he have a lump inside the abdomen?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
110. Did s/he have a **severe headache**?1=Yes, 0=No, 9=Don't know
111. Did s/he have a **stiff or painful neck**?1=Yes, 0=No, 9=Don't know
- 111a. For how long did s/he have a stiff or painful neck?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
112. Did s/he have **mental confusion**?1=Yes, 0=No, 9=Don't know
- 112a. For how long did s/he have mental confusion?www:d □□□:□
(If more than 3 weeks, enter only number of weeks and 0 for days; 997:7=NA, 999:9=Don't know)
113. Was s/he **unconscious for more than 24 hours**?1=Yes, 0=No, 9=Don't know
- 113a. Did the unconsciousness start suddenly, quickly (at least within a single day)?
1=Yes, 0=No, 7=NA, 9=Don't know

Completed by: □□□□

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122. Did s/he have **both feet swollen**? 1=Yes, 0=No, 9=Don't know

123. Did s/he have any **lumps**? 1=Yes, 0=No, 9=Don't know

123a. Did s/he have any lumps or lesions in the mouth? 1=Yes, 0=No, 9=Don't know

123b. Did s/he have any lumps on the neck? 1=Yes, 0=No, 9=Don't know

123c. Did s/he have any lumps on the armpit? 1=Yes, 0=No, 9=Don't know

123d. Did s/he have any lumps on the groin? 1=Yes, 0=No, 9=Don't know

124. Did s/he have **paralysis of one side of the body**? 1=Yes, 0=No, 9=Don't know

125. Did s/he have **difficulty or pain while swallowing liquids**? 1=Yes, 0=No, 9=Don't know

126. Did s/he have **yellow discolouration of the eyes**? 1=Yes, 0=No, 9=Don't know

127. Did her/his **hair colour change to reddish or yellowish**? 1=Yes, 0=No, 9=Don't know

128. Did s/he **look pale (thinning/lack of blood) or have pale palms, eyes or nail beds**?
..... 1=Yes, 0=No, 9=Don't know

129. Did s/he have **sunken eyes**? 1=Yes, 0=No, 9=Don't know

130. Did s/he **drink a lot more water than usual**? 1=Yes, 0=No, 9=Don't know

Section 10: Treatment and health service use for the final illness

131. Was s/he vaccinated? 1=Yes, 0=No, 9=Don't know

132. Did s/he receive medical treatment for the illness (or injury) that led to death? ... 1=Yes, 0=No, 9=Don't know

If NO or DON'T KNOW, score out Q132a-132g and go to Q133 (Section 11) →

132a. Did s/he receive oral rehydration salts? 1=Yes, 0=No, 9=Don't know

132b. Did s/he receive (or need) intravenous fluids (drip) treatment? 1=Yes, 0=No, 9=Don't know

132c. Did s/he receive (or need) a blood transfusion? 1=Yes, 0=No, 9=Don't know

132d. Did s/he receive (or need) treatment/food through a tube passed through the nose?

Completed by: □□□□

QA: □□□□

Data entry: □□□□

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137. Did s/he use motorised transport to get to the hospital or health facility?1=Yes, 0=No, 9=Don't know

138. Were there any problems during admission to the hospital or health facility?1=Yes, 0=No, 9=Don't know

138a. What were the problems (or enter NA)?

138b. Was it the waiting time?1=Yes, 0=No, 7=NA, 9=Don't know

138c. Was s/he turned away because s/he didn't have a referral letter?1=Yes, 0=No, 7=NA, 9=Don't know

138d. Was is an issue with the catchment area?1=Yes, 0=No, 7=NA, 9=Don't know

138e. Was it due to a shortage of staff?1=Yes, 0=No, 7=NA, 9=Don't know

138f. Was it due to a shortage of beds?1=Yes, 0=No, 7=NA, 9=Don't know

138g. Was it an issue with administrative processes?1=Yes, 0=No, 7=NA, 9=Don't know

138h. Was it due to a lack of identity document/passport?1=Yes, 0=No, 7=NA, 9=Don't know

138i. Was it something else?1=Yes, 0=No, 7=NA, 9=Don't know

138i1. Please specify (or enter NA): _____

139. Were there any problems with the way s/he was treated (medical treatment, procedures, interpersonal attitudes, respect, dignity) in the hospital or health facility?1=Yes, 0=No, 9=Don't know

139a. What were the problems (or enter NA)?

139b. Was it due to a shortage of treatment/medication?1=Yes, 0=No, 7=NA, 9=Don't know

139c. Was it to do with interpersonal attitudes?1=Yes, 0=No, 7=NA, 9=Don't know

139d. Was it to do with a lack of respect/dignity?1=Yes, 0=No, 7=NA, 9=Don't know

139e. Was it because s/he forgot her/his treatment card?.....1=Yes, 0=No, 7=NA, 9=Don't know

Completed by: □□□□

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140. Were there any problems getting medications or diagnostic tests in the hospital or health facility?.....

.....1=Yes, 0=No, 9=Don't know

141. Does it take more than 2 hours to get to the nearest hospital or health facility from the deceased's household?...

.....1=Yes, 0=No, 9=Don't know

142. In the final days before death, were there any doubts about whether medical care was needed?

.....1=Yes, 0=No, 9=Don't know

143. In the final days before death, was traditional medicine used?.....1=Yes, 0=No, 9=Don't know

144. In the final 24 hours before death, did anyone use a telephone or cell phone to call for help?

.....1=Yes, 0=No, 9=Don't know

145. Over the course of the illness, did the total costs of care and treatment prohibit other household payments?

.....1=Yes, 0=No, 9=Don't know

Section 13: History of mining

146. Had the deceased worked at a mine?.....1=Yes, 0=No, 9=Don't know

If NO or DON'T KNOW, score out Q146a-Q146e and go to Q147 (Section 14) →

146a. What type of mine?.....

- 1 = Gold
- 2 = Platinum
- 3 = Chromium
- 4 = Asbestos
- 6 = Other, specify _____
- 9 = Don't know

146b. What was the name/and or location of the mine? _____

146c. Was there any compensation received for the illness?1=Yes, 0=No, 9=Don't know

146d. How many years did the s/he work underground?

(97/997/9997=NA [didn't work underground], 999=Don't know)

146e. When did s/he last work underground?dd/mmm/yyyy / /

(97/997/9997=NA [didn't work underground], 99/999/9999=Don't know)

CONTINUE TO SECTION 14 →

Completed by:

QA:

Data entry:

Date Entered: / /



Ppt ID: - - Date of abstraction: / /

8. Other drug hx at admission (other than TB treatment/ARVs):

	Drug name (1)	Dose (2)	Frequency (3)
8a			
8b			
8c			
8d			
8e			
8f			
8g			
8h			

Please continue on CRF HN001A if needed

9. Admission observations (enter NA if not measured/recorded):

	Measurement	Value (1)	Units (2)
9a	Systolic BP		(mmHg)
9b	Diastolic BP		(mmHg)
9c	Heart rate/pulse		
9d	Respiratory rate		
9e	Temperature		
9f	Saturations		(%)
9g	Blood glucose		
9h	Glasgow Coma Score		(NA)
9i	Weight		
9j	Other 1		
9k	Other 2		

10. Examination findings on admission (peripheral/CVS/RS/CNS/Abdo/MSK/skin/other):

Completed by: Entered by: Date entered: / /

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Ppt ID: - - Date of abstraction: / /

11. Investigations & results:

	Investigation	Date (a)	Result (b)	Date (c)	Result (d)	Date (e)	Result (f)
11a FBC							
11a1	Hb						
11a2	WBC						
11a3	Neut						
11a4	Lymph						
11a5	Plt						
11a6	Hct						
11a7	MCV						
11a8	MCH						
11a9	MCHC						
11b U&E							
11b1	Na						
11b2	K						
11b3	U						
11b4	Cr						
11b5	eGFR						
11b6	Chloride						
11b7	Bicarbonate						
11b8	Anion gap						
11c LFT							
11c1	ALT						
11c2	AST						
11c3	Total bilirubin						
11c4	Conj bilirubin						
11c5	GGT						
11c6	ALP						
11c7	Alb						
11c8	Tot Prot						
11d Other venous blood							
11d1	CD4						
11d2	V/L						
11d3	CRP						
11d4	ESR						
11d5	LDH						
11d6	Amylase						
11d7	Serum CrAg						
11e Arterial blood gas							
11e1	pH						
11e2	H+						
11e3	HCO3						
11e4	PO2						
11e5	pCO2						

Completed by: Entered by: Date entered: / /

TBFT_Hosp_Deceased_v4.0_20150423



Ppt ID: - - Date of abstraction: / /

	Investigation	Date (a)	Result (b)	Date (c)	Result (d)	Date (e)	Result (f)
Arterial blood gas continue							
11e6	BE						
11e7	K+						
11e8	Na+						
11e9	Lactate						
11f Sputum							
11f1	Microscopy						
11f2	GXP						
11f3	Culture						
11g Urine							
11g1	pH						
11g2	Protein						
11g3	Blood						
11g4	Bilirubin						
11g5	Glucose						
11g6	Nitrates						
11g7	Ketones						
11g8	Urobilinogen						
11g9	Specific gravity						
11g10	Microscopy						
11g11	Culture						
11g12	Pregnancy						
11g13	LAM						
11h CSF							
11h1	CrAg						
11h2	Microscopy						
11h3	Gram stain						
11h4	India ink						
11h5	Protein						
11h6	WCC						
11h7	Lymphocytes						
11h8	Aerobic culture						
11h9	Fungal culture						
11h10	TB culture						
11i Miscellaneous							
11i1	ECG						
11i2	CXR						
11i3	CT						
11i4	Other 1						
11i5	Other 2						
11i6	Other 3						
11i7	Other 4						
11i8	Other 5						

Please continue on CRF HN001B if needed

Completed by: TBFT_Hosp_Deceased_v4.0_20150423

Entered by:

Date entered: / /



Ppt ID: - - Date of abstraction: / /

12. Major diagnoses: (see Appendix 1 for list of diagnoses; 997=NA)

12a. Major diagnosis 1:
 12ai. If other, specify: _____

12b. Major diagnosis 2:
 12bi. If other, specify: _____

13. Minor diagnoses: (see Appendix 1 for list of diagnoses; 997=NA)

13a. Minor diagnosis 1:
 13ai. If other, specify: _____

13b. Minor diagnosis 2:
 13bi. If other, specify: _____

13c. Minor diagnosis 3:
 13ci. If other, specify: _____

13d. Minor diagnosis 4:
 13di. If other, specify: _____

13e. Minor diagnosis 5:
 13ei. If other, specify: _____

14. Treatment(s) started on admission:

	Drug name (1)	Dose (2)	Route (3)	Frequency (4)	Date started (5)
14a					
14b					
14c					
14d					
14e					
14f					
14g					
14h					
14i					
14j					
14k					
14l					
14m					
14n					
14o					

Please continue on CRF HN001C if needed

Completed by: _____ Entered by: _____ Date entered: / /



Ppt ID: - - Date of abstraction: / /

15. Procedures performed during admission:

	Procedure (1)	Date (2)	Indication (3)	Outcome (4)
15a				
15b				

16. Ward admitted to: _____

17. Timeline of admission (overview: include changes to tx, significant results, major events, specialist reviews, etc):

Completed by: _____ Entered by: _____ Date entered: / /

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Ppt ID: - - Date of abstraction: / /

18. Outcome of admission:1=Discharged home; 2=Transferred out; 3=Died

19. Date of discharge/death:..... dd/MMM/yyyy / /

20. Follow-up planned?1=Yes; 0=No; 9=Don't know
If Q20=NO or DON'T KNOW, score out remaining questions and STOP, form is complete

Details of follow-up 1

20a. Date: dd/MMM/yyyy / /

20b. Site:
 1=Out-patient clinic at admitting hospital ; 2=Primary health care clinic
 6=Other, specify _____

20c. Type
 1=Review by general doctor; 2=Review by specialist; 3=Review by nurse; 4=Further investigation
 6=Other, specify: _____

20ci. If further investigation, give details: _____

Details of follow-up 2

20d. Date: dd/MMM/yyyy / /

20e. Site:
 1=Out-patient clinic at admitting hospital; 2=Primary health care clinic
 6=Other, specify _____

20f. Type
 1=Review by general doctor; 2=Review by specialist; 3=Review by nurse; 4=Further investigation
 6=Other, specify: _____

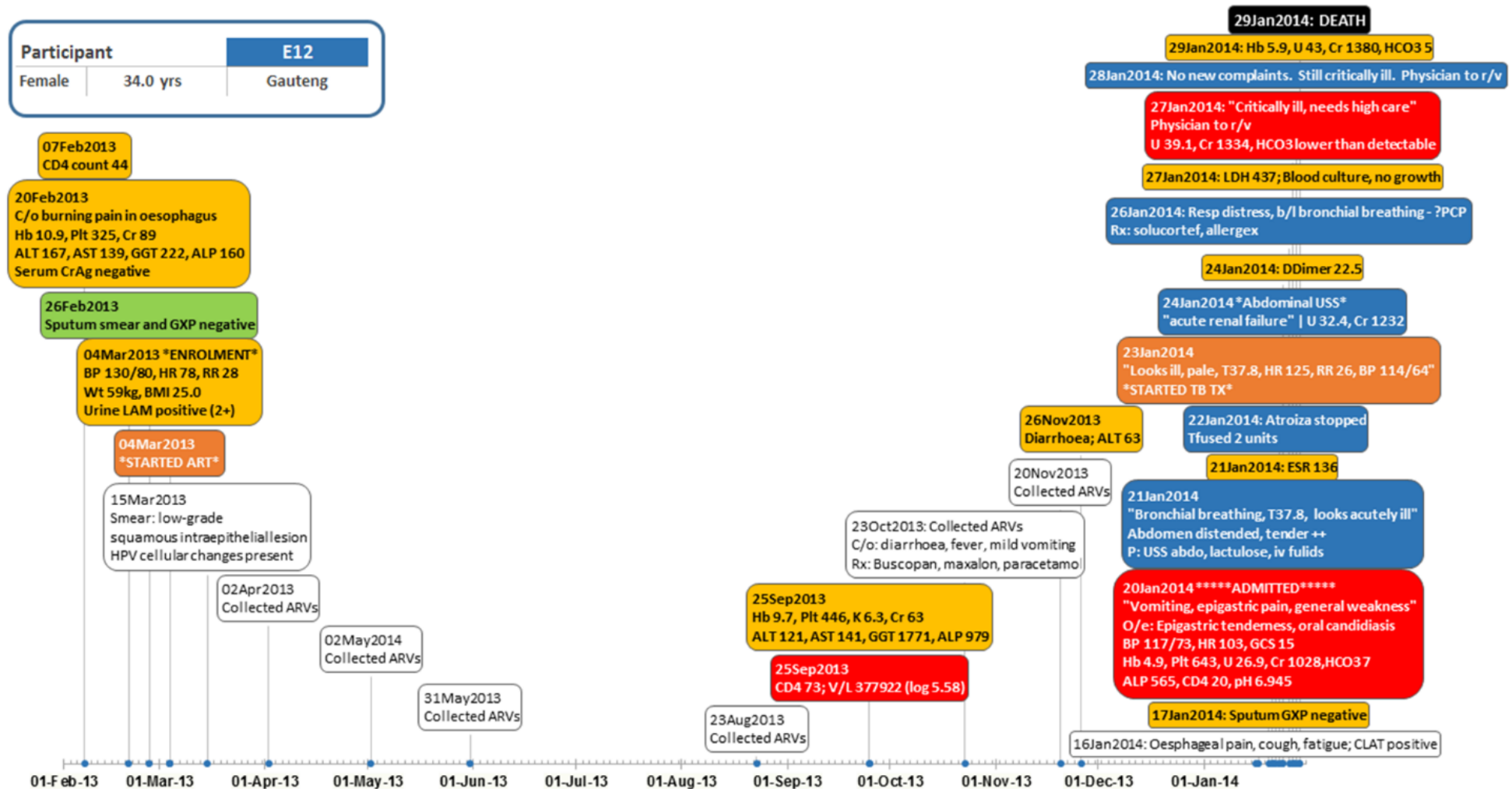
20fi. If further investigation, give details: _____

21. Prescription on discharge:

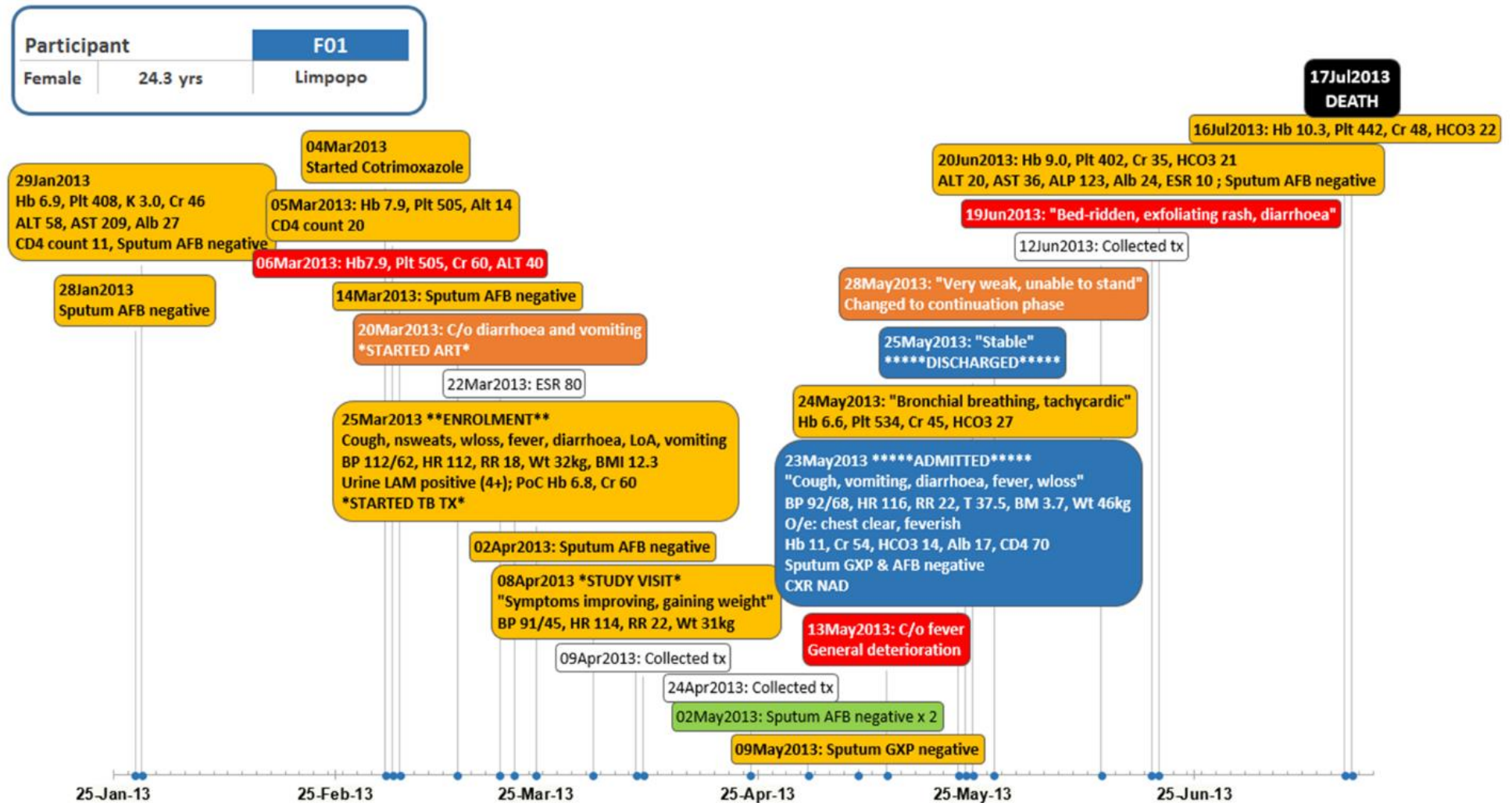
	Drug name (1)	Dose (2)	Frequency (3)	Duration (4)
21a				days
21b				days
21c				days
21d				days
21e				days
21f				days

Completed by: _____ Entered by: _____ Date entered: / /
 TBFT_Hosp_Deceased_v4.0_20150423

8.4.2. Sample timeline for clinical reviewers: 1



8.4.3. Sample timeline for clinical reviewers: 2



8.5. Appendix 5: Standard operating procedures and detailed methods

8.5.1. Minimally-invasive autopsy procedure (simplified procedure for non-clinical operator)

8.5.1.1 Before starting the autopsy

8.5.1.1.1 Documentation and permissions

1. Ensure you have all the appropriate documentation as described in the checklist
2. Ensure that formal informed consent has been obtained either from the participant or the appropriate family member
3. Ensure that the procedure has been discussed with the family at large and that at least verbal (if possible written) permission has been granted
4. Ensure that the mortuary/hospital have been informed of the procedure in as much time as possible, and that they have been in communication with the responsible member of the family

8.5.1.1.2 Safety principles

Personal protection

1. N95 mask
2. Goggles
3. Hat
4. Gown
5. Overshoes
6. Gloves at all times

Environmental

1. Remove potential slip/falling hazards
2. Extraction fan switched on if available
3. Establish a clear path between procedural site and sharps bin

Behavioural

1. Only one person using needles at any time
2. Vocal notification of sharps use
3. General sharps awareness
4. Use of sharps bin

8.5.1.2 Setting up

1. Ideally try to establish well lit, well ventilated and spacious working area (though frequently not possible; use head-torch if lighting insufficient)
2. Inform mortuary staff that they must be wearing an N95 mask if they are to observe or be in the same room
3. Set up large white bin bag using sello-tape in an **easily accessible location that does not compromise the sterile field**
4. Use 1 folding table to unpack and set up autopsy kit
5. Set up sterile working area on other folding table and pour povidone iodine (without touching field)
6. Label all CLS sample containers with the participant ID (*don't forget the blood culture bottle!*)
7. All NICD containers should also be labelled with participant ID, date of autopsy and sample type (e.g. blood, CSF, etc)
8. Pour sterile saline (using sterile method) into TB (blue-top) universal containers for lung tissue, liver tissue and spleen tissue
9. Pour formalin into the two histology universal containers (red-top)

8.5.1.3 Systemic examination

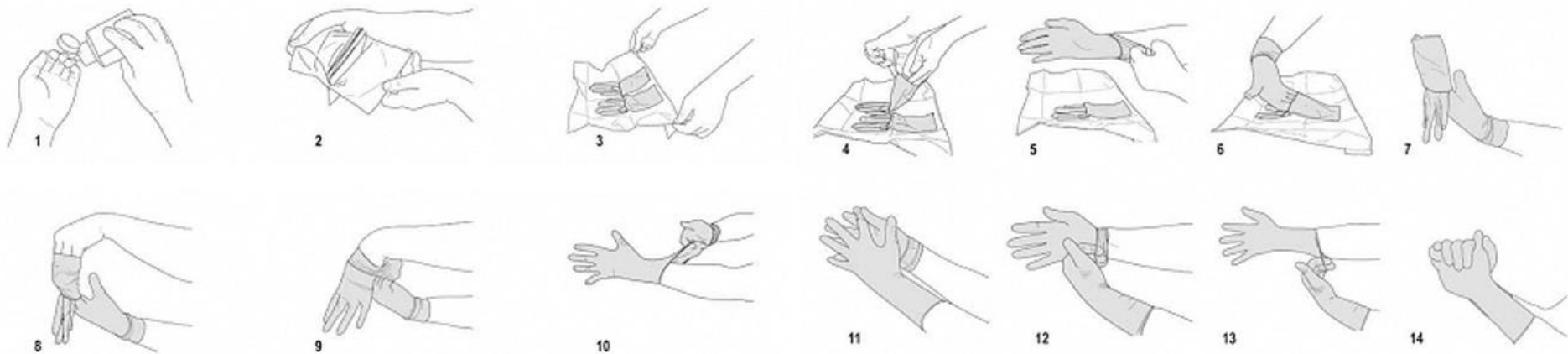
8.5.1.3.1 Observation

1. Put on a pair of large, non-sterile gloves
2. Make a formal observation of the body (head, arms, chest, abdomen, back, legs) paying particular attention to:
3. General appearance
4. Muscle mass (degree of wasting)
5. Hands & nails
6. Skin changes (evidence of rash, jaundice, trauma, coagulopathy, etc)

8.5.1.3.2 Examination for lymphadenopathy

1. Submandibular (under the jaw)
2. Cervical (neck)
3. Supraclavicular (above the clavicles)
4. Axillary (armpits)
5. Inguinal (groin)

Appendix figure 2. Guide to putting on sterile gloves



1. Perform hand hygiene before an "aseptic procedure" by handrubbing or hand washing.
2. Check the package for integrity. Open the first non-sterile packaging by peeling it completely off the heat seal to expose the second sterile wrapper, but without touching it.
3. Place the second sterile package on a clean, dry surface without touching the surface. Open the package and fold it towards the bottom so as to unfold the paper and keep it open.
4. Using the thumb and index finger of one hand, carefully grasp the folded cuff edge of the glove.
5. Slip the other hand into the glove in a single movement, keeping the folded cuff at the wrist level.
- 6-7. Pick up the second glove by sliding the fingers of the gloved hand underneath the cuff of the glove.
- 8-10. In a single movement, slip the second glove on to the ungloved hand while avoiding any contact/resting of the gloved hand on surfaces other than the glove to be donned (contact/resting constitutes a lack of asepsis and requires a change of glove).
11. If necessary, after donning both gloves, adjust the fingers and interdigital spaces until the gloves fit comfortably.
- 12-13. Unfold the cuff of the first gloved hand by gently slipping the fingers of the other hand inside the fold, making sure to avoid any contact with a surface other than the outer surface of the glove (lack of asepsis requiring a change of gloves).
14. The hands are gloved and must touch exclusively sterile devices or the previously-disinfected patient's body area.

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8.5.1.4 Liver biopsy

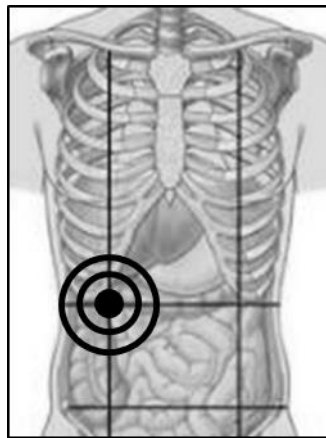
8.5.1.4.1 Equipment needed

1. Disposable scalpel x 1
2. Biopsy needle x 1
3. 18G hypodermic (pink) needle x 1
4. Pack of sterile gauze x 1
5. Alcohol swabs x 4
6. Pair of sterile gloves x 1
7. Histology (red-top) universal container (Label: 'R lung and liver') *pre-filled with formalin*
8. TB (blue-top) universal container (Label 'Tissue (liver)') *pre-filled with sterile saline*
9. Microbiology (white-top) universal container (Label: 'Tissue (liver)') *dry *
10. 'Liver' placemat

8.5.1.4.2 Procedure details

1. Put on a pair of large, non-sterile gloves
2. Open the sterile equipment onto the field (using sterile technique)
3. Biopsy needle
4. Scalpel
5. Hypodermic needle
6. Sterile gauze
7. Identify the appropriate point for needle insertion (**RIGHT** mid-clavicular line, just inferior to the costal margin [see Figure 1]) and mark the point with a felt pen

Appendix figure 3. Anatomical site for liver biopsy



8. Using the 'dirty hand/clean hand' method, clean the point and the surrounding area with at least four alcohol swabs, using the prescribed technique (start at the middle, move outwards in a clockwise motion)
9. Place a spare piece of absorbent material under the torso, where you are likely to have dripping iodine
10. Using the 'dirty hand/clean hand' method, clean the point and surrounding area with a cotton ball soaked in povidone iodine (removed carefully from the sterile area with the clean hand)
11. **BEFORE PUTTING ON STERILE GLOVES:**
 - **Make sure that the relevant pots (with lids removed) are on the appropriate placemats in an accessible location**
 - **Ensure that sharps bin is open and easily accessible**
 - **Double check that all necessary sterile equipment has been opened onto the field**
12. Put on a pair of sterile gloves using appropriate technique (see Section 2)

↓↓ **STERILE** ↓↓

13. Open one of the drapes contained in the sterile procedure pack and tear it in half, taking care to keep the folds away from the body and upper arms
14. Place one of the drape pieces back into the sterile field and fold the remaining piece in half longitudinally and then in half again
15. Tear a small hole in the sterile drape and dispose carefully of the waste, taking care not to contaminate yourself
16. Place the drape over the abdomen of the deceased, with the hole over the previously marked area
- 17. TAKE CARE NOT TO TOUCH THE BODY WHILE PLACING THE DRAPE**
18. Use sterile gauze to carefully clean away the residual iodine from the sterilised area
19. Using the scalpel, make a small nick in the skin in the area previously marked
20. Place a piece of sterile gauze in your new sterile field (on the abdomen of the deceased) and rest your hypodermic needle on it
21. Using your right hand, insert the biopsy needle (with the cutting blade withdrawn) into the nick made by your scalpel and direct it supero-infero-laterally until you feel the tip entering the liver
22. After you feel the tip of the needle enter the organ, push it in a further 1-2cm without changing the direction
23. Holding the body of the needle steady with your right hand, push the cutting blade forward, slowly, with your left hand until you feel it click closed
24. Holding the entire needle (body and cutting blade) closed, withdraw it from the abdomen, being particularly aware of people/items around you
25. Pull back the cutting blade to examine the sample
26. If you have obtained a satisfactory sample, transfer the biopsy needle to your left hand, pick up the hypodermic needle with your right hand and, taking care to maintain sharps safety and sterility, transfer the tissue sample into the appropriate container
27. Be careful not to touch any of the sample pots with either the biopsy needle or the hypodermic needle
28. Remember: for histology, the important thing is sample quality; for TB & micro, the important thing is sample quantity
29. Try to obtain at least 5 samples of high quality for histology and at least 8-10 samples (each) for microbiology and TB
30. When all samples have been obtained to your satisfaction, place the biopsy needle and the hypodermic needle into the sharps bin immediately

↑↑ **STERILE** ↑↑

31. Clean excess iodine from the area and dispose of the drape
32. Remove sterile gloves
33. Close the universal containers tightly and place the microbiology and TB containers immediately into an environment where they will be maintained at 2-8°C
34. Prepare for lung biopsies

8.5.1.5 Lung biopsies

8.5.1.5.1 Equipment needed

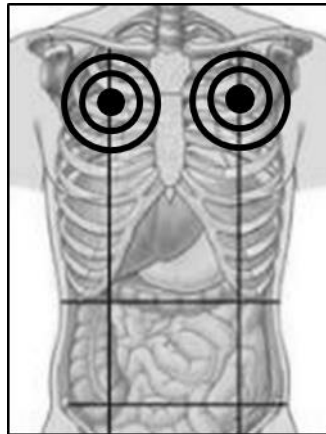
1. Disposable scalpel x 1 (re-use)
2. Biopsy needle x 1
3. 18G hypodermic (pink) needle x 2
4. Pack of sterile gauze x 1 (if needed)
5. Alcohol swabs x 8
6. Pair of sterile gloves x 1

7. Histology (red-top) universal container x 2 (Labels: 'R lung and liver' and 'L lung and spleen') *pre-filled with formalin*
8. TB (blue-top) universal container (Label 'Tissue (lung)') *pre-filled with sterile saline*
9. Microbiology (white-top) universal container (Label: 'Tissue (lung)') *dry *
10. 'Lungs' placemat

8.5.1.5.2 Procedure details

1. Put on a pair of large, non-sterile gloves
2. Open the sterile equipment onto the field (using sterile technique)
3. Biopsy needle x 1
4. Hypodermic needle x 2
5. Sterile gauze (if necessary)
6. Identify the appropriate points for needle insertion (**LEFT and RIGHT** mid-clavicular lines, 2nd intercostal space [see Figure 2]) and mark the points with a felt pen

Appendix figure 4. Anatomical sites for lung biopsies



7. Using the 'dirty hand/clean hand' method, clean the intended insertion points and the surrounding area with at least four alcohol swabs each, using the prescribed technique (start at the middle, move outwards in a clockwise motion)
8. Place a spare piece of absorbent material in the armpits and under the neck, where you are likely to have dripping iodine
9. Using the 'dirty hand/clean hand' method, clean the intended insertion points and surrounding areas with a cotton ball soaked in povidone iodine (removed carefully from the sterile area with the clean hand, *separate cotton ball for each site*)

10. BEFORE PUTTING ON STERILE GLOVES:

- **Make sure that the relevant pots (with lids removed) are on the appropriate placemats in an accessible location**
- **Ensure that sharps bin is open and easily accessible**
- **Double check that all necessary sterile equipment has been opened onto the field**

11. Put on a pair of sterile gloves using appropriate technique (see Section 2)

↓↓ **STERILE** ↓↓

12. Open one of the drapes contained in the sterile procedure pack and tear it in half (or use a remaining half drape), taking care to keep the folds away from the body and upper arms
13. Place one of the drape pieces back into the sterile field and fold the remaining piece in half, longitudinally, and then into thirds

14. Tear two small holes in the sterile drape, location depending on the size of the deceased's chest, and dispose carefully of the removed piece, taking care not to contaminate yourself
15. Place the drape over the chest of the deceased, with the holes over the area intended for needle insertion - ***TAKE CARE NOT TO TOUCH THE BODY WHILE PLACING THE DRAPE***
16. Use sterile gauze to carefully clean away the residual iodine from the sterilised areas (using a circular motion, as previously)
17. Using the scalpel, make a small nick in the skin in the insertion point for the **RIGHT** lung
18. Place a piece of sterile gauze in your new sterile field (on the chest of the deceased) and rest your hypodermic needle on it
19. Using your right hand, insert the biopsy needle (with the cutting blade **withdrawn**) into the nick made by your scalpel and direct it **postero-medially** until you feel a bit more resistance. Lung tissue tends to collapse post-mortem, and it can feel little like you are pushing a needle into a small pillow filled with straw...
20. After you feel the tip of the needle enter the lung, push it in a further 1-2cm without changing the direction
21. Holding the body of the needle steady with your right hand, push the cutting blade forward, **slowly** (this is particularly important with the lung biopsies), with your left hand until you feel it click closed
22. Holding the entire needle (body and cutting blade) closed, withdraw it from the abdomen, being particularly aware of people/items around you
23. Pull back the cutting blade to examine the sample
24. Use the hypodermic needle to try and stretch out your sample along the shaft of the needle. Lung tissue is very delicate and can get crushed in the biopsy process, so very often what looks like a very small sample can actually be very large!
25. If you have obtained a satisfactory sample, transfer the biopsy needle to your left hand, pick up the hypodermic needle with your right hand and, taking care to maintain sharps safety and sterility, transfer the tissue sample into the appropriate container (ensure that right and left lung samples go into the appropriate histology pots)
26. **Be careful not to touch any of the sample pots with either the biopsy needle or the hypodermic needle**
27. Remember: for **histology**, the important thing is sample **quality**; for **TB & micro**, the important thing is sample **quantity**
28. Try to obtain at least **5 samples** of high quality for histology and at least **8-10 samples (each)** for microbiology and TB from the right lung
29. When all right lung samples have been obtained to your satisfaction, use the scalpel to make a nick in the pre-marked position on the left chest wall and repeat the procedure (you may need a fresh piece of gauze at this stage)
30. It is suggested that you move to the left of the deceased to complete the left lung biopsies
31. Try to obtain at least **5 samples** of high quality for histology and at least **8-10 samples (each)** for microbiology and TB from the left lung
32. When all left lung samples have been obtained to your satisfaction, place the biopsy needle and the hypodermic needle into the sharps bin **immediately**

↑↑ **STERILE** ↑↑

33. Clean excess iodine from the area and dispose of the drape
34. Remove sterile gloves
35. Close the universal containers tightly and place the microbiology and TB containers immediately into an environment where they will be maintained at 2-8°C. The 'R lung and liver' histology container can also be placed into the insulated box at this stage
36. Prepare for spleen biopsy

8.5.1.6 Spleen biopsy

8.5.1.6.1 Equipment needed

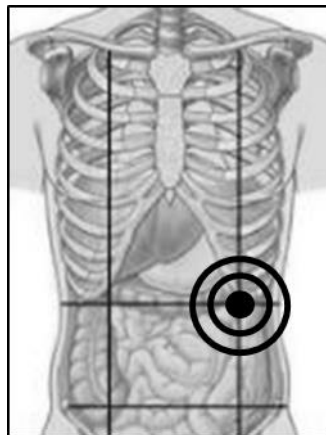
1. Disposable scalpel x 1 (re-use)
2. Biopsy needle x 1

3. 18G hypodermic (pink) needle x 1
4. Pack of sterile gauze x 1 (if needed)
5. Alcohol swabs x 4
6. Pair of sterile gloves x 1
7. Histology (red-top) universal container (Label: 'L lung and spleen') *pre-filled with formalin*
8. TB (blue-top) universal container (Label 'Tissue (spleen)') *pre-filled with sterile saline*
9. Microbiology (white-top) universal container (Label: 'Tissue (spleen)') *dry *
10. 'Spleen' placemat

8.5.1.6.2 Procedure details

1. Put on a pair of large, non-sterile gloves
2. Open the sterile equipment onto the field (using sterile technique)
3. Biopsy needle
4. Hypodermic needle
5. Sterile gauze (if necessary)
6. Identify the appropriate point for needle insertion (**LEFT** mid-clavicular line, below the costal margin [see Figure 3]) and mark the point with a felt pen
7. Using the 'dirty hand/clean hand' method, clean the intended insertion point and the surrounding area with at least four alcohol swabs, using the prescribed technique (start at the middle, move outwards in a clockwise motion)
8. Place a spare piece of absorbent material under the torso, where you are likely to have dripping iodine
9. Using the 'dirty hand/clean hand' method, clean the point and surrounding area with a cotton ball soaked in povidone iodine (removed carefully from the sterile area with the clean hand)

Appendix figure 5. Anatomical site for splenic biopsy



10. BEFORE PUTTING ON STERILE GLOVES:

- **Make sure that the relevant pots (with lids removed) are on the appropriate placemats in an accessible location**
- **Ensure that sharps bin is open and easily accessible**
- **Double check that all necessary sterile equipment has been opened onto the field**

11. Put on a pair of sterile gloves using appropriate technique (see Section 2)

↓↓ **STERILE** ↓↓

12. Open one of the drapes contained in the sterile procedure pack and tear it in half (or use a remaining half drape), taking care to keep the folds away from the body and upper arms

13. Place one of the drape pieces back into the sterile field and fold the remaining piece in half longitudinally and then in half again
14. Tear a small hole in the sterile drape and dispose carefully of the removed piece, taking care not to contaminate yourself
15. Place the drape over the abdomen of the deceased, with the hole over the area intended for needle insertion - ***TAKE CARE NOT TO TOUCH THE BODY WHILE PLACING THE DRAPE***
16. Use sterile gauze to carefully clean away the residual iodine from the sterilised area (using a circular motion, as previously)
17. Using the scalpel, make a small nick in the skin in the area previously marked
18. Place a piece of sterile gauze in your new sterile field (on the abdomen of the deceased) and rest your hypodermic needle on it
19. Using your right hand, insert the biopsy needle (with the cutting blade **withdrawn**) into the nick made by your scalpel and direct it **supero-postero-laterally** until you feel the tip entering the spleen
20. After you feel the tip of the needle enter the organ, push it in a further 1-2cm without changing the direction
21. Holding the body of the needle steady with your right hand, push the cutting blade forward, slowly, with your left hand until you feel it click closed
22. Holding the entire needle (body and cutting blade) closed, withdraw it from the abdomen, being particularly aware of people/items around you
23. Pull back the cutting blade to examine the sample
24. If you have obtained a satisfactory sample, transfer the biopsy needle to your left hand, pick up the hypodermic needle with your right hand and, taking care to maintain sharps safety and sterility, transfer the tissue sample into the appropriate container
25. **Be careful not to touch any of the sample pots with either the biopsy needle or the hypodermic needle**
26. Remember: for **histology**, the important thing is sample **quality**; for **TB & micro**, the important thing is sample **quantity**
27. Try to obtain at least **5 samples** of high quality for histology and at least **8-10 samples (each)** for microbiology and TB
28. When all samples have been obtained to your satisfaction, place the biopsy needle and the hypodermic needle into the sharps bin **immediately**

↑↑ **STERILE** ↑↑

29. Clean excess iodine from the area and dispose of the drape
30. Remove sterile gloves
31. Close the universal containers tightly and place the microbiology and TB containers immediately into an environment where they will be maintained at 2-8°C
32. Prepare to extract CSF

8.5.1.7 CSF sampling

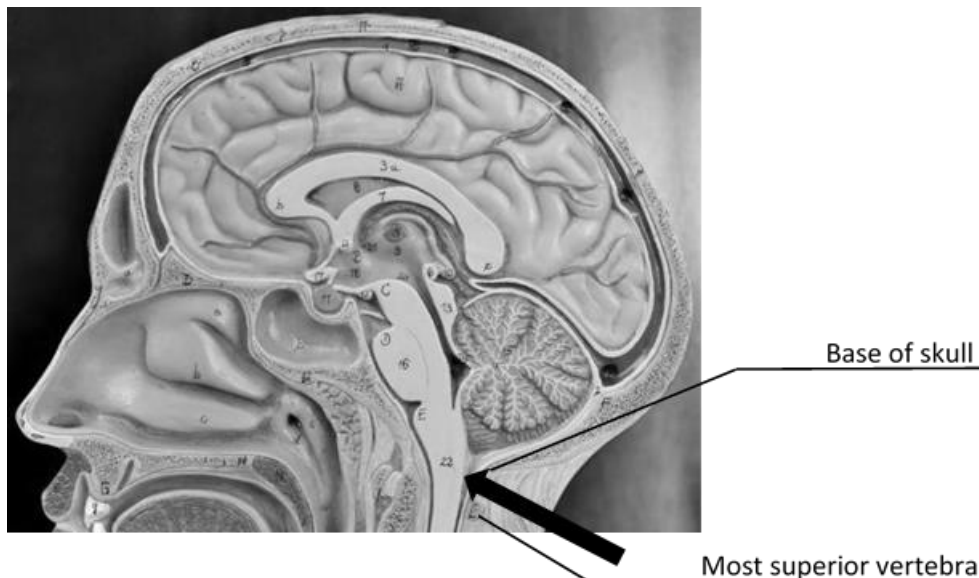
8.5.1.7.1 Equipment needed

1. Spinal needle x 1
2. Syringe x 1
3. Hypodermic (pink) needle x 1
4. Alcohol pads x 4
5. Pack of sterile gauze x 1
6. Pair of sterile gloves x 1
7. Beige-top vacutainers x 4 (2 for Micro, 2 for TB)
8. Red-top vacutainer x 1 (if CSF clear)
9. Purple-top (EDTA) vacutainer x 1 (if CSF bloodstained)
10. 'CSF' placemat

8.5.1.7.2 Procedure details

1. Put on a pair of large, non-sterile gloves
2. If possible, always approach from the right of the body, so that the top of the head is towards your left (non-dominant) hand
3. Turn the head towards the patient's left shoulder
4. You may need to apply some pressure to the left arm to move the left shoulder inferiorly
5. Palpate the spinous processes of the cervical spine, moving superiorly, until you find the gap between the superior-most vertebral body and the base of the skull (in the midline [see Figure 4])
6. Using the 'dirty hand/clean hand' method, clean the intended insertion point and the surrounding area with at least four alcohol swabs, using the prescribed technique (start at the middle, move outwards in a clockwise motion)
7. Place a spare piece of absorbent material under the head, where you are likely to have dripping iodine
8. Using the 'dirty hand/clean hand' method, clean the point and surrounding area with a cotton ball soaked in povidone iodine (removed carefully from the sterile area with the clean hand)
9. **BEFORE PUTTING ON STERILE GLOVES:**
 - **Make sure that the necessary vacutainers are on the appropriate placemats in an accessible location**
 - **Ensure that sharps bin is open and easily accessible**
 - **Double check that all necessary sterile equipment has been opened onto the field**
10. Put on a pair of sterile gloves using appropriate technique (see Section 2)

Appendix figure 6. Sagittal section of brain, showing entry point for CSF extraction



↓↓ **STERILE** ↓↓

11. Place a sterile sheet over the head and use your left hand to stabilise
12. Get onto your haunches to ensure better estimation of the needle's plane
13. Ensure that the needle's bevel is always facing **upwards** (see Figure 5)

Appendix figure 7. Diagram of needle tip showing ideal bevel position



14. Insert the spinal needle into the pre-identified spot, **directing it gently towards the middle of the eyes**
15. Once the needle is inserted to roughly 4/5 of its length, remove the cutting needle from the core and carefully attach the syringe, **taking care not to twist the needle**
16. Gently draw on the syringe to extract 20ml of CSF
17. Attach a hypodermic needle to the syringe and transfer the CSF into the 5 waiting vacutainers (red-top if clear, purple-top if bloodstained)

↑↑ **STERILE** ↑↑

18. Transfer the two parts of the spinal needle and the hypodermic needle into the sharps bin **immediately**
19. Use a swab to clean the remaining iodine and dispose of the sterile sheet
20. Remove sterile gloves
21. Transfer the 5 vacutainers to an environment of 2-8°C
22. Prepare to tidy up and complete autopsy

8.5.1.8 Finishing the autopsy (checklist)

8.5.1.8.1 Cadaver

- | | |
|---------------------|--------------------------|
| 1. Cleaned up | <input type="checkbox"/> |
| 2. Sutures | <input type="checkbox"/> |
| 3. Plasters | <input type="checkbox"/> |

8.5.1.8.2 Samples

- | | |
|-----------------------------|--------------------------|
| 1. TB labelled | <input type="checkbox"/> |
| 2. TB packed | <input type="checkbox"/> |
| 3. Micro labelled | <input type="checkbox"/> |
| 4. Micro packed | <input type="checkbox"/> |
| 5. Histology labelled | <input type="checkbox"/> |
| 6. Histology packed | <input type="checkbox"/> |
| 7. NICD labelled | <input type="checkbox"/> |
| 8. NICD packed | <input type="checkbox"/> |

8.5.1.8.3 Equipment

- | | |
|--|--------------------------|
| 1. Wiped down with cleaning fluid | <input type="checkbox"/> |
| 2. Packed | <input type="checkbox"/> |
| 3. White waste bag sealed and transferred to red waste bag (while still wearing protective clothing) | <input type="checkbox"/> |
| 4. Red waste bag sealed and disposed (ideally at health facility) | <input type="checkbox"/> |

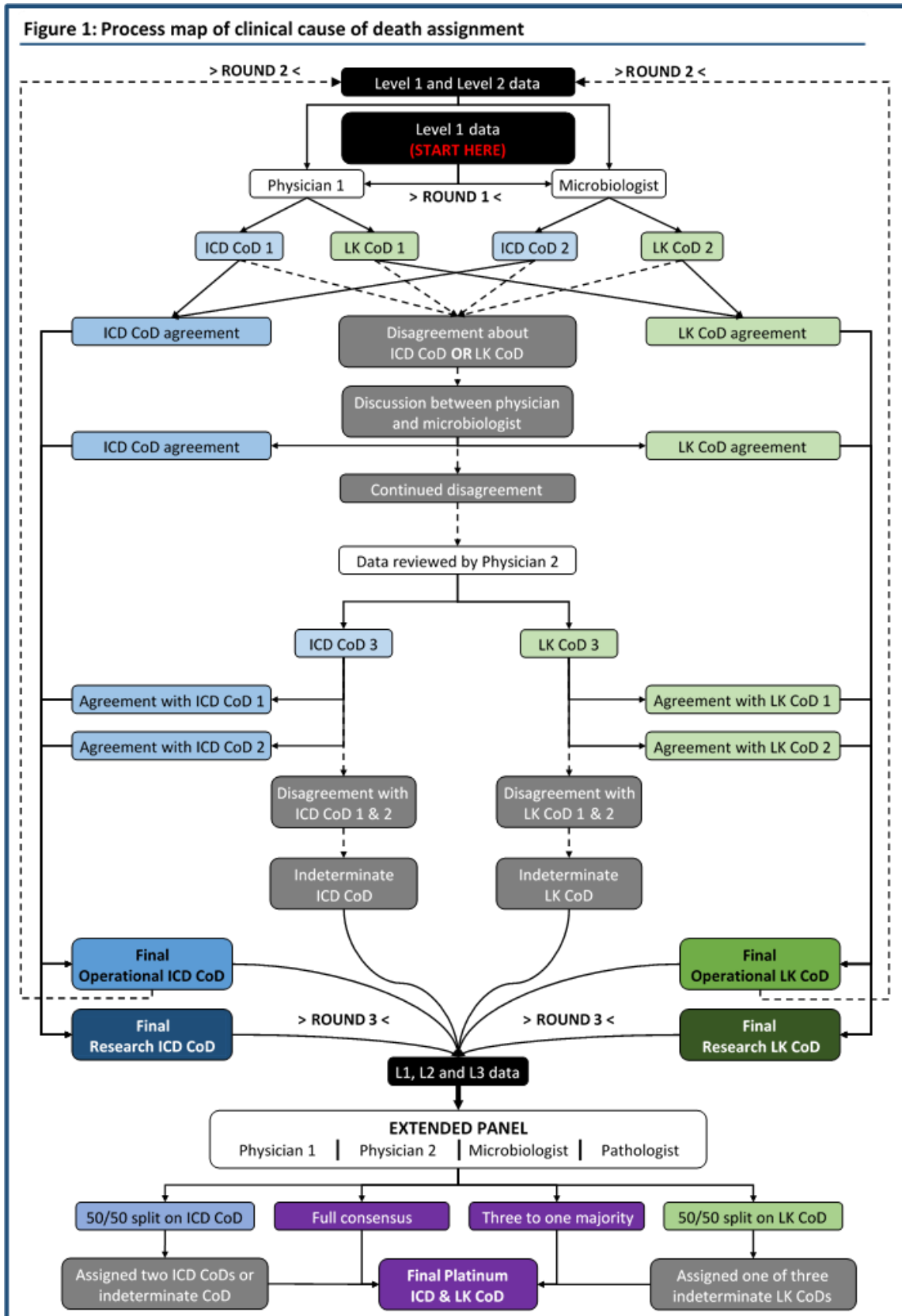
8.5.2. Summary of laboratory procedures (adapted from upgrading report)

Summary of samples obtained and laboratory tests done as part of minimally-invasive autopsy procedure

Sample type	Test type Looking for	Basic tests										Reflex tests			
		AFB	Fungi	Bacteria	Malignancy	<i>Pneumocystis jirovecii</i>	Aerobic bacteria	<i>Cryptococcus neoformans</i>	<i>Mycobacterium tuberculosis</i>	Bacterial species	Confirmation of AFB	ID TB complex +/- INH/RIF resistance	Non-tuberculous mycobacteria		
	Test	ZN stain	Grocot stain	B&H stain	IP stain	Immunofluorescence	Aerobic culture	Cryptococcal antigen	Cryptococcal culture	MGIT culture	Xpert® MTB/RIF	Bacterial ID (if +ve bacterial stain)	ZN stain (if +ve MGIT)	Hain GenoType MTBDRplus (if +ve MGIT)	Hain GenoType MTB CM/AS (if +ve MGIT & -ve MTB ID)
TB/ Microbiology	Lung biopsies (R&L)					X	✓	X	✓	✓	X		✓	✓	✓
	BAL					✓	✓	X	X	✓	✓		✓	✓	✓
	Liver				N/A	X	X	X	X	✓	X	N/A	✓	✓	✓
	Spleen (+/-LN)					X	✓	X	X	✓	X		✓	✓	✓
	CSF					X	X	✓	X	✓	X		✓	✓	✓
Histology	Block 1 (RL + liver)	✓	✓	10%	5%							✓			
	Block 2 (LL + spleen)	✓	✓	10%	5%			N/A				✓		N/A	
	(+/- Block 3)	✓	✓	10%	5%							✓			

+ve: positive; -ve: negative; AFB: Acid-fast bacilli; BAL: Bronchoalveolar lavage; CSF: Cerebrospinal fluid; G+ve: Gram positive; G-ve: Gram negative; ID: identification; INH=Isoniazid; IP: immunoperoxidase; L: Left; LL: Left lung; LN: Lymph node(s); MGIT: Mycobacteria growth indicator tube; MTB: *Mycobacterium tuberculosis*; R: Right; RIF: Rifampicin; RL: Right lung; TB: tuberculosis; ZN: Ziehl-Neelsen;

8.5.3. Assigning clinical causes of death: overview (adapted from upgrading appendix)



CoD: cause of death; ICD: International classification of diseases; L1: Level 1; L2: Level 2; L3: Level 3; LK: Lesedi Kamoso

8.5.4. Stata code for generation of MMDS input file from PCVA or clinical CoD

```
/*=====
Assigning 'underlying codes' using ACME software (generate input file structure for Super MICAR;
merge with CoD data and generate appropriate '.s10' file for import into SuperMICAR)
```

Author: AS Karat (aaron.karat@lshtm.ac.uk | aaron.s.karat@gmail.com)

Created: 11 May 2016
Last modified: 10 Sep 2016

```
=====*/
```

```
set more off
cd "~"
clear
// set x number of observations
set obs 10
```

```
// generate vars for SuperMicar
gen str4 ydeath=" "
gen str2 statedeath=" "
gen str6 certno=" "
gen str1 coder=" "
gen str4 lot=" "
gen str1 section=" "
gen str3 shipment=" "
gen str8 dreceipt=" "
gen str4 pgm=" "
gen str2 dodm=" "
gen str2 dodd=" "
gen str1 sex=" "
gen str1 ageunits=" "
gen str3 age=" "
gen str120 cod1a=" "
gen str20 cod1a2=" "
gen str120 cod1b=" "
gen str20 cod1b2=" "
gen str120 cod1c=" "
gen str20 cod1c2=" "
gen str120 cod1d=" "
gen str20 cod1d2=" "
gen str240 cod2=" "
gen str1 tobacco=" "
gen str1 pregnancy=" "
gen str1 iffemale=" "
gen str1 manner=" "
gen str8 dinjury=" "
gen str4 tinjury=" "
gen str1 tunits=" "
gen str50 placeinjury=" "
gen str1 injwork=" "
gen str250 howinjury=" "
gen str30 transport=" "
gen str1 autopsy=" "
gen str1 autopsyf=" "
gen str30 certifier=" "
gen str2 dsurgerym=" "
gen str2 dsurgeryd=" "
gen str4 dsurgeryy=" "
gen str1 incompletdata=" "
gen str1 line1bdel=" "
gen str1 line1cdel=" "
gen str1 line1ddel=" "
gen str1 line2del=" "
gen str1 activity=" "
gen str1 placeinjury2=" "
gen str12 auxstate=" "
gen str30 statespec=" "
```

```

// format to right length
format %1s coder section sex ageunits tobacco pregnancy iffemale manner /*
  */ injwork autopsy autopsyf incompletedata line1bdel line1cdel line1ddel /*
  */ line2del activity placeinjury2 tunits
format %2s statedeath dodm dodd dsurgerym dsurgeryd
format %3s shipment age
format %4s lot pgm tinjury dsurgeryy
format %6s certno
format %8s dreceipt dinjury
format %12s auxstate
format %20s cod1a2 cod1b2 cod1c2 cod1d2
format %30s transport certifier statespec
format %50s placeinjury
format %120s cod1a cod1b cod1c cod1d
format %240s cod2
format %250s howinjury

// Left align all strings
// (Code from: http://www.stata.com/statalist/archive/2005-02/msg00262.html)
ds, has(type string)
foreach v in `r(varlist)' {
  local type: type `v'
  local type: substr local type "str" ""
  format `v' %-%`type's
}

// Generate spaces to ensure all fields the right length
// (Code from: http://www.stata.com/statalist/archive/2009-01/msg00359.html)
ds, has(type string)
foreach v in `r(varlist)' {
  local spaces: display _dup(245) " "
  local length = substr("`': type `v'",4,.)
  replace `v'=`v' + substr("`spaces'",1,`length' - length(`v'))
}
save S10Structure.dta, replace

/*-----
Prep CoD data and merge with S10 structure file
-----*/
cd "~"
use [dataset with multiple causes of death].dta, clear

// create 6-digit identifier for Super-MICART purposes (essentially just 1-34 based on pptid
order)
sort pptid
gen cert=_n
format %06.0f cert
gen certno=string(cert, "%06.0f")
drop cert
order pptid certno

// A few that slipped through...
replace icodicdo="I64" if icodicdo=="164"
replace icodicdo="G00" if icodicdo=="G00"
replace icodicdo="D64" if icodicdo=="D54"
replace icodicdo="C22" if icodicdo=="L22"
replace ucodicdo="D64" if ucodicdo=="D46"

gen icodo1=substr(icodicdo, 1,3)
gen ucodo1=substr(ucodicdo,1,3)

// create string for all immediate cod and recode according to assigned icd/icdo
gen icodt=""
foreach v of var icodicd {
  replace icodt="Cholera" if `v'==1
  replace icodt="Typhus fever" if `v'==2
  replace icodt="Acute diarrhoea" if `v'==3
  replace icodt="Amoebiasis unspecified" if `v'==4
}

```

```
replace icodt="Other protozoal intestinal dx" if `v`==5
replace icodt="Other gastroenteritis" if `v`==6
replace icodt="Respiratory tuberculosis" if `v`==7
replace icodt="Respiratory tuberculosis" if `v`==8
replace icodt="Tuberculous meningitis" if `v`==9
replace icodt="Extra pulmonary tuberculosis" if `v`==10
replace icodt="Miliary tuberculosis" if `v`==11
replace icodt="Infection due to other mycobacteria" if `v`==12
replace icodt="Meningococcal infection" if `v`==13
replace icodt="Streptococcal sepsis" if `v`==14
replace icodt="Other sepsis" if `v`==15
replace icodt="Other bacterial disease" if `v`==16
replace icodt="Bacterial infection of unspecified location" if `v`==17
replace icodt="Viral meningitis" if `v`==18
replace icodt="Herpes simplex infections" if `v`==19
replace icodt="Varicella" if `v`==20
replace icodt="Herpes zoster" if `v`==21
replace icodt="Measles" if `v`==22
replace icodt="Other viral infections" if `v`==23
replace icodt="Unspecified viral infection" if `v`==24
replace icodt="Acute hepatitis A" if `v`==25
replace icodt="Acute hepatitis B" if `v`==26
replace icodt="Other acute viral hepatitis" if `v`==27
replace icodt="Chronic viral hepatitis" if `v`==28
replace icodt="Unspecified viral hepatitis" if `v`==29
replace icodt="HIV disease resulting in infection" if `v`==30
replace icodt="HIV disease resulting in malignancy" if `v`==31
replace icodt="HIV disease resulting in other specified" if `v`==32
replace icodt="HIV disease resulting in other and unspecified" if `v`==33
replace icodt="Unspecified HIV disease" if `v`==34
replace icodt="Cytomegaloviral disease" if `v`==35
replace icodt="Other viral diseases" if `v`==36
replace icodt="Coccidioidomycosis" if `v`==37
replace icodt="Histoplasmosis" if `v`==38
replace icodt="Plasmodium falciparum malaria" if `v`==39
replace icodt="Other malaria" if `v`==40
replace icodt="Unspecified malaria" if `v`==41
replace icodt="Toxoplasmosis" if `v`==42
replace icodt="Pneumocystosis" if `v`==43
replace icodt="Schistosomiasis" if `v`==44
replace icodt="Malignant neoplasm of oesophagus" if `v`==45
replace icodt="Malignant neoplasm of stomach" if `v`==46
replace icodt="Malignant neoplasm of colon" if `v`==47
replace icodt="Malignant neoplasm of pancreas" if `v`==48
replace icodt="Malignant neoplasm of other" if `v`==49
replace icodt="Malignant melanoma of skin" if `v`==50
replace icodt="Other malignant neoplasms" if `v`==51
replace icodt="Mesothelioma" if `v`==52
replace icodt="Kaposi sarcoma" if `v`==53
replace icodt="Malignant neoplasm of breast" if `v`==54
replace icodt="Malignant neoplasm of cervix" if `v`==55
replace icodt="Malignant neoplasm of ovary" if `v`==56
replace icodt="Cancer digestive system" if `v`==57
replace icodt="Cancer of breast unspecified" if `v`==58
replace icodt="Malignant neoplasm genital tract" if `v`==59
replace icodt="Other urinary malignancy" if `v`==60
replace icodt="Malignant neoplasm, without" if `v`==61
replace icodt="Hodgkin lymphoma" if `v`==62
replace icodt="Other and unspecified types non-Hodgkin's Lymphoma" if `v`==63
replace icodt="Iron-deficiency anaemia" if `v`==64
replace icodt="Unspecified diabetes mellitus" if `v`==65
replace icodt="Unspecified severe malnutrition" if `v`==66
replace icodt="Acute myocardial infarction" if `v`==67
replace icodt="Pulmonary embolism" if `v`==68
replace icodt="Subarachnoid haemorrhage" if `v`==69
replace icodt="Intracerebral haemorrhage" if `v`==70
replace icodt="Cerebral infarction" if `v`==71
replace icodt="Stroke" if `v`==72 | icodicdo=="I64"
replace icodt="Pneumonia due to Streptococcus" if `v`==73
```



```
replace icodt="Pneumonia due to Haemophilus" if `v'==74
replace icodt="Bacterial pneumonia" if `v'==75
replace icodt="Pneumonia due to other infective agents" if `v'==76
replace icodt="Pneumonia other" if `v'==77
replace icodt="Pneumonia organism unspecified" if `v'==78
replace icodt="Bronchiectasis" if `v'==79
replace icodt="Coalworker pneumoconiosis" if `v'==80
replace icodt="Intentional self-poisoning" if `v'==81
replace icodt="Intentional self-harm by hanging" if `v'==82
replace icodt="Intentional self-harm by jumping" if `v'==83
}

foreach v of var icod01 {
  replace icodt="Salmonellosis" if `v'=="A02"
  replace icodt="PML" if `v'=="A81"
  replace icodt="Nocardiosis" if `v'=="A43"
  replace icodt="Varicella" if `v'=="B01"
  replace icodt="Cryptococcosis" if `v'=="B45"
  replace icodt="Unspecified mycosis" if `v'=="B49"
  replace icodt="Malignant neoplasm of tongue" if `v'=="C02"
  replace icodt="Malignant neoplasm of nasopharynx" if `v'=="C11"
  replace icodt="Malignant neoplasm of lip, oral cavity, & pharynx" if `v'=="C14"
  replace icodt="Malignant neoplasm of liver & bile ducts" if `v'=="C22"
  replace icodt="Malignant neoplasm of larynx" if `v'=="C32"
  replace icodt="Malignant neoplasm of bronchus & lung" if `v'=="C34"
  replace icodt="Malignant neoplasm of bone" if `v'=="C41" | `v'=="C40"
  replace icodt="Malignant neoplasm of corpus uteri" if `v'=="C54"
  replace icodt="Malignant neoplasm of female genital organs" if `v'=="C57"
  replace icodt="Malignant neoplasm of prostate" if `v'=="C61"
  replace icodt="Malignant neoplasm of bladder" if `v'=="C67"
  replace icodt="Malignant neoplasm of eye" if `v'=="C69"
  replace icodt="Malignant neoplasm of brain" if `v'=="C71"
  replace icodt="Malignant neoplasm of ill-defined sites" if `v'=="C76"
  replace icodt="Metastases" if `v'=="C79"
  replace icodt="Malignant neoplasm, site unspecified" if `v'=="C80"
  replace icodt="Lymphoma" if `v'=="C83"
  replace icodt="Lymphoma NOS" if `v'=="C88"
  replace icodt="Myeloid leukaemia" if `v'=="C92"
  replace icodt="Leukaemia of unspecified cell type" if `v'=="C95"
  replace icodt="Unspecified lymphatic malignancy" if `v'=="C96"
  replace icodt="Neoplasm of oral cavity/digestive organs" if `v'=="D37"
  replace icodt="Neoplasm of central nervous system" if `v'=="D43"
  replace icodt="Iron deficiency anaemia" if `v'=="D50"
  replace icodt="Other anaemias" if `v'=="D64"
  replace icodt="ITP" if `v'=="D69"
  replace icodt="Immune disorder" if `v'=="D89"
  replace icodt="Other disorders of thyroid" if `v'=="E07"
  replace icodt="Type 1 Diabetes" if `v'=="E10"
  replace icodt="Type 2 Diabetes" if `v'=="E11"
  replace icodt="Hypoglycaemia" if `v'=="E16"
  replace icodt="Volume depletion" if `v'=="E86"
  replace icodt="Metabolic disorder" if `v'=="E87"
  replace icodt="Delirium" if `v'=="F05"
  replace icodt="Schizophrenia" if `v'=="F20"
  replace icodt="Bacterial meningitis" if `v'=="G00"
  replace icodt="Meningitis" if `v'=="G03"
  replace icodt="Meningitis" if `v'=="G04"
  replace icodt="Intracranial abscess" if `v'=="G07"
  replace icodt="Epilepsy" if `v'=="G40"
  replace icodt="Other disorders of brain" if `v'=="G93"
  replace icodt="Other diseases of spinal cord" if `v'=="G95"
  replace icodt="Other CNS disorders" if `v'=="G96"
  replace icodt="Hypertensive heart disease" if `v'=="I11"
  replace icodt="Essential hypertension" if `v'=="I15" | `v'=="I10"
  replace icodt="Chronic ischaemic heart disease" if `v'=="I25"
  replace icodt="Cardiomyopathy" if `v'=="I42"
  replace icodt="Heart failure" if `v'=="I50"
  replace icodt="Heart disease" if `v'=="I51"
  replace icodt="Unspecified intracranial haemorrhage" if `v'=="I62"
```

```
replace icodt="Peripheral vascular disease" if `v`=="I73"  
replace icodt="Arterial embolism & thrombosis" if `v`=="I74"  
replace icodt="Disorders of arteries, arterioles, capillaries" if `v`=="I79"  
replace icodt="Viral pneumonia" if `v`=="J12"  
replace icodt="Unspecified lower respiratory tract infection" if `v`=="J22"  
replace icodt="Status asthmaticus" if `v`=="J46"  
replace icodt="Respiratory conditions due to other external agents" if `v`=="J70"  
replace icodt="Pulmonary oedema" if `v`=="J81"  
replace icodt="Pleural effusion" if `v`=="J90"  
replace icodt="Respiratory failure" if `v`=="J96"  
replace icodt="Other respiratory disorders" if `v`=="J98"  
replace icodt="Gastric ulcer" if `v`=="K25"  
replace icodt="Peptic ulcer, site unspecified" if `v`=="K27"  
replace icodt="Gastritis & duodenitis" if `v`=="K29"  
replace icodt="Acute appendicitis" if `v`=="K35"  
replace icodt="Other appendicitis" if `v`=="K36"  
replace icodt="Other noninfective gastroenteritis/colitis" if `v`=="K52"  
replace icodt="Paralytic ileus" if `v`=="K56"  
replace icodt="Anal or rectal abscess" if `v`=="K61"  
replace icodt="Other diseases of intestine" if `v`=="K63"  
replace icodt="Alcoholic liver disease" if `v`=="K70"  
replace icodt="Toxic liver disease" if `v`=="K71"  
replace icodt="Hepatic failure" if `v`=="K72"  
replace icodt="Fibrosis & cirrhosis of liver" if `v`=="K74"  
replace icodt="Other diseases of liver" if `v`=="K76"  
replace icodt="Other diseases of biliary tract" if `v`=="K83"  
replace icodt="Other diseases of digestive system" if `v`=="K92"  
replace icodt="Cutaneous abscess, furuncle, carbuncle" if `v`=="L02"  
replace icodt="Cellulitis" if `v`=="L03"  
replace icodt="Other erythematous conditions" if `v`=="L53"  
replace icodt="Seropositive rheumatoid arthritis" if `v`=="M05"  
replace icodt="Other necrotizing vasculopathies" if `v`=="M31"  
replace icodt="SLE" if `v`=="M32"  
replace icodt="Other systemic connective tissue dx" if `v`=="M35"  
replace icodt="Myositis" if `v`=="M60"  
replace icodt="Osteomyelitis" if `v`=="M86"  
replace icodt="Nephrotic syndrome" if `v`=="N04"  
replace icodt="Drug-induced tubulo-interstitial conditions" if `v`=="N14"  
replace icodt="Acute renal failure" if `v`=="N17"  
replace icodt="Unspecified renal failure" if `v`=="N17/N18"  
replace icodt="Chronic renal failure" if `v`=="N18"  
replace icodt="Unspecified renal failure" if `v`=="N19"  
replace icodt="Disorder of urinary system" if `v`=="N39"  
replace icodt="Inflammatory disorders of male genitals" if `v`=="N49"  
replace icodt="Gestational hypertension" if `v`=="O13"  
replace icodt="Complications of the puerperium" if `v`=="O90"  
replace icodt="Congenital cardiac malformation" if `v`=="Q20"  
replace icodt="Haemorrhage from respiratory passages" if `v`=="R04"  
replace icodt="Abdominal and pelvic pain" if `v`=="R10"  
replace icodt="Nausea and vomiting" if `v`=="R11"  
replace icodt="Other symptoms involving the abdomen" if `v`=="R19"  
replace icodt="Shock" if `v`=="R57"  
replace icodt="Other sudden death, cause unknown" if `v`=="R96"  
replace icodt="Unknown" if `v`=="R99"  
replace icodt="Intracranial injury" if `v`=="S06"  
replace icodt="Unspecified injury of head" if `v`=="S09"  
replace icodt="Fracture of femur" if `v`=="S72"  
replace icodt="Injuries to spine and trunk" if `v`=="T09"  
replace icodt="Poisoning by other systemic anti-infectives" if `v`=="T37"  
replace icodt="Poisoning" if `v`=="T65"  
replace icodt="External cause" if `v`=="T81"  
replace icodt="Primarily systemic agents" if `v`=="Y43"  
replace icodt="Procedure for non-health purposes" if `v`=="Z41"  
replace icodt="Problems related to lifestyle" if `v`=="Z72"  
replace icodt="Coagulation defect" if `v`=="D68"  
replace icodt="Dysmenorrhoea" if `v`=="N94"  
replace icodt="Obstetric embolism" if `v`=="O88"  
replace icodt="Drug overdose" if `v`=="T96"  
replace icodt="Gunshot" if `v`=="W32"
```

```
replace icodt="Accidental poisoning" if `v'=="X49"  
replace icodt="Intentional self-harm by handgun" if `v'=="X72"  
replace icodt="Urethral stricture" if `v'=="N36"  
replace icodt="External trauma" if `v'=="Y41" | `v'=="Y85" | `v'=="V03" | `v'=="V49"  
}  
  
// repeat for underlying cod  
gen ucodt=""  
  foreach v of var ucodicd {  
    replace ucodt="Cholera" if `v'==1  
    replace ucodt="Typhus fever" if `v'==2  
    replace ucodt="Acute diarrhoea" if `v'==3  
    replace ucodt="Amoebiasis unspecified" if `v'==4  
    replace ucodt="Other protozoal intestinal dx" if `v'==5  
    replace ucodt="Other gastroenteritis" if `v'==6  
    replace ucodt="Respiratory tuberculosis" if `v'==7  
    replace ucodt="Respiratory tuberculosis" if `v'==8  
    replace ucodt="Tuberculous meningitis" if `v'==9  
    replace ucodt="Extra pulmonary tuberculosis" if `v'==10  
    replace ucodt="Miliary tuberculosis" if `v'==11  
    replace ucodt="Infection due to other mycobacteria" if `v'==12  
    replace ucodt="Meningococcal infection" if `v'==13  
    replace ucodt="Streptococcal sepsis" if `v'==14  
    replace ucodt="Other sepsis" if `v'==15  
    replace ucodt="Other bacterial disease" if `v'==16  
    replace ucodt="Bacterial infection of unspecified location" if `v'==17  
    replace ucodt="Viral meningitis" if `v'==18  
    replace ucodt="Herpes simplex infections" if `v'==19  
    replace ucodt="Varicella" if `v'==20  
    replace ucodt="Herpes zoster" if `v'==21  
    replace ucodt="Measles" if `v'==22  
    replace ucodt="Other viral infections" if `v'==23  
    replace ucodt="Unspecified viral infection" if `v'==24  
    replace ucodt="Acute hepatitis A" if `v'==25  
    replace ucodt="Acute hepatitis B" if `v'==26  
    replace ucodt="Other acute viral hepatitis" if `v'==27  
    replace ucodt="Chronic viral hepatitis" if `v'==28  
    replace ucodt="Unspecified viral hepatitis" if `v'==29  
    replace ucodt="HIV disease resulting in infection" if `v'==30  
    replace ucodt="HIV disease resulting in malignancy" if `v'==31  
    replace ucodt="HIV disease resulting in other specified" if `v'==32  
    replace ucodt="HIV disease resulting in other and unspecified" if `v'==33  
    replace ucodt="Unspecified HIV disease" if `v'==34  
    replace ucodt="Cytomegaloviral disease" if `v'==35  
    replace ucodt="Other viral diseases" if `v'==36  
    replace ucodt="Coccidioidomycosis" if `v'==37  
    replace ucodt="Histoplasmosis" if `v'==38  
    replace ucodt="Plasmodium falciparum malaria" if `v'==39  
    replace ucodt="Other malaria" if `v'==40  
    replace ucodt="Unspecified malaria" if `v'==41  
    replace ucodt="Toxoplasmosis" if `v'==42  
    replace ucodt="Pneumocystosis" if `v'==43  
    replace ucodt="Schistosomiasis" if `v'==44  
    replace ucodt="Malignant neoplasm of oesophagus" if `v'==45  
    replace ucodt="Malignant neoplasm of stomach" if `v'==46  
    replace ucodt="Malignant neoplasm of colon" if `v'==47  
    replace ucodt="Malignant neoplasm of pancreas" if `v'==48  
    replace ucodt="Malignant neoplasm of other" if `v'==49  
    replace ucodt="Malignant melanoma of skin" if `v'==50  
    replace ucodt="Other malignant neoplasms" if `v'==51  
    replace ucodt="Mesothelioma" if `v'==52  
    replace ucodt="Kaposi sarcoma" if `v'==53  
    replace ucodt="Malignant neoplasm of breast" if `v'==54  
    replace ucodt="Malignant neoplasm of cervix" if `v'==55  
    replace ucodt="Malignant neoplasm of ovary" if `v'==56  
    replace ucodt="Cancer digestive system" if `v'==57  
    replace ucodt="Cancer of breast unspecified" if `v'==58  
    replace ucodt="Malignant neoplasm genital tract" if `v'==59
```

```
replace ucodt="Other urinary malignancy" if `v'==60
replace ucodt="Malignant neoplasm" if `v'==61
replace ucodt="Hodgkin lymphoma" if `v'==62
replace ucodt="Other and unspecified types non-Hodgkin's Lymphoma" if `v'==63
replace ucodt="Iron-deficiency anaemia" if `v'==64
replace ucodt="Unspecified diabetes mellitus" if `v'==65
replace ucodt="Unspecified severe malnutrition" if `v'==66
replace ucodt="Acute myocardial infarction" if `v'==67
replace ucodt="Pulmonary embolism" if `v'==68
replace ucodt="Subarachnoid haemorrhage" if `v'==69
replace ucodt="Intracerebral haemorrhage" if `v'==70
replace ucodt="Cerebral infarction" if `v'==71
replace ucodt="Stroke" if `v'==72
replace ucodt="Pneumonia due to Streptococcus" if `v'==73
replace ucodt="Pneumonia due to Haemophilus" if `v'==74
replace ucodt="Bacterial pneumonia" if `v'==75
replace ucodt="Pneumonia due to other infective agents" if `v'==76
replace ucodt="Pneumonia other" if `v'==77
replace ucodt="Pneumonia organism unspecified" if `v'==78
replace ucodt="Bronchiectasis" if `v'==79
replace ucodt="Coalworker pneumoconiosis" if `v'==80
replace ucodt="Intentional self-poisoning" if `v'==81
replace ucodt="Intentional self-harm by hanging" if `v'==82
replace ucodt="Intentional self-harm by jumping" if `v'==83
}

foreach v of var ucod01 {
  replace ucodt="PML" if `v'=="A81"
  replace ucodt="Varicella" if `v'=="B01"
  replace ucodt="Cryptococcosis" if `v'=="B45"
  replace ucodt="Unspecified mycosis" if `v'=="B49"
  replace ucodt="Malignant neoplasm of tongue" if `v'=="C02"
  replace ucodt="Malignant neoplasm of nasopharynx" if `v'=="C11"
  replace ucodt="Malignant neoplasm of lip, oral cavity, & pharynx" if `v'=="C14"
  replace ucodt="Malignant neoplasm of liver & bile ducts" if `v'=="C22"
  replace ucodt="Malignant neoplasm of larynx" if `v'=="C32"
  replace ucodt="Malignant neoplasm of bronchus & lung" if `v'=="C34"
  replace ucodt="Malignant neoplasm of bone" if `v'=="C41"
  replace ucodt="Malignant neoplasm of corpus uteri" if `v'=="C54"
  replace ucodt="Malignant neoplasm of female genital organs" if `v'=="C57"
  replace ucodt="Malignant neoplasm of prostate" if `v'=="C61"
  replace ucodt="Malignant neoplasm of bladder" if `v'=="C67"
  replace ucodt="Malignant neoplasm of eye" if `v'=="C69"
  replace ucodt="Malignant neoplasm of ill-defined sites" if `v'=="C76"
  replace ucodt="Metastases" if `v'=="C79"
  replace ucodt="Malignant neoplasm, site unspecified" if `v'=="C80"
  replace ucodt="Lymphoma" if `v'=="C83"
  replace ucodt="Lymphoma NOS" if `v'=="C88"
  replace ucodt="Myeloid leukaemia" if `v'=="C92"
  replace ucodt="Leukaemia of unspecified cell type" if `v'=="C95"
  replace ucodt="Unspecified lymphatic malignancy" if `v'=="C96"
  replace ucodt="Neoplasm of oral cavity/digestive organs" if `v'=="D37"
  replace ucodt="Iron deficiency anaemia" if `v'=="D50"
  replace ucodt="Other anaemias" if `v'=="D64"
  replace ucodt="ITP" if `v'=="D69"
  replace ucodt="Immune disorder" if `v'=="D89"
  replace ucodt="Other disorders of thyroid" if `v'=="E07"
  replace ucodt="Type 1 Diabetes" if `v'=="E10"
  replace ucodt="Type 2 Diabetes" if `v'=="E11"
  replace ucodt="Hypoglycaemia" if `v'=="E16"
  replace ucodt="Endocrine disorder" if `v'=="E35"
  replace ucodt="Metabolic disorder" if `v'=="E87"
  replace ucodt="Delirium" if `v'=="F05"
  replace ucodt="Schizophrenia" if `v'=="F20"
  replace ucodt="Bacterial meningitis" if `v'=="G00"
  replace ucodt="Meningitis" if `v'=="G03"
  replace ucodt="Meningitis" if `v'=="G04"
  replace ucodt="Epilepsy" if `v'=="G40"
  replace ucodt="Parpalegia" if `v'=="G82"
}
```

```
replace ucodt="Other disorders of brain" if `v`=="G93"  
replace ucodt="Other diseases of spinal cord" if `v`=="G95"  
replace ucodt="Other CNS disorders" if `v`=="G96"  
replace ucodt="Rheumatic heart disease" if `v`=="I09"  
replace ucodt="Hypertensive heart disease" if `v`=="I11"  
replace ucodt="Essential hypertension" if `v`=="I15" | `v`=="I10"  
replace ucodt="Chronic ischaemic heart disease" if `v`=="I25"  
replace ucodt="Cardiomyopathy" if `v`=="I42"  
replace ucodt="Heart failure" if `v`=="I50"  
replace ucodt="Heart disease" if `v`=="I51"  
replace ucodt="Unspecified intracranial haemorrhage" if `v`=="I62"  
replace ucodt="Peripheral vascular disease" if `v`=="I73"  
replace ucodt="Arterial embolism & thrombosis" if `v`=="I74"  
replace ucodt="Disorders of arteries, arterioles, capillaries" if `v`=="I79"  
replace ucodt="Unspecified lower respiratory tract infection" if `v`=="J22"  
replace ucodt="Respiratory conditions due to other external agents" if `v`=="J70"  
replace ucodt="Pulmonary oedema" if `v`=="J81"  
replace ucodt="Pleural effusion" if `v`=="J90"  
replace ucodt="Gastric ulcer" if `v`=="K25"  
replace ucodt="Peptic ulcer, site unspecified" if `v`=="K27"  
replace ucodt="Gastritis & duodenitis" if `v`=="K29"  
replace ucodt="Acute appendicitis" if `v`=="K35"  
replace ucodt="Other appendicitis" if `v`=="K36"  
replace ucodt="Other noninfective gastroenteritis/colitis" if `v`=="K52"  
replace ucodt="Paralytic ileus" if `v`=="K56"  
replace ucodt="Anal or rectal abscess" if `v`=="K61"  
replace ucodt="Other diseases of intestine" if `v`=="K63"  
replace ucodt="Alcoholic liver disease" if `v`=="K70"  
replace ucodt="Toxic liver disease" if `v`=="K71"  
replace ucodt="Hepatic failure" if `v`=="K72"  
replace ucodt="Fibrosis & cirrhosis of liver" if `v`=="K74"  
replace ucodt="Other diseases of liver" if `v`=="K76"  
replace ucodt="Other diseases of biliary tract" if `v`=="K83"  
replace ucodt="Other diseases of digestive system" if `v`=="K92"  
replace ucodt="Cutaneous abscess, furuncle, carbuncle" if `v`=="L02"  
replace ucodt="Cellulitis" if `v`=="L03"  
replace ucodt="Other erythematous conditions" if `v`=="L53"  
replace ucodt="Seropositive rheumatoid arthritis" if `v`=="M05"  
replace ucodt="Other necrotizing vasculopathies" if `v`=="M31"  
replace ucodt="SLE" if `v`=="M32"  
replace ucodt="Other systemic connective tissue dx" if `v`=="M35"  
replace ucodt="Osteomyelitis" if `v`=="M86"  
replace ucodt="Drug-induced tubulo-interstitial conditions" if `v`=="N14"  
replace ucodt="Acute renal failure" if `v`=="N17"  
replace ucodt="Unspecified renal failure" if `v`=="N17/N18"  
replace ucodt="Chronic renal failure" if `v`=="N18"  
replace ucodt="Unspecified renal failure" if `v`=="N19"  
replace ucodt="Disorder of urinary system" if `v`=="N39"  
replace ucodt="Inflammatory disorders of male genitals" if `v`=="N49"  
replace ucodt="Gestational hypertension" if `v`=="O13"  
replace ucodt="Complications of the puerperium" if `v`=="O90"  
replace ucodt="Post-partum cardiomyopathy" if `v`=="O99"  
replace ucodt="Congenital cardiac malformation" if `v`=="Q20"  
replace ucodt="Abdominal and pelvic pain" if `v`=="R10"  
replace ucodt="Nausea and vomiting" if `v`=="R11"  
replace ucodt="Other symptoms involving the abdomen" if `v`=="R19"  
replace ucodt="Other sudden death, cause unknown" if `v`=="R96"  
replace ucodt="Unknown" if `v`=="R99"  
replace ucodt="Unspecified injury of head" if `v`=="S09"  
replace ucodt="Fracture of femur" if `v`=="S72"  
replace ucodt="Injuries to spine and trunk" if `v`=="T09"  
replace ucodt="Poisoning by other systemic anti-infectives" if `v`=="T37"  
replace ucodt="External cause" if `v`=="T81"  
replace ucodt="Primarily systemic agents" if `v`=="Y43"  
replace ucodt="Procedure for non-health purposes" if `v`=="Z41"  
replace ucodt="Problems related to lifestyle" if `v`=="Z72"  
replace ucodt="Coagulation defect" if `v`=="D68"  
replace ucodt="Dysmenorrhoea" if `v`=="N94"  
replace ucodt="Obstetric embolism" if `v`=="O88"
```

```

replace ucodt="Drug overdose" if `v'=="T96"
replace ucodt="Gunshot" if `v'=="W32"
replace ucodt="Urethral stricture" if `v'=="N36"
replace ucodt="External trauma" if `v'=="Y41" | `v'=="Y85" | `v'=="V03" | `v'=="V49"
replace ucodt="Motor-vehicle accident" if `v'=="V89"
}

drop icodicd icodicdo ucodicd ucodicdo

// Find those with HIV underlying that aren't necessarily classified as such
gen nonhivc=1 if (icodt!="HIV disease resulting in infection" & /*
*/ icodt!="HIV disease resulting in malignancy" & /*
*/ icodt!="HIV disease resulting in other specified" & /*
*/ icodt!="HIV disease resulting in other and unspecified" & /*
*/ icodt!="Unspecified HIV disease")
recode nonhivc .=0

gen nonhivuc=1 if (ucodt!="HIV disease resulting in infection" & /*
*/ ucodt!="HIV disease resulting in malignancy" & /*
*/ ucodt!="HIV disease resulting in other specified" & /*
*/ ucodt!="HIV disease resulting in other and unspecified" & /*
*/ ucodt!="Unspecified HIV disease")
recode nonhivuc .=0

gen hivadduc=1 if nonhivuc==1 & nonhivc==1 & (lkcod>=1 & lkcod<=4) & (ucodt!="")
recode hivadduc .=0

// bring in age/sex vars
merge 1:m pptid using All_Dem_Detail.dta
drop if _merge==2
keep pptid certno icodt ucodt hivadduc sex dodfinal agedeath
rename sex sex1

// merge with s10 structure file
merge 1:m certno using S10Structure.dta
drop if _merge==2
drop _merge

// updating s10 variables for Super-Micar
// Sex
replace sex="M" if sex1==1
replace sex="F" if sex1==2

// Date of death
gen yod=year(dodfinal)
gen mod=month(dodfinal)
gen dod=day(dodfinal)
format %02.0f dod mod
gen yodst=string(yod, "%04.0f")
gen modst=string(mod, "%02.0f")
gen dodst=string(dod, "%02.0f")
replace ydeath=yodst
replace dodm=modst
replace dodd=dodst

// Age
format %2.0f agedeath
gen age2=string(agedeath,"%03.0f")
replace ageunits="1"
replace age=age2

// Causes of death - immediate to cod1a and underlying to cod2
replace cod1a=icodt
replace cod1b=ucodt if hivadduc==1
replace cod2=ucodt if hivadduc==0
replace cod2="Unspecified HIV disease" if hivadduc==1

// Get rid of non-s10 vars
drop dodfinal sex1 yod dod mod yodst dodst modst agedeath age2 icodt ucodt pptid hivadduc

```

```
// Fill in the remaining required S10 vars
replace statedeath="XX"
replace coder="9"
replace lot="9999"
replace section="9"
replace shipment="999"
replace dreceipt="01012016"
replace pgm="1110"
replace iffemale="0"
replace manner="A" if (cod1a=="Pedestrian in collision" | cod1a=="Gunshot" | /*
  */ cod1a=="Injuries to spine and trunk" | cod1b=="Transport accident" )
  replace manner="C" if cod1a=="Other sudden death, cause unknown"
  replace manner="N" if manner==" "
replace certifier="D"
replace activity="9"

save CC3_ForACME.dta, replace

/* ----- EXPORT AS A TEXT FILE ----- */
cd "~"
use CC3_ForACME.dta, clear
gen str1 end="."
order ydeath statedeath certno coder lot section shipment dreceipt pgm dodm dodd /*
  */ sex ageunits age cod1a cod1a2 cod1b cod1b2 cod1c cod1c2 cod1d cod1d2 cod2 /*
  */ tobacco pregnancy iffemale manner dinjury tinjury tunits placeinjury injwork /*
  */ howinjury transport autopsy autopsyf certifier dsurgerym dsurgeryd /*
  */ dsurgeryy incompletedata line1bdel line1cdel line1ddel line2del activity /*
  */ placeinjury2 auxstate statespec end

// run 'generate space' bit of code again, for some reason these revert back after the merge
ds, has(type string)
  foreach v in `r(varlist)' {
    local spaces: display _dup(250) " "
    local length = substr("`': type `v'",4,.)
    replace `v'=`v' + substr("`spaces'",1,`length' - length(`v'))
  }

// Export CCoD to txt file [SuperMICAR can only process deaths from the same year at once, so export
according to year of death]
export delimited using /*
  */ "~/CC3_013.s10" /*
  */ if ydeath=="2013", delimiter("_") novarnames datafmt replace

export delimited using /*
  */ "~\CC3_014.s10" /*
  */ if ydeath=="2014", delimiter("_") novarnames datafmt replace

export delimited using /*
  */ "~\CC3_015.s10" /*
  */ if ydeath=="2015", delimiter("_") novarnames datafmt replace

/*
Further action needed:
--> In a text editor, find and replace all "_" with nothing, and find and replace all "." with
nothing
--> save/copy into the MMDS 'DATA' folder
*/

// ===== END ===== //
```

8.5.5. Stata code for conversion of WHO 2012 data into appropriate format for SmartVA-Analyze

```

/*=====
Convert WHO 2012 VA data to input .csv file for SmartVA-Analyze
Author: AS Karat (aaron.karat@lshtm.ac.uk | aaron.s.karat@gmail.com)

Created:      09Aug2016
Last modified: 09Sep2016 (v4.3)
=====*/

set more off

// use file derived from PHMRC instrument (v3-13) for structure
use ODK_Full.dta, clear
gen str SubmissionDate=""
save SmartVAInputStructure_Blank.dta, replace

// Append to existing VA data in 2012 form
append using $VADData$.dta
order pptid SubmissionDate interviewstarttime interviewdate
sort pptid

// MERGE W CLEAN DEMOGRAPHIC DATASET
merge 1:m pptid using >>DemographicData<<.dta
drop _merge

/*-----
CLEAN AND GEN INTERMEDIATE VARIABLES
-----*/

// GENERAL CLEANING
// clean death certificate spellings *NO COMMAS - WILL BE TREATED AS SEPARATE FIELDS WHEN
CONVERT TO CSV*
foreach v of var registeredcauseofdeath contributingcauses underlyingcauses {
  replace `v'="HIV/AIDS" if /*
    */ `v'=="Natural causes-Hiv/Aids" | `v'=="Vitima de sida vms (hiv)" | /*
    */ `v'=="Hiv an aids" | `v'=="Retroviral disease" | `v'=="Retroviral diseases" | /*
    */ `v'=="Hiv disease" | `v'=="RVD" | `v'=="HIV stage 4" | `v'=="Rnd" | /*
    */ `v'=="NIV Human Immunodeficiency Virus"

  replace `v'="Immunosuppression" if /*
    */ `v'=="Immunesupresstow" | `v'=="Immuno compromised " | `v'=="Tmmononoppression"

  replace `v'="Pulmonary tuberculosis" if /*
    */ `v'=="Cardiorespiratory arrest,pulmonary TB" | `v'=="Polmonary tb" | /*
    */ `v'=="pulmonary T.B." | `v'=="Palmonary tb" | `v'=="Pulmonary tb" | /*
    */ `v'=="Pulmonary TB" | `v'=="Pulmonary turbercolis" | `v'=="PTB"

  replace `v'="Disseminated tuberculosis" if `v'=="Disseminated TB"
  replace `v'="Abdominal tuberculosis" if `v'=="Abdominal TB"
  replace `v'="Metabolic acidosis" if `v'=="Metabokcaadimsis"
  replace `v'="Lower respiratory tract infection" if /*
    */ `v'=="Lower respuratory track infection"

  replace `v'="Cough and chest pain" if `v'=="Cought chest pain"
  replace `v'="Chest pain" if `v'=="Chest pains/ natural"
  replace `v'="Cardiorespiratory arrest" if /*
    */ `v'=="Cardiorespiratory arrest" | `v'=="Cardio respiratory arrest"

  replace `v'="Meningitis" if `v'=="Managitis"
  replace `v'="N/A" if /*
    */ `v'=="Abridged" | `v'=="." | `v'=="Na" | `v'=="We did not check tag" | /*
    */ `v'=="Not determined" | `v'=="NA" | `v'=="N/a" | `v'=="Na " | `v'=="None" | /*
    */ `v'=="na"

  replace `v'="Acute gastroenteritis" if `v'=="Age"

```



```

replace `v'="Pneumonia" if /*
  */ `v'=="Pneynomia" | `v'=="Respiratory pneumonia" | `v'=="Multilobar pneumonia"

replace `v'="Community-acquired pneumonia" if `v'=="Community acquired pneummonia"
replace `v'="Bicytopenia" if `v'=="Bicyotmpcenia"
replace `v'="Sepsis and meningitis" if `v'=="Sepsis, meningitis (sgih herdytitis"
replace `v'="Hypernatraemia" if `v'=="Hypernatreamra"
replace `v'="Immune reconstitution inflammatory syndrome" if `v'=="Iris"
replace `v'="HIV encephalopathy" if `v'=="NIV encephalopathy"
replace `v'="Natural causes" if /*
  */ `v'=="Natural cause" | `v'=="Natura Causes" | `v'=="Natural" | /*
  */ `v'=="Natural Cause" | `v'=="Natural Causes" | `v'=="Natural courses" | /*
  */ `v'=="Natural cause " | `v'=="Natural causes " | `v'=="Natural course" | /*
  */ `v'=="Natural courses" | `v'=="Natural cause" | `v'=="Natural death" | /*
  */ `v'=="NaturalCauses" | `v'=="natural causes" | `v'==" natural causes"

replace `v'="Cardiac failure" if /*
  */ `v'=="Cardiac failure fluid overload" | `v'=="Recurring cardiovascular problems"

replace `v'="HIV/AIDS; tuberculosis" if `v'=="Hiv and tb"
replace `v'="Tuberculosis" if `v'=="Tb"
replace `v'="Renal failure" if `v'=="Renal Failure"
replace `v'="Lung cancer; pneumonia; tuberculosis" if /*
  */ `v'=="Lung cancer, pneumonia and TB"

replace `v'="Diabetes mellitus" if /*
  */ `v'=="Diabetes meaulus" | `v'=="dM2"

replace `v'="Intracranial haemorrhage" if `v'=="Intracranal haemorrhage"
replace `v'="T-cell lymphoma" if `v'=="T cell lymphoma hpt"
}

// spelling error
rename smoketabacco smoketobacco

// CLEAN AND REFORMAT ALL LENGTHS (currently in a www:d format, stored as str5)
foreach v of var lengthillness lengthfever lengthnightsweats lengthcough /*
  */ lengthfastbreathing lengthbreathlessness lengthdiarrhoea lengthabdominalpain /*
  */ lengthprotrudingabdomen lengthabdomenlumpresent lengthstifforpainfulneck /*
  */ lengthmentalconfusion lengthskinrash {

replace `v'="000:0" if `v'=="0"
replace `v'="000:1" if (`v'=="1 day" | `v'=="000:1" | `v'=="000,1" | /*
  */ `v'=="0:1" | `v'=="000.1")

replace `v'="000:2" if (`v'=="2 days" | `v'=="0:2" | `v'=="000-2")
replace `v'="000:3" if (`v'=="3 days" | `v'=="0:3" | `v'=="000-3" | `v'=="3days")
replace `v'="000:4" if (`v'=="4 days" | `v'=="000-4" | `v'=="0:4")
replace `v'="000:5" if `v'=="5 days"
replace `v'="000:6" if `v'=="6 days"
replace `v'="001:0" if (`v'=="1 week" | `v'==" 1:00" | `v'=="001" | /*
  */ `v'=="1" | `v'=="1:0" | `v'=="1 " | `v'=="01:00" | `v'=="7 days" | `v'=="000:7")

replace `v'="001:1" if (`v'=="8 days" | `v'=="000:8")
replace `v'="001:2" if (`v'=="9 days" | `v'=="1 week 2days" | `v'=="1:2")
replace `v'="001:3" if `v'=="10 days"
replace `v'="001:4" if (`v'=="11 days" | `v'=="1:4")
replace `v'="001:5" if `v'=="12 days"
replace `v'="001:6" if `v'=="13 days"
replace `v'="002:0" if (`v'=="2 weeks" | `v'=="2 wks" | `v'=="2" | /*
  */ `v'==" 2:00" | `v'=="2:0" | `v'=="02" | `v'=="002" | `v'=="14 days" | `v'=="001:7")

replace `v'="002:5" if `v'=="2:5"
replace `v'="003:0" if (`v'=="3 weeks" | `v'=="3weeks" | /*
  */ `v'==" 3 weeks" | `v'=="3" | `v'=="003:2" | `v'=="003")

replace `v'="004:0" if (`v'=="4 weeks" | `v'=="4" | `v'=="004" | /*
  */ `v'=="30 days" | `v'=="F4 weeks" | `v'=="4weeks")
replace `v'="005:0" if (`v'=="5 weeks" | `v'=="5" | `v'==" 5:06" | `v'=="5:6")

```

```

replace `v'="006:0" if (`v'=="6 weeks" | `v'=="6" | `v'=="006;0" | `v'=="40 days")
replace `v'="007:0" if (`v'=="7 weeks" | `v'=="007:5")
replace `v'="008:0" if (`v'=="8 weeks" | `v'=="8" | `v'=="0080" | /*
    */ `v'==" 8:04" | `v'=="8:4" | `v'=="8weeks" | `v'=="008" | `v'=="8 wks")

replace `v'="009:0" if (`v'=="9 weeks" | `v'=="9" | `v'=="009" | /*
    */ `v'=="09:00:00" | `v'=="70 days")

replace `v'="010:0" if (`v'=="10 weeks" | `v'=="10" | `v'=="10.0" | /*
    */ `v'=="010" | `v'=="010.0")

replace `v'="011:0" if `v'=="11 weeks"
replace `v'="012:0" if (`v'=="12 weeks" | `v'=="12" | `v'=="12:0")
replace `v'="013:0" if `v'=="13 weeks"
replace `v'="014:0" if (`v'=="14 weeks" | `v'=="14")
replace `v'="015:0" if `v'=="15 weeks"
replace `v'="016:0" if (`v'=="16 weeks" | `v'=="016" | `v'=="16" | /*
    */ `v'=="016" | `v'=="016.0" | `v'=="016:0")

replace `v'="017:0" if (`v'=="17" | `v'=="17 weeks")
replace `v'="018:0" if `v'=="18 weeks"
replace `v'="019:0" if `v'=="19 weeks"
replace `v'="020:0" if (`v'=="20 weeks" | `v'=="20" | `v'=="20:00:00")
replace `v'="021:0" if `v'=="21 weeks"
replace `v'="022:0" if `v'=="22 weeks"
replace `v'="023:0" if `v'=="23 weeks"
replace `v'="024:0" if (`v'=="24 weeks" | `v'=="24:0")
replace `v'="025:0" if `v'=="25 weeks"
replace `v'="026:0" if `v'=="26 weeks"
replace `v'="027:0" if `v'=="27 weeks"
replace `v'="028:0" if `v'=="28 weeks"
replace `v'="029:0" if `v'=="29 weeks"
replace `v'="030:0" if `v'=="30 weeks"
replace `v'="031:0" if `v'=="31 weeks"
replace `v'="032:0" if `v'=="32 weeks"
replace `v'="033:0" if `v'=="33 weeks"
replace `v'="034:0" if `v'=="34 weeks"
replace `v'="035:0" if `v'=="35 weeks"
replace `v'="036:0" if (`v'=="36 weeks" | `v'=="36")
replace `v'="037:0" if `v'=="37 weeks"
replace `v'="038:0" if (`v'=="38 weeks" | `v'=="38")
replace `v'="039:0" if `v'=="39 weeks"
replace `v'="040:0" if (`v'=="40 weeks" | `v'=="40")
replace `v'="041:0" if `v'=="41 weeks"
replace `v'="044:0" if (`v'=="44 weeks" | `v'=="44")
replace `v'="049:0" if `v'=="049"
replace `v'="052:0" if (`v'=="52 weeks" | `v'=="52")
replace `v'="060:0" if `v'=="60"
replace `v'="080:0" if `v'=="80"
replace `v'="094:0" if `v'=="94"
replace `v'="120:0" if `v'=="120"
replace `v'="999:9" if (`v'=="15:08:59" | `v'=="13:09:00" | `v'=="00:00:00" | /*
    */ `v'=="Dont know" | `v'=="999" | `v'=="997:9" | `v'=="999:8" | /*
    */ `v'=="997:7" | `v'=="999;9" | `v'=="999:5" | `v'=="999:3")
}

// Do the same for length of convulsions
foreach v of var lengthconvulsions {
    replace `v'="00:10" if `v'=="001:0"
    replace `v'="00:07" if `v'=="007:00"
    replace `v'="00:10" if `v'=="10:00"
    replace `v'="00:11" if `v'=="11:00"
    replace `v'="99:99" if `v'=="00:00"
}

// Generate a decimal point for conversion to numeric variables
gen str1 dot="."

// Destring lengths

```

```

foreach v of var lengthillness lengthfever lengthnightsweats lengthcough /*
  */ lengthfastbreathing lengthbreathlessness lengthdiarrhoea /*
  */ lengthabdominalpain lengthprotrudingabdomen lengthabdomenlumpresent /*
  */ lengthstifforpainfulneck lengthmentalconfusion lengthskinrash {
  gen str3 `v'_w=substr(`v',1,3)
  gen str1 `v'_d=substr(`v',5,1)
  gen `v'_str=`v'_w + dot + `v'_d
  destring `v'_str, gen(`v'int)
  format %03.1f `v'int
  destring `v'_w, replace
  destring `v'_d, replace
  recode `v'_w 999=.
  recode `v'_d 9=.
  gen `v'_m=`v'_w/4
  format %03.0f `v'_m
  gen `v'_y=`v'_w/52
  format %03.0f `v'_y
}

foreach v of var length*_m length*_y {
  replace `v'=0 if `v'<1
}

// DEMOGRAPHICS
// date of interview into strings
gen dvastr=string(dem_dvafinal, "%tdDD.NN.CCYY")
gen dvastr2=string(dem_dvafinal, "%tdd-m-y")

// Gen seconds for time
gen str3 timezero=":00"

// gen random string for var gen_4_1 -

// requires the RALPHA module, available from: https://ideas.repec.org/c/boc/bocode/s457277.html
ralpha random1, lower l(10)

// for vars gen_6_3 and gen_6_7
ralpha random2, lower l(10)
ralpha random3, lower l(10)

// for metainstance ID and KEY
ralpha random4, lower l(20)
ralpha random5, lower l(20)

// Dob to string to gen d/m/y vars
gen dobstr=string(dem_dob, "%tdDD.NN.CCYY")
gen doby=substr(dobstr,7,4)
destring doby, replace

gen dobm=substr(dobstr,4,2)
destring dobm, replace

gen dobd=substr(dobstr,1,2)
destring dobd, replace

// Dod to string to gen d/m/y vars
gen dodstr=string(dem_dodfinal, "%tdDD.NN.CCYY")
gen dody=substr(dodstr,7,4)
destring dody, replace

gen dodm=substr(dodstr,4,2)
destring dodm, replace

gen dodd=substr(dodstr,1,2)
destring dodd, replace

// Generate age at death
gen ageddeath=(dem_dod-dem_dob)/365.25

```

```

format %02.0f ageddeath
recast byte ageddeath, force

// WOMEN'S HEALTH
// in case any errors
foreach v of var breastswelling vaginalbleedingbetweenmenstruati /*
  */ naturalmenopause bleedingaftermenopause pregnant {
  replace `v'= . if dem_sex==1
}

save SmartVAInputStructure_CleanVAData.dta, replace

/*-----
GENERATE SMARTVA INPUT
-----*/
use SmartVAInputStructure_CleanVAData.dta, clear

// DECEASED DETAILS
// replace date and time vars
replace SubmissionDate=dvustr + " 12:00:00"
drop interviewstarttime interviewdate sid
gen str interviewstarttime=dvustr + " " + startinterview + timezero
gen str interviewdate=dvustr
gen str sid=pptid

// date of birth
gen gen_1_1d=dobd
gen gen_1_1m=dobm
gen gen_1_1y=doby

// sex
gen gen_1_5=dem_sex

// date of death
gen gen_1_6d=dodd
gen gen_1_6m=dodm
gen gen_1_6y=dody

// age at death
gen gen_1_7a=agedeath

// marital status
gen gen_1_8=.
recode gen_1_8 . =1 if maritalstatus==1
recode gen_1_8 . =2 if maritalstatus==2
recode gen_1_8 . =3 if maritalstatus==5
recode gen_1_8 . =4 if maritalstatus==4
recode gen_1_8 . =5 if maritalstatus==3
recode gen_1_8 . =9

// highest education
gen gen_1_9=.
recode gen_1_9 . =1 if education==1
recode gen_1_9 . =2 if education==2 | education==3
recode gen_1_9 . =3 if education>=4 & education<=6
recode gen_1_9 . =4 if education==7 | education==8
recode gen_1_9 . =9

// RESPONDENT DETAILS
replace gen_3_1=1
drop gen_4_1
gen str gen_4_1=random1
replace gen_4_2=9

// relationship to deceased
foreach v of var gen_4_3 {
  replace `v'=1 if relationshiptodeceased<3 | /*

```

```

    */ relationshiptodeceasedspecify=="Stepmother" | /*
    */ relationshiptodeceasedspecify=="Parents"

replace `v'=2 if relationshiptodeceasedspecify=="Father, sister,aunt"
replace `v'=3 if relationshiptodeceased==5 | /*
    */ relationshiptodeceasedspecify=="Family members grandmother,brother, partner,
nephew."

replace `v'=5 if relationshiptodeceasedspecify=="Aunt" | /*
    */ relationshiptodeceasedspecify=="Aunty" | /*
    */ relationshiptodeceasedspecify=="aunt"

replace `v'=6 if relationshiptodeceasedspecify=="Uncle"
replace `v'=7 if dem_sex==2 & (relationshiptodeceased==3 | /*
    */ relationshiptodeceasedspecify=="Partner")

replace `v'=8 if (dem_sex==1 & (relationshiptodeceased==3 | /*
    */ relationshiptodeceasedspecify=="Partner")) | /*
    */ relationshiptodeceasedspecify=="Girlfriend"

replace `v'=9 if relationshiptodeceasedspecify=="Brother"
replace `v'=10 if relationshiptodeceased==4 | /*
    */ relationshiptodeceasedspecify=="Younge sister" | /*
    */ relationshiptodeceasedspecify=="Sister"

replace `v'=12 if relationshiptodeceasedspecify=="Son" | /*
    */ relationshiptodeceasedspecify=="Family friend" | /*
    */ relationshiptodeceasedspecify=="Friend" | /*
    */ relationshiptodeceasedspecify=="Brother inlaw" | /*
    */ relationshiptodeceasedspecify=="Boyfriend" | /*
    */ relationshiptodeceasedspecify=="nephew"

replace `v'=13 if relationshiptodeceasedspecify=="Child" | /*
    */ relationshiptodeceasedspecify=="Dougter" | /*
    */ relationshiptodeceasedspecify=="Dougther" | /*
    */ relationshiptodeceasedspecify=="DAughter" | /*
    */ relationshiptodeceasedspecify=="Daughter" | /*
    */ relationshiptodeceasedspecify=="Care giver" | /*
    */ relationshiptodeceasedspecify=="Caregiver" | /*
    */ relationshiptodeceasedspecify=="Khayelihle old age home" | /*
    */ relationshiptodeceasedspecify=="Brothers partner" | /*
    */ relationshiptodeceasedspecify=="Cousin" | /*
    */ relationshiptodeceasedspecify=="Daughter in law" | /*
    */ relationshiptodeceasedspecify=="relative" | /*
    */ relationshiptodeceasedspecify=="Niece" | /*
    */ relationshiptodeceasedspecify=="Nierce" | /*
    */ relationshiptodeceasedspecify=="Sister in law" | /*
    */ relationshiptodeceasedspecify=="Sister inlaw" | /*
    */ relationshiptodeceasedspecify=="Daughter-in-law" | /*
    */ relationshiptodeceasedspecify=="Neighbour" | /*
    */ relationshiptodeceasedspecify=="Home based care nurse"

}

// specify respondent
drop gen_4_3b gen_4_3c
gen str gen_4_3b=""
gen str gen_4_3c=""
replace gen_4_3b=relationshiptodeceasedspecify if gen_4_3==12
replace gen_4_3c=relationshiptodeceasedspecify if gen_4_3==13

// INFORMATION ABOUT THE DECEASED
drop gen_5_0
gen str gen_5_0="NA"

// DoB
replace gen_5_1a=doby
replace gen_5_1b=dobm

```

```

replace gen_5_1c=dobd
// replace birthday=dobstr // Cancel this for now

// Sex
replace gen_5_2=dem_sex

// DoD
replace gen_5_3a=dody
replace gen_5_3b=dodm
replace gen_5_3c=dodd

// Age
replace gen_5_4a=agedeath

// age group (3=adult)
replace gen_5_4d=3

replace UKagehoursA=((agedeath*365.25)*24)
replace UKagedaysA=(agedeath*365.25)
replace UKagemonthsA=(agedeath*12)
replace UKageyearsA=agedeath

// marital status
replace gen_5_5=gen_1_8

// education
replace gen_5_6=gen_1_9

// ADULT MODULE 1: DIAGNOSES
replace adult_1_1a=asthma
replace adult_1_1b=9 // not in WHO 2012
replace adult_1_1c=cancer
replace adult_1_1d=tbdagnosis
replace adult_1_1e=dementia
replace adult_1_1f=depression
replace adult_1_1g=diabetes
replace adult_1_1h=epilepsy
replace adult_1_1i=heartdisease
replace adult_1_1j=highbloodpressure
replace adult_1_1k=9 // not in WHO 2012
replace adult_1_1l=stroke
replace adult_1_1m=copd
replace adult_1_1n=hivaidsdiagnosis

// ADULT MODULE 2: FINAL ILLNESS
// For all the lengths, first part of question (adult_2_x) defines the units (1=years,
2=months, 3=weeks, 4=days, 5=hours)
// Sub-questions are for the numbers of specified units; generally _xa years, _xb months, _xc
weeks, etc, *but not always*

// length of illness (years, months, weeks, or days)
replace adult_2_3=1 if lengthillness_y!=0 & lengthillness_y!=.
replace adult_2_3=2 if /*
*/ (lengthillness_y==0 | lengthillness_y=.) & /*
*/ (lengthillness_m!=0 & lengthillness_m!=.)
replace adult_2_3=3 if /*
*/ (lengthillness_y==0 | lengthillness_y=.) & /*
*/ (lengthillness_m==0 | lengthillness_m=.) & /*
*/ (lengthillness_w!=0 & lengthillness_w!=.)
replace adult_2_1=4 if /*
*/ (lengthillness_y==0 | lengthillness_y=.) & /*
*/ (lengthillness_m==0 | lengthillness_m=.) & /*
*/ (lengthillness_w==0 | lengthillness_w=.) & /*
*/ (lengthillness_d!=0 | lengthillness_d!=.)
replace adult_2_1=9 if lengthillness_str=="999.9"

replace adult_2_1a=lengthillness_y if adult_2_3==1

```

```
replace adult_2_1b=lengthillness_m if adult_2_3==2
replace adult_2_1c=lengthillness_w if adult_2_3==3
replace adult_2_1d=lengthillness_d if adult_2_3==4

// fever
replace adult_2_2=fever

// length fever (days only)
replace adult_2_3=4 if fever==1
  replace adult_2_3=9 if fever==1 & lengthfever_str=="999.9"
  replace adult_2_3=. if fever==0

gen lengthfever_d2=(((lengthfever_w)*7)+(lengthfever_d)) if lengthfever_w!=.
  replace adult_2_3a=lengthfever_d2 if adult_2_3==4

// adult_2_4 & adult_2_5 not in WHO 2012

// night sweats
replace adult_2_6=night sweats

// skin rash
replace adult_2_7=skinproblem

// length of rash (days only)
replace adult_2_8=4 if skinproblem==1
  replace adult_2_8=9 if skinproblem==1 & lengthskinrash_str=="999.9"

gen lengthskinrash_d2=(((lengthskinrash_w)*7)+(lengthskinrash_d)) if lengthskinrash_w!=.
  replace adult_2_8a=lengthskinrash_d2 if adult_2_8==4

// adult_2_9/9a not in WHO 2012

// body sores
replace adult_2_10=bodysores
  replace adult_2_11=9 if bodysores==1 // not in WHO 2012

// adult_2_12 not in WHO 2012

// foot sores
replace adult_2_13=footsores
  replace adult_2_14=9 if footsores==1
  replace adult_2_15=9 if footsores==1

// adult_2_16 & 17 not in WHO 2012

// weight loss
replace adult_2_18=weightloss
replace adult_2_19=3 if severeweightloss==1
  replace adult_2_19=9 if weightloss==1 & severeweightloss!=1

// anaemia
replace adult_2_20=paleappearance

// jaundice
replace adult_2_21=eyesyellowdiscolouration
  replace adult_2_22=9 if adult_2_21==1 // not in WHO 2012

// pedal oedema
replace adult_2_23=bothfeetswollen
  replace adult_2_24=9 if adult_2_23==1 // not in WHO 2012

// facial oedema
replace adult_2_25=facialswelling
  replace adult_2_26=9 if adult_2_25==1 // not in WHO 2012

// adult_2_27 & 28 not in WHO 2012

// lymphadenopathy
replace adult_2_29=necklumps
```

```
replace adult_2_30=armpitlumps
replace adult_2_31=groinlumps

// cough
replace adult_2_32=cough

// length of cough (months or days)
replace adult_2_33=2 if cough==1 & /*
  */ (lengthcough_y==0 | lengthcough_y==.) & /*
  */ lengthcough_m!=. & lengthcough_m!=0

replace adult_2_33=4 if cough==1 & /*
  */ (lengthcough_y==0 | lengthcough_y==.) & /*
  */ (lengthcough_m==0 | lengthcough_m==.) & /*
  */ (lengthcough_w==0 | lengthcough_w==.) & /*
  */ lengthcough_d!=. & lengthcough_d!=0

replace adult_2_33=9 if cough==1 & lengthcough_str=="999.9"
replace adult_2_33a=lengthcough_d if adult_2_33==4
replace adult_2_33b=lengthcough_m if adult_2_33==2

// productive cough
replace adult_2_34=productivecough
replace adult_2_35=coughblood

// breathing problems
replace adult_2_36=breathlessness

// length breathing problems (months or days)
replace adult_2_37=2 if breathlessness==1 & /*
  */ (lengthbreathlessness_y==0 | lengthbreathlessness_y==.) & /*
  */ lengthbreathlessness_m!=. & lengthbreathlessness_m!=0

replace adult_2_37=4 if breathlessness==1 & /*
  */ (lengthbreathlessness_y==0 | lengthbreathlessness_y==.) & /*
  */ (lengthbreathlessness_m==0 | lengthbreathlessness_m==.) & /*
  */ (lengthbreathlessness_w==0 | lengthbreathlessness_w==.) & /*
  */ lengthbreathlessness_d!=. & lengthbreathlessness_d!=0

replace adult_2_37=9 if breathlessness==1 & lengthbreathlessness_str=="999.9"
replace adult_2_37a=lengthbreathlessness_d if adult_2_37==4

replace adult_2_37b=lengthbreathlessness_m if adult_2_37==2

// fast breathing
replace adult_2_40=fastbreathing

// length of fast breathing
replace adult_2_41=2 if fastbreathing==1 & /*
  */ (lengthfastbreathing_y==0 | lengthfastbreathing_y==.) & /*
  */ lengthfastbreathing_m!=. & lengthfastbreathing_m!=0

replace adult_2_41=4 if fastbreathing==1 & /*
  */ (lengthfastbreathing_y==0 | lengthfastbreathing_y==.) & /*
  */ (lengthfastbreathing_m==0 | lengthfastbreathing_m==.) & /*
  */ (lengthfastbreathing_w==0 | lengthfastbreathing_w==.) & /*
  */ lengthfastbreathing_d!=. & lengthfastbreathing_d!=0
replace adult_2_41=9 if fastbreathing==1 & lengthfastbreathing_str=="999.9"

replace adult_2_41a=lengthfastbreathing_d if adult_2_41==4

replace adult_2_41b=lengthfastbreathing_m if adult_2_41==2

// wheezing
replace adult_2_42=wheezing

// chest pain
replace adult_2_43=severechestpain
```



```

foreach v of var adult_2_44 adult_2_45 adult_2_46 {
  replace `v`=9 if severechestpain==1
}

// diarrhoea
replace adult_2_47=diarrhoea

// length diarrhoea (days only)
replace adult_2_48=4 if diarrhoea==1
  replace adult_2_48=9 if diarrhoea==1 & lengthdiarrhoea_str=="999.9"

  gen lengthdiarrhoea_d2=(((lengthdiarrhoea_w)*7)+(lengthdiarrhoea_d)) if
lengthdiarrhoea_w!=.
  replace adult_2_48a=lengthdiarrhoea_d2 if adult_2_48==4

// adult_2_49 not in WHO 2012

// blood in stools
replace adult_2_50=bloodinstools
  replace adult_2_51=9 if bloodinstools==1 // not in WHO 2012

// urine, vomiting, dysphagia
replace adult_2_52=passnourine
replace adult_2_53=vomit
  replace adult_2_54=9 if vomit==1 // not in WHO 2012

replace adult_2_55=vomitblood
replace adult_2_56=vomitblood
replace adult_2_57=difficultyswallowingliquids
  replace adult_2_58=9 if difficultyswallowingliquids==1 // not in WHO 2012

replace adult_2_59=2 if difficultyswallowingliquids==1
replace adult_2_60=difficultyswallowingliquids

// abdominal pain
replace adult_2_61=severeabdominalpain

// length abdominal pain (hours, days, or months)
replace adult_2_62=2 if severeabdominalpain==1 & /*
  */ (lengthabdominalpain_y==0 | lengthabdominalpain_y==.) & /*
  */ lengthabdominalpain_m!=. & lengthabdominalpain_m!=0

replace adult_2_62=4 if severeabdominalpain==1 & /*
  */ (lengthabdominalpain_y==0 | lengthabdominalpain_y==.) & /*
  */ (lengthabdominalpain_m==0 | lengthabdominalpain_m==.) & /*
  */ (lengthabdominalpain_w==0 | lengthabdominalpain_w==.) & /*
  */ lengthabdominalpain_d!=. & lengthabdominalpain_d!=0

replace adult_2_62=9 if severeabdominalpain==1 & lengthabdominalpain_str=="999.9"

replace adult_2_62b=lengthabdominalpain_d if adult_2_62==4
replace adult_2_62c=lengthabdominalpain_m if adult_2_62==2

replace adult_2_63=9 if severeabdominalpain==1 // not in WHO 2012

// protruding abdomen
replace adult_2_64=protrudingabdomen

// length protruding abdomen (months or days)
replace adult_2_65=2 if protrudingabdomen==1 & /*
  */ (lengthprotrudingabdomen_y==0 | lengthprotrudingabdomen_y==.) & /*
  */ lengthprotrudingabdomen_m!=. & lengthprotrudingabdomen_m!=0

replace adult_2_65=4 if protrudingabdomen==1 & /*
  */ (lengthprotrudingabdomen_y==0 | lengthprotrudingabdomen_y==.) & /*
  */ (lengthprotrudingabdomen_m==0 | lengthprotrudingabdomen_m==.) & /*
  */ (lengthprotrudingabdomen_w==0 | lengthprotrudingabdomen_w==.) & /*
  */ lengthprotrudingabdomen_d!=. & lengthprotrudingabdomen_d!=0

```

```
replace adult_2_65=9 if protrudingabdomen==1 & lengthprotrudingabdomen_str=="999.9"

replace adult_2_65a=lengthprotrudingabdomen_d if adult_2_65==4
replace adult_2_65b=lengthprotrudingabdomen_m if adult_2_65==2

replace adult_2_66=9 if protrudingabdomen==1 // not in WHO 2012

// lump in abdomen
replace adult_2_67=lumpinsideabdomen

// length of abdominal lump (days or months)
replace adult_2_68=2 if lumpinsideabdomen==1 & /*
  */ (lengthabdomenlumppresent_y==0 | lengthabdomenlumppresent_y==.) & /*
  */ lengthabdomenlumppresent_m!=. & lengthabdomenlumppresent_m!=0

replace adult_2_68=4 if lumpinsideabdomen==1 & /*
  */ (lengthabdomenlumppresent_y==0 | lengthabdomenlumppresent_y==.) & /*
  */ (lengthabdomenlumppresent_m==0 | lengthabdomenlumppresent_m==.) & /*
  */ (lengthabdomenlumppresent_w==0 | lengthabdomenlumppresent_w==.) & /*
  */ lengthabdomenlumppresent_d!=. & lengthabdomenlumppresent_d!=0

replace adult_2_68=9 if lumpinsideabdomen==1 & lengthabdomenlumppresent_str=="999.9"

replace adult_2_68a=lengthabdomenlumppresent_d if adult_2_68==4
replace adult_2_68b=lengthabdomenlumppresent_m if adult_2_68==2

// headache
replace adult_2_69=severeheadache
replace adult_2_70=9 if severeheadache==1 // not in WHO 2012
replace adult_2_71=9 if severeheadache==1 // not in WHO 2012

// meningism
replace adult_2_72=stifforpainfulneck

// length of stiff neck (days or months)
replace adult_2_73=2 if stifforpainfulneck==1 & /*
  */ (lengthstifforpainfulneck_y==0 | lengthstifforpainfulneck_y==.) & /*
  */ lengthstifforpainfulneck_m!=. & lengthstifforpainfulneck_m!=0

replace adult_2_73=4 if stifforpainfulneck==1 & /*
  */ (lengthstifforpainfulneck_y==0 | lengthstifforpainfulneck_y==.) & /*
  */ (lengthstifforpainfulneck_m==0 | lengthstifforpainfulneck_m==.) & /*
  */ (lengthstifforpainfulneck_w==0 | lengthstifforpainfulneck_w==.) & /*
  */ lengthstifforpainfulneck_d!=. & lengthstifforpainfulneck_d!=0

replace adult_2_73=9 if stifforpainfulneck==1 & lengthstifforpainfulneck_str=="999.9"

replace adult_2_73a=lengthstifforpainfulneck_d if adult_2_73==4
replace adult_2_73b=lengthstifforpainfulneck_m if adult_2_73==2

// unconsciousness
replace adult_2_74=unconscious
replace adult_2_75=1 if suddenunconsciousness==1
replace adult_2_76=9 if unconscious==1 // not in WHO 2012
replace adult_2_77=9 if unconscious==1 // not in WHO 2012

// confusion
replace adult_2_78=mentalconfusion

// length confusion (days or months)
replace adult_2_79=2 if mentalconfusion==1 & /*
  */ (lengthmentalconfusion_y==0 | lengthmentalconfusion_y==.) & /*
  */ lengthmentalconfusion_m!=. & lengthmentalconfusion_m!=0

replace adult_2_79=4 if mentalconfusion==1 & /*
  */ (lengthmentalconfusion_y==0 | lengthmentalconfusion_y==.) & /*
  */ (lengthmentalconfusion_m==0 | lengthmentalconfusion_m==.) & /*
  */ (lengthmentalconfusion_w==0 | lengthmentalconfusion_w==.) & /*
  */ lengthmentalconfusion_d!=. & lengthmentalconfusion_d!=0
```

```
replace adult_2_79=9 if mentalconfusion==1 & lengthmentalconfusion_str=="999.9"

replace adult_2_79a=lengthmentalconfusion_d if adult_2_79==4
replace adult_2_79b=lengthmentalconfusion_m if adult_2_79==2

replace adult_2_80=9 if mentalconfusion==1 // not in WHO 2012

// adult_2_81 not in WHO 2012

// convulsions & paralysis
replace adult_2_82=convulsions
replace adult_2_83=9 if convulsions==1 // asked differently in WHO 2012
replace adult_2_84=convulsionunconsciousness
replace adult_2_85=paralysedoneside
replace adult_2_86=9 if paralysedoneside==1 // not in WHO 2012
replace adult_2_87=9 if paralysedoneside==1 // not in WHO 2012

// ADULT MODULE 3: WOMEN'S HEALTH
replace adult_3_1=breastswelling
replace adult_3_2=9 if breastswelling==1 // not in WHO 2012
replace adult_3_3=naturalmenopause
  recode adult_3_3 7=9

replace adult_3_4=bleedingaftermenopause
  recode adult_3_4 7=9

replace adult_3_5=9 // not in WHO 2012
  // adult_3_6,
replace adult_3_7=9 // not in WHO 2012
  // adult_3_8, & 9 not in WHO 2012
replace adult_3_10=pregnantatdeath
  recode adult_3_10 7=9

  // adult_3_11 not in WHO 2012
  // adult_3_12 not in WHO 2012
replace adult_3_13=vaginalbleedingpregnancy
  recode adult_3_13 7=9

replace adult_3_14=excessivevaginalbleedinglabour
  recode adult_3_14 7=9

replace adult_3_15=deathlabour
  recode adult_3_15 7=9
replace adult_3_16=9 if deathlabour==1 // not in WHO 2012

replace adult_3_17=deathfirstorsecondtrimester
  recode adult_3_17 7=9

replace adult_3_18=deathsixweeksafterchildbirth
  recode adult_3_18 7=9

replace adult_3_19=vaginalbleedingafterdelivery
  recode adult_3_19 7=9

replace adult_3_20=vaginaldischargepregnancy
  recode adult_3_20 7=9

* just in case
foreach v of var adult_3_* {
  replace `v'=. if dem_sex==1
}

// ADULT MODULE 4: LIFESTYLE
replace adult_4_1=smoketobacco
replace adult_4_2=1 if smoketobacco==1
  // adult_4_3 not in WHO 2012
```

```
replace adult_4_4=9 if smoketobacco==1 // not in WHO 2012
replace adult_4_5=alcohol
  recode adult_4_5 7=9

replace adult_4_6=9 if adult_4_5==1 // not in WHO 2012

// ADULT MODULE 5: ACCIDENTS & INJURIES
replace adult_5_1=1 if roadtrafficaccident==1
  replace adult_5_1=2 if fall==1
  replace adult_5_1=3 if drowning==1
  replace adult_5_1=4 if poisoning==1
  replace adult_5_1=5 if biteorsting==1
  replace adult_5_1=6 if burns==1
  replace adult_5_1=7 if firearm==1
  replace adult_5_1=8 if injuryoraccident==0

replace adult_5_2=1 if suicide==1
replace adult_5_3=1 if intentionalinjury==1
// adult_5_4 not in WHO 2012

// HEALTH SERVICE USE & DEATH CERTIFICATE
replace adult_6_1=medicaltreatment
replace adult_6_2=9 if medicaltreatment==1
  // adult_6_3 not in WHO 2012
replace adult_6_4=9 // not in WHO 2012

// death certificate
replace adult_6_9=1 if deathcertificate==1 | deathcertificate==2
  replace adult_6_9=0 if deathcertificate==0
replace adult_6_10=1 if deathcertificate==1

// Sort out death cert fields
drop adult_6_10b adult_6_11 adult_6_12 adult_6_15
gen str5 adult_6_10b="1 2 3"
gen str adult_6_11=registeredcauseofdeath if deathcertificate==1
gen str adult_6_12=underlyingcauses if deathcertificate==1
gen str adult_6_15=contributingcauses if deathcertificate==1

// FINAL ADMIN
drop interviewendtime
gen str interviewendtime=endinterview + timezero
gen str metainstanceID=random4
gen str KEY=random5

// DROP NON-SMARTVA VARS
keep SubmissionDate interviewstarttime interviewdate sid monthsfill daysfill agehours /*
  /*/ agedays ageweeks agemonths ageyears UKagehoursN UKagedaysN UKageweeksN /*
  /*/ UKagemonthsN UKageyearsN UKagehoursC UKagedaysC UKageweeksC UKagemonthsC /*
  /*/ UKageyearsC UKagehoursA UKagedaysA UKagemonthsA UKageyearsA Child2 breathing_dif /*
  /*/ fast_dif fever_dif cold_dif stools_dif interviewendtime metainstanceID KEY /*
  /*/ child_* gen_* adult_*

// ORDER NEW VARS
order SubmissionDate interviewstarttime interviewdate
order sid, after(gen_2_2a)
order gen_1_1d gen_1_1m gen_1_1y gen_1_5 gen_1_6d gen_1_6m gen_1_6y /*
  /*/ gen_1_7a gen_1_8 gen_1_9, after(gen_1_1)

order gen_4_1, after(gen_3_1)
order gen_4_3b gen_4_3c gen_5_0, after(gen_4_3a)
order adult_6_10b adult_6_11 adult_6_12 adult_6_15, after(adult_6_10ab)

// TEMPORARY VARS FOR .CSV
gen str1 START="''''''"
```

```
gen str1 STOP=`''''`
order START
order STOP, last

// EXPORT AS .xlsx
export excel using /*
*/ "-----.xlsx", sheetreplace firstrow(varlabels) nolabel

/* FURTHER MANUAL ACTIONS NEEDED
Save .xlsx as .csv
Open .csv in text editor
  Replace START, and ,STOP with "
  Replace all ''''', and ,'''''' with "
  Replace all , with ", "
Save and import to SmartVA-Analyze
*/

// ===== END ===== //
```

8.6. Appendix 6: Miscellaneous

8.6.1. Response to peer reviewer for research paper 2 (Chapter 4)

Dr Pere-Joan Cardona

Academic Editor

PLOS ONE

02 September 2016

Dear Dr Cardona,

Re: PONE-D-16-18440

Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa

Thank you for the opportunity to respond to the reviewer's comments. Her/his comments are shown in **bold** and our responses in normal font. All page and line numbers referenced below correspond to the document named RevisedManuscriptWithTrackedChanges.docx. Text from the original manuscript is shown in grey, dashed boxes, and text from the revised manuscript in solid, black boxes.

Reviewer #1:

1. The study background identifies causes of high early mortality among HIV-positive adults starting ART as a key issue the study wanted to address. However, in the decedents, only 74% were actually on ART at some point after enrolment. What happened to the other 26% and was there any correlation with autopsy findings?

Thank you for your comment. Existing literature documents that early mortality is high both among HIV-positive people who start ART and among those with advanced disease who have not yet started ART. In our study, causes of death in both groups were of interest. In the parent clinical trial, TB Fast Track, we recruited people with advanced disease at the point their CD4 count was known, reflecting the reality of clinical practice, because we were interested in the potential for empirical TB treatment to influence clinical outcomes if implemented at the earliest opportunity. The evidence cited in the background includes reference to mortality among people who have not started ART.

To explain this better, we have added a reference (Ref 3: Bassett et al. JAIDS 2016 Apr) and made a minor amendment to the text (page 4, lines 64-66).

Original text

Mortality in the first year of antiretroviral therapy (ART) remains high, particularly among those with advanced HIV disease in resource-constrained settings [1].

Revised text (page 4, lines 64-66)

Mortality among HIV-positive adults prior to starting and in the first year of antiretroviral therapy (ART) remains high, particularly among those with advanced disease in resource-constrained settings [1-3][22–24].

TB Fast Track was a pragmatic study. In the intervention arm, study staff were guided by the study algorithm concerning when participants started TB treatment or ART, but final decisions were at the discretion of the clinic doctor; in the control arm, participants were managed according to routine practice. Six (66%) of the nine decedents who underwent MIA and did not receive ART prior to death were started on TB treatment prior to death and died a median 11.5 days after enrolment, so were unlikely to have had time to start ART (according to South African practice, usually started two weeks after the start of TB treatment). The remaining three individuals died one, 14, and 48 days after enrolment (decedents 30, 17, and 08, respectively; S2 Table). According to verbal autopsy narratives from relatives of the deceased, decedent 17 was thought to have TB by clinic staff, but did not return to the clinic to start treatment; and decedent 08 was being investigated for TB by the clinic and referral hospital, but encountered delay waiting for tests.

With a total sample of 34 decedents, we have very low power to compare between groups, so we have not made comparisons for example between ART status and autopsy findings (of the 25 decedents who started ART, 14 [56%] had autopsy evidence of TB and 11 [44%] did not). Summary data, including length of time on ART prior to death, are included in the supplementary material (S2 table).

We have not changed the manuscript with regards to the second part of the reviewer's comment.

2. What is the correlation between full autopsy and MIA in terms of sensitivity and specificity for TB (or other infections)? I don't think this is adequately discussed?

At present there is only one study which has compared MIA to full autopsy (Ref 18: Cox et al. JAIDS 2014;67(2):169-76). Results from a large study comparing full autopsy to MIA are awaited (Bassat et al. Lancet Glob Health 2013; 1:e125-e126).

We have added more detail to the methods concerning the performance of MIA compared to full autopsy (page 4, lines 82-90).

Original text

Full pathological autopsy with sampling of all organs remains the gold standard for assigning cause of death [15,16], but it is expensive, time consuming, and not well accepted by families, who are often required to provide consent [17]. There is growing evidence that minimally invasive autopsy (MIA) can provide useful information relevant to cause of death, particularly with regards to infectious diseases [16,18].

Revised text (page 4, lines 82-90)

Full pathological autopsy with visualisation and sampling of all organs remains the gold standard for assigning cause of death [17,18], but it is expensive, time consuming, and not well accepted by families, who are often required to provide consent [19]. There is growing evidence that minimally invasive autopsy (MIA) can provide useful information relevant to cause of death, particularly with regards to TB and other infectious diseases [20,21]; one study, involving 96 HIV-positive adults in Uganda, compared histology from MIA to that from full autopsy and found that MIA was 71% sensitive and 100% specific in detecting TB [18]. Adding culture and/or other bacteriological modalities to MIA would likely improve its sensitivity in this regard.

3. For those selected to have an MIA, there does seem to be some selection bias in that the decedents who did not have an MIA (the overwhelming majority) were much less likely to be on TB treatment, and therefore may well have significantly different of TB detectable at autopsy if they had received autopsy. The study could well underestimate the true rate of TB in this population and maybe this needs to be discussed further?

Thank you for raising this. As stated in the methods, we conducted MIA on everyone possible, but a potential explanation for this difference in TB treatment may be that those taking TB treatment were more likely to have regular contact with clinics, increasing the likelihood of our ascertaining their death in time for MIA. We agree that because individuals who underwent MIA were more likely to have been treated for TB compared to those who did not undergo MIA, we could have underestimated TB prevalence at death in the overall study population.

We have now addressed this in the text (page 13, lines 320-326).

Original text

Those who had MIA and those who did not were largely similar with regard to characteristics at enrolment and during follow up. In this group, we found the autopsy prevalence of active TB to be almost 50%.

Revised text (page 13, lines 320-326)

Those who had MIA and those who did not were largely similar with regard to characteristics at enrolment and during follow up, although those with MIA were more likely to have been treated for TB between enrolment and death. This, together with the likelihood that MIA will miss some cases that may be identified by full autopsy [18], suggests that the prevalence of TB at death among the entire population of decedents in TB Fast Track may have been even higher than the almost 50% found in this sample.

4. Does the overall study population selected into TB Fast Track exhibit some selection bias compared to the “at-risk” population? Were certain people more likely to have participated or not?

TB Fast Track criteria were very inclusive: adults, with CD4 \leq 150 cells/ μ L, not on TB treatment or ART at the time of enrolment, and well enough to not require referral to hospital (respiratory rate <30/minute, pulse <120/minute and systolic blood pressure >90mmHg). As such, only 38/3091 (1.2%) individuals were screened and not enrolled, 26/38 (68.4%) because they declined to take part. Of the 3053 individuals enrolled, a further 31 (1.0%) were subsequently excluded, leaving 3022 for final analysis (NB: this was previously stated as 3032 and has been corrected [page 7, line 176]). We are therefore reasonably confident that the overall study population is representative of adults with advanced HIV, not on ART or TB treatment, attending public primary health clinics in these parts of South Africa.

We have not changed the manuscript.

5. Line 94 – maybe identify public service PHCs versus private? This is important in that public health services cater for uninsured people of lower socio-economic status. This is the “at-risk” population from which the study population is derived.

Thank you, we have amended the text so that PHCs are now referred to as ‘public sector’ (page 4, line 93; page 5, line 100).

6. Could silicosis play a confounding role if the study population contains many former miners? Ex-miners in Southern Africa are known to have high prevalence of silica dust exposure and silicosis. Thus the high prevalence of TB at MIA could really be only applicable to this type of population. It’s not clear in reference 21. There is no reference to occupation for those employed. With this in mind, how generalizable are these findings?

Information on occupation was unfortunately not collected at enrolment to TB Fast Track. However, the majority of study sites (16/24) were in areas (Ekurhuleni and Tshwane) not known as labour-sending areas for mines and over half of those enrolled to the study were women, who rarely work underground. Questions around mining history were included in verbal autopsies, which were administered to the families of 212 TB Fast Track decedents (including the 34

who underwent MIA); nine (4.2%) reported that their relative had worked underground and only one of these decedents underwent MIA, where no evidence of TB was found (decedent 25, S2 table). None of the lung samples obtained as part of MIA showed any evidence of silicosis on histological examination. We therefore believe that our findings are generalisable to other adults with advanced HIV receiving the majority of their care from community facilities in low- and middle-income countries with a high TB burden.

We have not changed the manuscript.

7. Line 44 – it seems from the methods section some were followed up for more than 6 months.

Thank you, this was an error. We have amended the text to reflect that participants had ‘at least’ six months’ follow-up (page 3, line 44; page 5, line 106).

8. Line 51 and 160 and 335 – are the bacterial results clinically relevant still 5 days after death on average? Could these be artefacts or bacteria that have thrived since death rather than represent a clinical infection that contributed to death? Although this is mentioned, I don’t think this has been adequately discussed. Is there any other literature to reference around this?

This is still a contested issue, but there is some evidence to suggest that a delay between death and autopsy should not have a major impact on the validity of results, as long as the body is kept refrigerated.

We have now discussed this in more detail (page 15, lines 372-384) and added additional references, including a review of over 5000 autopsies (Ref 43: Morris et al. J Clin Path 2006;59(1):1-9).

Original text

The interpretation of culture results from autopsy specimens is not straightforward and is complicated further when the procedure is conducted many days after death, providing greater opportunity for tissue autolysis and translocation of organisms to compartments they did not occupy in life [36,37]. Pathologists have suggested that the translocation of organisms to CSF and blood is inevitable and may even be facilitated by post-mortem examination [38]. In our study, samples from all sites, except CSF, grew some organisms that were likely artefact (S1 Table).

Revised text (page 15, lines 372--384)

The interpretation of culture results from autopsy specimens is not straightforward and may be complicated further when the procedure is conducted many days after death, potentially providing greater opportunity for tissue autolysis and translocation of organisms to compartments they did not occupy in life [40-42]. However, there is evidence to show that the time from death to autopsy does not have a major effect on false-positive bacterial results if the body is kept refrigerated [43-44]. One study, conducted among 507 infants with sudden and unexpected deaths, even suggests that a longer interval may allow for fewer false-positive results [45]; and there are reports of pathogens recovered from bodies in states of advanced decomposition [46,47]. In our study, samples from all sites, except CSF, grew some organisms that were likely artefact (S1 Table); data from each decedent were reviewed individually by a microbiologist to decide on likely contaminants.

9. Line 59 – this paper could maybe suggest further research is needed to define the structured pathway. For instance, the algorithm used to assign TB treatment at enrolment seems to miss many cases of active TB found at autopsy; only 63% with TB at autopsy were on TB treatment between enrolment and death.

Thank you for raising this. We should have mentioned that of the six individuals with TB at autopsy who were not started on TB treatment, five were enrolled to the control arm of TB Fast Track and were therefore not assessed using the study algorithm.

We appreciate that this was not clear enough in the text and have amended the manuscript appropriately (page 9, lines 235-237).

Original text

Six (38%) individuals with evidence of TB at autopsy were not started on TB treatment ante-mortem.

Revised text (page 9, lines 235-237)

Six (38%) individuals with autopsy evidence of TB were not started on TB treatment ante mortem, five of whom were enrolled to the control arm of TB Fast Track.

10. Line 75 – however most decedents died in hospital in this study (74%). Maybe the point is not where they died but where they received most of their care?

Although a high proportion of individuals in this study did die in hospital, we consider the 26% that died outside of hospital to be a vital part of this study. Almost all previous autopsy studies in sub-Saharan Africa have included only individuals who die in hospitals; data on causes of death those dying outside of this setting are sparse and it may be

that there are important differences in causes of death depending on the location of death. Although our sample size is too small to draw any conclusions in this regard, our sample was drawn from a trial population broadly inclusive of adults with advanced HIV disease in primary care and is not biased by place of death. We have made minor amendments to the background to add clarity (page 4, lines 73-77).

Original text

Pathological autopsy studies provide the most accurate estimates, but the vast majority have included only hospitalised adults and do not necessarily represent the broader population of people who receive out-patient care from primary care clinics and may die outside of hospitals [13].

Revised text (page 4, lines 73-77)

Pathological autopsy studies provide the most accurate estimates, but almost all have only included individuals recruited after admission to hospital and do not necessarily represent the broader population who receive the majority of their care from primary health clinics and may die outside of hospitals [15].

11. Line 170 – were any of the 2401 lost to follow up and is there any source of selection bias here?

Overall loss to follow up was very low in TB Fast Track, with vital status at six months ascertained using various sources, including patient interview, case note abstraction, and use of the South African ID number. Vital status at 150 days post-enrolment was known for 2972/3022 (98.3%) individuals.

NB: The number of individuals enrolled to TB Fast Track after the autopsy sub-study started was 2396, rather than the previously stated 2401. This error has now been corrected (page 7; lines 176-179).

Among the 2396 who were asked for permission for autopsy at enrolment, 226 individuals died; vital status at 150 days was known for 2346/2396 (97.9%) individuals.

We would suggest that the relatively low loss to follow-up among those recruited after August 2013 was unlikely to have biased selection for autopsy. We have not made changes to the manuscript.

12. Line 216 – is it unexpected that only 5 / 16 tested Xpert MTB/RIF positive? I would have thought his unusual or worth commenting on?

Xpert® MTB/RIF was conducted only on BAL samples, compared to culture and histology on almost all other samples. This was due to budget limitations, but also because we were primarily interested in recovering viable MTB, whereas Xpert® MTB/RIF also identifies dead bacilli. In addition, two individuals with autopsy evidence of TB did not have BAL

samples obtained, so the proportion with positive Xpert® MTB/RIF is actually 35.7% (5/14). Regardless, we do agree that this issue requires more attention and have discussed further the potential use of Xpert® MTB/RIF in autopsy studies, including a reference to recently published work from Mozambique (Ref 21: García-Basteiro, Scientific Reports 2016;6:20703; page 16, lines 393-404).

Original text

Combining BAL Xpert® MTB/RIF with BAL mycobacterial culture would have detected nine of our TB cases; adding mycobacterial culture of lung tissue would add a further two; and histological examination of lung tissue a further three; accounting for 88% of the TB seen in our decedents.

Revised text (page 16, lines 393-404)

Combining BAL Xpert® MTB/RIF with BAL mycobacterial culture would have detected nine of our TB cases; adding mycobacterial culture of lung tissue would add a further two; and histological examination of lung tissue a further three; accounting for 88% of the TB seen in our decedents. The role of Xpert® MTB/RIF as part of the autopsy process is not established: a recent study testing post mortem liver, lung, and brain tissue found that it detected TB in 7/8 (87.5%) cases, although the gold standard used was another PCR technique, rather than liquid culture [21]. In our study, only 5/14 (35.7%) decedents with BAL specimens and evidence of TB had a positive Xpert® MTB/RIF, though this test was done only on BAL specimens, whereas culture and histology were conducted on specimens from many other anatomical sites, many of them extrapulmonary. There were three decedents whose BAL specimens were Xpert® MTB/RIF negative, but culture positive for MTB (decedents 2, 4, and 33; S2 table). More work is needed to establish the optimum use of this and other molecular techniques in post mortem specimens.

13. Line 318 to 322 – is it worth expanding a little more on the formidable challenges?

We agree and have expanded our description (page 14, lines 345-352).

Original text

WHO now recommends Xpert® MTB/RIF for use on extrapulmonary samples [32] and, though there are formidable challenges in establishing alternative diagnostic algorithms and care pathways for those at highest risk of extrapulmonary disease, particularly in primary care in resource-limited settings, these data suggest that they are obstacles that must be overcome in order to achieve any meaningful reduction in TB mortality.

Revised text (page 14, lines 345-352)

WHO recommends Xpert® MTB/RIF for use on extrapulmonary samples [35]. However, there are formidable practical and financial challenges in establishing alternative diagnostic algorithms and care pathways in primary care in resource-limited settings, in particular, the lack of a sensitive, cost-effective, point-of-care diagnostic test, and the difficulties in obtaining suitable specimens samples in individuals who cannot produce sputum. Regardless, the data suggest that these are obstacles that must be overcome in order to achieve any meaningful reduction in TB mortality.

14. Line 333 and 334 – is it worth mentioning the latest guidelines to start ART on initial HIV diagnosis?

These are now referred to (page 15, lines 366-368).

15. Line 497 – close bracket

Many thanks. This has been amended accordingly (page 19, line 564).

Other alterations made to the manuscript

1. We can confirm that separate ethical approvals were received for the parent study and the autopsy sub-study. Line 153 of the manuscript and the submission ethics statement have been amended accordingly
2. Minor changes to overall numbers of TB Fast Track participants enrolled (page 7, lines 176-179)
3. Minor rewording to the description of reference 34 (Lawn 2015; page 14, lines 340-345)
4. Addition of a more detailed description of reference 4 (Bates 2015; page 15, lines 358-361)
5. Addition of an extra figure (new Figure 1) which provides examples of some of the histological changes observed at autopsy
6. Added references:
 - a. Ref 3: Bassett et al. JAIDS 2016 Apr
 - b. Ref 21: Garcia-Basteiro et al. Nature 2016
 - c. Ref 37: WHO 2016 ART guidelines
 - d. Ref 43: Morris et al. J Clin Path. 2006;59(1):1-9
 - e. Ref 44: Christoffersen et al. Forensic Sci Int. 2015;250:27-32
 - f. Ref 45: Weber et al. Forensic Sci Int. 2010; 198(1-3):121-5
 - g. Ref 46: Maujean et al. J Forensic Sci. 2013; 58(4): 1069-70
 - h. Ref 47: Palmiere et al. J Forensic Leg Med. 2015;30:21-4

Thank you again for your review of this article. We trust that these revisions are satisfactory and look forward to hearing from you.

Kind regards,

Aaron Karat

Corresponding author

8.6.2. Response to peer reviewers for research paper 3 (Chapter 5)

Dr Petros Isaakidis
Academic Editor
PLoS One

03 March 2017

Dear Dr Isaakidis,

Re: PONE-D-16-43976

Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: comparing verbal autopsy, minimally-invasive autopsy, and research data; Karat AS, et al.

Thank you for the opportunity to respond to the reviewers' comments. Their comments are shown in **bold** and our responses in normal font. All page and line numbers referenced below correspond to the document named RevisedManuscriptWithTrackedChanges.docx. Text from the original manuscript is shown in grey, dashed boxes, and text from the revised manuscript in solid, black boxes.

Reviewer #1

1. Population versus Individual level accuracy. The findings suggest better VA performance at population level. It can be argued that improved performance at the population level is still valuable information for policy decisions. Given the pertinence of the finding in this regards, readers will likely anticipated a discussion on; particularly given the poor performance at the individual level.

Thank you for this comment. We agree that this issue required further discussion and, as a combined response to this and the point raised by Reviewer #2, we have added to the text the sub-section below.

Amendment #1 (Discussion)

Added text (page 19, lines 486–514)

Moving forward

In the absence of robust, validated CRVS data, there are few alternatives to VA that are both feasible and cost-effective to generate estimates of cause-specific mortality in countries with high HIV and TB prevalence [65,66]. Although, in this study, VA methods performed poorly in assigning individual CoD, it should be noted that VA is primarily intended to generate population-level estimates [18], and that performance in this regard was better. However, when using study-

defined codes, which were designed to allow for the differentiation of HIV-associated TB from other HIV-associated causes, the population-level accuracy of PCVA was still sub-optimal (ρ_c 0.70 and CSMF accuracy 0.71 compared to L2 standard [n=259]; Table 3), confirming the difficulty of making this distinction.

The challenges of diagnosing HIV-associated TB disease are well documented [67,68] and, as found in a recent systematic review of autopsy studies [4], in the absence of new diagnostics it is likely that clinicians will continue to underdiagnose TB, which will have important implications for measuring progress towards the WHO targets described above [16]. Improvements are needed to TB surveillance methods, which, at present, consist mostly of enumerating individuals already diagnosed and started on treatment [69–71]. Minimally-invasive autopsy is a useful technique for estimating the prevalence of infectious diseases [72,73], is acceptable to a high proportion of families [26,74], and could be used periodically for surveillance at sentinel sites [75], allowing for more accurate evaluation of the impact of disease-focused interventions.

Population-level estimates of cause-specific mortality are extremely valuable and improving the accuracy of VA-generated estimates would be of benefit, regardless of whether or not VA is used to assign individual CoD. The continued development and sharing of gold standard datasets that include pathological autopsy data, better reflecting the high proportions of HIV-associated mortality seen in high-burden countries and including both hospital and community deaths in different populations, would allow for greater standardisation in future validation studies. The parallel development of a structured, standardised process for CoD assignment, similar to that described in the Coding Causes of Death in HIV (CoDe) project [76], but assigning CoD matched to ICD codes [77], would increase the value of this exercise.

2. VA methods will ultimately be as good as the information gathered during the interviews. What role could recall bias have played in the quality of information given that the median time to VA after death was quite (...but typically so) long i.e 146 (IQR 82-290) days

Thank you for the comment. Although the median time from death to VA was just outside the ‘ideal’ window described by previous authors, it was well within the maximum 12 months recommended by WHO. In addition, recent work by colleagues at Agincourt HDSS, South Africa, using VA data in WHO 2012 format, showed only minor differences in cause-specific mortality fractions (CSMFs) generated by InterVA-4 for adults with VA conducted 0–5 months after death compared with those with VA conducted 6–12 months after death (Hussain-Alkhateeb L, et al. *Emerg Themes Epidemiol.* 2016;13: 1–6). Specifically, the CSMF ratios between the two groups for the 3759 deaths attributed to ‘HIV/AIDS’ or ‘pulmonary TB’ were 1.03 (99% confidence interval [CI] 0.93–1.13) and 1.07 (99% CI 0.97–1.19), respectively.

The small impact of recall period on causes of death assigned by VA is corroborated by work from colleagues at the Institute of Health Metrics and Evaluation (Serina P, et al. *Popul Health Metr.* 2016;14: 40). This evaluation of 1394 adult deaths in India and the Philippines, using the Tariff 2.0 method (the method employed by SmartVA-Analyze),

found that the probability of obtaining a 'correct' VA-assigned cause of death (compared with the gold standard used in the study) decreased by around 0.6% per month; the probability of accuracy of VAs conducted 4–11 months after death was 95.9% of those conducted within three months after death. As such, we do not feel that the time from death to VA, and therefore recall bias, will have had a substantial influence on our outcomes.

We have amended the 'limitations and strengths' sub-section of the manuscript as detailed below and have cited the two studies described above.

Amendment #1 (Discussion)

Original text

This study had limitations: physicians who reviewed clinical and VA data were aware...

Amended text (page 19, lines 517–520)

This study had limitations: the median time from death to VA was slightly longer than the ideal three months that some recommend, but was well within the maximum 12 months recommended by WHO and was therefore considered unlikely to have had a substantial effect on VA-generated estimates [78,79]; physicians who reviewed clinical and VA data were aware...

3. On page 4 of 23, it is indicated that the WHO 2012 VA instrument was used, with additional questions around treatment for TB and HIV..... What informed the choice of these questions and how could they have impacted on the overall findings, relative to studies that will strictly apply the WHO instrument?

Our apologies that this was not described clearly enough in the methods. Although these questions were added to the instrument and used in interviews, the answers were not provided to the physicians or software assigning CoD, all of which were provided only with data consistent with the WHO 2012 instrument. It is possible that the use of these questions affected the way that respondents relayed or interviewers recorded information in the free narrative section of the instrument.

These two points have now been included in the manuscript, as described below.

Amendment #1 (Methods)

Original text

VA data were interpreted using both physician-certified verbal autopsy (PCVA) and computer-coded verbal autopsy (CCVA) methods.

Revised text (page 6, lines 142–143)

VA data included in the WHO 2012 VA instrument (i.e., excluding data from study-specific added questions around ART use, treatment for TB, health beliefs, or health service use) were interpreted using both physician-certified verbal autopsy (PCVA) and computer-coded verbal autopsy (CCVA) methods.

Amendment #2 (Discussion)

Original text

...the reference CoD assigned represent our best estimates using the data available, the true CoD may still differ. InterVA-4 and SmartVA-Analyze are designed for use with the WHO 2014 and PHMRC VA instruments, respectively, therefore...

Revised text (page 20, lines 525–528)

...the reference CoD assigned represent our best estimates using the data available, the true CoD may still differ. Questions on ART and TB treatment, added to the VA instrument by the study team, may have led to changes in how events were reported in the free narrative section; the answers to the questions themselves, however, were not provided to reviewing physicians or to either software. InterVA-4 and SmartVA-Analyze are designed for use with the WHO 2014 and PHMRC VA instruments, respectively, therefore...

Reviewer #2

1. The authors conclude safely that more accurate methods are needed to directly estimate HIV associated TB. Agreed. But what about the subject of the paper? Is there any hope for VA to fill this data gap with modified questions? Or should we admit the limitations of VA and invest in other methods of measurement? If so, what should these be to measure at least population level mortality accurately?

Thank you for your comment. We agree that this issue required more discussion and, as a combined response to this and the first point raised by Reviewer #1, we have added the sub-section 'Moving Forward' to the discussion (page 19, lines 486–514 of the manuscript; provided on pages 1–2 of this letter).

Other alterations made to the manuscript

1. Minor amendments to author affiliations (page 1)
2. Minor changes to punctuation and grammar throughout the manuscript
3. Hyphens replaced with en dashes throughout manuscript, as appropriate

4. Minor changes to formatting of all tables (pages 9, 11, 12, 14, and 15)
5. Addition of Prof Lucas and Dr Morris-Jones to the acknowledgments (page 21, lines 593_594)
6. Minor updates to specific references:
 - a. Ref. 14: Appendix to 2015 WHO Global TB report replaced with appendix to 2016 WHO Global TB report (Glaziou P, et al.)
 - b. Ref. 26: Article described as 'in press' has now been published; reference updated accordingly (Karat AS, et al.)
7. Updates of all references to include DOI and/or PMID, where available (pages 21–26)
8. New references added:
 - a. Ref. 65: Garenne M, et al. Bull WHO 2006
 - b. Ref. 66: de Savigny D, et al. Glob Health Action 2017
 - c. Ref. 67: Dheda K, et al. Respirology 2013
 - d. Ref. 68: Lawn SD, et al. Lancet Infect Dis 2013
 - e. Ref. 69: Maher D, et al. Int J Tuberc Lung Dis 2005
 - f. Ref. 70: Auld SC, et al. Int J Tuberc Lung Dis 2013
 - g. Ref. 71: Podewils LJ, et al. BMC Public Health 2015
 - h. Ref. 72: Castillo P, et al. PLoS Med 2016
 - i. Ref. 73: Martinez MJ, et al. Diagn Microbiol Infect Dis 2016
 - j. Ref. 74: Wong EB, et al. PLoS One 2012
 - k. Ref. 75: Byass P. PLoS Med 2016
 - l. Ref. 76: CoDe Working Group 2013
 - m. Ref. 77: Korenromp EL, et al. Int J Tuberc Lung Dis 2009
 - n. Ref. 78: Serina P, et al. Pop Health Metrics 2016
 - o. Ref. 79: Hussain-Alkhateeb L, et al. Emerg Themes Epidemiol 2016

Thank you again for your review of this article. We trust that these revisions are satisfactory and look forward to hearing from you.

Yours sincerely,

Aaron Karat

Corresponding author