Improving tuberculosis mortality estimates

An evaluation of data sources, strategies and new diagnostics in a high tuberculosis and HIV burden setting

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Most scientific literature on tuberculosis, as this thesis, begin with remarks and preambles about the human, social or economic burden attributable to tuberculosis. Mortality, indisputably one of the main public health indicators, is often highlighted in order to attract attention and justify the relevance of the fight against tuberculosis in today’s plural disease landscape. Indeed, tuberculosis is likely the infectious disease (potentially the disease irrespective of the cause), which has been responsible for most deaths in mankind’s recent history.[1] Nonetheless, our certainty about its true burden in general and the mortality toll in particular is subject of debate until today, and the main driver of this thesis.[2,3]

As with other poverty-related diseases, the lack of precision of the mortality burden stems from the fact that most deaths due to tuberculosis occur in countries with fragile health systems, including poor disease surveillance systems and limited resources for ascertaining TB diagnosis at death. Poor surveillance systems (among others) lead to a) a considerable proportion of cases of disease and death remaining undiagnosed, b) deaths not reported and/or not included in formal registries and c) deaths reported without a diagnosis (or incorrect diagnosis) of the cause. The limited resources for ascertaining TB as cause of death affect the latter, and the degree of misclassification varies depending on the setting, the tools and the strategies used.[4] The lack of generalized high-quality TB mortality surveillance systems justifies the need for a variety of modelling strategies that try to come up with the best possible estimates that could then be used by health authorities for health policy and planning.

The present thesis provides new evidence on the limitations and strengths of different traditional and novel methods, tools and strategies used to estimate TB mortality and ascertain TB as cause of death. It does so by using an interdisciplinary approach, which combines population-based data, analysis from publicly available burden of disease data repositories and clinical-diagnostic studies. We analyzed TB-associated and TB-attributable mortality in a sub-rural district and a reference hospital in Mozambique, a high TB, TB/HIV and MDR-TB burden country.[5] This thesis also evidences the need for greater efforts to improve TB mortality estimates in specific countries, where mortality figures vary considerably depending on the institution which estimates them. The solutions to come up with more precise estimates are unlikely to be similar for all countries, but rather setting-specific depending on the TB epidemiology and general disease profile, quality of the health system and overall social and economic context. Likewise, some of the conclusions presented here might only apply to countries or settings with conditions similar to Mozambique. Nonetheless, findings from this thesis have the potential to open up a debate on which data sources might be most adequate or inadequate for TB mortality estimation. The results on the use of new molecular tools to ascertain TB at death are tantalizing and open up new avenues to assign TB more precisely as cause of death.

Here, the overall conclusions of this thesis, grouped by the main themes and research questions, are presented. The main limitations and strengths for the research included in this thesis are also discussed. The chapter closes with considerations on future perspectives in the field, knowledge gaps and recommendations for future research.
A CLOSER LOOK AT GLOBAL TB MORTALITY DATA. HOW DOES GLOBAL AND NATIONAL TB MORTALITY BURDEN DIFFER BY THE INSTITUTION WHICH ESTIMATES THEM?

This thesis presents the first peer-reviewed comparison showing the differences in TB mortality estimates of two renowned institutions, WHO and IHME, at global, regional and local level (Chapter 2). Despite a striking difference of more than 450,000 deaths for the year 2015, the analysis shows that most of the absolute differences relate to only a few countries, most of them high TB burden countries with poor disease surveillance systems in place. The discrepancy in absolute TB mortality estimates in Nigeria, Bangladesh and Tanzania clearly calls for close attention to the data sources used and the modelling strategy itself. In addition, the analysis aimed at establishing where the methodological approaches differed the most, adjusting for the size of the TB epidemic in each country. The results highlight some other countries (i.e. Azerbaijan, Egypt, Macedonia, Papua New Guinea, Timor-Leste, or Marshall Islands), in which the differences in estimates are substantial for the magnitude of their TB burden. We attempted to explore which factors could be associated with the fact that TB mortality estimates from WHO and IHME differed considerably for some countries. We found that the use of prevalence surveys and CDR estimates seem to play a role in the differences observed. However, the analysis about potential factors explaining the differences was performed at an ecological level, with all the inherent limitations of such approach. In fact, further unknown factors might be behind the findings. Indeed, prevalence surveys are generally conducted in countries with a presumed low CDR, which could make these two factors (CDR and use of prevalence surveys) associated with each other. Nonetheless, the way in which WHO determined the CDR for many countries, i.e. by expert opinion, or applying CFR estimates from systematic reviews of TB patient series from the pre-chemotherapy era to patients detected in prevalence surveys (i.e. regardless of the presence of symptoms), might result in overestimating TB mortality and need to be revised. Likewise, and as some of the data presented in chapters 6 and 8 suggest, the use of verbal autopsies as a data source for TB mortality estimation (method used by IHME) seems inappropriate. The last iteration of the GBD project excludes VA data from countries where HIV prevalence is higher than 5% in order to minimize bias. The lack of large validation studies of VA using CDA as gold standard remains a knowledge gap and the extent to which VA can play any role for TB mortality estimation still needs to be elucidated.

TB BURDEN INDICATORS IN A RURAL DISTRICT IN SOUTHERN MOZAMBIQUE. LIMITATIONS OF USING DATA FROM NATIONAL TB CONTROL PROGRAMS FOR TB MORTALITY ESTIMATION

Our data on breakdown of TB notification rates by HIV status (Chapter 3) are hard to obtain given that population-level estimates of HIV prevalence are usually not available. An HIV survey recently conducted in the district of Manhiça provided an estimate of the HIV prevalence. We
also used population estimates from the HDSS at CISM and from the Mozambican National Statistics Institute. The laboratory-confirmed TB case notification rate was very high, especially among HIV positive men, in the age band of 38–47 years. The true incidence rate of TB might be much higher given the likely low CDR.[7] If it were similar to the estimates of WHO, Southern Mozambique might constitute one of the main hotspots of TB/HIV in the world.

TB CFR derived from NTP data and population estimates from health and demographic surveillance systems (HDSS) can be used to estimate TB associated mortality but have limitations. Our analyses of the main TB burden indicators in the district of Manhiça over a sixteen-year period (chapter 4) showed a strikingly low treatment success rate (below 70%) with over 15% of patients started on treatment dying before its completion. The main limitation of using NTP data as an approach to estimating TB associated mortality is that both the proportion of patients reported as lost to follow up who died and the proportion of patients with death as reported treatment outcome who died of non-TB related causes are unknown. A specific autopsy study to determine the causes of death among patients on TB treatment could potentially be used to model TB mortality using the CFR calculated from outcome data provided by NTPs. Despite the limitation of being setting-specific, it could likely be an approach to consider for estimating TB mortality in various Southern African settings. This design would also contribute to identify other concomitant (perhaps treatable) diseases which might explain the high observed mortality among treated TB patients. The study described in chapter 4 also shows a clear disparity between women and men with regards to TB notifications (much higher in men), and this requires further attention. Although this difference has been widely reported for other African countries,[8] in our setting it is remarkably high. Accessibility and health seeking behavior have been reported as drivers of the difference,[9,10] but these findings merit further qualitative analysis.[11] Further limitations of the use of NTP data concern completeness of information (use of ART, age, date of HIV diagnosis, CD4 counts, among others), data quality and unavailability of relevant variables (clinical and laboratory data, X ray, among others) which could contribute to explaining some of the results and ultimately shape specific control measures.

Despite the abovementioned limitations, we tried to deepen the assessment of treatment outcomes and TB-associated mortality in the district of Manhiça with a specific analysis presented in chapter 5. It aimed to estimate the proportion of patients in the district who died of natural causes while being on TB treatment as well as to analyse the drivers of mortality during TB treatment. We found the contribution of TB-associated deaths to the overall mortality profile for non-violent causes (thus, excluding homicides, suicides and accidents) to be very high. Unfortunately, we could not rank TB-related deaths and compare them to other causes of death, given the lack of similar data for other prevalent diseases, but the study suggests that TB is one of the main causes of death in the district of Manhiça. The fact that TB was the main cause of death in neighbouring South Africa supports this claim.[12] The study also showed that HIV infection, being male or lacking laboratory confirmation were factors associated with increased likelihood of dying during TB treatment. Importantly the one-stop model for HIV/TB care, which implies full HIV care at the NTP facilities while on TB treatment, was implemented during
2012. The impact of this innovation in patient management on TB and HIV outcomes needs to evaluated to quantify its benefits in a setting where a large majority of TB patients are HIV-positive. The high case fatality rate in the district of Manhiça cannot be solely explained by HIV, given the high mortality also observed in HIV-negative patients. Studies with specific designs, for example mixed-methods approaches, could explore the determinants of dying while on TB treatment. This knowledge could be used to refine the tools and health interventions aimed at reducing this unacceptable high CFR.

**ACCURACY OF CLINICAL (PREMORTEM) DIAGNOSIS TO DETERMINE TB DISEASE AS DEATH**

Hospital-based mortality data using pre-mortem clinical diagnoses have been used to estimate cause-specific mortality, despite the limitations of its generalizability to the overall population.[4,13] These estimates could be useful in combination with other data sources such as vital registration systems, DSS or mortality surveys. We found a high proportion of clinico-pathological "major discrepancies" between pre- and post-mortem diagnoses, as defined by Goldman and Battle (clinical diagnostic errors in which knowledge of the diagnosis before death would have led to changes in clinical management that could have prolonged survival or cured the patient; chapter 6).[14] The proportion of these discrepancies among all patients dying of TB was 65%. The study, thus, highlights the importance of increasing clinical awareness of TB and other infectious diseases in the hospital setting. Poor diagnostic algorithms and lack of use of available sensitive diagnostic assays might also hinder the diagnosis of fatal infections/diseases and in consequence, the prevention of premature mortality associated with them. If the clinical diagnosis (often done by medical specialists) has such a low sensitivity to diagnose TB as the cause of death, verbal autopsies might perform even worse. This assumption is justifiable given the limited quality of the data derived from the interview with the relatives or caregivers of the deceased person, often occurring months after the death. Unfortunately, in this study (chapter 6) we were unable to assess the accuracy of verbal autopsies for determining the cause of death, which would have allowed a head-to-head comparison of both types of diagnosis (clinical vs VA) against the gold standard. As mentioned before, this is a clear knowledge gap that needs to be addressed specifically for TB. Data from this study needs to be interpreted cautiously, given the specific profile of patients dying in hospitals, both in terms of the access to hospital based health care and disease profile in reference hospitals.

**DIAGNOSTIC PERFORMANCE OF XPERT MTB/RIF TO DETECT M. TUBERCULOSIS IN POST-MORTEM TISSUES**

In 2011, Xpert MTB/RIF (Xpert) was recommended by WHO for diagnosing TB and different guidelines on its use were released subsequently.[15] It is now well accepted that Xpert MTB/
RIF should be used on pulmonary and extrapulmonary specimens in patients reporting TB-compatible signs and symptoms.[16] However, little was known about its potential utility in post-mortem tissues. We showed high sensitivity and specificity when Xpert was used on lung tissue samples collected during CDA (chapter 7). Xpert results also correlated well in other tissues such as brain or liver, although the sample size for these other tissues was limited. Mycobacterial culture would have been preferred as gold standard for a formal diagnostic performance comparison, given that is the most sensitive tool available, but the design of the study did not consider it. In fact, our composite gold standard for TB disease (with histological and microbiological (in house PCR or LAMP) elements) could be questionable. However, since the other main molecular tools (in-house PCR and LAMP) could be as sensitive as Xpert, and no culture was available, the addition of histological endpoints allowed to obtain a more robust reference standard for presence of TB disease. Although our sample size was limited to 30 deaths, further evidence published afterwards confirm the value of Xpert in (post-mortem) tissue samples.[16-18] It would avoid the use of traditional methods which are time consuming, expensive, slow or much more operator-dependent.

TB BURDEN IN A POST-MORTEM STUDY AT A REFERENCE HOSPITAL IN MOZAMBIQUE. IMPLICATIONS OF USE OF HIGHLY SENSITIVE MOLECULAR ASSAYS

From the evidence obtained in chapter 7, we aimed at using the new version of Xpert, Xpert Ultra, to conduct a detailed characterization of the burden of TB in deaths occurring at Maputo Central Hospital. Findings from this study may be considered as evidence to support the idea that latent TB infection (potentially in an unstable phase of replication of *M. tuberculosis* bacilli) [19] can be detected through direct methods, which is against the current scientific opinion,[20] at least for the living. Eighteen cases had positive results to two molecular tools (in-house PCR and Xpert Ultra) without histological evidence of TB disease. Several explanations could be plausible. Given the procedures performed in the CDA, these findings could be due to sampling error. Since all tissue from all organs was not analyzed, small granulomas with few containing bacilli might have been missed. However, practically the exact same tissue sample was analysed under the microscope and tested microbiologically, so those few bacilli might have been present with poorer granuloma formation.[21] These findings could be compatible with the concept of incipient TB or even unstable latent TB infection, but the possibility of *M. tuberculosis* contamination, although thought to be highly unlikely, cannot be ruled out entirely. This study suggests that the use of highly sensitive tools for post-mortem TB diagnosis might contribute to detect cases of TB disease which might not be the cause of death, as well as cases of very early forms of TB. The role of these forms of TB as a contributory cause of death needs to be elucidated.

In addition, we found the overall burden of TB among hospitalized patients to be very high, and the use of Xpert Ultra in the diagnostic algorithm prompted the revision of some of
the histological samples and helped to identify a considerable amount of cases of TB disease that would have otherwise been missed (15 cases). One of the limitations of the study design concerns the diagnostic algorithm used, in which Xpert Ultra was not systematically used in HIV-positive patients or in all lung samples (as originally was done with in-house PCR), and thus, some incipient cases of TB might have been missed. The use of culture would have helped to identify some false-positive results, given that Xpert Ultra might detect *M. tuberculosis* non-viable DNA in patients previously treated for TB (even treated up to 5 years earlier).[22,23] Unfortunately culture was not originally considered in this study. The study also evidences the high rate of misdiagnosis of TB as a concomitant disease at the time of death.

As one main conclusion of this thesis, it is quite clear that in Southern Mozambique, for deaths occurring in hospital, clinical diagnosis is not a good indication of TB as cause of death, and that many cases of TB disease among those who die are missed. Thus, this study corroborates earlier findings from Mozambique and other settings in Sub Saharan Africa[17,24,25] that hospital mortality based on pre-mortem diagnosis is an inadequate data source for TB mortality estimation.

**NEW POSTMORTEM STRATEGIES COMBINED WITH NEW DIAGNOSTIC TOOLS. COULD THIS BE A WAY FORWARD FOR TB ASCERTAINMENT AT DEATH?**

Our evaluation of the usefulness of Xpert MTB/RIF Ultra in post-mortem tissues obtained through MIA samples (chapter 9) is probably the first study to attempt using this combination of tools to diagnose TB as the cause of death. It shows that Xpert Ultra could be very useful to rule in or rule out TB in single tissues or in combination of tissues in a fast and simple fashion. Although the highest sensitivity was obtained in combined lung and brain tissues, the highest specificity was obtained in serum samples. People who die of TB often have disseminated disease, and if mycobacterial DNA is detected in serum, it is likely that TB is the cause of death. Indeed, the PPV was also highest for serum, together with lung. Nonetheless the estimates of PPV and NPV may be biased given their dependence on the prevalence of TB. In our study TB prevalence as cause of death is overestimated due to our inability to use Xpert Ultra in all MIA samples of the large post-mortem validation of MIAs. Importantly, the use of highly sensitive molecular diagnosis could obviate the need for histopathological examination which requires specific expertise. The combined use of both tools (MIAs and Xpert Ultra) could therefore ease post-mortem TB diagnosis and our results would support its use in sentinel sites for improved TB mortality surveillance. In addition, these results may have implications for pre-mortem TB diagnosis of severely ill hospitalized patients, in whom TB might or might not be suspected and who might be too weak to provide adequate sputum samples. Thus, applying this highly sensitive molecular tool in an easily accessible sample could alert the clinician to disseminated TB and perhaps prompt him/her to earlier medical action that could impact the patient’s prognosis.
FUTURE DIRECTIONS

It is clear that modeling strategies to estimate TB mortality depend on the quality of different data sources that are used. However, we show that that existing data sources pose major limitations to TB mortality estimation in a high burden setting such as southern Mozambique, where VR-based mortality data are not available. In our setting, hospital diagnosis and, by extension, verbal autopsies, are not good tools to use for TB mortality measurement. However, it is important that validation studies of verbal autopsies are conducted, including full CDAs as reference standard with appropriate study sample sizes. Future attempts to estimate TB mortality in settings with high TB/HIV prevalence should not rely on verbal autopsy data. WHO or IHME, the two main institutions estimating TB mortality globally need to take a closer look at specific countries, mostly in sub-Saharan Africa, and to a lesser extent in South East Asia, and disregard some of the data points based on these sources. The argument “bad quality data might be better than no data” is understandable but the scenario of limited available data sources in places with indirect signs of high mortality should prompt policy makers and the international community to invest in epidemiological research to better characterize the TB mortality burden.

MIAs have shown to be useful for post-mortem diagnosis of TB as cause of death. In this thesis we show that easy-to-perform, rapid and highly sensitive molecular tools to detect *M. tuberculosis*, such as Xpert Ultra, could be used with MIAs for TB diagnosis, obviating the need for histopathological examination. We believe these two tools (MIAs coupled with Xpert Ultra testing) could potentially be performed at a decentralized level of care and would be well accepted by the community in rural areas. Thus, MIA-based TB mortality surveillance should be explored as a new tool for TB mortality estimation. Nonetheless, there is a need to answer the following questions: Are MIAs feasible and equally accurate for postmortem TB diagnosis in children and adult deaths at district hospital level of care (as shown in validation studies[26-28])? If so, could a network of sentinel sites be established in high TB burden countries without VR systems in order to monitor population based TB mortality?

The limitations of case fatality rates based on treatment outcomes from NTPs have been discussed in this theses. Future autopsy studies in people who died while on TB treatment could potentially provide evidence on how to adjust crude data. Nonetheless, these programmatic data could be useful to monitor trends or alert about gross changes in TB associated mortality.

The evidence presented in this thesis, beyond methodological discussions on the appropriateness of specific data sources, clearly shows a very high mortality associated with TB and due to TB in Mozambique. Thus two areas need to be prioritized. First, there is an urgent need to narrow the gap of missed TB cases, whose CFR is assumed to be the highest.[29] This could be done with a combination of using improved diagnostic tests (for both triage and diagnosis) and targeted and sustained active case finding strategies among high risk groups.[30] Secondly, there is a need to understand why mortality is so high among those on TB treatment. Both qualitative and quantitative approaches will be needed to fully understand why TB patients (both HIV positive and negative) have unacceptable rates of fatal outcomes. Many factors could play a role in this observed high mortality (HIV infection and its management, overall patient...
management, sociodemographic and behavioural factors, health system related ones), but they need to be clearly characterized for the Mozambican setting. Knowing what the causes of this high mortality are might allow to act upon them. Ultimately, new tools (at the prevention, diagnostic and treatment fronts) that bring down TB incidence will consequently also impact mortality.
REFERENCES

23. García-Basteiro AL, Saavedra B, Cobelens F. The Good, the Bad and the Ugly of the Next-Generation Xpert Mtb/Rif Ultra Test for Tuberculosis Diagnosis. *Archivos de bronconeumología* 2017; published online July 10. DOI:10.1016/j.arbres.2017.05.023.


