PhD defence of Willy Ssengooba

Consequences of Mycobacterium tuberculosis genetic diversity in the context of HIV co-infection for laboratory diagnosis of tuberculosis in Africa

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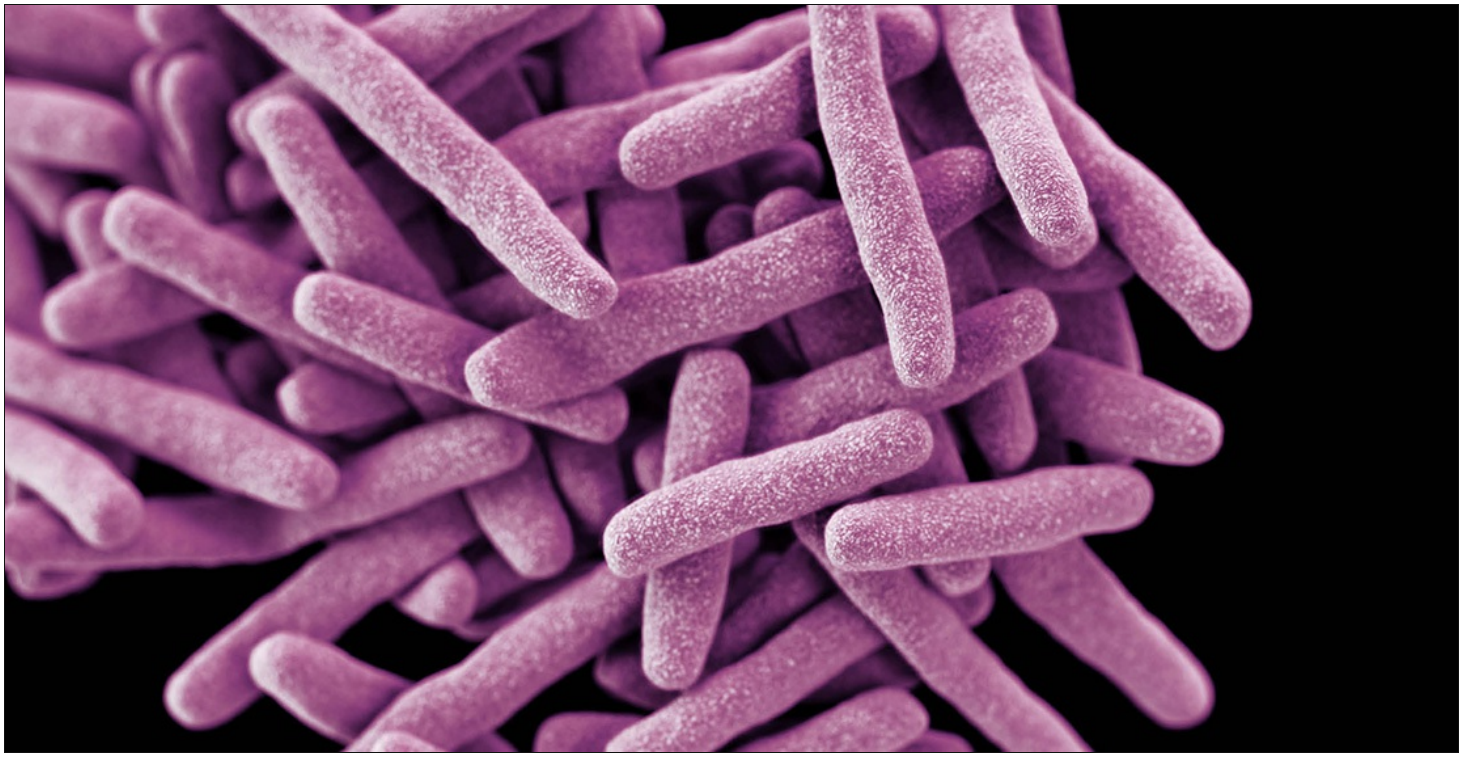

Dit is de omschrijving

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Summary:

Efforts towards TB control in Africa are still facing diagnostic and treatment challenges. The high HIV/AIDS burden in sub-Saharan Africa (sSA) further complicates these challenges, as people infected with HIV not only are at a greater risk of pulmonary TB, but also have difficult to diagnose forms of TB, including drug resistance. This means that control of TB in sSA faces a dual challenge of both diagnosis and treatment against the background of HIV. We present our main findings, suggest potential solutions and draw some conclusions towards the future about diagnosis and drug resistance of TB in the context of *M. tuberculosis* genetic diversity and HIV in Africa. The findings are presented in two parts;

Part I: Laboratory diagnosis of *M. tuberculosis* and its genetic diversity

In **Study 1** evaluated the performance of molecular diagnostics in sSA settings. We compared the diagnostic yield and costs from Xpert testing among HIV-positive individuals when used as a stand-alone test, versus in combination strategies with existing methods. We found low sensitivity of a single sputum smear examination from HIV-positive individuals (32-44% relative to culture) compared to the high sensitivity (76%) of Xpert testing. Changing from a replacement- to an add-on strategy, the number of Xpert cartridges needed was reduced by approximately 10%. In this population of HIV-positive individuals, doing smear microscopy prior to an Xpert assay in add-on fashion (doing Xpert among smear negatives) only identified a few additional TB cases. These findings opens up future discussions on the impact of Xpert on reducing mortality given the clinical practices in most of the affected settings.

The Xpert test generates additional informative data, which could be used to inform TB patient management and programs. This includes the cycle threshold (Ct) values which have been found to correlate with bacterial burden in sputum. In **Study 2**, we assessed whether Ct values could generate information useful for monitoring TB control programs, in particular as a readily available indicator for diagnostic delay. We found in a study in a mostly rural setting in Mozambique that Xpert Ct values did not predict patient delay for TB diagnosis and cannot be used as an indicator of TB control program performance. Ct values showed no correlation with delay (R^2=0.001, p=0.621), nor any association with long delays. We did find that rural residence and being HIV-negative predicted diagnostic delays.

The type of presentation of TB in an individual affects not only the clinical but also the laboratory diagnosis. For example, people living with HIV/AIDS (PLWHA) with low CD4 cell counts commonly have disseminated TB. This form of TB is associated with early mortality and can be misdiagnosed due to lower rates of sputum smear positivity. Therefore documented predictors of mycobacteremia in combination with the LAM test that may guide patient management among smear negative patients with HIV-advanced immunosuppression. In **Study 3**, in 10% of smear-negative HIV-infected Ugandan patients, we identified mycobacteremia, which was associated with

higher mortality compared with smear-negative patients without mycobacteremia. We found that advanced HIV disease (CD4 < 100 cells/mm^3), male gender and positive lateral flow urine TB LAM test predicted mycobacteremia in HIV-infected smear-negative presumptive TB patients in this high prevalence TB/HIV setting. This indicates that LAM test could offer significant contribution to reduction of mortality among HIV-positive individual who are seriously ill and immunosuppressed, more so when alternative diagnostic measures are absent. Whether this group of patients who present with MTB bacteremia, given their high mortality, benefit from adapted or intensified TB treatment, such as an increase of the rifampicin dose given the high mortality, requires future studies.

PLWHA with low CD4 cell count may have different strains of *M. tuberculosis* in blood as compared to sputum. This occurrence has been found to have clinical as well as treatment implications, as different strains have been found to exhibit different phenotypes which may affect disease presentation and treatment responses. In **Study 4** we compared genotypes of MTB strains concurrently isolated from blood and sputum of HIV-infected individuals with low CD4 cell counts in Uganda. We report that more than half of the patients have discordant MTB genotypes concomitantly in both compartments. This may pose diagnostic challenges in case of mixed infections of drug-susceptible and drug resistant strains as one may be easily missed if only one compartment is sampled. This has been reported for sputum testing with Xpert MTB/RIF assay in which mixed infection caused false-negative results for rifampicin resistance. Future studies are needed on the optimization of molecular diagnostics to detect minority populations, including mixed infections.

Although the main site of TB infection is the lungs, TB may also progress from blood and/or lymphatic system to the lungs. While in immunosuppressed individuals mycobacteremia is common, little is known about the rate of microevolution of MTB strains during dissemination, and on the direction in which such infection typically spreads between blood and sputum compartments. **Study 5,** builds on the work in Study 4 by analysing the strains that were categorized as identical using conventional genotyping methods with the technique with the highest possible resolution: whole genome sequencing. We reported three (23%) of 13 patients having SNPs separating paired isolates from blood and sputum compartments, indicating evidence of microevolution. Using a phylogenetic approach to identify the ancestral compartment, we found that, in two (15%) patients the blood isolate was ancestral to the sputum isolate, in one (8%) it was the opposite; the remaining ten (77%) pairs were identical. We concluded that, in this patient population with advanced immunosuppression, microevolution does occur in a few patients and that the lack of a consistent direction of infection suggests that these two compartments are highly connected. This has been also evidenced in a recent study with a larger sample size and more body compartments sampled. Studies on the clinical impact of the within-host microevolution and consequences for development or use of (molecular) diagnostics are warranted.

Part II: *M. tuberculosis* drug resistance and HIV

The emergence of multi drug resistant TB (MDR-TB) has provided challenges to efforts towards TB control. Previous studies have found HIV-positive individuals to be at a higher risk of getting infected with MDR-TB as they are more susceptible to infections. Several studies have documented associated risk factors of MDR-TB with different estimates. **Study 6**, presents results from a systematic review and meta-analysis conducted to document the prevalence of drug resistant tuberculosis and its determinants in sSA apart from the Republic of South Africa, among new and previously treated TB patients. We reported low prevalence of drug resistant tuberculosis in sSA compared to WHO estimates and found that at the population level MDR-TB in this region does not seem to be driven by the high HIV prevalence rates. The low levels of MDR-TB can probably be attributed to the restricted access to anti-TB drugs, mostly limited to the national programs, the late introduction of rifampicin in sSA, and the widespread use of fixed drug combinations.

Evolutionary changes in MTB strains are known to influence patient-relevant outcomes of infection and treatment through affecting clinical virulence and transmissibility. These changes are also known to have consequences for laboratory diagnostics, drug development, as well as TB clinical presentation and treatment results. For example, the usefulness of emerging rapid point-of-care molecular TB assays largely depend on detection of conserved TB specific sequences in combination with known drug resistance mutations. In **Study 7** we analysed by whole genome sequencing isolates from the two drug resistance surveys done in Uganda for common and rare mutations to first and second line TB drugs among 90 patients who had isolates that were phenotypically resistant to rifampicin and/or isoniazid. We also compared test agreement to first line drugs using WGS and a phenotypic reference standard. In this study we found the agreement between phenotypic and genotypic methods to be high for rifampicin, followed by isoniazid, and poor for ethambutol and streptomycin. This suggested that rapid genotypic methods targeting rifampicin and isoniazid can be reliable is this setting. The genome sequences furthermore allowed us to estimate resistance to second line and novel TB drugs, which was fortunately low. No resistance was found to fluoroquinolones, and little 4/90 (4.4%) to injectable drugs, indicated that drug resistance has not yet advanced towards XDR-TB in Uganda, befitting clinical trials for novel TB drugs. Phylogenetic analysis indicated only one MDR-TB cluster in the South Western part of Uganda, indicating that MDR-TB in Uganda is not due to a dominant clone. The NTP should evaluate the TB program, including rapid and targeted surveillance mechanisms, such as Xpert-based, in Western Uganda, to prevent more MDR-TB clusters to interrupt on-going MDR-TB transmission.

The alterations in the make-up of the *M. tuberculosis* genome may target essential bacterial function and hence affect the bacterial fitness in terms of transmission and acquiring more resistance. Some drug resistance-conferring mutations have been found to have a fitness cost whereas others do not or compensate the fitness cost through compensatory mutations. Moreover, resistance-conferring mutations with fitness cost have been postulated to be more frequent among HIV-positive individuals in HIV-endemic settings. In **Study 8,** we tested the hypothesis that HIV-positive individuals are more likely to have TB drug resistance conferring mutations with fitness cost compared to HIV-negative individuals with TB. Our findings did not support the hypothesis, however, we noted that TB patients at the national referral hospital TB unit had higher odds of non-531 *rpoB* mutations (that are associated with fitness cost) and this require further studies. Moreover, isolates with non-531 *rpoB* mutations are known to grow slowly and most likely to be missed by phenotypic DST interpreted at two rather than six weeks, allowing these mutations to spread, although we had only one (*rpoB*.L511P) missed by LJ-based DST method used in this survey. Sequencing of all MTB isolates, including a sample of the survey isolates that were phenotypically sensitive to rifampicin and/or isoniazid may yield more power for our conclusions.

All our studies generated a compressive understanding of the diagnostics and tuberculosis presentation dynamics in context of HIV- coinfection which may impact efforts for TB control. The studies generate recommendations and future remarks which may be useful for TB patient management as well as management of drug resistant TB mainly in sSA settings.

The studies included in this thesis were mostly initiated from Uganda at Makerere University College of Health Sciences at the Infectious Diseases Institute, Kampala Uganda and the Supra National TB Reference laboratory, Kampala, Uganda. Further analyses were performed at the Institute of Tropical Medicine, Antwerp, Belgium, at the Department of Global Health and Amsterdam Institute of Global Health and Development, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, Netherlands and at the Barcelona Institute for Global Health (ISGlobal), Centro de Investigação em Saúde de Manhiça, CISM; Maputo Mozambique.