The HOST DETERMINANTS *of* TREATMENT OUTCOMES *of* MULTI-DRUG RESISTANT PULMONARY TUBERCULOSIS



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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) has been an emerging public health problem. MDR-TB is a fatal disease whilst treatment of MDR-TB is prolonged but less effective and toxic. Inter-individual variations in clinical presentation and treatment response have been reported in tuberculosis (TB). Identifying host determinants for MDR-TB is important in the search for adjunctive therapy to improve treatment outcome for MDR-TB. This thesis aims to evaluate the impacts of three host factors of leukotriene A4 hydrolase (*LTA4H*) genotype, diabetes mellitus (DM) and vitamin D on clinical presentation, treatment response and treatment outcome of pulmonary TB patients, particularly MDR-TB.

The first results chapter, **chapter 3**, illustrates the picture of increasing MDR-TB in Ho Chi Minh City, Vietnam, from 2011 to 2015 and its risk factors for poor treatment outcomes.

In **chapter 4,** *LTA4H* genotype showed no significant association with clinical presentation, bacterial burden, extent of lung damage, treatment response or outcome in 312 MDR-TB and 600 drug susceptible (DS) TB patients. Although a potential marker for host-targeted therapy for TB meningitis, this genotype does not appear to be such for pulmonary TB.

In **chapter 5**, the detrimental effect of DM on clinical presentation, bacterial load, disease severity, increased inflammation and treatment response was clearly observed in pulmonary TB. Although not a significant risk factor for mortality and poor treatment outcome, DM prolonged sputum culture conversion of MDR-TB and should be considered as an important host factor to adjust for.

Chapter 6 reveals the association between vitamin D and extent of lung damage, severity and inflammatory response through blood biomarkers among 309 MDR-TB and 301 DS-TB patients. Vitamin D deficiency caused an adverse effect on MDR-TB, but not on DS-TB during treatment.

The work of this thesis has provided more evidence on host factors in efforts to improve treatment outcomes for pulmonary MDR-TB.

DECLARATIONS

I declare that this thesis is my own work. The original ideas for this thesis was developed in discussions with my supervisors - Prof. Guy E. Thwaites and Assoc. Prof. Nguyễn Thụy Thương Thương. I was responsible for obtaining ethical approvals, designing, conducting the studies, performing statistical analysis, interpreting findings and writing the thesis. Nonetheless, I would not have been able to reach to this stage without great statistical support of Prof. Ronald B. Geskus, Dr. Lê Thanh Hoàng Nhật (chapter 3), Dr. Dư Hồng Đức (chapter 4, 5 and 6), Mr. Hoàng Thanh Hải and Dr. Đào Nguyễn Vĩnh (for assisting with complex R codes) and Dr. Võ Thanh Sơn (for mapping of figure 3-4). I confirm that this thesis has not been submitted for a degree or other qualification to this or any other university.

Parts of **chapter 3** of this thesis have been published in BMC Infectious Diseases (Risk factors for poor treatment outcomes of 2266 multidrug-resistant tuberculosis cases in Ho Chi Minh City: a retrospective study. *BMC Infect Dis* **20,** 164 (2020). https://doi.org/10.1186/s12879-020-4887-1). I was the lead investigator on this study. I designed the study and involved in clinical data collection, entry, quality check. I performed data analysis and wrote the final manuscript that was reviewed by all authors. Guy E. Thwaites and Nguyễn Thụy Thương Thương conceptualized, designed and revised the manuscript. Nguyễn Hữu Lân, Trương Văn Vĩnh, Nguyễn Thị Mai Trang and Đặng Thị Minh Hà participated in the design and data collection. Nguyễn Thị Hạnh, Phan Triệu Phú and Võ Thanh Sơn coordinated and assisted data collection. Đào Nguyễn Vĩnh and Lê Thanh Hoàng Nhật assisted in statistical analysis and revised the manuscript.

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ABBREVIATIONS

µg	Microgram
µL	Microliter
µM	Micromol
95% CI	95% confidence interval
AFB	Acid fast bacilli
AG	Albumin globulin ratio
Am	Amikacin
aOR	Adjusted odds ratio
Bdq	Bedaquiline
BMI	Body mass index
Cfx	Clofazimine
CRyPTIC	The Comprehensive Resistance Prediction for Tuberculosis: an International Consortium
Cs	Cycloserine
CXR	Chest X-ray
Dlm	Delamanid
DM	Diabetes Mellitus
DOTS	Directly observed treatment short course
DS	Drug susceptible
DST	Drug susceptibility testing
DTU	District Tuberculosis Unit
E	Ethambutol
Eto	Ethionamide
FPG	Fasting plasma glucose

H	Isoniazid
HbA1C	Glycosylated hemoglobin
HCMC	Ho Chi Minh City
IL-1β	Interleukin 1β
IFN-γ	Interferon γ
IQR	Interquartile range
LAM	Lipoarabinomannan
LAMP	TB-Loop-isothermal Mediated Amplification
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LED-FM	Light-emitting diode fluorescence microscopy
Lfx	Levofloxacin
LJ	Lowenstein-Jensen
LPA	Line probe assay
LTA4H	Leukotriene A4 hydrolase
Lzd	Linezolid
M.tb	Mycobacterium tuberculosis
MDR	Multidrug resistant
Mean CT	Mean cycle threshold
Mfx	Moxifloxacin
MGIT	Mycobacteria growth indicator tube
MIC	Minimum inhibitory concentration
MODS	Microscopic observation drug susceptibility
NAAT	Nucleic acid amplification test
NLR	Neutrophil:Lymphocyte ratio
NTM	Non –Tuberculosis Mycobacteria
NTP	National Tuberculosis Programme

OR	Odds ratio
OUCRU	Oxford University Clinical Research Unit
OxTREC	Oxford Tropical Research Ethics Committee
PAS	Para aminosalicylic acid
PCR	Polymerase chain reaction
Pto	Prothionamide
R	Rifampicin
RCT	Randomized clinical trial
RMPT	Restricted mean positive time
RMST	Restricted mean survival time
S	Streptomycin
SNP	Single nucleotide polymorphism
TB	Tuberculosis
TBM	Tuberculosis meningitis
TNF-α	Tumor necrosis factor α
UV	Ultraviolet
WGS	Whole genome sequencing
WHO	World Health Organization
XDR	Extensively drug resistant
Z	Pyrazinamide

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Chapter 1

INTRODUCTION

1.1 TUBERCULOSIS AND MULTIDRUG RESISTANT TUBERCULOSIS

1.1.1 History

Tuberculosis (TB) is known to be one of the oldest diseases in humankind, caused by *Mycobacterium tuberculosis* (*M.tb*). Mycobacteria are hypothesized to have existed for around 150 million years. Egyptian mummies dating back to more than 5000 years ago were found to have *M.tb* DNA in their tissues and skeletal deformities of tuberculosis (1). Hippocrates described "phthisis" as a fatal disease of young adults "from eighteen to thirty-five" with "constant sweats, but not diffused over the whole body". He also noted the differences between "phythisis" and seasonal respiratory infections in the winter (2). The name "tuberculosis" was introduced in 1834 by Johann Lukas Schonlein in replacing for the terms "consumption" and "phthisis" (3).

The infectious nature of this disease was first proposed in 1720 in a publication of Benjamin Martin, but needed more than a century to be proven. In 1882 Robert Koch successfully isolated the tubercle bacillus and later reproduced the disease in the laboratory animals (1). Even before the infectiousness of this disease was discovered, patients with "consumption" receiving cod liver oil experienced remarkable improvement, which was first reported in 1849 by Dr. Chapman JB Williams, 73 years before vitamin D was discovered (4). In the late 1890s, Dr. Neils Finsen successfully treated many patients with lupus vulgaris, a form of cutaneous tuberculosis, by exposing them to sun light, and then light from electric arc lamp. This work led to the Nobel Prize for Physiology and Medicine award for Dr. Finsen in 1903. In 1940s, high doses of vitamin D for six to nine months was used to treat lupus vulgaris and other form of TB, including pulmonary and pleural TB (5). Cod liver oil and sun bathing, the two common sources of vitamin D, quickly became the treatment for many TB patients during the pre-antibiotics era. Treatment of TB mainly focused on improving host immunity through bed rest, diets, and sun bathing in sanatoriums. Other therapeutic interventions such as artificial pneumothorax, thoracoplasty, plombage surgery and lung resection were also used to treat TB (6).

Once the pathogen of the disease and mode of transmission were identified, prevention of TB has been searched and practiced. In 1921, BCG, a vaccine originally used to prevent TB in cows, began to be used in humans. However, BCG could not help to eliminate this infectious disease. In a meta-analysis, the efficacy of BCG was 51% and widely varied between studies and populations (7). Despite its modest efficacy and high variations between populations, BCG is the only licensed vaccine for prevention of TB to date.

The invention of antibiotic in 1928 brought hope for infectious diseases. However, penicillin has shown no effect on *Mycobacterium tuberculosis*. The lipid-rich thick cell wall has made it resistant to many antibiotics. Streptomycin was discovered by Schatz, Bugie and Waksman in January 1944 and the first TB patient to receive streptomycin in November 1944 got cured. Also in 1944, para-aminosalicylic acid (PAS) was proved to effectively treat TB (6). Widespread use of antibiotics to treat TB significantly decreased the prevalence. In 1948, the British Medical Research Council conducted the first randomized clinical trial using streptomycin to treat TB and proved that streptomycin was effective. Unfortunately, soon after recovery from TB, many patients relapsed and drug resistance was observed. The British Medical Research Council concluded that active TB should not be treated with a single agent. Later, in 1951, isoniazid, one of the most potent anti-tuberculosis drugs, was discovered. Combination of isoniazid, streptomycin and PAS for 18 to 24 months cured most of TB patients. The role of vitamin D and sanatoriums quickly subsided with the discovery of effective chemotherapy for TB.

Rifampin, discovered in 1957 in Italy, is now still the corner stone of TB treatment. Its combination with isoniazid and pyrazinamide shortens the treatment from 18-24 months to 6 months. In 1960, study in India showed that TB could effectively be treated in out-patient settings, in which adherence took a crucial role in treatment success (8). Directly observed treatment, short course (DOTS) has been a widely used strategy to manage TB in many countries since 1994 to minimize unfavorable outcomes related to non-compliance (9).

Since the start of treating TB with streptomycin, *M.tb* began to evolve resistance to anti-tuberculosis drugs. The first national survey in 1955-1956 for drug resistant prevalence among new pulmonary TB in Britain revealed 5.1% strains with resistance to at least one of three anti-tuberculosis drugs (10). Even with the invention of rifampicin and combination therapy, drug resistant tuberculosis still persisted and increased.

1.1.2 Epidemiology of TB and multidrug resistant TB

In 1957, just more than ten years after the invention of chemotherapy for TB, Wallace Fox *et al.* found different patterns of drug resistance among newly diagnosed TB patients, and suggested a robust screening for drug resistance of all pre-treatment sputum samples (10). Since then, drug resistant TB has been a threat for patients' health and TB control globally. With effective chemotherapy for TB for more than 50 years since then, this communicable disease still kills more people than any other single infectious disease. *M.tb* was estimated to cause 10 million new cases of TB and 1.4 million deaths in 2019 (11). Three point five percent of new TB cases and 18% of previously treated cases were MDR or rifampicin resistant (RR) TB. The number of patients with bacteriologically confirmed TB who were tested for rifampicin resistance was increasing from 51% in 2017 to 61% in 2019. Around 465,000 incident TB cases were estimated to have rifampicin resistance, 78% of whom had MDR-TB (11). A total of 206,030 MDR/RR-TB cases were detected in 2019, which were less than half of the estimated number of MDR/RR-TB. Only 56% of MDR/RR-TB who got treatment reached a successful outcome (11). There has been gaps between prevalent cases and diagnosed cases of both TB and MDR-TB, leading to on-going transmission of this fatal disease. As majority of MDR-TB are caused by transmission rather than acquisition, early diagnosis and efficient treatment are crucial in MDR-TB control (12).

Furthermore, 1.4 million incident TB cases were estimated to have isoniazid resistance, of whom almost 1.1 million were not concurrently resistant to rifampicin in 2019 (11). With the drug resistant screening strategy prioritizing the identification of rifampicin resistance, a substantial number of

mono-isoniazid resistant TB patients were under-diagnosed for their resistance. Those rifampicin susceptible isoniazid resistant patients received first line drugs and might be exposed to rifampicin monotherapy in the continuation phase. This inappropriate treatment caused a 28.9% failure or relapse within two years (13). WHO recommended a modified regimen for rifampicin susceptible, isoniazid resistant TB, however, implementation of a systematic identification of this group has not been a priority in many countries, especially in resource-limited settings (14).

Vietnam, located in the South East Asia, has a surface area of 331,210 square kilometres with a population of 96 million in 2018. It is ranked 12th among 20 high TB burden countries and also the 14th in the high MDR TB burden countries. The estimated incidence of TB in 2017 was 176 (112-255) per 100,000 people (15). The National Institute for TB in Ha Noi was the headquarter for TB control in Vietnam from 1975 to 1986. Vietnam's National Tuberculosis Program (NTP) was founded in 1986 in response to increasing demand for TB control. A national survey in Vietnam in 2010 showed that the prevalence of any drug resistant tuberculosis was 32.7% in new cases and 54.2% in previously treated cases; the prevalence of MDR-TB was 4% in new patients and 23.3% in previously treated patients (16). These prevalences of MDR-TB among TB patients in Vietnam are similar to the average prevalence at global level (17).

1.2 DIAGNOSIS

1.2.1 Tuberculosis

1.2.1.1 Sputum based tests

Microscopy:

Acid fast stain was first used in 1882 by Dr Paul Ehlich to identify the pathogen of TB and has soon become the first line method to diagnose TB (18). Acid-fast bacilli (AFB) smear microscopy remains the most widely used, affordable test but has a low detection rate. The sensitivity of testing with one sputum sample was 53.8%, and increased to 64.9% from with two sputum samples, and

67.2% with three sputum samples (19). An estimated 10,000 bacilli per milliliter of specimen are required for a positive result, with fewer causing false negative results. Hawken *et al.* showed a wide variation in smear results between readers, in which 26% of smear negative TB patients were found positive when carefully re-examination (20). Light-emitting diode fluorescence microscopy (LED-FM) with auramine staining enables visualization at lower magnifications than conventional Ziehl-Neelsen smear, leading to lesser time to read. LED-FM was recommended by WHO in 2011 as it adds 6% more sensitive compared to Ziehl-Neelsen method with specificity of 98%, and requires less maintenance and facilities than conventional fluorescence microscopy (21). With higher positivity rate, LED-FM had similar cost and reading time with Ziehl-Neelsen method (22). However, implementation of LED-FM requires additional training and quality control.

Culture:

The gold standard in diagnosing TB is to detect viable bacteria through culturing on solid and liquid media. Solid culture using egg-based medium, such as Lowenstein-Jensen (LJ), Ogawa, have been used to identify *M.tb* isolates since 1932. However, an average of two to eight weeks is required to identify the presence of mycobacteria which leads to delay in diagnosis. In 1958, Middlebrook and Cohn introduced agar-based medium that can detect *M.tb* faster than LJ media (23).

Liquid culture systems, invented in 1969, indirectly detect mycobacterial growth by measuring the CO_2 production or O_2 consumption. The BACTEC Mycobacterial Growth Indicator Tube 960 (MGIT) (Becton Dickinson, USA) is an automatic system that incubates and detects the growth of mycobacteria through a fluorescent quenching-based oxygen sensor at the bottom of the tube. Mycobacteria consumes oxygen and generates orange fluorescence, which is monitored continuously in the system. The BACTEC 960 will give results within four to 21 days (manual MGIT). MGIT gives a higher yield of mycobacterial, with a faster, however higher contamination rate results for *M.tb* than LJ culture (23) (24).

Molecular tests:

Rapid and accurate diagnosis is crucial to improve outcome of TB patients. Nucleic acid amplification tests (NAATs) satisfies this need when it detects components of *M.tb* genes in a timely manner. There are several NAATs, among which GeneXpert MTB/RIF and its newer generation GeneXpert Ultra are rolled out to screen TB in many countries.

The GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is an automated polymerase chain reaction (PCR) assay detecting DNA of *M.tb* and mutations in 81-bp region of *rpoB* gene which account for more than 95% of rifampicin resistance in two hours. GeneXpert can detect *M.tb* at a very low level of 114 bacterial colony forming units per milliliter of specimen (25). This assay has a pooled sensitivity of 88% and specificity of 99% for presumed pulmonary TB. *M.tb* strains with RR are often also resistant to isoniazid, making resistance to rifampicin a marker for MDR-TB. Since 2010, WHO endorsed the use of Xpert MTB/RIF in replacing smear microscopy for TB diagnosis (26). To improve the diagnostic performance, a new generation GeneXpert Ultra has been implemented to detect *M.tb* at a much lower threshold of 16 bacterial colony forming units per milliliter, which leads to an increase of 17% sensitivity to detect smear negative-culture positive TB patients compared to GeneXpert MTB/RIF (25). Both generations of GeneXpert have 98.7% specificity (27). Those GeneXpert assays are operator-independent and do not require significant laboratory infrastructure, making them the point of care test in many countries in the world, especially in resource-limited settings.

The GenoType MTBDR*plus* line probe assay (LPA) (Hain Life Sciences, Nehren, Germany), another NAAT, was also endorsed by WHO since 2008 for rapid detection of *M.tb* complex and susceptibilities to rifampicin and isoniazid. The MTBDR*plus* detects mutations in the *rpoB* gene for rifampicin resistance and *inhA* and *katG* genes for isoniazid resistance. However, the version 1 of GenoType MTBDR*plus* required DNA extraction from clinical samples and positive cultures, which limited its application to AFB positive samples. The version 2 of this test can be applied for both smear positive and negative specimens. Comparing to GeneXpert MTB/RIF, the GenoType

MTBDR*plus* version 2 had similar sensitivity and specificity. This assay also provides information on isoniazid susceptibility (28), which is the advantage of GenoType MTBDR*plus* compared to GeneXpert MTB/RIF. However, the GenoType MTBDR*plus* needs more sophisticated laboratory infrastructure than does GeneXpert, which prevents the scale-up of this test in the resource-limited settings.

Another NAATs is the TB-Loop-isothermal Mediated Amplification (LAMP) assay (Eiken Chemical Co., Japan). The TB-LAMP is a simple and rapid diagnostic test that targets *IS6110* region specific for MTB complex and *rpoB* region specific for both MTB complex and non-tuberculosis mycobacteria (NTM). This test amplifies the nucleic acid under isothermal conditions of 65ºC for 60 minutes and then 80ºC for 5 minutes. The results can be read manually under ultraviolet light. In a meta-analysis, the sensitivity and specificity of this assay is similar to those of GeneXpert MTB/RIF (29). Kim *et al.* designed a new multiplex LAMP assay to detect both MTB and NTM. In a cohort of 72 *M.tb*, 123 NTM and 138 negative samples, multiplex LAMP showed specificity of 100% and sensitivity of 100% for *M.tb* and 98.4% for NTM (30). Since 2016, WHO recommended the use of TB-LAMP to replace sputum smear microscopy or to follow a negative smear test in both central and peripheral laboratories (31).

1.2.1.2 Non sputum based tests

Despite the progress in diagnostics for TB, still 2.9 million people were either undiagnosed or underreported (11). Diagnosis for pauci bacillary pulmonary TB and extra pulmonary TB is even more challenging and urgently needs better diagnostic tools. To facilitate detecting TB in those patients, especially in severely ill or pediatric patients, non-sputum based assays have been rigorously studied to overcome this problem.

Urine Lipoarabinomannan (LAM) test:

Lipoarabinomannan (LAM) is a lipopolysaccharide of the outer cell wall of *M.tb* that is extracted by the kidney to urine. LAM is heat-stable and has the potential to stimulate immune response of the host. LAM can be detected by ELISA technique through the use of polyclonal (AlereLAM, Abbott, Chicago, IL, USA) or monoclonal antibodies (SILVAMP TB LAM, Fujifilm, Tokyo, Japan). The main advantage of LAM test is the ease of obtaining urine samples. This point of care test can be simply performed, especially in severely ill patients who have difficulty in expectorating sputum or who have extra-pulmonary TB. The astonishing impact of the first generation urine LAM test, Alere LAM, was to improve mortality in hospitalized HIV patients (32). In a meta-analysis by WHO, sensitivity of AlereLAM for patients with CD4 count less than 100/ µl was 56% (33). Albeit suboptimal sensitivity, this test improves survival when performed for advanced HIV patients. Therefore, WHO recommended the use of lateral flow LAM assay for patients with HIV with CD4 count less than 100 per µl (34).

A newer generation assay to detect urine LAM epitopes was using monoclonal antibodies under silver-amplification. With this technique, urine SILVAMP TB LAM test (Fujifilm, Tokyo, Japan) was able to detect LAM antigen at much lower concentration than the first generation assay. The sensitivity of FujiLAM (70.4%) was higher than Alere LAM (42.3%) among HIV patients, and the specificity remained high for both of the assays (90.8% for FujiLAM, 95.0% for AlereLAM) (35). This suggested that urine LAM test, especially FujiLAM, could serve as an easy-to-use test to diagnose TB among HIV patients.

Blood host biomarkers

Revolutionaries in biology have enabled researchers to study genomics (entire genomes), transcriptomics, proteomics and metabolomics to understand the pathogenesis of many diseases including TB. The omics data identifies host bio-signatures that can differentiate TB patients and non-TB and provides a platform to develop potential diagnostic markers to triage active TB.

A study by Chendi *et al.* evaluated 54 host biomarkers to identify a combination of five plasma proteins that was able to diagnose TB with sensitivity of 72.7% and specificity of 90.5% (36). Transcriptomics has shed lights on the expression of genomes through transcriptions in response to diseases. Transcriptomic data indicates combination of gene expressions can discriminate TB from other diseases. In a retrospective meta-analysis of Paxgene stored blood in seven cohorts, Sweeney *et al.* discovered that a set of three gene expression (GBP5, DUSP3 and KLF2) was able to separate active TB from other diseases, latent TB and healthy controls. The expression of the three-gene in TB patients declined with treatment and was not affected by HIV status, bacterial resistant status or BCG vaccination (37). Cepheid (Sunnyvale, CA, USA) developed the set of three gene expression into a point of care GeneXpert-MTB-HR-Prototype cartridge that can be performed on GeneXpert machine. Sodersten *et al.* showed that this test had an area under the curve of 0.94 to diagnose TB against GeneXpert MTB-RIF in adult patients with HIV (38). Penn-Nicholson *et al.* discovered transcriptomic signature that can predict incident TB, and developed a simple, capillary blood-based PCR-device to predict incident TB (39). Those studies highlighted the feasibility of applying omics technology to develop non-invasive point of care test for TB.

Electronic nose (E-nose) and Volatile Organic Compounds (VOCs)

In 2013, Bruins *et al.* reported a novel tool, the DiagNose (C-it BV, Zutphen, The Netherlands) detecting breathprints of TB through volatile biomarkers (40). This point of care test can differentiate between TB and healthy control with sensitivity of 97.9% and specificity of 98.5%. Yet, the performance of the DiagNose was much poorer when comparing TB and non-TB patients, with sensitivity of 50.0% and specificity of 60.0%. After excluding cases of misclassification, the sensitivity increased to 76.5% and specificity to 87.2% for differentiating between TB and non-TB cases. This device also poses potential errors as it collects exhaled air into bags, the material of which might interact with the VOCs. In order to reduce the interaction between the material of the collecting bags, exhaled VOCs was directly collected by the portable Aeonose (the Enose BV,

Zutphen, The Netherlands) (41) (42). Exhaled air goes through a mouth piece to pass three sensor surfaces and VOCs are measured. This device had both high sensitivity (88%) and specificity (92%) in diagnosing TB in a cohort of 110 participants in Paraguay (41). In a larger cohort of 360 suspected TB patients and 40 healthy controls in Indonesia, sensitivity was 85% in calibration phase and 78% in validation phase. The specificity was 55% in calibration phase and 42% in validation phases (42). This simple, fast and non-invasive assay can be implemented in rural areas but still needs more improvement to become a point of care test to screen TB.

Circulating cell-free DNA (cfDNA) in biological fluid

Circulating cell-free DNA is defined as fragments of nucleic acids found in various body fluids including blood, saliva and urine. Extracellular *M.tb* DNA fragments in pleural fluid achieved higher sensitivity (79.5%) than GeneXpert (38.5%) and culture (27.1%) and had 100% specificity in a cohort of 286 suspected TB pleural effusion patients (43). Diagnosis of TB meningitis remains challenging even with the application of GeneXpert Ultra. The diagnostic performance of cfDNA in cerebrospinal fluid was evaluated in a cohort of 68 patients including 46 TB meningitis and 22 without TB meningitis. The overall sensitivity was 56.5%, which increased to 100% in definite TB meningitis, while its specificity was 100% (44). Plasma cfDNA in diagnosing pulmonary TB showed a sensitivity of 54.2% and specificity of 96.5% (45). CfDNA is a promising non-invasive test for diagnosing smear negative pulmonary and extrapulmonary TB.

1.2.2 Drug resistant tuberculosis

Drug resistance occurs when *M.tb* is able to survive under the effect of anti-tuberculosis drug(s). Alteration of drug target, activation of efflux pump, production of inactivating enzymes or disruption of drug activation can lead to drug resistance (12) (46). Identification of drug resistance requires drug susceptibility testing (DST), which is divided into phenotypic and genotypic tests. Phenotypic DST identifies the growth or inhibition of *M.tb* in anti-tuberculosis drug containing

media, either solid or liquid method. Genotypic DST predicts resistance to a specific drug by identifying mutation(s) of related genes.

1.2.2.1 Phenotypic drug susceptibility testing (DST)

Phenotypic DST identifies drug resistance through the growth of *M.tb* in anti-tuberculosis drug containing media, either solid or liquid method. *M.tb* strains collected in cultures are inoculated with culture media containing anti-tuberculosis drug, and resistance is defined when growth is detected. Conventional solid media phenotypic DST can be performed with proportion method, resistance ratio method and absolute concentration method. The conventional culture and DST method usually takes 8 to 12 weeks and might lead to delay in diagnosing and appropriate treating for drug resistant TB (47). This method also is restricted in facilities with laboratory infrastructure allowing culturing *M.tb*. Manipulation of live strain of *M.tb*, with potential MDR and extensive drug resistant (XDR) strains, should be carried out in high-risk laboratory facility, such as biosafety level 3.

Phenotypic DST in automated liquid-based culture systems such as BACTEC MGIT 960 system can reduce the time to resistant detection to 7 to 12 days (12). The automated MGIT DST has been endorsed by WHO for both smear positive samples (direct) and positive cultures (indirect method) (47). The indirect method has been widely implemented. The positive culture suspension is inoculated in drug containing MGIT tubes and then incubated into the BACTEC MGIT 960 instrument. The susceptibility of rifampin, rifabutin, isoniazid, streptomycin, pyrazinamide, amikacin and capreomycin showed high concordance between BACTEC MGIT 960 and conventional resistance ratio method (48). WHO has issued critical concentrations of anti-tuberculosis drugs to identify drug resistance and susceptibility of *M.tb* for both solid and liquid DST (***Table 1.1***) (49). With a shorter turnover time compared to solid DST, the BACTEC MGIT DST has become the most commonly used phenotypic DST.

Table 1.1. Critical concentrations of first line and second line anti-tuberculosis drugs in LJ and MGIT DST

Drugs	Lowenstein Jensen	BACTEC MGIT
Isoniazid	0.2 mg/L	0.1 µg/ml
Rifampicin	40.0 mg/L	1.0 µg/ml
Ethambutol	2.0 mg/L	5.0 µg/ml
Pyrazinamide	-	100 µg/ml
Levofloxacin	2.0 mg/L	1.0 µg/ml
Moxifloxacin	1.0 mg/L	0.25 µg/ml
Amikacin	30.0 mg/L	1.0 µg/ml
Streptomycin	4.0 mg/L	1.0 µg/ml

The 14 drug microtiter plate is a novel indirect method to measure minimum inhibitory concentration (MIC) for a wide range of anti-tuberculosis drugs used to treat drug resistant and susceptible TB. The Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC Consortium) modified the 12 drug Sensititre MYCOTB 96-well microtiter plate (Thermo Fisher Scientific, Massachusetts, USA) into 14 drug UKMYC5 plate to establish the new method for MIC measurement for rifampicin, rifabutin, isoniazid, ethambutol, kanamycin, amikacin, ethionamide, PAS, levofloxacin, moxifloxacin, linezolid, clofazimine, bedaquiline and delamanid (50). The UKMYC5 was optimally read by an automatic Vizion system, which could produce results after 14 days of incubation. This study has set foundation to develop MIC measurement standard to be applied in clinical use.

Microscopic observation drug susceptibility (MODS)

Microscopic observation drug susceptibility is a liquid culture based drug susceptibility testing. This method of phenotypic DST uses an inverted microscope to observe the early growth of micro-colony and typical cord-formation of *M.tb* in drug-free and drug-containing liquid media. Drug resistance is indicated by the growth of *M.tb* in drug-containing media, with high sensitivity and

specificity (51) (52). Agarwal *et al.* showed that the median time for culture positivity in MODS was 10.3 days, compared to 13.8 days in MGIT and 30.5 days in LJ. As culture and DST are concurrent in MODS, the median for DST was also 10.3 days, which was much faster than DST using MGIT (23.6 days) and LJ (61.2 days) (52). Although labor consuming in preparation, MODS is an inexpensive and simple method to detect *M.tb* and drug resistance that can be deployed in resource-limited laboratory settings.

1.2.2.2 Genotypic DST

Genotypic DST detects mutations of *M.tb* that predict drug resistance. When no mutations is detected, the genotypic DST usually reports as no resistance. Genotypic DST can be performed by non-sequencing and sequencing method.

Non-sequencing method

Genotypic testing can quickly detect mutations that associate with drug resistance. Xpert MTB/RIF is an automated PCR-based test that detects *M.tb* and *rpoB* gene mutations that account for 96% resistance to rifampicin in 2 hours (53). WHO recommended the use of Xpert MTB/RIF to simultaneously detect TB and RR to guide treatment (54). Among RR-TB patients, 78% had MDR-TB (11). In order to guide treatment for MDR/RR-TB, the Xpert MTB/XDR was developed to detect common resistant mutations to isoniazid, fluoroquinolone, ethionamide and aminoglycosides. With phenotypic DST as reference, the Xpert MTB/XDR has specificity more than 95% for all tested drugs and sensitivity of 98.3% for isoniazid resistance, 91.4% for fluoroquinolone resisitance, 91% for amikacin resistance, 98.1% for kanamycin resistance and 65.4% for ethionamide resistance (55). This assay is a reflex test for samples with *M.tb* positive and provides additional drug susceptibility information that is not covered in GeneXpert MTB/RIF.

LPA is another NAAT that simultaneously detects *M.tb* and drug resistance. The MTBDR*plus* (Hain Lifescience, Nehren, Germany) is a rapid test endorsed by WHO for detecting rifampicin and isoniazid resistance with an average laboratory turn-around-time of 5 hours (47). The MTBDR*plus*

detects *rpoB* mutations for rifampicin resistance, and *katG* and *inhA* mutation for isoniazid resistance. Its sensitivity was 98.1% and specificity of 98.7% when performed on culture isolates (56). The GenoType MTBDR*sl* version 1.0 was the first available molecular test for XDR-TB diagnosis, which predicted resistance to fluoroquinolones, aminoglycosides and ethambutol. The new generation MTBDR*sl* version 2.0 extended the range of loci for the detection of resistance to fluoroquinolones and aminoglycosides. Compared to phenotypic DST, the sensitivity of MTBDR*sl* version 2.0 was 100% for ofloxacin, 93.8% for amikacin, 89.2% for kanamycin, 86.2% for capreomycin. The specificity for all the tested drugs was higher than 95% (57).

Drug resistance can be the consequence of mutations in one or multiple genes or multiple loci in one gene. Non-sequencing molecular DST tests limited number of loci. Hence, it emphasizes the need for sequencing method to predict drug resistance and guide treatment for drug resistance patients.

Sequencing method

Sequencing method emerges as a promising technique to broaden knowledge on genetics of pathogen, the mutations leading to drug resistance and transmission in a timely manner. Sequencing method can provide sequence information for specified gene regions with targeted sequencing, or for whole genomes with whole genome sequencing (WGS). Targeted sequencing can be performed on clinical samples and focuses on detecting known gene mutations related to drug resistance. WGS requires DNA extracted from cultured isolates and maps organism's genome to reference genome. WGS is able to identify both pre-defined and novel mutations. Enormous efforts have been made to link genotypic and phenotypic DST to build a data platform for drug resistance. The ReSeqTB project and CRyPTIC Consortium have contributed to a catalogue of mutations to predict drug resistance (58) (59). The sequencing technique has become more affordable and rapidly been applied for diagnosis in clinical and public health settings (60). Once advanced bioinformatics was required to analyze results, WGS can now be applied in clinical settings with ready-to-use softwares

such as MykrobeTB Predictor, QuantTB or SP3 (61, 62). However, WGS still requires sophisticated laboratory infrastructure, which limits its application in research institutes and laboratories in high income countries.

1.3 TREATMENT

Treatment of tuberculosis aims at curing individual patients and rapidly reducing transmission to community. Patients receive the regimen depending on the drug susceptibility of *M.tb*. WHO classified drug resistant TB into a more detailed category for appropriate treatment (Table 1.2) (63).

Table 1.2. Classification of drug resistant TB

Mono-resistance	Resistance to only one first-line anti-TB drug.
Rifampicin resistance	Resistance to rifampicin with or without resistance to other anti-tuberculosis drugs.
Poly-resistance	Resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin.
Multidrug resistance	Resistance to at least both isoniazid and rifampicin.
Extensive drug resistance	MDR-TB that has extend resistance to any fluoroquinolone and at least one of bedaquiline or linezolid
Totally drug resistance	Resistance to all the first-line and second-line anti-TB drugs that can be tested for.

1.3.1 Drug susceptible tuberculosis

Patients with rifampicin susceptible TB are treated with 6 month regimen, including 2 months with 4 first line drugs in the intensive phase and 4 months of rifampicin and isoniazid in the continuous phase, as described below:

2HRZE/4HR

H: isoniazid; R: rifampicin; Z: pyrazinamide; E: ethambutol

Patients with isoniazid resistance but not rifampicin resistance also need a modified regimen with levofloxacin (14).

1.3.2 Drug resistant tuberculosis

While the treatment of drug susceptible TB requires 6 months of 4 first line oral drugs, the treatment of MDR/RR-TB often requires at least 18 months. According to WHO guidelines for treatment of MDR-TB in 2016, the regimen for MDR/RR-TB should have at least five effective drugs in the intensive phase (64). Empiric standardized regimens used in programmatic MDR-TB management typically consists of a twenty-month regimen. There have been dramatic changes in the treatment of MDR-TB since 2016 when a shorter nine-month regimen with injectable agent was recommended by WHO (64). In 2018, kanamycin and capreomycin were advised to be substituted by amikacin as they were associated with increased risk for failure and relapse. Since 2020, WHO has recommended the implementation of a new shorter all oral drug regimen for eligible MDR-TB patients (14). The bedaquiline, pretomanid and linezolid (BPaL) regimen might be used to treat MDR-TB patients who have additional fluoroquinolone resistance and no existing WHO recommended regimen is possible. This regimen was first proposed in the Nix-TB study (65). Until now, there has been no data on efficacy and safety of this regimen on larger population, and the BPaL regimen has not been recommended for the routine programmatic use.

Longer regimen

The longer regimen required the use of at least 5 drugs including injectable agent in the 8-month intensive phase and oral drugs in 12-month continuation phase. Vietnam has implemented the standardized twenty-month regimen since 2009. This regimen now are used for patients who are not eligible for shorter regimen. The following drug code of the standardized longer regimen is being used in Vietnam programmatic management of drug resistant TB:

8 Km(Cm)-Lfx-Pto-Cs-Z/12Lfx-Pto-Cs-Z

Km: Kanamycin; Cm: Capreomycin; Lfx: levofloxacin; Pto: Prothionamide; Cs: Cycloserine; Z: Pyrazinamide

In 2020, WHO recommended the use of longer regimen that constitutes with all three Group A agents and at least one Group B agent (Table 1.3). Each patient receives a personalized regimen based on DST results and history of TB treatment. The total duration of longer regimen is 18-20 months, and can be modified to continue for 15-17 months after culture conversion (14). At least four second-line anti-TB drugs are used in the intensive phase. At least three agents are used for the rest of treatment when bedaquiline is stopped. Kanamycin and capreomycin are not recommended because of their association with high failure and relapse rate.

Table 1.3. Grouping of drugs for longer MDR-TB regimens (14)

Groups and steps	Medication	Abbreviation
Group A: Include all three medicines	Levofloxacin or Moxifloxacin	Lfx or Mfx
	Bedaquiline	Bdq
	Linezolid	Lzd
Group B: Add one or both medicines	Clofazimine	Cfz
	Cycloserine or terizidone	Cs or Trd
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol	E
	Delamanid	Dlm
	Pyrazinamide	Z
	Imipenem-cilastatin or meropenem	Ipm-Cln or Mpm
	Amikacin (or streptomycin)	Am (or S)
	Ethionamide or prothionamide	Eto or Pto
	P-aminosalicylic acid	PAS

Shorter regimen

Poor adherence due to long duration and potential adverse effects is a major problem of the standardized twenty-month regimen. To improve treatment success rate, a shorter regimen was first

used in Bangladesh and then named “Bangladesh regimen” (66). The shorter regimen of 9 to 11 months duration was recommended by WHO in 2016 for new RR-TB/MDR-TB patients who are not pregnant and do not have or suspect to have resistance to fluoroquinolones and second-line injectable agents (67) (68).

The shorter regimen consists of 4 to 6 months intensive phase containing injectable agents, and 5 months of continuous phase as below:

4-6Km-Lfx/Mfx-Pto-Cfz-Z-$H_{high\text{-}dose}$-E/5Mfx-Cfz-Z-E

Km: Kanamycin; Lfx: Levofloxacin; Mfx: Moxifloxacin; Pto: Prothionamide; Cfz: Clofazimine; Z: Pyrazinamide; $H_{high\text{-}dose}$: high dose isoniazid; E: Ethambutol

Shorter all oral bedaquiline-containing regimen

In 2020, WHO has recommended the use of new fully oral drug regimen to replace the previously recommended shorter regimen. The shorter all oral bedaquiline containing regimen replaces injectable agents of the shorter regimen with 6 months of bedaquiline. This shorter all oral drug regimen has a comparable treatment outcomes to an injectable-free longer regimen containing bedaquiline. This regimen is indicated for newly diagnosed MDR/RR patients who do not have concurrent fluoroquinolone resistance.

6Bdq-4-6Lfx/Mfx-Pto-Cfz-Z-$H_{high\text{-}dose}$-E/5Mfx-Cfz-Z-E

Bdq: Bedaquiline; Lfx: Levofloxacin; Mfx: Moxifloxacin; Pto: Prothionamide; Cfz: Clofazimine; Z: Pyrazinamide; $H_{high\text{-}dose}$: high dose isoniazid; E: Ethambutol

The bedaquiline, pretomanid and linezolid (BPaL) regimen

This regimen can be used in MDR-TB patients with additional resistance to fluoroquinolones, who had no previous exposure to bedaquiline and linezolid or have been exposed for no more than two weeks (14). This regimen consists of six to nine months of three drugs: bedaquiline, pretomanid and linezolid.

1.4 HOST DETERMINANTS OF MULTIDRUG RESISTANT TUBERCULOSIS TREATMENT OUTCOMES

1.4.1 Treatment outcomes of MDR-TB

Treatment success rate for new and relapse TB patients was 85% in 2018, and greatly varied between countries due to their capacities of providing correct treatment and support. Treatment success rate for MDR-TB was much lower at 57%. Mortality rate was 15%, failure was 7%, and 16% lost to follow-up. For MDR-TB patients with concurrent fluoroquinolone resistance, 24% died, 11% failed the treatment, and only 47% reached treatment success (11). The treatment success rate in Vietnam was reported to be 74% in MDR/RR-TB, compared to 92% for new and relapse TB (69).

With the development of new drugs and the use of repurposed drugs, treatment for MDR-TB can be shortened from 20 months to 9 months. Trials in Bangladesh regimen showed that shorter regimen resulted in a comparable outcomes and relapse rate with longer regimen (66). This shorter regimen, recommended by WHO in 2016, was more acceptable for MDR-TB patients when the intensive phase with injectable agent significantly reduced into half duration compared to the conventional MDR-TB regimen. In an individual participant data meta analysis, treatment success of MDR-TB patients receiving shorter regimen is 84% and significantly higher than 62% of those receiving longer regimen (68).

In 2020, WHO recommend a new fully oral regimen to replace the previous shorter regimen (14). The programmatic data in South Africa showed a higher treatment success rate of all oral bedaquiline containing regimen (73%) compared to standardized shorter regimen (60%). This regimen is more acceptable to patients when the daily injectable agent is removed, and had an odds ratio of 0.5 for lost to follow up when compared to shorter regimen with injectable agent (14). The implementation of the all oral drug regimen requires the access to second line drug DST before the

commencement of treatment, and the availability of bedaquiline. Those requirements restricts the use of this regimen in low and middle income countries with limited resources.

The BPaL regimen used in the Nix-TB study to treat XDR-TB and complicated MDR-TB showed impressing treatment success rate of 90% (65). Although this 26 week regimen had high rate of adverse events, mostly from the long term use of linezolid, its high rate of treatment success has brought hope for XDR and complicated MDR-TB patients.

1.4.2 Host determinants of MDR-TB treatment outcomes

Unfavorable outcomes still account for up to 40% of MDR-TB cases (11), yet the determinants of the treatment response and outcome are still poorly understood. When the effect of drugs is lessened in MDR-TB, host immunity becomes more important in controlling the infection. Host factors and comorbidities have been known as risk factors for unfavorable outcomes (70) (71) (72) (73). Notably, HIV-co-infection significantly worsened treatment outcome of MDR-TB. Diabetes mellitus (DM) is a common co-morbidity in TB patients. DM increases risk for developing TB (74) and MDR-TB (75) by impairing immune response to *M.tb* (76). DM is associated with more severe disease, presenting as more cavities and higher smear grade (77). Furthermore, DM complicates treatment and contributes to poor treatment outcome of TB (78) (79). However, effect of DM on MDR-TB outcomes is still unclear. A study in Pakistan did not find association between DM and poor outcome (80) while in Korea, DM decreased treatment success rate in MDR-TB (81). Understanding whether and how DM influences presentations and treatment outcomes of MDR-TB could help establish a better management for MDR-TB patients with DM.

Besides co-morbidities, host immunity determined by genotypes is a key factor for heterogeneity response to treatment. In zebrafish model, leukotriene A4 hydrolase (*lta4h*) genotype regulates the secretion of pro-inflammatory tumor necrosis factor (TNF), both the excess and deficiency of which can lead to hypersusceptibility to TB (82). Targeted therapy based on *lta4h* genotype to normalize zebrafish immunity led to reduction of infection burden. In human, *LTA4H* genotypes associated

with different inflammatory response (83, 84). A single nucleotide polymorphism at rs17525495 C/T of *LTA4H* genotype also associated with survival of TB meningitis patients and defined response to adjunctive dexamethasone treatment (85). Study in TB meningitis patients by Thuong *et al.* showed *LTA4H* SNP rs17525495 correlated with intracerebral cytokine concentrations and predicted survival in HIV-uninfected patients (86). The role of *LTA4H* genotype was further proven by analyzing the association between this genotype and inflammatory response. The *LTA4H* TT genotype was associated with elevated inflammatory response, which explained its response to adjunctive dexamethasone treatment (87). The effect of this genotype was robustly studied in TB meningitis, the most severe form of TB. However, there has been no data on the effect of this genotype on pulmonary TB, the most common form of TB. Information on the effect of *LTA4H* genotype on pulmonary MDR-TB might provide foundation for host targeted therapy in MDR-TB patients.

Since 1849, before the infectious nature of TB was understood, vitamin D was used to treat this disease. In the pre-antibiotic era, vitamin D, in the form of cod liver oil, sun light and then supplementations, was one of the cornerstone of TB treatment (4). Vitamin D is now considered an immunomodulator that regulates monocyte-macrophage activity and stimulates antimycobacterial response (88). Polymorphisms in vitamin D receptor gene associated with rate of sputum conversion in MDR-TB patients (89). In vitamin D deficient patients, supplementation of vitamin D accelerated host inflammatory response, clinical and radiographic improvement (90) and ameliorated treatment outcome in the homozygotes of vitamin D receptor genotype group (83). Association between vitamin D receptor polymorphism and lower serum level of 25(OH) vitamin D were observed in MDR-TB compared to drug susceptible TB and healthy participants (91). However, clinical trials of vitamin D showed conflicting results (92) (93) (94) (95). The role of vitamin D in treatment outcome of MDR-TB is not fully understood and needs further investigation.

1.5 AIMS AND OBJECTIVES

Differences in host immunity, influenced by both genetic, non-genetic factors, and co-morbidities, can lead to different levels of disease susceptibility, progression and treatment response. Attempt to shorten treatment TB has rigorously tested since the development of TB drugs. A clinical trial of 4 month regimen failed to show non-inferiority to 6 month regimen, although patients treated with 4 month regimen had more rapid sputum conversion (96). Non-cavitary pulmonary TB patients treated with 4 month regimen experienced greater relapse rate, indicating that non-cavitation was not a sufficient predictor for success with the shortened regimen in drug susceptible TB patients (97).

Personalized therapy or precision medicine has been developed to tailor treatment for individuals to improve outcome, notably in cardiovascular diseases and cancer. Treatment of infectious diseases, yet, still focuses the pathogens and their sensitivities to antibiotics. Host factors, including innate and adaptive immunity, has governed the progression, treatment response and outcome of infectious diseases (98). Corticosteroid and other anti-inflammatory agents including non-steroidal anti-inflammatory drugs, leukotriene inhibitor, have been showing conflicting results in other infectious diseases, and their role in shortening and improving treatment outcome of TB remains unknown. In preclinical studies, host-directed-therapy might enhance immunity and improve treatment outcome of TB (98) (99) (100). Despite various studies to shorten treatment of MDR-TB, host-directed adjunctive therapy to accelerate recovery for pulmonary MDR-TB is still lacking. Identifying host determinants of MDR-TB treatment outcomes is crucial for development of host-directed therapy in TB.

To understand the epidemiology and characteristics of MDR-TB, a retrospective study was conducted in Ho Chi Minh City, Vietnam. I hypothesize that host *LTA4H* genotypes, DM and vitamin D at baseline influence patients' immune response and treatment response of MDR-pulmonary TB. In this thesis, I conducted a prospective study to include MDR- pulmonary TB

patients to understand the host factors on clinical manifestations and treatment outcome. I also evaluated the effects of those host factors in a cohort of drug susceptible pulmonary TB to understand the different impacts of host factors on MDR and drug susceptible pulmonary TB.

The aims of my thesis are listed below:

1. To understand the epidemiology of drug resistant TB in Ho Chi Minh City from 2011 to 2015;
2. To investigate the impact of *LTA4H* genotypes on presentation severity, blood cytokines, treatment response and outcome of pulmonary TB;
3. To estimate the impact of DM and pre-diabetes mellitus on clinical manifestations, blood cytokines, treatment response and treatment outcomes of pulmonary TB;
4. To evaluate the influence of vitamin D on clinical manifestation, blood cytokines, treatment response and treatment outcomes of pulmonary TB.

Chapter 2

METHODS

2.1 SUMMARY OF STUDIES

All studies in this thesis were conducted in Ho Chi Minh City (HCMC), Vietnam. This city is the economic center of Vietnam, and the center for tuberculosis (TB) and multidrug resistant (MDR) TB management in the South of Vietnam. Its TB incidence was 150/100,000 population in 2018 and 146/100,000 population in 2019 (annual TB report in 2019 of HCMC, Vietnamese).

A retrospective study was carried out to answer the questions in chapter 3. Two prospective studies of MDR-TB adult patients and drug susceptible (DS) TB adult patients were used for chapter 4, 5 and 6. The details of three cohorts are described below.

2.1.1 Retrospective study

2.1.1.1 Study design and participants

A retrospective cohort study was conducted to include all patients who initiated treatment with second-line drugs in HCMC under programmatic management of drug resistant TB from January 2011 to December 2015. To ensure that all treatment outcomes could be captured, more recently diagnosed cases were excluded. Patients were excluded if 1) MDR-TB was diagnosed by a molecular assay but DST reported rifampicin or isoniazid susceptibility; 2) they were enrolled in the STREAM trial (101) to receive a 9-month regimen; 3) they did not start treatment.

2.1.1.2 Study setting

Patients were hospitalized in Pham Ngoc Thach hospital, the tertiary hospital for TB and lung diseases in HCMC, for 7 to 14 days to initiate treatment, and then referred to district TB units (DTUs) for outpatient follow-up. Treatment modalities did not change during the study time, with the standardized combination of 6 drugs, including an injectable agent for at least 6 months in the intensive phase, with a total of 18 to 24 months of treatment (16).

Sputum samples from patients with MDR/RR-TB detected by XpertMTB/RIF (Cepheid, USA) or line probe assay (LPA) were sent to the laboratory in Pham Ngoc Thach hospital to confirm MDR-

TB by phenotypic drug susceptibility testing (DST). Culture and DST in solid and liquid media were the main initial diagnostic method prior 2012. Although pyrazinamide and ethambutol were components of standardized MDR-TB regimen, detecting these drugs' resistance were challenging because of unreliable phenotypic DST assays (102). XpertMTB/RIF and GenoType MTBDRplus (Hain Lifescience GmbH, Germany) were used to detect MDR/RR-TB since 2010 and 2012, respectively. I later use the term "DST" to refer to phenotypic DST in our results.

2.1.1.3 Data collection

Demographic and clinical information, radiographs, acid fast bacilli (AFB) staining, DST results, treatment regimens and treatment outcomes were recorded into structured paper forms. To improve reliability, I collected data from both NTP standardized paper-based treatment cards and electronic records and verified data during the data collection, entry and analysis processes. All data from paper forms were inputted into electronic CliRes system (www.clires2.oucru.org) and verified by data officers in Oxford University Clinical Research Unit (OUCRU). CliRes system, developed by OUCRU team in 2012, is a web-based data management system that allows secure access from everywhere. All access to data is restricted by using password only for principle investigators and specified research staff. All participants were recorded in CliRes system with a unique study code without any identifying detail.

2.1.2 Multidrug resistant tuberculosis study

2.1.2.1 Sample size justification

One of the main aims of this study is to evaluate the effect of leukotriene A4 hydrolase (*LTA4H)* genotype on MDR pulmonary TB treatment response and outcomes. A study in China showed 3-month sputum culture conversion predicted treatment outcomes of MDR-TB (103). There was no published data on impact of *LTA4H* genotype on pulmonary TB. Previous studies of impact of *LTA4H* genotypes on TB meningitis showed heterozygotes responded faster than homozygotes (85)

and ratio of heterozygotes:homozygotes in Vietnamese TB meningitis patients was approximately 1:1 (86). I speculated that 3 month sputum sterility probability of all MDR-TB patients was 40%, I would have 80% power (α=0.05) to detect a difference in 3-month sputum sterility of 49.6% vs. 30.4% between homozygotes and heterozygotes in 202 MDR patients. I increased the sample size to 300 to account for possible smaller effect sizes of diabetes and vitamin D status and to overcome some of the power lost by multiple testing.

2.1.2.2 Study design and participants

A prospective cohort study to recruit new MDR/RR-TB patients was conducted in 16 DTUs and Pham Ngoc Thach Hospital in HCMC between March 2017 and October 2020. Eligible adult participants included pulmonary MDR-TB patients confirmed by GeneXpert MTB/RIF, LPA or DST with age ≥ 18 years. Patients were excluded if they had previous MDR-TB treatment, or had signs of TB meningitis, or had received more than 7 days of drug resistant anti-TB treatment, or were pregnant. Study staff approached potential participants to explain the study and obtain written consent.

2.1.2.3 Treatment

Patients were treated with the standardized MDR-TB regimen according to the Vietnam national TB program (NTP) guideline. Patients who initiated treatment from March 2018 to July 2019 received longer regimen for 20 months. Patients who initiated treatment after July 2019 received either longer or shorter regimen according to NTP guidelines (Table 2.1).

Treatment for diabetes mellitus (DM) and other co-morbidities was at the decision of specialists.

2.1.2.4 Data and samples collection

Detailed clinical characteristics, history and physical examination were recorded into structured paper forms. Information on socio-demographics, TB history, smoking, alcohol use and previous DM history were collected using a questionnaire. Clinical assessments were performed every two

weeks in the first month, monthly until treatment completion and then every six months until the end of study. All data in the case research forms were entered into CliRes system and verified during study period.

Pretreatment posteroanterior chest X-rays (CXRs) were collected and read by a radiologic expert. Blood samples and sputum were collected before or within seven days of treatment. Aliquots of plasma, serum and whole blood were stored at -80^{o}C. Sputa were collected every two weeks in the first month, then monthly until patients could not produce sputum or completed their treatment. Patients were followed up until completion of treatment.

2.1.3 Rifampicin susceptible prospective study

2.1.3.1 Sample size justification

One of the main aims of this study was to evaluate the effect of leukotriene A4 hydrolase (*LTA4H)* genotype on DS pulmonary TB treatment response and outcomes. Previous study of Thuong *et al.* showed the frequency of heterozygote CT in Vietnamese TB meningitis patients was 45.2%, and CT genotype responded faster to TB meningitis treatment than homozygotes of CC and TT (86). I speculated that 2 month sputum sterility probability of all DS patients was 70%, I would have 80% power (α=0.05) to detect a difference in 3-month sputum sterility of 75.7% vs. 64.3% between homozygotes (CC and TT) and heterozygotes (CT) in 504 DS-TB patients. I increased the sample size to 600 to account for possible smaller effect sizes of diabetes mellitus and to overcome some of the power lost by multiple testing.

The effect of vitamin D on DS pulmonary TB was unclear. I aimed to explore the impact of vitamin D status MDR and DS pulmonary TB, therefore, I included 300 DS patients in the study of vitamin D status to have 1:1 ratio of MDR:DS-TB.

2.1.3.2 Study design and participants

Another prospective cohort of rifampicin susceptible pulmonary TB was conducted in 13 DTUs, which were all in 16 DTUs of the MDR-TB study, from April 2018 to July 2020. Eligible participants included pulmonary TB patients aged more than 18 years with no rifampicin resistant detected by either Xpert MTB/RIF, LPA or DST. Patients were excluded if they already initiated anti-tuberculosis treatment for more than three days or had symptoms of TB meningitis. Other extra-pulmonary TB was not an exclusion criteria.

2.1.3.3 Treatment

Patients received first line anti-tuberculosis treatment for six months (category IA regimen) or eight months (category II regimen) according to NTP guidelines (Table 2.1).

Table 2.1. Standardized TB treatment used in Vietnam during study time

Regimen	Drugs and duration	Indications
Category IA	**2RHZE/4RHE**	For new TB patients without rifampicin resistance
Category II	**2SRHZE/1RHZE/5RHE** or **2SRHZE/1RHZE/5$R_3H_3E_3$**	For TB patients with relapse, retreatment after lost to follow-up, unknown history of treatment AND without rifampicin resistance
Shorter regimen (9 month regimen)	**4-6 Km Lfx Pto Cfz Z $H_{high\ dose}$ E/5 Lfx Cfz Z E**	New pulmonary MDR/RR-TB patients with no history of previous MDR-TB treatment or with less than one month of second line drugs usage.
Longer regimen (20 month regimen)	**8 Km(Cm) Lfx Pto PAS Z E/12 Lfx Pto PAS Z E**	All MDR/RR-TB patients treated before July 2018, or MDR/RR-TB patients who did not qualify for shorter regimen

S: Streptomycin; R: Rifampicin; H: Isoniazid; Z: Pyrazinamide; E: Ethambutol; Km: Kanamycin; Cm: Capreomycin; Lfx: Levofloxacin; Pto: Protionamid; Cfz: Clofazimine; PAS: Para Aminosalicylic Acid

2.1.3.4 Data collection

Detailed clinical characteristics, history and physical examination were recorded into structured paper forms. Sputum were collected before commencement of treatment, every two weeks in the first month, then monthly until patients could not produce sputum or completed their treatment and on month 12 if they could produce sputum. Blood samples were collected within the first seven days since treatment initiation and were stored at -80ºC. Patients were followed for 12 months, which included four to six months after treatment completion.

2.2 MEASUREMENTS

2.2.1 Chest Xray (CXR) score

Pretreatment CXRs were assessed to calculate Timika's score (104). Briefly, the presence of cavities was reported, and the total percentage of affected lung was estimated. A cavity was reported when gas-filled space with wall thicker than 1mm in the lung field was identified. Affected lung percentage was estimated by dividing the lung fields into four quadrants using imaginary horizontal line through the hila. In each quadrant, the percentage of affected lung was estimated. Then the average affected lung was calculated for the total four quadrants. Timika's score was calculated by the percentage (0-100%) of affected lung plus 40 if cavity was present. All CXRs of two prospective cohorts were read by a radiologist who was blinded to all clinical characteristics, HIV status, DM status and treatment outcomes.

Patients were classified as having high lung damage when their Timika scores were equal or greater than 71, and having low lung damage when their Timika scores were less than 71.

2.2.2 *M.tb* bacterial burden

M.tb bacterial burden were assessed by using GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), an automated real-time PCR test that reports semi-quantitative results of high, medium, low and very low *M.tb* load. Sputa collected at entry of the study were decontaminated by NALC-

NaOH, and added with 1ml of phosphate buffer. The mixture were incubated with 2ml GeneXpert reagent for 15 minutes, then transferred into GeneXpert cartridge, and analyzed by GeneXpert machine (105).

I classified patients into binary level of bacterial burden with high burden when patients had either high or medium *M.tb* load, and low burden when patients had either low, very low or *M.tb* not detected by GeneXpert.

GeneXpert also provides the number of cycles needed to cross the detection threshold, called CT value. A CT value of 40 was assigned if *M.tb* was not detected. I calculated the mean CT value of five probes after excluding delayed value of the probe caused by rifampicin resistance.

2.2.3 Severity scores

To evaluate the total effect of host determinants on TB presentation, scoring systems were required to classify patients into mild and severe disease, rather than simply accessing the influence of host factors on individual signs and symptoms.

I used a previously defined symptom scoring system to differentiate mild and severe symptomatic TB patients (106) (107). TB related symptoms consisting of cough, low-grade fever, weight loss, night sweats, anorexia, hemoptysis and malaise were assessed. One point was given for the presence of each symptom to generate a symptom score from zero to seven. In this study, an arbitrary cut off point of four was used to differentiate between mild and severe symptomatic patients. Patients were considered to have mild symptoms when their symptom score was equal or less than four and severe symptoms when their symptom score was greater than four.

In addition to symptom score, I also modified a composite TB score developed by Gil-Santana *et al*. to assess TB disease severity (106). The authors combined symptom score, the presence of cavity on CXR and presence of acid-fast bacilli positive sputum smear. In this study, instead of the presence of cavity and positive smear, Timika's score (extent of lung damage on CXR) and GeneXpert level of sputum collected at diagnosis were used to compute the clinical score. One

point was given for severe lung damage (Timika's score greater than 71) and for high bacterial burden (medium or high GeneXpert level). The total TB severity score ranged from zero to nine and classified patients into mild (clinical score equal or less than five) and severe disease (clinical score greater than five) (106).

2.2.4 LTA4H genotyping

EDTA blood collected at entry to the study was stored in -80ºC, and then extracted genomic DNA at the end of recruitment. *LTA4H* genotyping was done according to the manufacturer manual. Briefly, *LTA4H* rs17525495 (C/T) polymorphism was analyzed by TaqMan genotyping assay and confirmed by sequencing. Real-time polymerase chain reaction (PCR) analysis by Light Cycler 480, Roche was used for Taqman genotyping assay. Sequencing was analyzed with the Bigdye Terminator v3.1 Cycle Sequence kit (Applied BioSystems, Thermo Fisher Scientific, Massachusetts, USA) on the Genetic Analyzer machine (Applied BioSystems, Thermo Fisher Scientific, Massachusetts, USA) (86).

2.2.5 Cytokines measuring

A panel of 8 cytokines including tumor necrosis factor α (TNF- α), interferon γ (IFN-γ), interleukin 1β (IL-1β), IL-2, IL-6, IL-10, IL-13 and IL-12p70 was measured using 50µl serum stored at -80ºC. The concentrations of cytokines were quantified using magnetic bead-based multiplex method with Human Premixed Multi-Analyte Kit-8 cytokines (RnD Systems, Minnesota, USA) according to the manufacturer instruction (108). Briefly, flourescent dyed-beads, coated with monoclonal antibodies, were used to quantify the concentrations of 8 cytokines in the serum samples. The bead-cytokine-antibody complex was incubated with streptavidin-PE and the flourescent intensity produced were recorded by multiplex array reader of FlexMAP 3D (Luminex, Texas, USA). The concentrations were calculated by xPONENT Software (Luminex, Texas, USA).

2.2.6 Vitamin D concentration measuring

25(OH)vitamin D is an inactive form of vitamin D that is stable with long half-life. It is considered to be the best indicator for vitamin D status. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been the most accurate method to measure 25(OH)vitamin D (109). 100µl plasma collected at baseline were stored at -80^{o}C until the assay were performed after recruitment completion. Levels of 25(OH)vitamin D_2 and D_3 were measured by LC-MS/MS technique using UHPLC-MS/MS-8050 Shimadzu system. Inter-assay and spiked recoveries for 25(OH) vitamin D_2 and D_3 were performed using control plasma. Non-zero calibrators varied within 5% concentrations for all 5 points in the calibration curve and the correlation coefficient R^2>0.99 for validation. The limit of detection and limit of quantification for 25(OH)vitamin D were 0.83ng/ml and 2.48ng/ml, respectively.

2.2.7 Glycosylated hemoglobin (HbA1C) and fasting plasma glucose (FPG)

Venous blood samples were collected to measure HbA1C and FPG within seven days of TB treatment and analyzed within four hours after sampling. HbA1C was measured using high-performance liquid chromatography method.

2.2.8 Mycobacterial culture

All sputum samples were decontaminated by NALC-NaOH and then mixed with 1ml of phosphate buffer. 200µl of the mixture were cultured using BACTEC Mycrobacteria growth indicator tube (MGIT) 960 (Becton Dickinson Diagnostics, New Jersey, USA). MGIT culture were considered to be positive if it detected *M.tb* growth within 56 days, negative if no detection of *M.tb* growth after 56 days (105). The *Mycobacterial* strains collected in the MGIT cultures were re-cultured in Lowenstein-Jensen (LJ) solid media to confirm the existence of *M.tb*. The strains suspected to have contaminated in MGIT were checked by Ziehl Neelsen smears for morphology. If AFB were found in Ziehl Neelsen smear of MGIT, stored sputum were defrosted, decontaminated and re-cultured.

Culture result was classified as “positive” if the result of LJ was positive, “negative” if either LJ or MGIT was negative.

2.2.9 Time to sputum culture conversion

Culture conversion for MDR-TB was defined as two consecutive negative cultures taken at least 30 days apart. The time to sputum culture conversion was the duration in days from the time of treatment initiation to the first of two consecutive negative cultures in MDR-TB patients.

Culture conversion for DS-TB was defined as at least one negative culture, and time to culture conversion for DS-TB was the duration in days from the time of treatment initiation to the date of culture conversion. Censor was defined when patients did not have culture conversion until study time completion. Patients who either lost to follow-up or died before culture conversion were also considered as “censor”.

2.2.10 Proportions of culture conversion

Proportions of culture conversion at specified time points were used to assess the effect of host factors on early treatment response. Sputa collected within 14±3 days were used to calculate the proportion of conversion at day 14. Sputa collected within 30±7 days, 60±7 days and 90±7 days were used to assess the proportion of conversion at month 1, 2, and 3, respectively. Proportions of culture conversion was assessed at day 14, month 1, month 2 and month 3 for MDR-TB, and day 14, month 1 and month 2 for DS-TB.

MDR-TB patients who had at least two consecutive negative cultures at the observed time point were defined as “converted”. DS-TB patients just needed one negative culture to be considered “converted”. Patients were classified as “not converted” when they did not reach the criteria for conversion, or already converted but reversed to positive at the specified time points. Patients without previous conversion who either died or lost to follow-up or did not have sputa for at least 2 months before the observed time point were excluded for the analysis of that time point.

2.2.11 Genotypic drug resistance profile

200µl of decontaminated sputum collected at the beginning of treatment were cultured using BACTEC MGIT 960 (Becton Dickinson Diagnostics, New Jersey, USA). The *Mycobacterial* strains collected in the MGIT cultures were cultured in LJ media. The strains collected in LJ media were extracted DNA using cetyltrimethylammonium bromide method. Nextera XT DNA library preparation kit (Illumina, San Diego, California, USA) was used to prepare library from the genomic DNA according to the manufacturer manual (https://genome.med.harvard.edu/documents/libraryPrep/IlluminaNexteraXTProtocol.pdf). The 150-bp paired end sequencing with MiSeq reagent kits on the Nova sequencing platform (Illumina) was performed on the library to generate FASTQ data which were analyzed by Mykrobe predictor TB platform to identify the genotypic drug resistance profile (61) (110).

2.3 DEFINITIONS

2.3.1 MDR-TB registration groups

The Vietnam national TB program classified patient into registration groups as:

i) New: a patient who received none or less than one month of anti-tuberculosis treatment (can be called primary resistant TB case)

ii) Relapse: a patient whose most recent treatment outcome was “cured” or “treatment complete” and who is currently diagnosed with MDR-TB

iii) Treatment after default: a patient who returns to treatment after more than two consecutive months of interruption and is diagnosed with bacteriologically-positive TB by sputum smear microscopy or culture

iv) Failure of Category I: a patient who has received Category I treatment for TB and whose sputum smear remains positive at 5 months or later of the treatment

v) Failure of Category II: a patient who has received Category II treatment for TB and whose sputum smear remains positive at 5 months or later of the treatment

vi) Transfer in: A patient who received MDR treatment from another unit and has transferred in to continue treatment

vii) Other: Patients who do not fit into any of the above categories or who do not know their previous treatment outcome

2.3.2 Treatment outcomes for tuberculosis patients

Treatment outcomes were defined according to WHO designated definitions as below:

2.3.2.1 Drug susceptible tuberculosis:

Vietnam uses standard WHO definitions for DS-TB treatment outcomes as (111):

i) Cured: A patient whose sputum smear or culture was positive at the beginning of the treatment but who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.

ii) Treatment completed: A patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion.

iii) Failed: A patient whose sputum smear or culture is positive at 5 months or later during treatment.

iv) Died: A patient who died from any cause during treatment.

v) Lost to follow-up: A patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.

vi) Not evaluated: A patient for whom no treatment outcome is assigned. (This includes cases “transferred out” to another treatment unit and whose treatment outcome is unknown)

Treatment success: a sum of cured and completed treatment.

In this thesis, sputum culture results were used to classify treatment outcomes of DS-TB patients.

2.3.2.2 Multidrug resistant tuberculosis:

According to Vietnam NTP guidelines, MDR-TB treatment outcomes were defined as (63):

i) Cured: Treatment completed as recommended by the national guidelines without evidence of failure AND three (for 20 month regimen)/two (for 9 month regimen) or more consecutive cultures taken at least 30 days apart are negative after the intensive phase

ii) Treatment completed: Treatment completed as recommended by the national guidelines without evidence of failure BUT no record that three (for 20 month regimen)/two (for 9 month regimen) or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.

iii) Failed: Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: − lack of conversion by the end of the intensive phase , or − bacteriological reversion in the continuation phase after conversion to negative, or − evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs, or − adverse drug reactions (ADRs)

iv) Died: A patient who died from any cause during treatment

v) Lost to follow-up: A patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more

vi) Not evaluated: A patient for whom no treatment outcome is assigned. (This includes cases “transferred out” to another treatment unit and whose treatment outcome is unknown)

2.3.3 Diabetes and prediabetes

Participants’ DM status was identified using American Diabetes Association criteria (112). Participants were diagnosed with DM if either FPG ≥ 7 mmol/l at 2 different time points or HbA1C ≥ 6.5% and FPG ≥ 7 mmol/l; and prediabetes if HbA1C 5.7-6.4% or FPG 5.6-6.9mmol/l (112).

Those who met only one DM criteria (either HbA1C ≥ 6.5% or FPG ≥ 7 mmol/l) were invited to come back for a second FPG test to confirm the diagnosis. Those who had only one abnormal result of either HbA1C or FPG but failed to meet the criteria of DM diagnosis will be classified as prediabetes. Participants with history of DM whose HbA1C and FPG were in normal range were still considered having DM.

Information of anti-diabetes medications of DM patients was collected to further categorize DM patients into four groups: "non-DM", "prediabetes", "DM with metformin use" and "DM without metformin use".

DM patients whose HbA1C < 7% were considered having good glycemic control, and poor glycemic control when their HbA1C ≥ 7% (112).

2.3.4 Body mass index categories

The Asian cut-off points for BMI was used to identify severe underweight (<16kg/m^2), moderate underweight (16 - <17kg/m^2), mild underweight (17 - <18.5kg/m^2), normal weight (18.5- < 23kg/m^2), overweight (23 - <27kg/m^2), and obese (≥27kg/m^2) (113).

2.3.5 Vitamin D deficiency

According to the Endocrine society, vitamin D deficiency, insufficiency and normal levels were defined as total concentrations of 25(OH) vitamin D2 and D3 < 20ng/ml, 20-30ng/ml and > 30ng/ml, respectively (114). In this thesis, patients were classified into two groups for vitamin D status: deficiency when their vitamin D concentration < 20ng/ml, and sufficiency when their vitamin D concentration ≥ 20ng/ml.

2.4 STATISTICAL ANALYSIS

Data analysis was performed using R program version 3.5.2 (115). Graphs were performed using GraphPad Prism version 5.0.0 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

The baseline characteristics were summarized as number of cases and percentage for categorical variables and median with interquartile range (IQR) for continuous variables.

2.4.1 Statistical analysis in chapter 3

I used Kruskal Wallis test to identify the change of median of age and body mass index (BMI) over 5 years, and Wilcoxon test to compare median BMI of diabetes mellitus and non-diabetes patients. Chi squared test was used to compare categorical variables.

Multivariate logistic regression was used to identify risk factors contributing to poor treatment outcome. Treatment outcome was binary, defined as "success" (cured, completed) or "non-success" (death, failure, lost to follow-up) as in (63). The covariates were all the preceding proven risk factors. The model adjusted for potential risk factors of male gender, age and diabetes status. For variable age and BMI, the univariate analysis of the effect of age and BMI on treatment outcomes suggested to model them by index variable for age less than 60 years old and BMI greater than 21 kg/m2, and linear patterns for age greater than 60 years old and BMI less than 21 kg/m2. To minimize bias caused by missing data, I imputed them by using multiple imputation by chained equation ("mice" package in R (116)) and performed multivariate logistic regression models using both imputed data analysis and complete case analysis.

OpenRefine (https://openrefine.org) was used to clean patients' ward and district information and QGIS (www.qgis.org) was used to plot the geographic distribution of MDR-TB cases in HCMC.

2.4.2 Statistical analysis in chapter 4, 5 and 6

Mann-Whitney U test was used to compare skewed continuous variables, and Chi squared test to compare categorical variables. Univariate logistic regression was performed to evaluate the association between host factors and TB symptoms, severity, proportion of sputum culture conversion and treatment outcomes. Multivariate logistic regression models for proportion of sputum culture conversion at early time point (day 14, month 1, month 2 and month 3) were adjusted

for confounding factors, which were identified by directed acyclic graphs, generated through a web-based tool at http://www.dagitty.net/dags.html.

Kaplan-Meier survival curves were built to visualize the differences of time to sputum culture conversion between host genotypes, DM and vitamin D status. Restricted mean survival time (RMST) (117) ("survRM2" package in R) was used to calculate the average culture positive time from the start of treatment to day 30, 60, 120 and day 180.

Biomarkers with more than 50% of samples above detectable levels were analyzed as continuous variables. Log-transformation of cytokine concentrations and time to sputum conversion was performed to adjust for skewness. Linear regression was performed to evaluate the correlation between vitamin D concentration and other continuous variables (mean CT value, Timika score, symptom score, clinical score, levels of TNF-α and IL-6).

2.5 ETHICAL APPROVAL

The three aforementioned studies were approved by Institutional Review Boards at Pham Ngoc Thach Hospital, HCMC, Vietnam (Reference number: 546/NCKH-PNT and 547/NCKH-PNT) and Oxford Tropical Research Ethics Committee (OxTREC Reference: 24-17 and 33-17). Individual written informed consent for retrospective study was waived by the Institutional Review Board because this is a retrospective collection and data were recorded and analyzed anonymously. Written informed consent was obtained from all patients, or their representatives if patients were incapacitated, of prospective studies of MDR-TB and rifampicin susceptible TB.

Chapter 3

MULTIDRUG RESISTANT TUBERCULOSIS IN HO CHI MINH CITY

3.1 BACKGROUND

Ho Chi Minh City (HCMC), located in the south of Vietnam, is the most populous city in Vietnam with more than ten million inhabitants and 24 administrative districts in 2020. HCMC is also the center for TB and drug-resistant TB management in southern Vietnam. In 2009, the Vietnam NTP initiated the Programmatic Management of Drug-resistant TB under the support of Global Fund (Geneva, Switzerland) to provide free treatment and support for MDR-TB patients (16). Pham Ngoc Thach hospital is a tertiary referral center for lung diseases and TB patients in Southern Vietnam, which provided treatment for 81% of registered MDR-TB patients in Vietnam in 2010 (118). From 2011, all 24 district TB units (DTUs) of HCMC participated in the MDR-TB management. To facilitate MDR-TB management, a web-based tool called e-TB Manager was used in parallel with paper-based records. The number of patients enrolled in Programmatic Management of Drug-resistant TB rapidly increased from 2010 to 2014, 74% of whom had favourable outcomes, which included cured and treatment completed (16). Still a fourth had poor treatment outcomes, which was either died, failed the treatment or lost to follow-up. However, until now, there has been no information about the trend, treatment outcome and factors associated with poor treatment outcomes of MDR-TB patients in HCMC. In this study, I retrospectively investigated the demographic characteristics, trend, treatment outcomes and risk factors for poor treatment outcomes of MDR-TB in HCMC from 2011 to 2015. I also further dissected the characteristics of MDR-TB with diabetes mellitus (DM) and HIV co-infection, the two main comorbidities of TB.

3.2 RESULTS

The methods for this chapter were previously described in section 2.4.1 of chapter 2. Briefly, the descriptive analysis for MDR-TB patients with success and non-success outcome, between DM and non-DM, HIV and non-HIV was based on χ^2 test for category variables and Mann-Whitney test for continuous variables. Kruskal Wallis test was used to detect the trend of age

and BMI. Geographic distribution of MDR-TB cases in HCMC was plotted with OpenRefine (https://openrefine.org) and QGIS (www.qgis.org). Univariate logistic regression and multivariate logistic regression with complete case analysis and multiple imputation were used to assess the total effect of risk factors on MDR-TB treatment outcomes.

3.2.1 Characteristics of MDR/RR-TB patients

A list of 2,395 patients who received second line drugs in the NTP database was extracted using the search criteria of location of treatment inside HCMC and date of treatment initiation from January 2011 to the end of December 2015. As paper-based records contained more detailed information than web-based records, I collected all paper-based records in 24 DTUs whose treatment initiation dates were during study time. A total of 1,652 records were able to retrieve in DTUs, in which 27 cases received treatment in January 2016 and were excluded. Another 288 records were retrieved in Pham Ngoc Thach Hospital. One thousand nine hundred and thirteen records were included. Two nurses independently collected data from the paper-based records into structured paper forms and checked each other for accuracy at the end of the day. I checked every form for the first 100 forms, and then every 10 out of 100 for the next 300 forms. Afterwards, when errors were less than 5% of the cases, random check 10 out of 200 was done for the rest of paper-based records. All forms were inputted into electronic database. Data verification was done during data entry and analysis.

Sixty four patients in the STREAM trial (101) were excluded as they received different regimen (the shorter regimen) and might introduce bias for our treatment outcome analysis. I further excluded 61 mono and poly drug resistant cases together with one case of confirmed NTM and three patients who did not start treatment either at tertiary Pham Ngoc Thach Hospital or DTUs. Finally, a total of 2,266 cases were included for demographic analysis, and 2,240 cases for treatment outcome analysis after excluding 26 cases without treatment outcome information (Figure 3-1).

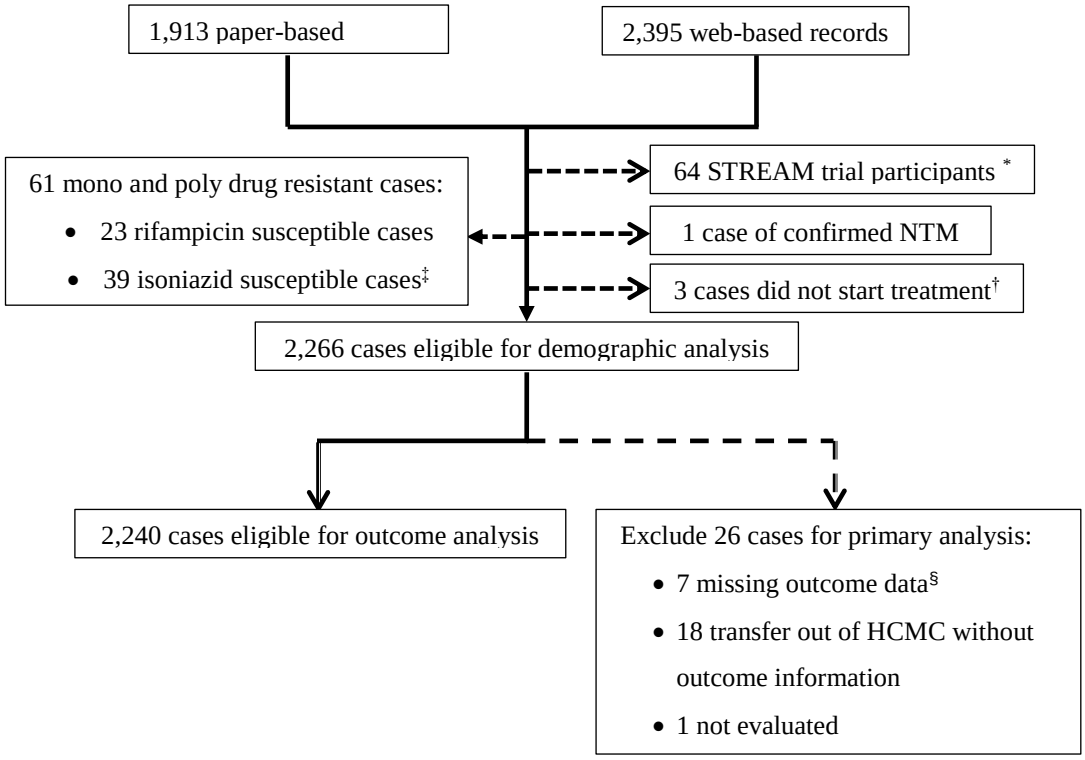


Figure 3-1 Flow diagram for eligible cases

* 64 STREAM trial participants from 2012 and 2015 were excluded as they received 9-month regimen and were not enrolled in the programmatic management of drug resistant TB.

† 3 patients died before MDR-TB treatment

‡ 39 isoniazid susceptible TB patients received MDR-TB treatment, including 38 cases with mono rifampicin resistance and 1 case with mono streptomycin resistance (susceptible to both isoniazid and rifampicin).

§ Missing outcome data: both treatment outcome and culture results were not recorded.

A total of 2,266 MDR/RR-TB cases were included. Twenty-three patients had two episodes of treatment during the study time, including eight relapse cases, 13 retreatment after lost to follow-up cases, and two retreatment after failure cases. Median age of MDR-TB patients was 43 years (IQR: 33-53 years) and did not change between 2011- 2015 (p = 0.481), 1,716 (75.7%) were male, 128 (5.6%) were registered as new patients, 678 (29.9%) were relapse cases, 1,364

(60.2%) were failure of either category I or II regimen (***Table 3.1***). A total of 204 patients (9.6% of tested patients) were HIV co-infected. Among MDR-TB patients with HIV co-infection, 33 (16.1%) registered as new patients, 21 (10.3%) had extra-pulmonary MDR-TB including 10 (4.9%) patients with MDR-TB meningitis. MDR-TB meningitis was more common in patients with HIV co-infection than in patients without HIV co-infection (p<0.001). Among 1,815 cases for whom BMI data were available, 57.8% was classified as underweight and 25.1% severely underweight. Median BMI did not significantly change over 5 years (p value for trend = 0.966). DM status was available for 1,189 patients (52.5%), 359 of whom (30.2%) had DM. Median BMI among patients with DM (20.0 kg/m2) was higher than among patients without DM (17.8 kg/m2) (p<0.001) and HIV co-infection in patients with DM (0.9%) was lower than among patients without DM (9.8%) (p<0.001).

Among 2,240 MDR-TB patients with treatment outcomes available, patients who had success treatment outcomes were heavier (BMI: 18.36 kg/m^2 (IQR:16.34-20.38) vs 16.44 [14.72-18.160] in non-success, p<0.001), less likely to have HIV co-infection (6.5% in success vs 17.9% in non-success, p<0.001) and more likely to have smear negative TB at the time of diagnosis (22.5% in success vs 16.2% in non-success, p=0.002) (***Table 3.1***).

Table 3.1. Characteristics of 2,266 MDR-TB patients in HCMC from 2011 to 2015, and of 2,240 MDR-TB patients stratified by treatment outcomes

Characteristic	**All patients N= 2,266**	**Patients with treatment outcomes N=2,240**		
		Success n=1642	**Non-success n=598**	**P value***
Age at diagnosis (years)	43 [33-53]	43 [32.5-53.5]	43 [34-52]	0.138
≥ 60 years old	197 (8.7%)	130 (7.9%)	66 (11%)	
18-60 years old	2,037 (89.9%)	1,488 (90.6%)	525 (87.8%)	

Characteristic	All patients N= 2,266	Patients with treatment outcomes N=2,240		
		Success n=1642	Non-success n=598	P value*
≤18 years old	32 (1.4%)	24 (1.5%)	7 (1.2%)	
Male	1,715 (75.7%)	1,231 (75.0%)	471 (78.8%)	0.071
BMI at diagnosis (kg/m^2)	17.86 [15.76-19.96]	18.36 [16.34-20.38]	16.44 [14.72-18.16]	<0.001
Registration group				
New	128 (5.6%)	79 (4.8%)	46 (7.7%)	
Relapse	678 (29.9%)	512 (31.2%)	156 (26.1%)	
Failure of regimen 1	512 (22.6%)	403 (24.5%)	104 (17.4%)	
Failure of regimen 2	852 (37.6%)	613 (37.3%)	233 (39%)	
Treatment after lost to follow-up	47 (2.1%)	16 (1%)	31 (5.2%)	
Transfer	1 (0%)	1 (0.1%)	0 (0%)	
Other	46 (2%)	18 (1.1%)	28 (4.7%)	
Site of disease				0.463
Pulmonary	2,237 (98.7%)	1,626 (99%)	590 (98.7%)	
Extra pulmonary	66 (3%)	41 (2.5%)	19 (3.2%)	
Multi-organ **	37 (1.7%)	25 (1.5%)	11 (1.8%)	
HIV positive	204/2,136 tested for HIV (9.6%)	102/1558 tested for HIV (6.5%)	99/554 tested for HIV (17.9%)	<0.001
DM	359/1,189 (30.2%)	285/837 (34.1%)	71/334 (21.3%)	<0.001
Unknown history of DM	1,077 (47.5%)	805 (49.0%)	264 (44.1%)	

Characteristic	All patients N= 2,266	Patients with treatment outcomes N=2,240		
		Success n=1642	Non-success n=598	P value*
AFB smear at baseline				0.002
Positive	1,748 (77.1%)	1,244 (75.8%)	490 (81.9%)	
< 1+	158 (9%)	126 (10.1%)	31 (6.3%)	
1+	895 (51.1%)	662 (53.2%)	228 (46.5%)	
2+	357 (20.5%)	249 (20%)	104 (21.2%)	
3+	275 (15.7%)	167 (13.4%)	105 (21.4%)	
Unknown grade	63 (3.7%)	40 (3.2%)	22 (4.5%)	
Negative	475 (21%)	369 (22.5%)	97 (16.2%)	
Not recorded	43 (1.9%)	29(1.8%)	11 (1.8%)	
Culture at diagnosis				
Positive	1,371 (60.5%)	1,017 (61.9%)	339 (56.7%)	0.028
Negative	178 (7.8%)	139 (8.5%)	39 (6.5%)	0.157
*Non-Tuberculosis Mycobacterium (but Xpert positive)***	4 (0.2%)	3 (0.2%)	1 (0.2%)	1.000
Contaminated	19 (0.8%)	16 (1%)	3 (0.5%)	0.413
Not recorded	694 (30.6%)	467 (28.4%)	216 (36.1%)	<0.001

* P value for comparing characteristics of success and non-success patients

** involved both pulmonary and extrapulmonary TB

*** All four cases of culture positive for Non-Tuberculosis Mycobacterium also had GeneXpert detected Mycobacterium tuberculosis.

Value are presented as number (%) for category variables and median [IQR] for continuous variables

3.2.2 MDR-TB with comorbidities

HIV co-infection and DM was known to be the risk factors for poor treatment outcomes in TB in previous studies. In this cohort, DM was the most common comorbidity. I further explored the characteristics of MDR-TB patients with HIV co-infection and DM.

3.2.2.1 Diabetes mellitus

During 2011 to 2015, DM was not routinely tested for MDR-TB patients. Random blood glucose was indicated in patients hospitalized to initiate MDR-TB treatment. However, there were 1,069 cases without recorded DM status. Among 1,189 cases with recorded DM status, 359 (30.2%) MDR-TB patients had DM. DM was associated with older age (median age 51 vs 41, p<0.001), higher median BMI (median BMI 20.0 vs 17.8, p<0.001) and more likely to have an AFB smear positive (85.4% vs 77.5%, p=0.003) compared to non-DM MDR-TB (p<0.001). The prevalence of HIV in DM patients (0.9%) was significantly lower than that in non-DM patients (9.77%) (p<0.001) (***Table 3.2***). MDR-TB patients with and without DM had similar rate of previous TB treatment (96.4% in DM vs 93.7% in non-DM, p=0.085) and of previous MDR-TB treatment (2.6% in DM vs 3.0% in non-DM, p=0.900).

Table 3.2. Characteristics of DM and non-DM MDR-TB patients in HCMC, 2011-2015

Characteristics	**DM** ***N=359***	**Non-DM** ***N=830***	**p value**
Age (Median [IQR])	51.0 [45.0-57.0]	41.0 [30.0;51.0]	<0.001
Male gender	260 (72.4%)	629 (75.8%)	0.249
BMI (Median [IQR])	20.0 [17.7-22.2]	17.8 [15.8-19.9]	<0.001
HIV positive	3 (0.9%)	77 (9.8%)	<0.001
Extra-pulmonary MDR-TB	19 (2.3%)	3(0.8%)	0.136
MDR-TB meningitis	8 (0.9%)	0 (0.0%)	0.115
Previous TB treatment	346 (96.4%)	773 (93.7%)	0.085

Characteristics	DM *N=359*	Non-DM *N=830*	p value
Previous MDR-TB treatment	9 (2.6%)	22 (3.0%)	0.900
AFB smear grade at diagnosis			<0.001
Negative	52 (14.6%)	178 (22.5%)	
Positive < 1+	30 (8.4%)	36 (4.6%)	
Positive 1+	164 (45.9%)	297 (37.5%)	
Positive 2+	67 (18.8%)	129 (16.3%)	
Positive 3+	39 (10.9%)	99 (12.5%)	
Positive unknown grade	5 (1.4%)	53 (6.7%)	

Value are presented as number (%) for category variables and median [IQR] for continuous variables

3.2.2.2 HIV co-infection

HIV was routinely tested in MDR-TB patients; however, there were 130 (5.7%) cases without recorded HIV status, increasing from 1.48% in 2011 to 6.13% in 2015. Among 2,137 cases with HIV status, the prevalence of HIV increased from 5.76% in 2011 to 13.1% in 2015 (p value for trend < 0.001).

Table 3.3 described the characteristics of MDR-TB HIV co-infected patients. Among 204 HIV co-infected patients, 172 (84.3%) were male, with a median age of 34 years (IQR: 30-38) and median of BMI of 17 kg/m^2 (IQR: 15.6-18.7). MDR-TB HIV co-infected patients were found to have significantly higher rates of no previous history of TB treatment (16.2% were registered as new in HIV vs 4.2% in non-HIV, p<0.001), of extra-pulmonary MDR-TB (10.7% vs 1.97%, p<0.001), especially TB meningitis (4.88% vs 0.36%, p<0.001), of smear negative at the time of diagnosis (35% vs 20.2%, p<0.001). Eighty-four (41.2%) HIV patients were treated with ART prior MDR-TB treatment. One hundred and twenty two MDR-TB patients who were diagnosed with HIV infection at the time of TB diagnosis received ART treatment after at least 2 months of MDR-TB treatment.

Table 3.3. Characteristics of MDR-TB patients with and without HIV co-infection

Characteristics	HIV *N=204*	Non-HIV *N=1932*	p value
Age (Median [IQR])	34.0 [30.0-38.0]	44.0 [32.0;53.0]	<0.001
Male gender	172 (84.3%)	1444 (74.7%)	0.003
BMI (Median [IQR])	17.0 [15.6-18.7]	18.0 [16.0-20.4]	<0.001
DM comorbidity	3 (3.75%)	330 (31.7%)	<0.001
Registration group			<0.001
New	33 (16.2%)	82 (4.25%)	
Relapse	73 (35.8%)	568 (29.4%)	
Failure of regimen 1	31 (15.2%)	458 (23.7%)	
Failure of regimen 2	54 (26.5%)	754 (39.1%)	
Retreatment after lost to follow-up	9 (4.41%)	34 (1.76%)	
Extra-pulmonary MDR-TB	21 (10.3%)	38 (1.97%)	<0.001
MDR-TB meningitis	10 (4.9%)	7 (0.36%)	<0.001
History of previous TB treatment	170 (83.3%)	1849 (95.7%)	<0.001
History of MDR-TB treatment	8 (4.73%)	38 (2.09%)	0.900
AFB smear grade at diagnosis			<0.001
Negative	70 (34.7%)	383 (20.2%)	
Positive < 1+	6 (2.97%)	146 (7.2%)	
Positive 1+	72 (35.6%)	755 (39.9%)	
Positive 2+	28 (13.9%)	310 (16.4%)	
Positive 3+	20 (9.9%)	241 (12.7%)	
Positive unknown grade	6 (2.97%)	57 (3.0%)	

Value are presented as number (%) for category variables and median [IQR] for continuous variables

3.2.3 Drug resistant profile

During the study time, both solid and MGIT culture were used for DST. A total 502 DST results of 490 patients were retrieved. Ten patients had two DST results at different time points, and one patient had three DST results. Among the 490 patients with one DST result, 55.0% and 63.1% had resistance to pyrazinamide and ethambutol, respectively. Resistance to fluoroquinolones and injectable agents was seen in 12.7% and 8.1% of isolates, respectively (***Table 3.4***). Among 378 patients with DST to second-line drugs, 63 (16.7%) had pre extensively drug-resistant (XDR) TB and 8 (2.1%) had XDR-TB.

Table 3.4. Frequency of first- and second-line drug resistance of MDR-TB in HCMC, 2011-2015

Drug resistance	n/ total tested (%)
Patients with DST result*	490
First-line drugs	
Pyrazinamide	210/382 (55.0%)
Ethambutol	298/472 (63.1%)
Streptomycin	438/455 (96.3%)
Second-line drugs	
Fluoroquinolones†	48/378 (12.7%)
Any injectable agents†	31/384 (8.1%)
All injectable agents	9/115 (7.8%)
Cycloserine	2/240 (0.8%)
Ethionamide/Prothionamide	21/223 (9.4%)

* A total of 502 DST of 490 patients were retrievable

†fluoroquinolones include moxifloxacin, levofloxacin, ofloxacin

‡ injectable agents include kanamycin, amikacin and capreomycin

3.2.4 MDR-TB trend

I explored the trend of MDR-TB over five years to visualize its burden (Figure 3-2). Number of notified MDR/RR-TB patients decreased by 9% between 2011-2012, and increased an average of 15.9% annually from 2012 to 2015. From 2011 to 2015, the number of MDR-TB

cases and notification rate increased 41.0% and 24.7%, respectively. In 2011, the notification rate of MDR-TB was 5.61 per 100,000 population, and increased to 6.97 per 100,000 population in 2015.

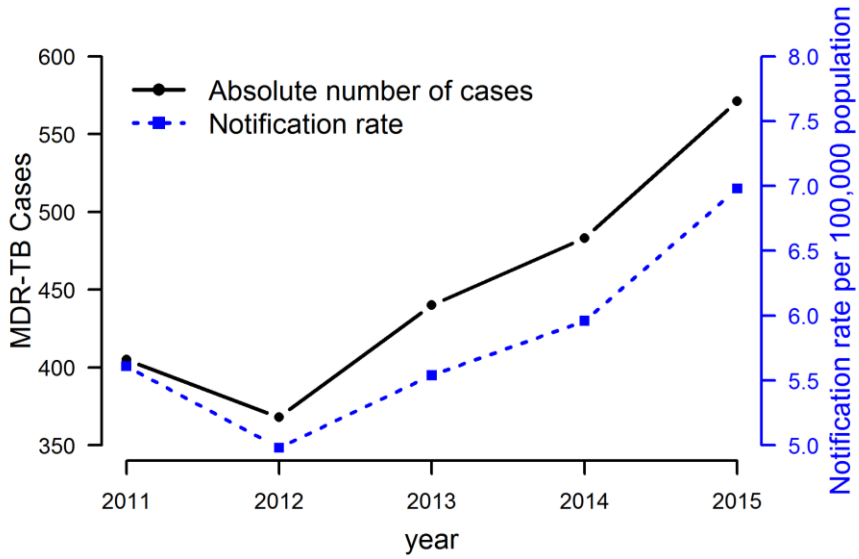


Figure 3-2: MDR-TB trend for a five year period. The absolute number of MDR-TB cases are shown in the solid line, and the notification rate per 100,000 population in the dashed line.

HIV co-infection and DM were two most important comorbidities in TB patients and were associated with poor treatment outcomes of TB and MDR-TB in previous studies. The prevalence of HIV and DM was plotted to observe the trend of those comorbidities to explore their impacts on MDR-TB. Prevalence of DM increased 1.7 fold from 20.2% in 2011 to 34.5% in 2015 (p for trend <0.001) and HIV increased 2.3 fold from 5.76% in 2011 to 13.1% in 2015 (p for trend <0.001) while number of patients without record of HIV tests increased from 6 (1.48%) in 2011 to 35 (6.13%) in 2015 (Figure 3-3).

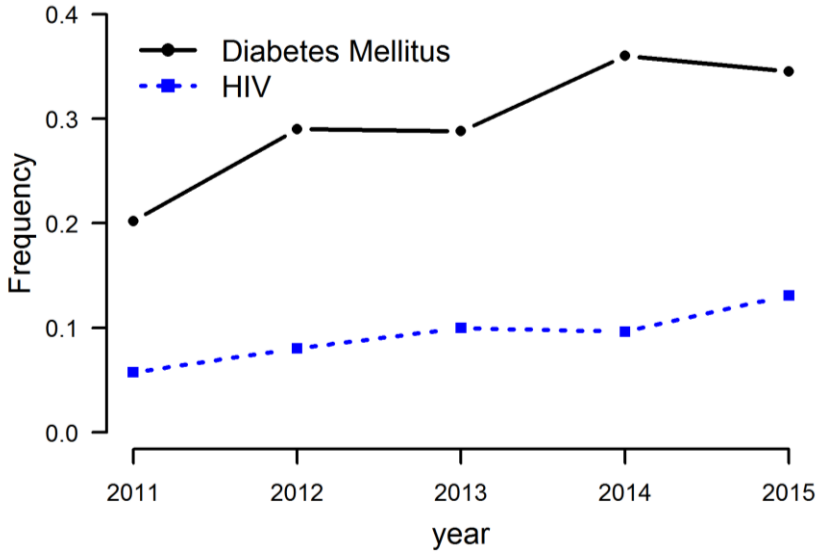


Figure 3-3. Increasing HIV and DM prevalence among MDR-TB patients. Prevalence of DM among MDR-TB patients is shown in black solid line, and that of HIV in blue dashed line.

3.2.5 Dynamics of MDR over total TB cases

Identifying spatial temporal pattern of MDR over total TB cases might be of public health importance in order to appropriately allocate facilities and efforts. I calculated the total MDR-TB cases treated in each DTUs, and then divided by number of total TB cases in each DTUs that were extracted from annual report of TB program in HCMC. The proportion of MDR over total TB cases was plotted using color scale, with yellow for < 2%, pale orange for 2 – <4%, orange for 4 – <6% and red for area with ≥ 6%.

There was an increasing trend of MDR proportion among most districts, notably those in the center of HCMC (Figure 3-4). The proportion of MDR over total TB cases was 3.45% for the period of 2011 to 2015. This proportion was increasing from 2.89% in 2011 and 2.80% in 2012 to 3.46% in 2013, 3.74% in 2014 and 4.43% in 2015. Areas with proportion of MDR/TB ≥ 4% (with red color) were mainly in the center of HCMC in 2011 and steadily spreading to much larger areas in 2015. The areas with MDR proportion ≥ 4% included only four districts (district 1, 5, 11 and Binh Thanh), and increased to 17 districts in 2015. In 2011, only district 1 had MDR/TB proportion > 6%. In 2015, five other districts reached this threshold, making a total

of six districts had the proportion > 6%. For a period of 5 years, district 1 (downtown of HCMC) had the highest mean proportion of 5.96%, followed by district 10 (4.95%), district 6 (4.95%).

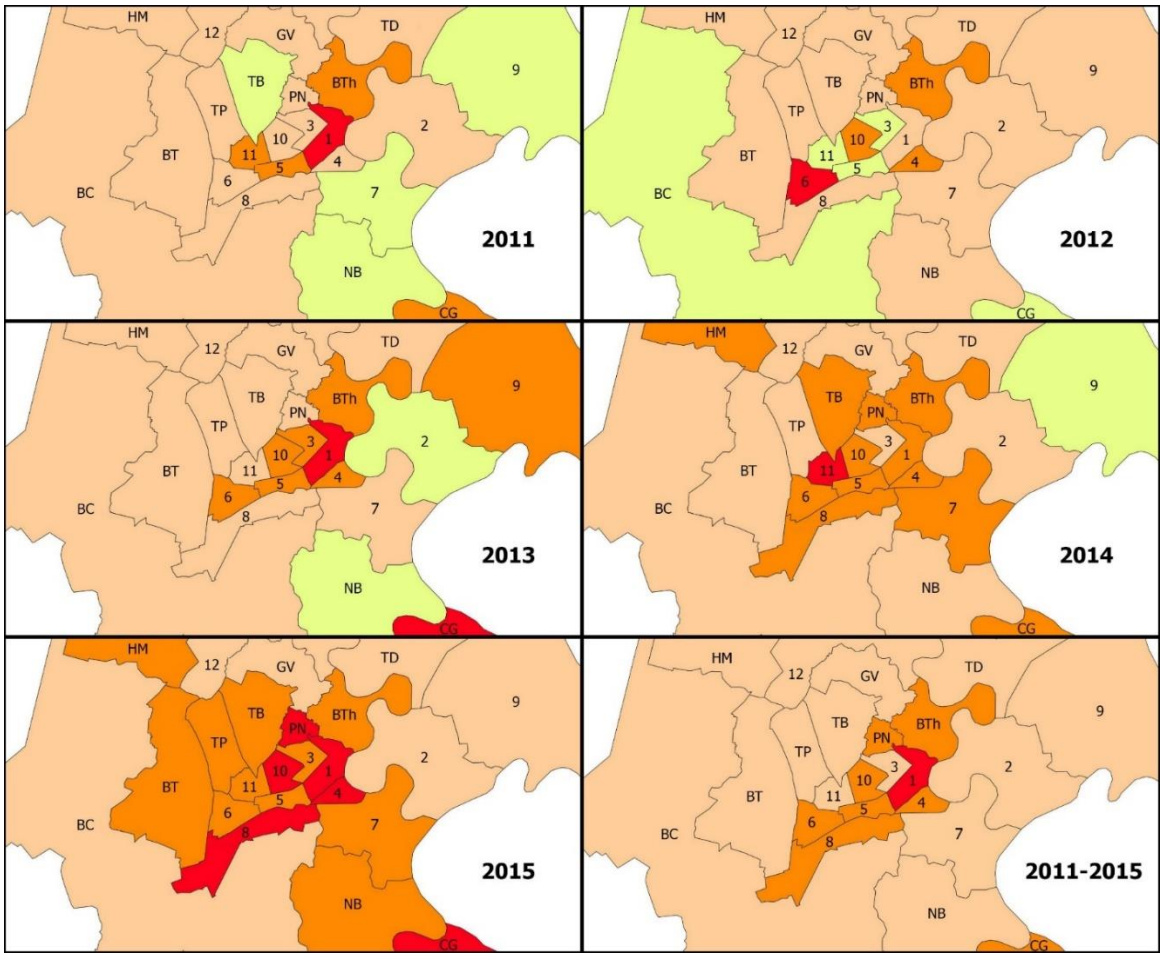


Figure 3-4. Increasing proportion of MDR over total TB from 2011 to 2015

Abreviations for district names: TB: Tan Binh, TP: Tan Phu, BT: Binh Tan, BTh: Binh Thanh, HM: Hoc Mon, GV: Go Vap, TD: Thu Duc, NB: Nha Be, CG: Can Gio

3.2.6 Treatment outcomes

Of 2,240 MDR-TB patients whose treatment outcomes were retrievable, successful outcomes were achieved in 1,641 (73.3%) patients, including 55.6% who were cured and 17.7% who completed treatment but for whom data on cure were unavailable (***Table 3.5***). Among those with

unsuccessful outcomes, 10.1% died, 5% failed treatment and 11.6% were lost to follow-up. Ten of 21 (47.6%) MDR-TB patients with TB meningitis had successful outcomes, while nine (42.6%) died and two (9.5%) were lost to follow-up.

In this cohort, 64 patients were diagnosed with pre-XDR-TB, of whom 53.1% had a successful outcome, 14.1% died, 23.4% failed treatment and 7.8% were lost to follow-up. Of 8 XDR-TB patients, 1 (12.5%) was cured with a bedaquiline-containing regimen, 2 (25%) died, including 1 who received a bedaquiline-containing regimen, and 5 (62.5%) failed.

Table 3.5. Treatment outcomes of 2,240 MDR-TB patients in HCMC, 2011-2015

Treatment outcome	2011 n = 405	2012 n=365	2013 n=438	2014 n=476	2015 n=556	Total N=2,240
Cured (n, %)	246 (60.7%)	190 (52.1%)	225 (51.4%)	289 (60.7%)	296 (53.2%)	1,246 (55.6%)
Completed	64 (15.8%)	80 (21.9%)	85 (19.4%)	71 (14.9%)	96 (17.3%)	396 (17.7%)
Died	27 (6.67%)	39 (10.7%)	45 (10.3%)	51 (10.7%)	64 (11.5%)	226 (10.1%)
Failed	19 (4.69%)	20 (5.48%)	23 (5.25%)	22 (4.62%)	29 (5.22%)	113 (5%)
Lost to follow-up	49 (12.1%)	36 (9.86%)	60 (13.7%)	43 (9.03%)	71 (12.8%)	259 (11.6%)

3.2.6.1 Treatment outcomes of MDR-TB with HIV and DM

Figure 3-5 showed total treatment outcomes of MDR-TB patients, and then MDR-TB patients with and without HIV, and with and without DM. There was a significant decrease in success outcome in MDR-TB patients with HIV co-infection, but not in patients with DM.

Among 201 (9.5%) MDR-TB patients with HIV co-infection, 49 (23.0%) patients with HIV died, compared to 155 (8.1%) in non-HIV ($p<0.001$), and 42 (20.5%) HIV co-infected patients were lost to follow-up compared to 201 (10.5%) in non-HIV ($p<0.001$). Although cured rate was significantly lower in HIV (33.8% in HIV vs 58.0% in non-HIV, $p<0.001$), failure rate was similar (4.0% in HIV vs 5.2% in non-HIV, $p=0.569$) (***Table 3.6***).

Treatment success rate of 346 (30.4%) MDR-TB patients with DM was 80% with 214 (60.1%) cured and 71 (19.9%) completed the treatment, compared to 441 (54.1%) cured and 111

(13.6%) completed in non-DM patients (p=0.066 for cured rate, p=0.008 for treatment completed). Loss to follow-up was significant lower in DM patients (6.2% in DM vs 13.7% in non-DM, p<0.001). There was no significant difference in death and failure rate between the two groups (death rate: 9.6% in DM vs 11.9% in non-DM, p=0.258; failure rate: 4.2% in DM vs 6.5% in non-DM, p=0.160) (***Table 3.6***).

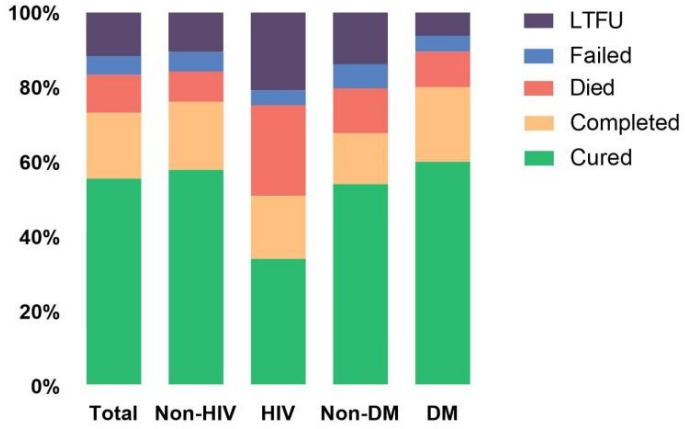


Figure 3-5. Treatment outcomes of MDR/RR TB patients, stratified by HIV status and DM mellitus

Table 3.6. Treatment outcomes of MDR-TB with DM and HIV

Treatment outcome	**Diabetes** ***N=356***	**Non-Diabetes** ***N=815***	**P value**
Cured	214 (60.1%)	441 (54.1%)	0.066
Completed	71 (19.9%)	111 (13.6%)	0.008
Died	34 (9.6%)	98 (11.9%)	0.258
Failed	15 (4.2 %)	53 (6.5%)	0.160
Lost to follow-up	22 (6.2%)	112 (13.7%)	<0.001

Treatment outcome	**HIV** ***N=201***	**Non-HIV** ***N=1911***	**P value**
Cured	68 (33.8%)	1108 (58.0%)	<0.001
Completed	34 (16.9%)	348 (18.2%)	0.721
Died	49 (24.4%)	155 (8.1%)	<0.001

Treatment outcome	HIV *N=201*	Non-HIV *N=1911*	P value
Failed	8 (4.0%)	99 (5.2%)	0.569
Lost to follow-up	42 (20.9%)	201 (10.5%)	<0.001

3.2.6.2 Characteristics of lost to follow-up patients

Lost to follow-up accounted for 11.6%, whose true treatment outcomes were not unknown. Given the free treatment and support for MDR-TB patients, those who failed to adhere the therapy might have poor treatment outcome and might be sources of infection to others, especially their caregivers and household contacts. Of 259 lost to follow-up patients, median treatment duration was 200 days (IQR: 60-340) with 56% lost during intensive phase, 32% had positive AFB smear and 35.9% had culture positive prior the time of lost to follow-up. Compared to patients with a successful outcome, lost to follow-up patients were more likely to have history of previous MDR-TB treatment (6.4% vs 1.2%, p<0.001), had higher rate of HIV co-infection (17.3% vs 6.6%, p<0.001) and lower rate of DM (16.4% vs 34.1%, p<0.001) (***Table 3.7***).

Table 3.7. Characteristics of lost to follow-up patients, compared to successfully treated patients.

Characteristics	Lost to follow-up *N=259*	Success *N=1642*	P value
Age (Median [IQR])	39.0 [32.0-48.0]	43.0 [31.0-52.0]	0.020
Male gender	207 (79.9%)	1231 (75.0%)	0.099
BMI (Median [IQR])	18.4 [16.5-20.6]	16.9 [15.4-18.7]	<0.001
HIV positive	42 (17.3%)	102 (6.55%)	<0.001
Previous TB treatment	240 (93.4%)	1560 (95.1%)	0.306
History of MDR-TB treatment	15 (6.4%)	18 (1.17%)	<0.001
Diabetes mellitus	22 (16.4%)	285 (34.1%)	<0.001
AFB smear grade at baseline			0.012

Characteristics	Lost to follow-up	Success	P value
	N=259	*N=1642*	
Negative	41 (16.1%)	369 (22.9%)	
Positive low grade†	119 (46.9%)	788 (48.8%)	
Positive high grade‡	84 (33.1%)	416 (25.8%)	
Positive unknown grade	10 (3.9%)	40 (2.48%)	

† low smear grade: AFB scanty or 1+

‡ high smear grade: AFB 2+ or 3+

3.2.7 Risk factors for poor outcomes

I evaluated the association between poor treatment outcome (death, failure and lost to follow-up) and predefined risk factors of HIV status, history of previous MDR-TB treatment, AFB smear grade, BMI (70) (119) and potential risk factors of gender, age and DM status.

The generalized additive models of age and BMI on treatment outcomes were plotted to diagnose their non-linear effects. This model suggested to treat them by index variable for age less than 60 years old and BMI greater than 21 kg/m^2, and linear patterns for age greater than 60 years old and BMI less than 21 kg/m^2 (Figure 3-6).

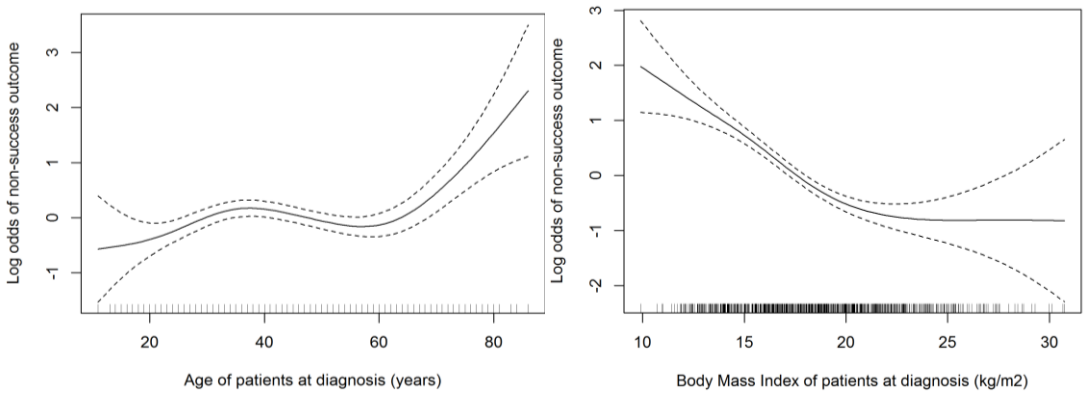


Figure 3-6: Effect of age and BMI on treatment outcome of MDR-TB

In univariate logistic regression model, MDR-TB patients with HIV co-infection increased risk of poor outcome by 3.08 times (95%CI: 2.29-4.13). During 2011-2015, MDR-TB patients who already had previous MDR-TB treatment were treated with the same regimen. Among 56 patients with previous MDR-TB treatment, 16 (28.6%) were cured, two (3.6%) completed the

treatment, 13 (23.2%) died, 10 (17.9%) failed the treatment and 15 (26.9%) lost to follow-up. Having history of previous MDR-TB treatment significantly increased risk of poor treatment outcomes (OR: 6.4, 95%CI: 3.68-11.58). Patients older than 60 years had 1.79 times (95%CI: 1.26-2.54) risk of poor treatment outcome for every increase of 5 years. Malnutrition with low BMI and AFB smear high grade were also associated with increased risks of poor treatment outcome (***Table 3.8***).

In multivariate models that included risk factors of age, DM, HIV co-infection, smear grade, BMI and history of previous MDR-TB treatment, the impact of older age, HIV, high smear grade, low BMI and history of previous MDR-TB treatment remained significant. The odds of having poor outcome in patients with previous MDR-TB treatment drastically increased from 6.4 (95%CI: 3.68-11.58) in univariate logistic regression model to 20.37 in multivariate logistic regression model (95%CI: 5.52-75.17). The impact of HIV co-infection in this multivariate model remained significant (aOR: 3.12, 95%CI: 1.66-5.84), and so did high AFB smear grade (aOR: 2.25, 95%CI: 1.28-3.93), older age (for patients > 60 years: aOR: 1.75, 95%CI: 1.28-2.39 per 5 year increase) and malnutrition with low BMI (for patients with BMI $\leq$ 21kg/m^2, aOR: 0.82, 95%CI: 0.76-0.89). Patients with DM had higher success rate, leading to decrease risk of poor treatment outcome in univariate model (OR: 0.52, 95%CI: 0.39-0.70), but this effect was insignificant in multivariate model (aOR: 0.84, 95%CI: 0.5-1.31) (***Table 3.8***).

Table 3.8. Complete case univariate analysis and multivariate analysis of risk factor for non-success outcome

Risk factors	**Success**	**Non-success**	**Univariate analysis**		**Multivariate analysis**	
	n (%)	**n (%)**	**OR**	**95% CI**	**aOR**	**95% CI1**
Gender: male	1232 (75.0%)	474 (78.9%)	1.24	0.99-1.55	1.55	0.99-2.42
Age $\leq$ 60 years (per 5y increase)			1.00	0.99-1.01	1.00	0.98-1.01
Age > 60 years (per 5y increase)			1.42	1.20-1.68	1.75	1.28-2.39
DM	285 (34.1%)	71 (21.1%)	0.52	0.39-0.70	0.84	0.55-1.31

HIV positive	103 (6.6%)	101 (18.1%)	3.08	2.29-4.13	3.12	1.66-5.84
Previous MDR-TB treatment	18 (1.2%)	38 (7.0%)	6.40	3.68-11.58	20.37	5.52-75.17
Low smear grade†	788 (48.8%)	259 (44.1%)	1.25	0.97-1.64	1.72	1.00-2.95
High smear grade‡	416 (25.8%)	209 (35.6%)	1.92	1.45-2.54	2.25	1.28-3.93
AFB positive unknown	40 (2.5%)	22 (3.7%)	2.10	1.18-3.67	2.70	1.08-6.78
BMI $\leq$ 21 (per 1kg/m^2 increase)			0.77	0.73-0.81	0.82	0.76-0.89
BMI > 21 (per 1kg/m^2 increase)			1.04	0.91-1.17	0.96	0.81-1.15

† low smear grade: AFB scanty or 1+, AFB smear negative as referent group

‡ high smear grade: AFB 2+ or 3+

OR: odds ratio, CI: confidence interval, aOR: adjusted odds ratio

To overcome the bias caused by missing data, I decided to perform multiple imputation analysis. Missing data was high among 2,240 MDR-TB cases with treatment outcomes. There was 442 (19.5%) cases missing data on BMI, 128 (5.6%) cases missing HIV status, 1,078 (47.5%) cases missing DM status, causing 1,469 observation deletions in multivariate analysis. The missing data in this cohort was missing at random. Therefore, I imputed missing data of DM mellitus status, weight, height, history of MDR-TB treatment and smear grade based on existing data of those variables and HIV status, patients' registration group, using multiple imputation by chained equation. There were only small differences in the results between complete case and multiple imputation analysis, except the odds of having poor treatment outcome in patients with previous MDR-TB treatment significantly decreased from 20.37 in complete case analysis to 5.53 in multiple imputation analysis (***Table 3.9***).

Table 3.9. Comparison of multivariate logistic regression models using complete case and multiple imputation analysis

Risk factor	Success n (%)	Non-success n (%)	Complete case analysis*		Multiple imputation analysis	
			aOR	95% CI	aOR	95% CI
Gender: male	1231 (75.0%)	471 (78.8%)	1.55	0.99-2.42	1.10	0.84-1.44
Age ≤ 60 years (per 5y increase)			0.98	0.90-1.07	1.01	0.96-1.06
Age > 60 years (per 5y increase)			1.79	1.26-2.54	1.45	1.14-1.79
DM	285 (34.1%)	71 (21.3%)	0.84	0.55-1.31	0.81	0.61-1.08
HIV positive	102 (6.5%)	99 (17.9%)	3.12	1.66-5.84	2.94	2.07-4.16
Previous MDR-TB treatment	18 (1.2%)	38 (7.0%)	20.37	5.52-75.17	5.53	2.85-10.72
Low smear grade†	788 (48.9%)	259 (44.1%)	1.72	1.00-2.95	1.47	1.08-2.00
High smear grade‡	416 (25.8%)	209 (35.6%)	2.25	1.28-3.93	2.06	1.49-2.87
AFB positive unknown	40 (2.5%)	22 (3.7%)	2.70	1.08-6.78	2.80	1.47-5.36
BMI < 21 (per 1kg/m^2 increase)			0.82	0.76-0.89	0.83	0.79-0.87
BMI ≥ 21 (per 1kg/m^2 increase)			0.96	0.81-1.15	1.06	0.93-1.2

† low smear grade: AFB scanty or 1+, AFB smear negative as referent group

‡ high smear grade: AFB 2+ or 3+

aOR: adjusted odds ratio, CI: confidence interval

Figure 3-7 outlined the results of multivariate logistic regression model using multiple imputation by chained equation. Independent risk factors of poor outcomes were older age (aOR for every increase of 5 years when patients are older than 60: 1.47, 95% CI: 1.19-1.80), HIV co-infection (aOR: 2.92, 95% CI: 2.06-4.14), having history of MDR-TB treatment (aOR: 5.65, 95% CI: 2.93-10.93), AFB positive (aOR: 1.48 for low smear grade, 95%CI: 1.08-2.03,

and aOR: 2.07 for high smear grade, 95%CI: 1.49-2.89), low BMI (aOR: 0.84 for every increase of 1kg/m2 for patients with BMI<21, p<0.001).

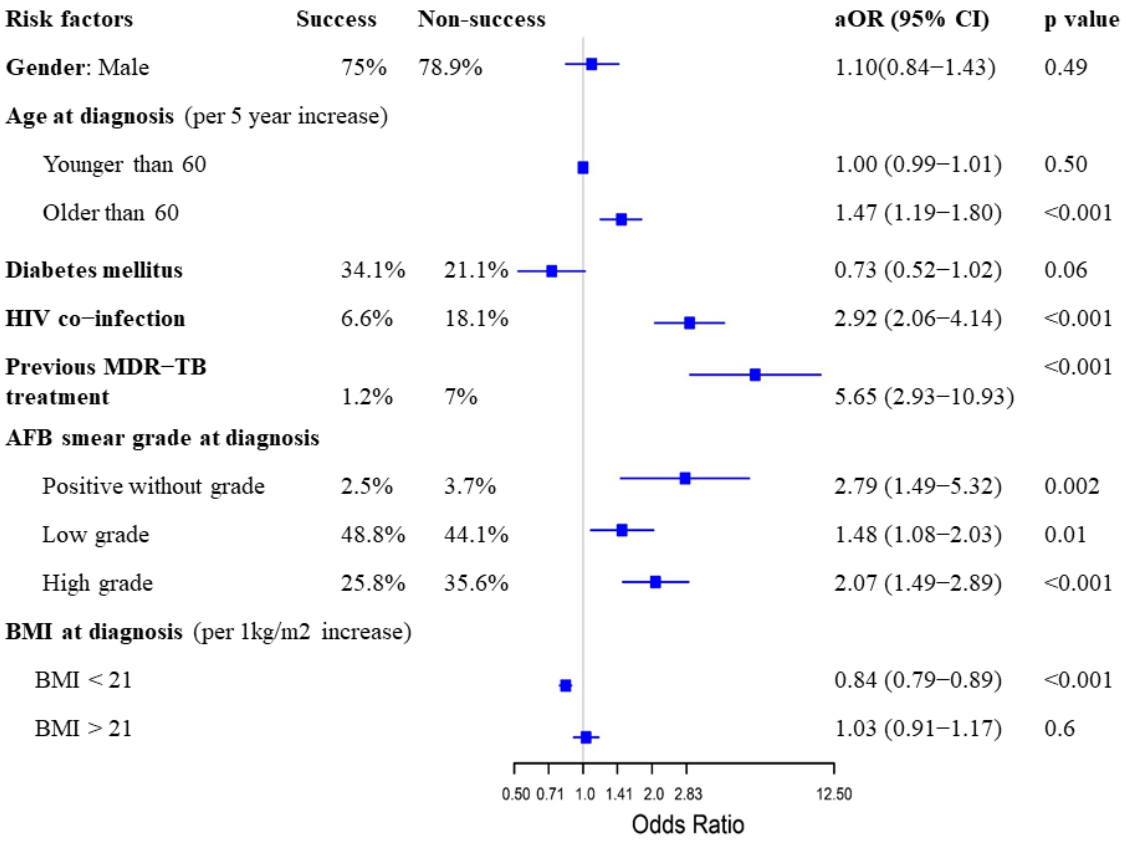


Figure 3-7: Forrest plot of multivariate multiple imputation logistic regression model showing risk factors for non-success outcome.

aOR, adjusted odds ratio; CI, confidence interval

I further performed multinomial multivariate logistic regression model to better understand about the effect of risk factors on death, failure and lost to follow-up. With treatment success as the referent group, risk factors for death included age older than 60 years (OR: 1.8, 95%CI: 1.27-2.58 per 5 year increase), HIV co-infection (OR: 5.5, 95%CI: 2.3-12.1), previously treated for MDR-TB (OR: 21.65, 95%CI: 4.96-92.7), AFB positive but unknown grade (OR: 4.03, 95%CI: 1.23-13.2), low BMI (OR: 0.74 for per 1kg/m^2 increase) (Table 3.10). Risk factors for failure were old age (OR: 1.68, 95%CI: 1.04-2.72), having history of previous MDR-TB treatment (OR: 26.46, 95%CI: 6.03-116.07) and high AFB smear grade (OR: 3.75, 95%CI: 1.22-11.54). Patients with HIV co-infection, history of previous MDR-TB treatment and low BMI increased risk of lost-to-follow-up. DM comorbidity did not increase risk of death, failure or lost to follow-up.

Table 3.10. Complete case multinomial multivariate logistic analysis of treatment outcomes

Predictor	Death*		Failure*		Lost to follow up*	
	aOR	95%CI	aOR	95%CI	aOR	95%CI
Male gender	1.68	0.86-3.28	1.31	0.61-2.80	1.71	0.88-3.30
Age ≤ 60 years (per 5y increase)	1.02	1.00-1.05	0.99	0.96-1.02	0.98	0.96-1.00
Age > 60 years (per 5y increase)	1.80	1.27-2.58	1.68	1.04-2.72	1.51	0.93-2.45
HIV positive	5.5	2.30-12.11	0.45	0.06-3.49	3.43	1.61-7.30
DM	1.26	0.68-2.31	0.67	0.30-1.49	0.64	0.32-1.28
Previous MDR-TB treatment	21.65	4.96-92.70	26.46	6.03-116.07	15.71	3.56-69.24
Low smear grade†	1.33	0.60-2.93	2.34	0.76-7.24	1.89	0.88-4.07
High smear grade‡	1.90	0.86-4.19	3.75	1.22-11.54	2.00	0.89-4.47
AFB positive unknown grade	4.03	1.23-13.21	1.28	0.13-12.47	2.38	0.64-8.82
BMI <21 (per 1kg/m^2 increase)	0.74	0.66-0.83	0.89	0.77-1.02	0.87	0.77-0.97
BMI > 21 (per 1kg/m^2 increase)	0.99	0.74-1.32	1.04	0.80-1.36	0.84	0.60-1.19

* Treatment success, including cured and completed, was the referent group for non-success outcome (death, failure, lost to follow-up).

† low smear grade: AFB scanty or 1+, AFB smear negative as referent group

‡ high smear grade: AFB 2+ or 3+

OR: adjusted odds ratio, CI: confidence interval

3.3 DISCUSSION

This is the first study to describe the characteristics and identify the risk factors for poor outcomes of MDR-TB in HCMC, Vietnam. MDR-TB cases and the proportion of MDR over total TB in HCMC, the largest city of Vietnam, increased from 2011 to 2015 although the incidence of TB in Vietnam has been declining (17). The improvement in diagnostic technologies with the introduction of GeneXpert in the end of 2012 and changes in MDR-TB diagnostic policies might be part of the reasons for the increasing numbers of notified cases, together with the ongoing transmission of drug-resistant TB.

The spatial temporal dynamics of MDR-TB over total TB cases showed the expanding pattern of MDR-TB during the study period. It reflected the tendency of increased MDR-TB, although the incidence of TB was decreasing (120), and strengthened the hypothesis of on-going transmission of MDR-TB in HCMC. The Programmatic Management of Drug resistant TB should explore the potential reasons for this increase in proportion of MDR. If this increase was due to improvement of diagnostic policies, a homogeneity of dynamics all over 24 DTUs should be observed. The map pointed that the central districts with dense populations had the highest proportion of MDR over total TB. Therefore, I suspected that there were clusters and on-going transmission of MDR-TB in those crowded areas.

High rates of failure of regimen 1 (22.6%) and regimen 2 (37.6%) in MDR-TB patients reflect the insufficient screening for drug resistance prior treatment of new and retreated patients, and highlight the need for drug resistance screening for all TB patients regardless of their TB history.

I found high rates of resistance to pyrazinamide (55.0%) and ethambutol (63.0%) in our MDR-TB cohort, as reported by other studies (121, 122). This may reflect the fact that the majority of MDR-TB patients (94.3%) already had exposure to first line anti-TB drugs and might have developed resistance to pyrazinamide and ethambutol during previous treatment. This questions the effectiveness of emperical use of these two drugs in the standardized MDR-TB regimen

(64) and emphasizes the need for an approved genotypic DST to rapidly detect pyrazinamide resistance.

Resistance rates to fluoroquinolones (12.7%) and injectable agents (8.1%) were comparable to those of the survey in Vietnam in 2011 (123) but lower than in South Korea (121) and average global rates (17). Resistance to second-line drugs were high although they are not used in the regimen 1 and 2. It might partly due to easy access to antibiotics in the community without prescription in Vietnam (124).

Patients who had previous MDR-TB treatment significantly increased the risk of death (OR: 21.65, 95%CI: 4.96-92.7), failed the treatment (OR: 26.46, 95%CI: 6.03-116.07) and lost to follow-up (OR: 15.71, 95%CI: 3.56-69.24). This high rate of unfavourable outcomes might be due to prolonged illness and the extended drug resistance.

Patients who were lost to follow-up might have more severe disease, as they had higher smear grade, higher rate of HIV co-infection and lower BMI at diagnosis. One third of them still had AFB smear positive at the time of lost to follow up, which posed a risk for on-going transmission to the community. Patients who were HIV co-infected and had history of previous MDR-TB episodes were at risk of lost to follow-up. Early identification and intensified support for this group might help to improve treatment adherence and outcome.

The prevalence of DM in our cohort (30.2%) was double that of other TB patients (13.7%) in Hanoi, Vietnam (118) and was almost 6 times higher that of general Vietnamese population in 2013 (5.4%) (125). The prevalence of DM comorbidity increased 1.7 times over 5 years. It showed association between DM and MDR-TB, and reflected the growing prevalence of DM in HCMC and also in Vietnam (125, 126) (127). However, there was no systematic screening for DM in MDR-TB patients as 47.5% of our cohort did not have record of DM status. Although DM was a known risk factor for poor treatment outcome of TB, developing MDR-TB and reducing sputum conversion rate during MDR-TB treatment (77, 78, 128), whether DM also leads to poor treatment outcome of MDR-TB is still controversial (77, 81, 129). DM MDR-TB

patients had higher BMI and 10 times lower prevalence of HIV co-infection than non-DM patients. Patients with DM were more likely to complete their treatment with significantly lower rate of lost to follow up (6.2% vs 13.7%, $p<0.001$), which agreed with study of Fisher-Hoch et al in Texas and Mexico (130). However, DM patients just showed an insignificant tendency to lower the odds of lost to follow-up (OR: 0.64, 95%CI: 0.32-1.28) after adjusted for gender, age, HIV status, history of previous MDR-TB treatment, smear grade and BMI. Moreover, in our cohort DM was not an independent risk for poor outcomes after adjusted for those factors, which agrees with pooled data analysis from cohorts in Latvia, Korea and Italy (131). Due to the unavailability of DM treatment information, I do not know whether the effect of DM on MDR-TB treatment was influenced by the use of metformin, a hypoglycemic agent that might improve TB treatment outcomes (132, 133). Despite this limitation, my study showed that DM is a common but neglected comorbidity in MDR-TB patients and should be screened for prior MDR-TB treatment.

The prevalence of HIV co-infection in MDR-TB patients (9.6%) was 2.4 times higher than that of HIV co-infection in new and relapse TB patients (17). Although HIV patients were younger, they were more malnourished, indicating that they had more severe disease than patients without HIV co-infection. They were more likely to get MDR-TB for their first TB episode (16.2% registered as new vs 4.25% in non-HIV group) and more likely to develop extra-pulmonary MDR-TB, especially TB meningitis. Death rate in HIV co-infected patients (24.4%) was 3 times higher than in non-HIV patients (8.1%). HIV co-infection was a major risk factor for death and lost to follow-up in our cohort. AFB smear, the initial test for TB in Vietnam and in many high burden lower middle income countries, is not sufficient to rule out TB in HIV patients as 35% of HIV patients had negative smear at the time of diagnosis. This might lead to delay in diagnosis and therefore worsen outcomes. Patients who were diagnosed with HIV and treated with ART before commencing MDR-TB treatment had lower risks of death and lost to

follow-up than HIV patients not on ART therapy at the start of MDR-TB. It emphasized the need to integrate HIV treatment and MDR-TB program.

There were only small differences in the results of multivariate logistic regression models using complete case analysis and multiple imputation. Under multiple imputation logistic regression analysis, HIV co-infection, positive baseline AFB smear, older age and previous treatment with second-line drugs are main risk factors for poor treatment outcomes in our cohort, which were also observed in cohorts in Estonia, Latvia, Philippines, Russia, Peru (119), Ukraine (70). Malnutrition was common (57.8%) and also a risk factor for poor outcome (OR: 0.81 for every 1kg/m^2 increase of BMI). Low BMI might be a consequence of severe disease and low social-economic status, which are well-known risk factors for poor outcome of TB. Programmatic Management of Drug resistant TB should focus on nutrition support to improve treatment outcomes.

The study has several limitations. It was a retrospective study and some records were irretrievable at the study time. Demographic information and records of smear, culture and DST was not completely recorded in the electronic database. The majority of patients (78.4%) did not have DST results, and I could not include drug resistance information into multivariate logistic regression models. Only the patients treated under the Programmatic Management of Drug resistant TB were included in this study. The MDR-TB patients treated in the private sectors were not included due to unavailable data. As MDR-TB diagnosis were only provided by DTUs and the tertiary Pham Ngoc Thach Hospital, I argued the patients treated in the private sectors were a portion of lost to follow up cases reported here. Therefore, I expected that our study reflected the true situation of MDR-TB in this city. Finally, the information on smoking and alcohol use were not available in our cohort, although they are known risk factors for poor outcome (71, 134). As retrospective study results are limited by missing data and other unrecorded factors, prospective studies are needed to comprehensively explore risk factors for poor treatment outcomes of MDR-TB.

3.4 CONCLUSION

This study emphasized the increasing trends of MDR-TB, HIV co-infection and DM comorbidity in HCMC between 2011 and 2015. There was also an expanding of MDR-TB in the total TB in Vietnam.

Rapid molecular DST is needed as more than half of patients with DST available had resistance to pyrazinamide and ethambutol, the two drugs used in MDR-TB regimen. DM was prevalent among MDR-TB patients, which urges for a systematic screening for DM. Patients with HIV, high smear grade, malnutrition and history of previous MDR-TB treatment are at high risk of poor outcomes and should receive additional medical care.

Chapter 4

IMPACT OF *LTA4H* GENOTYPE ON PULMONARY TUBERCULOSIS

4.1 INTRODUCTION

Inter-individual variation in disease manifestation and response to treatment has long been observed in many diseases. Advances in biomedicine have partially unveiled why some people are more susceptible to some diseases, and why some patients develop more severe diseases and rapidly progress towards poorer outcomes than others. Personalized therapy based on host factors to select an appropriate treatment is a novel and promising approach. In the scope of infectious diseases, antibiotics are usually indicated depending on the pathogens and their sensitivities. Host immune response regulating by genes plays an important role in pathogenesis and treatment outcomes, yet are mostly unexplored.

Tuberculosis meningitis (TBM) is the most severe form of TB with high mortality and neurological disabilities. Adjunctive dexamethasone therapy improved survival (135) and has become a standard care for TBM patients. Prasad *et al* reviewed nine randomized controlled trials to find that corticosteroids reduced mortality by 25% for TBM (136). It raised the question of whether the more common but less severe pulmonary TB also needs adjunctive corticosteroid therapy to improve treatment outcomes. Drug susceptible (DS) TB had much lower mortality and better treatment outcomes than multidrug resistant (MDR)-TB, where chemotherapy has lesser effects on *Mycobacterium tuberculosis (M.tb)*. Therefore, MDR-TB might be the form of pulmonary TB that needs adjunctive therapy. Understanding the impact of host genetics on pulmonary TB, especially MDR-TB, might help to identify the target for adjuvant therapy to improve treatment outcomes.

One of the potential host genetic factors is leukotriene A4 hydrolase *(LTA4H)* genotype. The forward genetic model in zebrafish-*Mycobacterium marinum* has revealed that *lta4h* genotype regulates the production of *lta4h*, which catalyzes the conversion of leukotriene A4 into pro-inflammatory leukotriene B4, and then determines the production of pro-inflammatory cytokine TNF-α. This *lta4h* genotype is associated with TB hyper-susceptibility (137). In humans, Tobin *et al* found six intronic *LTA4H* single nucleotide polymorphisms (SNPs) had protective effect

on TB susceptibility for the heterozygotes compared to homozygotes. After adjusting for multiple comparisons, heterozygotes of the two SNPs at rs1978331 and rs2660898 were associated with significantly lower TB incidence in a Vietnamese population. Heterozygotes of those two SNPs were also associated with lower mortality in TBM patients in Vietnam, and with lower incidence of severe leprosy without erythema nodosum leprosum in Nepal (137). This finding has led to studies in other territories and ethnicities with different results. No association was found between *LTA4H* genotype of two SNPs, rs1978331 and rs2660898, and TB susceptibility in a larger population in Russia (138). In Mozambique, *LTA4H* genotype of these SNPs did not correlate with pulmonary TB in 102 patients compared to 456 healthy control (139).

Tobin *et al* later found that an rs17525495 C/T lying upstream of *LTA4H* regulates *LTA4H* transcription and influences LTA4H protein levels from lymphoblastoid cell lines. This SNP was associated with mortality and dexamethasone response in TBM patients (85). To understand how *LTA4H* genotype influence inflammatory response and treatment outcomes of TBM patients, Thuong *et al* prospectively analyzed a cohort of 764 Vietnamese TBM patients and found that genotypes at the *LTA4H* SNP rs17525495 were associated with intracerebral cytokine concentrations and predicted mortality in HIV-uninfected patients (86). The homozygous TT genotype had increased survival and higher cytokine expression compared to CC. However, no association was found between *LTA4H* genotypes of this SNP and mortality of 608 TBM patients in Indonesia (140). There were several explanations for this discrepancy between the two cohorts (141). Differences in environmental factors can lead to variations in genetic expression. The rs17525495 SNP might be the causative SNP for *LTA4H* gene expression in Vietnamese but not in Indonesian population. But the most plausible reason is that Indonesian TBM patients were more severe than Vietnamese patients and that the impact of *LTA4H* genotype varied on disease severity. Later, Whitworth *et al* reanalyzed the three cohorts including two Vietnamese studies and Indonesian TBM patients by using Bayesian

analysis to prove that survival of TT genotype was higher than non-TT with dexamethasone treatment in both Vietnamese and Indonesian patients (142). In Indonesian cohort, the effect of TT was significant in grade 1 and grade 2 but not in grade 3 TBM patients. Whitworth *et al* also reanalyzed the cytokine data of HIV-uninfected TBM Vietnamese patients to confirm the finding that *LTA4H* TT genotype was associated with increased inflammatory response, which explained its benefit to dexamethasone (87).

Although a proven predictor for response to dexamethasone in TBM, the role of *LTA4H* genotype in pulmonary TB is still unknown. Understanding the relationships between *LTA4H* genotype and TB presentations and treatment outcome might provide evidence for adjunctive corticosteroids therapy for pulmonary TB, especially MDR-TB. Furthermore, exploring the relationship between this genotype and cytokine expression is also important to understand the mechanism of adjunctive host targeted therapy in improving treatment outcomes for pulmonary MDR-TB patients. I hypothesized that a C/T promoter variant at rs17525495 of *LTA4H* gene influences host inflammatory response, hence, has impacts on disease severity and treatment response of pulmonary MDR-TB. Two prospective studies in Ho Chi Minh City, Vietnam, were conducted to evaluate 1) the impacts of *LTA4H* genotype on MDR and DS-TB manifestation, severity and bacterial burden before treatment, 2) the association between *LTA4H* genotype and early bacterial clearance of MDR and DS-TB patients, 3) the association between *LTA4H* genotype and treatment outcomes of MDR and DS-TB patients, and 4) the relationships between *LTA4H* genotype and blood cytokines in MDR-TB patients before treatment.

4.2 RESULTS

4.2.1 Characteristics of MDR/RR-TB and DS-TB

Between March 2018 and October 2019, 312 patients with newly confirmed pulmonary MDR-TB treated in 16 DTUs in HCMC, aged ≥ 18 years and without previous drug resistant TB treatment were recruited into a MDR-TB cohort. Most patients were initially diagnosed with

MDR/RR-TB by GeneXpert, except for 10 identified by DST and 14 by Hain test. GeneXpert assays were performed for 24 MDR-TB patients who were diagnosed by other methods in order to obtain the semi-quantitative levels and mean CT value for all 312 patients.

A cohort of 600 patients with newly confirmed drug (rifampicin) susceptible pulmonary TB treated in 13 DTUs in HCMC, aged ≥ 18 years were included during April 2018 and April 2019. DS-TB patients were diagnosed by positive AFB smear and rifampicin resistance not detected by GeneXpert, LPA or DST. Ten DS-TB patients with negative GeneXpert results of sputum obtained before treatment had *M.tb* detected and rifampicin resistance not detected by GeneXpert in culture samples.

Culture of samples collected within 14 days of treatments were positive for 284 (91%) MDR-TB and 576 (96%) DS-TB. Patients with positive culture at entry of study were included for culture conversion analysis.

All 600 DS-TB patients had final treatment outcome information. Of 312 MDR-TB patients, 13 were still on treatment at the time of analysis, making a total of 299 MDR-TB patients available for treatment outcome analysis (Figure 4-1).

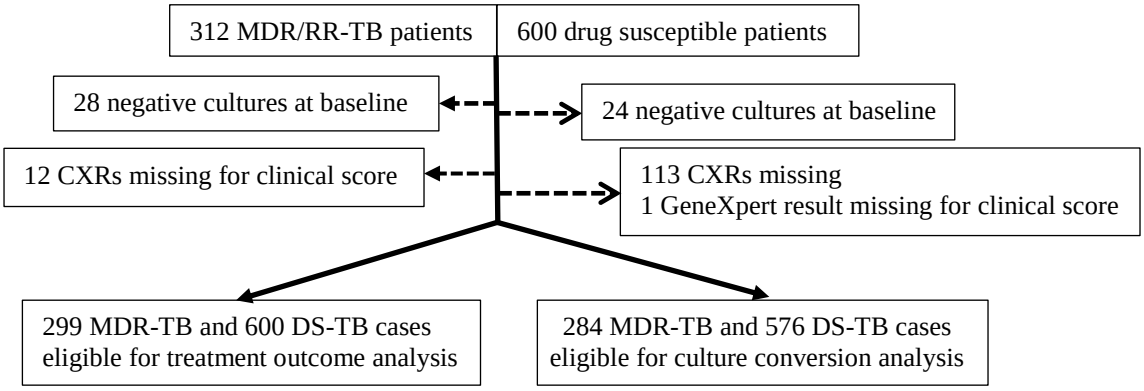


Figure 4-1. Flow diagram of eligible cases for analysis. Baseline and symptom severity analysis were for 312 MDR-TB and 600 DS-TB. Clinical severity analysis was for 300 MDR-TB and 486 DS-TB patients. Sputum culture conversion analysis was for 284 MDR-TB and 576 DS-TB with positive culture at baseline. Treatment outcome analysis was for 299MDR-TB and all 600 DS-TB patients. Solid lines represented inclusion and dashed lines for exclusion.

Table 4.1 summarizes the characteristics of 312 MDR-TB and 600 DS-TB patients included in this study. Median age was 42 years (interquartile range (IQR): 32-51) in MDR-TB and 43.5 (IQR: 31-52) in DS-TB, male was predominant in both MDR (236 (75.6%)) and DS-TB (478 (79.7%)), 21(6.75%) were HIV co-infected in MDR-TB vs 18 (3.0%) in DS-TB (p=0.014), 174(55.8%) had previous TB treatment in MDR-TB vs 76 (12.7%) in DS-TB (p<0.001). Median BMI of MDR-TB patients was 19.1 vs 19.4 in DS-TB (p=0.099). More MDR-TB patients smoked (35.9% in MDR vs 22.3% in DS-TB, p<0.001) and drank alcohol than DS-TB (34.9% in MDR-TB vs 29.0% in DS-TB, p<0.001). The frequency of three *LTA4H* genotype was similar among MDR and DS-TB patients (p=0.721). Ninety five (10.4%) TB patients had TT genotype, 440 (48.2%) had CT and 377 (41.3%) had CC.

To comprehensively assess the severity of disease, I modified a symptom severity score and clinical severity score (106, 107). The symptom score consists of seven TB related symptoms (cough, hemoptysis, fever, night sweat, anorexia, weight loss, malaise), ranging from zero to seven. The clinical severity score consists of symptom score, Timika score of chest X-ray (CXR) and bacterial load by GeneXpert level, ranging from zero to nine. Patients were classified as severe symptom and disease if their symptom score ≥5 or clinical score ≥6. One hundred and four (33.3%) MDR-TB and 165 (27.5%) DS-TB patients had severe symptoms.

Clinical score could not be computed for 126 patients, including 12 MDR-TB and 113 DS-TB patients with missing CXRs, and one DS-TB patients missing GeneXpert level result. MDR-TB patients presented with more severe disease (p=0.023), and more extended lung damage on CXR (p=0.001) but lower bacterial burden indicated by higher mean CT value (p=0.021) than DS-TB patients (***Table 4.1***).

Table 4.1. Characteristics of MDR and DS TB

	MDR-TB *N=312*	**DS-TB** *N=600*	**P value**
Age	42.0 [32.0-51.0]	43.5 [31.0-52.0]	0.870
Male	236 (75.6%)	478 (79.7%)	0.189
HIV positive	21 (6.75%)	18 (3.00%)	0.014
History of previous TB	174 (55.8%)	76 (12.7%)	<0.001
Alcohol drinker	109 (34.9%)	174 (29.0%)	<0.001
Regular smoker	112 (35.9%)	134 (22.3%)	<0.001
BMI	19.1 [17.0-20.9]	19.4 [17.6-21.6]	0.099
Cavity on CXR	133 (44.3%)	198 (40.5%)	0.323
Timika score	37.5 [12.5-65.0]	25.0 [10.0-55.0]	0.001
Symptom duration	20.0 [11.0-30.0]	18.0 [12.0-30.0]	0.762
Severe symptom	104 (33.3%)	165 (27.5%)	0.079
Severe clinical disease	102 (34.0%)	127 (26.1%)	0.023
Mean CT value	20.9 [17.9-26.0]	20.2 [16.8-24.4]	0.021
Anemia	166 (60.4%)	317 (59.1%)	0.795
LTA4H genotype			0.721
CC	134 (42.9%)	243 (40.5%)	
CT	148 (47.4%)	292 (48.7%)	
TT	30 (9.6%)	65 (10.8%)	

Value were presented as number (%) for category variables and median [IQR] for continuous variables. P value was based on χ^2 test for category variables and Mann-Whitney test for continuous variables.

4.2.2 *LTA4H* genotype and baseline characteristics

In order to evaluate the effect of *LTA4H* genotype on clinical presentations, the baseline characteristics of MDR (***Table 4.2***) and DS-TB (***Table 4.3***) patients were compared between the three genotypes. No significant differences were observed regarding patients' age, gender and rate of HIV co-infection in both MDR and DS-TB. MDR-TB patients with TT genotype were

heavier than two other genotype (p<0.001). More MDR-TB patients with CC genotype had previous TB episode(s) compared to patients with heterozygote CT (p=0.050), but there was no significant difference between TT and CT (p=1.000) and between TT and CC (p=0.325).

DS-TB patients presented with similar baseline characteristics for all three genotype (***Table 4.3***). There were no significant difference in BMI, history of previous TB, duration of symptoms before diagnosis among three genotype. More TT patients (47.5%) had anemia than CC (42.6%) and CT (37.9%), but the difference did not reach significance (p=0.323).

Table 4.2: Characteristics of MDR patients stratified by LTA4H genotype

	MDR-TB			
	CC ***N=134***	**CT** ***N=148***	**TT** ***N=30***	**P value**
Age (years)	41.0 [30.2-48.0]	44.5 [34.0-53.0]	41.0 [32.2-50.0]	0.144
Male	106 (79.1%)	109 (73.6%)	21 (70.0%)	0.425
HIV co-infection	10 (7.46%)	10 (6.80%)	1 (3.33%)	0.846
Drinking alcohol	45 (33.6%)	54 (36.5%)	10 (33.3%)	0.873
Regular smoker	50 (37.3%)	50 (33.8%)	12 (40.0%)	0.732
History of previous TB	86 (64.2%)	73 (49.3%)	15 (50.0%)	0.034
Symptoms duration (days)	20 [12-30]	20[10-30]	30 [14-30]	0.981
Cough	123 (91.8%)	142 (95.9%)	30 (100%)	0.159
Sputum	119 (88.8%)	132 (89.2%)	27 (90.0%)	1.000
Weight loss	59 (44.0%)	79 (53.4%)	11 (37.9%)	0.154
Low grade fever	63 (47.0%)	79 (53.4%)	15 (50.0%)	0.565
Haemoptysis	22 (16.4%)	14 (9.46%)	2 (6.67%)	0.157
Chest pain	46 (34.3%)	52 (35.1%)	10 (33.3%)	0.978
Malaise	82 (61.2%)	93 (62.8%)	19 (63.3%)	0.951
Dyspnea	43 (32.1%)	49 (33.1%)	8 (26.7%)	0.788
BMI (kg/m^2)	19.2 [17.5-20.8]	18.5 [16.8-20.7]	21.5 [18.9-23.5]	0.001

	MDR-TB			
	CC	**CT**	**TT**	**P value**
	N=134	***N=148***	***N=30***	
BMI group				0.019
Normal	66 (49.3%)	62 (41.9%)	15 (50.0%)	
Overweight	16 (11.9%)	13 (8.78%)	8 (26.7%)	
Underweight	52 (38.8%)	73 (49.3%)	7 (23.3%)	
Cavity	63 (49.6%)	59 (40.7%)	11 (39.3%)	0.287
Timika score	45.0 [15.0-65.0]	40.0 [15.0-65.0]	25.0 [9.38-66.2]	0.586
GeneXpert				
Not Detected	10 (7.46%)	7 (4.73%)	1 (3.33%)	
Very Low	18 (13.4%)	13 (8.78%)	6 (20.0%)	
Low	24 (17.9%)	32 (21.6%)	3 (10.0%)	
Medium	54 (40.3%)	63 (42.6%)	14 (46.7%)	
High	28 (20.9%)	33 (22.3%)	6 (20.0%)	
Mean CT*	21.3 [17.9-27.8]	20.7 [17.8-24.9]	20.0 [17.5-26.4]	0.497
Anemia	45 (37.5%)	54 (42.2%)	10 (37.0%)	0.721

*: bacterial load was accessed by mean CT value of GeneXpert

Value were presented as number (%) for category variables and median [IQR] for continuous variables.

Table 4.3. Characteristics of DS-TB with LTA4H genotype

	DS-TB			
	CC	**CT**	**TT**	**P value**
	N=243	***N=292***	***N=65***	
Age (years)	44.0 [31.5-52.0]	44.0 [30.0-52.0]	41.0 [34.0-54.0]	0.729
Male	191 (78.6%)	233 (79.8%)	54 (83.1%)	0.726
HIV co-infection	9 (3.70%)	8 (2.74%)	1 (1.54%)	0.826
Drinking alcohol	72 (29.6%)	85 (29.2%)	17 (26.2%)	0.119
Regular smoker	58 (23.9%)	63 (21.6%)	13 (20.0%)	0.729

	DS-TB			
	CC	**CT**	**TT**	**P value**
	N=243	***N=292***	***N=65***	
History of previous TB	26 (10.7%)	42 (14.4%)	8 (12.3%)	0.441
Symptoms duration (days)	17 [12-30]	17 [12-30]	19 [13-53]	0.240
Cough	234 (96.3%)	274 (93.8%)	60 (92.3%)	0.244
Sputum	212 (87.2%)	245 (83.9%)	55 (84.6%)	0.546
Weight loss	107 (44.0%)	134 (45.9%)	28 (43.1%)	0.871
Low grade fever	137 (56.4%)	140 (47.9%)	30 (46.2%)	0.105
Haemoptysis	20 (8.23%)	28 (9.59%)	8 (12.3%)	0.591
Chest pain	68 (28.0%)	101 (34.6%)	25 (38.5%)	0.143
Malaise	124 (51.0%)	143 (49.0%)	32 (49.2%)	0.889
Dyspnea	34 (14.0%)	55 (18.8%)	8 (12.3%)	0.213
BMI (kg/m^2)	19.5 [17.6-22.0]	19.2 [17.5-21.5]	19.5 [17.9-21.3]	0.564
BMI group				0.459
Normal	112 (46.1%)	142 (48.6%)	35 (53.8%)	
Overweight	39 (16.0%)	33 (11.3%)	7 (10.8%)	
Underweight	92 (37.9%)	117 (40.1%)	23 (35.4%)	
Cavity	63 (49.6%)	59 (40.7%)	11 (39.3%)	0.960
Timika score	25.0 [10.0-60.0]	25.0 [10.0-52.5]	27.5 [10.0-56.2]	0.921
GeneXpert				
Not Detected	3 (1.24%)	6 (2.05%)	1 (1.54%)	
Very Low	16 (6.61%)	31 (10.6%)	7 (10.8%)	
Low	58 (24.0%)	60 (20.5%)	11 (16.9%)	
Medium	99 (40.9%)	120 (41.1%)	30 (46.2%)	
High	66 (27.3%)	75 (25.7%)	16 (24.6%)	
Mean CT*	20.1 [16.6-24.1]	20.4 [17.0-24.6]	20.1 [17.0-24.5]	0.558
Anemia	92 (42.6%)	99 (37.9%)	28 (47.5%)	0.323

*: bacterial load was accessed by mean CT value of GeneXpert

Value were presented as number (%) for category variables and median [IQR] for continuous variables.

MDR-TB patients showed a trend of lower BMI than DS-TB patients (***Table 4.1***). However, when stratified by *LTA4H* genotype, TT patients with MDR-TB were heavier than TT patients with DS-TB. Patients with TT genotype were the heaviest among the three genotypes in MDR-TB (***Figure 4-2***).

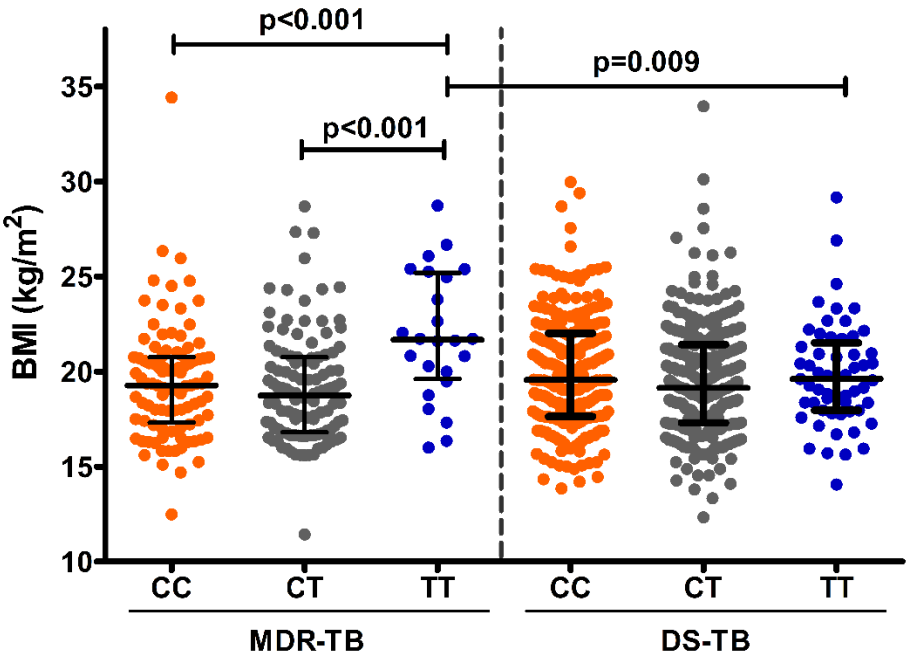


Figure 4-2. Differences in BMI between TB patients with LTA4H genotype. Data was for 312 MDR-TB patients with 134 CC, 30 TT and 148 CT patients, and for 600 DS-TB including 243 CC, 65 TT and 292 CT. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

4.2.2.1 TB signs and symptoms

Univariate logistic regression was used to explore the associations between *LTA4H* genotype and individual signs and symptoms of pulmonary MDR (**Figure 4-3**) and DS-TB patients (**Figure 4-4**). Patients from both MDR and DS-TB mainly presented with cough as their chief complaint (249 (79.3%) MDR-TB and 519 (85.4%) DS-TB). *LTA4H* genotype did not associate

with any individual sign or symptom of TB. For patients with MDR-TB, all 30 (100%) TT patients coughed, compared to 142 (95.9%) CT and 123 (91.8%) CC (p=0.159). Patients with TT and CT genotype insignificantly reduced the risk of haemoptysis (TT genotype: OR: 0.36, 95%CI: 0.08-1.64; CT genotype: OR: 0.53, 95%CI: 0.26-1.09) (**Figure 4-3**). Eleven (37.9%) TT, 59 (44.0%) CC and 79 (53.4%) CT patients experienced weight loss, leading to a slight reduction in risk of weight loss in TT compared to CC (OR: 0.78, 95%CI: 0.34-1.77).

For patients with DS-TB, patients with TT genotype had lower odds of cough (OR: 0.46, 95%CI: 0.15-1.43), fever (OR: 0.66, 95%CI: 0.38-1.15), high lung damage on CXR (OR: 0.63, 95%CI: 0.18-2.25) compared to CC genotype (**Figure 4-4**).

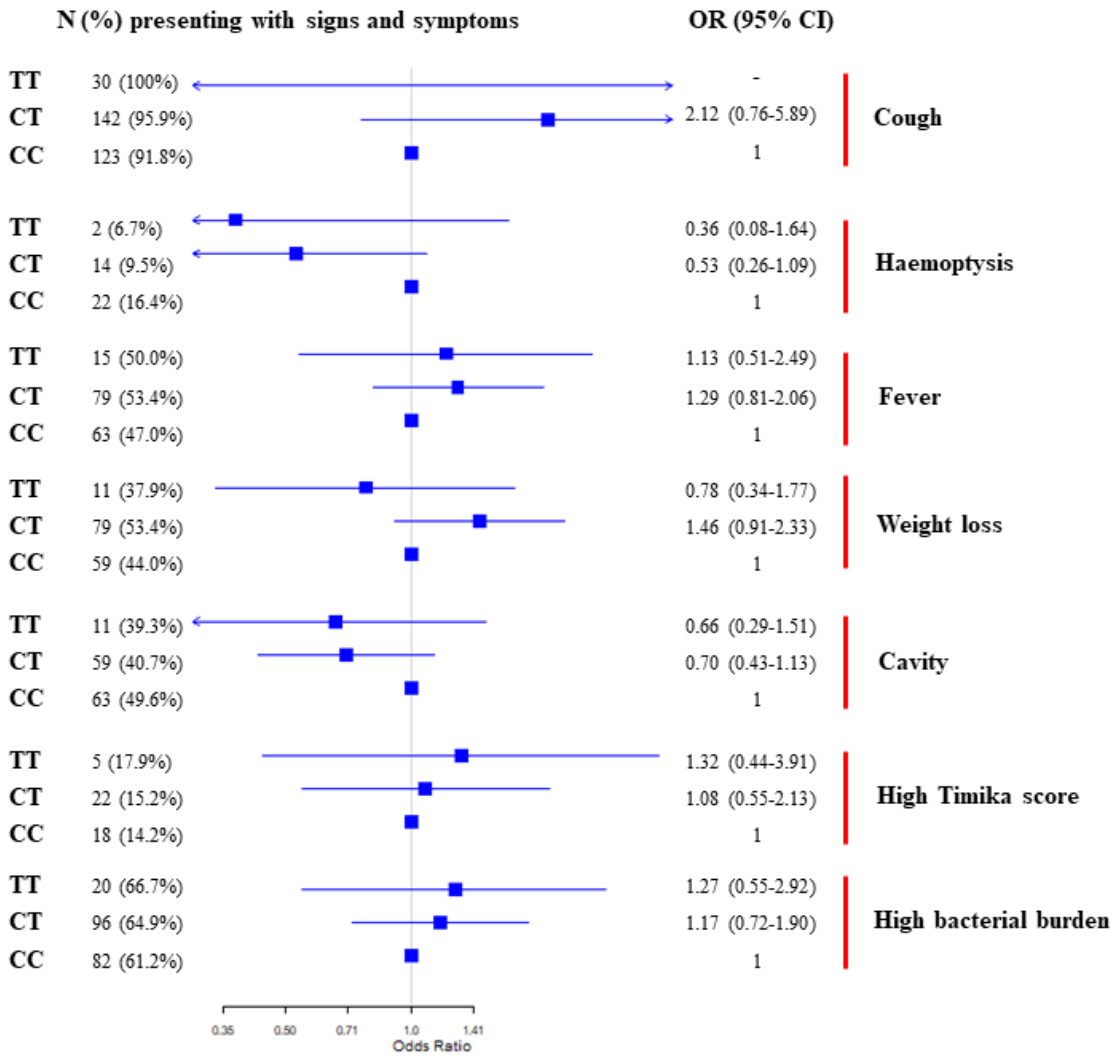


Figure 4-3. Forest plot of univariate logistic regression of LTA4H genotype and MDR-TB signs and symptoms. Data of TB symptoms and bacterial burden was for 312 MDR-TB patients. Data of cavity and Timika score was for 300 MDR-TB patients with CXR. OR: odds ratio; 95%CI: 95% confidence interval.

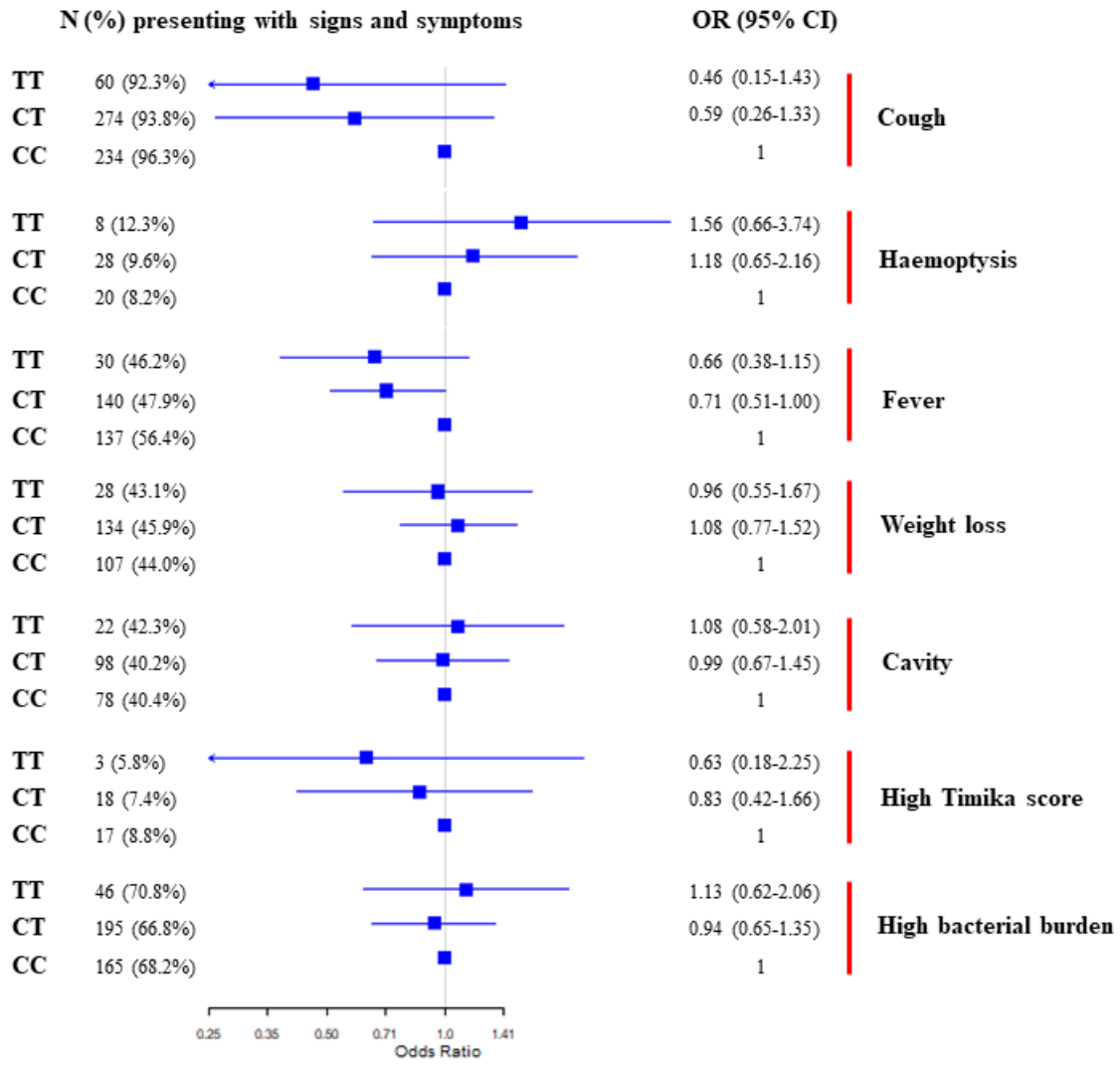


Figure 4-4. Associations between LTA4H genotype and DS-TB signs and symptoms. Data of TB symptoms was for 600 DS-TB patients. Data of cavity and Timika score was for 487 DS-TB patients with CXR. Data of bacterial burden was for 599 DS-TB patients. OR: odds ratio; 95%CI: 95% confidence interval.

4.2.2.2 *M.tb* bacterial burden

Univariate logistic regression was used to evaluate the effect of *LTA4H* genotype on *M.tb* bacterial burden. GeneXpert results were classified into high (*M.tb* detected high or medium in GeneXpert) and low bacterial burden (*M.tb* detected low or very low or not detected in GeneXpert). Among MDR-TB, TT patients slightly increased risk of high bacterial burden by

1.27 times (95%CI: 0.55-2.92) and CT by 1.17 (95%CI: 0.72-1.90) compared to CC patients (***Figure 4-3***). In DS-TB, *LTA4H* genotype did not significantly influence bacterial burden.

Mean CT value was calculated as the mean cycles of five probes needed to cross the detection threshold in GeneXpert assay (section 2.2.9 in chapter 2). The higher mean CT values reflected the lower bacterial load. Patients, who had GeneXpert results as *M.tb* not detected, were assigned mean CT value of 40. There was no difference in mean CT value between *LTA4H* genotype in both MDR-TB and DS-TB (***Table 4.2*** and ***Table 4.3***). However, MDR-TB patients had higher mean CT value (20.9, IQR: 17.9-26.0) which reflected a lower bacterial burden than DS-TB (20.2, IQR: 16.8-24.4) (p=0.021). ***Figure 4-5*** showed that among CC genotype patients, MDR-TB had higher mean CT value than DS-TB patients (21.3 [17.9-27.8] in MDR-TB vs 20.1 [16.6-24.1] in DS-TB, p=0.008) but other genotype did not show any significant differences (TT genotype: 20.0 [17.5-26.4]in MDR-TB vs 20.1 [17.0-24.5] in DS-TB, p=0.583; CT genotype: 20.7 [17.8-24.9] in MDR-TB vs 20.4 [17.0-24.6] in DS-TB, p=0.550).

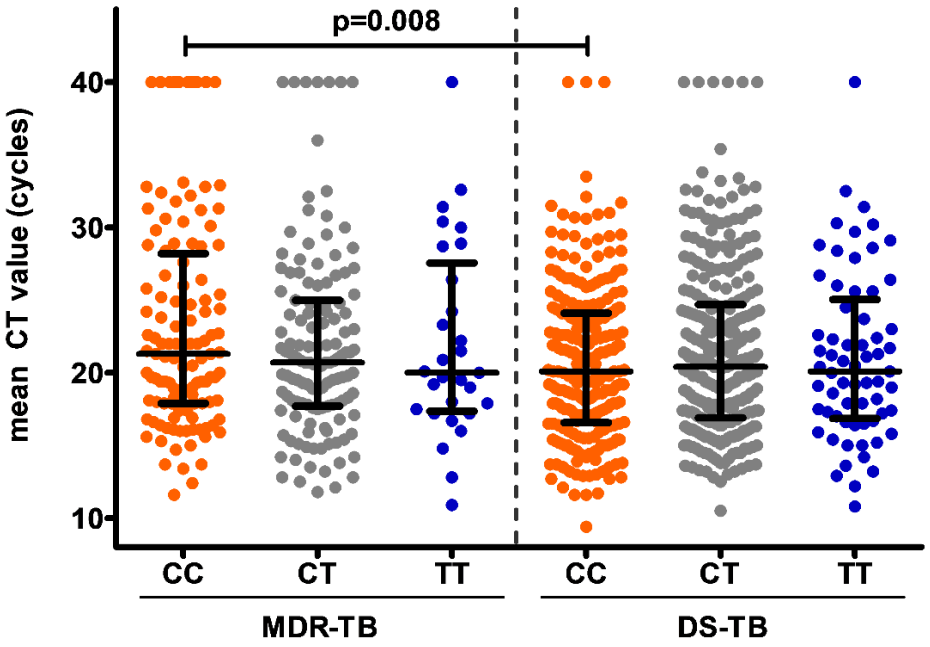


Figure 4-5. Differences in mean CT value of MDR and DS-TB stratified by LTA4H genotype. Data was for 280 MDR-TB patients with available mean CT values including 120 CC, 29 TT and 131 CT

patients, and for 598 DS-TB including 242 CC, 65 TT and 291 CT. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

4.2.2.3 Chest X-ray cavity and severity Timika score

The extent of lung damage on CXR was evaluated by using Timika score (section 2.2.1 in chapter 2), with high lung damage when Timika score ≥ 71 and low lung damage when Timika score < 71. Among 300 MDR-TB and 487 DS-TB patients with baseline CXR available, *LTA4H* genotype did not significantly increase the risk of having cavity or high lung damage by Timika score (***Figure 4-3***). Compared to CC genotype, TT and CT genotype slightly decreased the risk of having cavity (in MDR-TB: OR: 0.66, 95%CI: 0.29-1.51 for TT; OR: 0.70, 95%CI: 0.43-1.13 for CT) (in DS-TB: OR: 1.08, 95%CI: 0.58-2.01 for TT; OR: 0.99, 95%CI: 0.67-1.45 for CT). Median Timika scores were similar among genotype in DS-TB (p=0.921) and were insignificantly lower in TT among MDR-TB patients (p=0.586) (***Table 4.2***). MDR-TB patients had higher Timika score (median: 37.5, IQR: 12.5-65.0) than DS-TB patients (median: 25.0, IQR: 10.0-55.0) (p=0.001). When stratified by *LTA4H* genotype, MDR-TB patients had higher Timika score than DS-TB patients for CC genotype (45.0 [15.0-65.0] in MDR-TB vs 25.0 [10.0-60.0] in DS-TB, p=0.019) and CT genotype (40.0 [15.0-65.0] in MDR-TB vs 25.0 [10.0-52.5] in DS-TB, p=0.018), but not for TT genotype (p=0.968) (***Figure 4-6***).

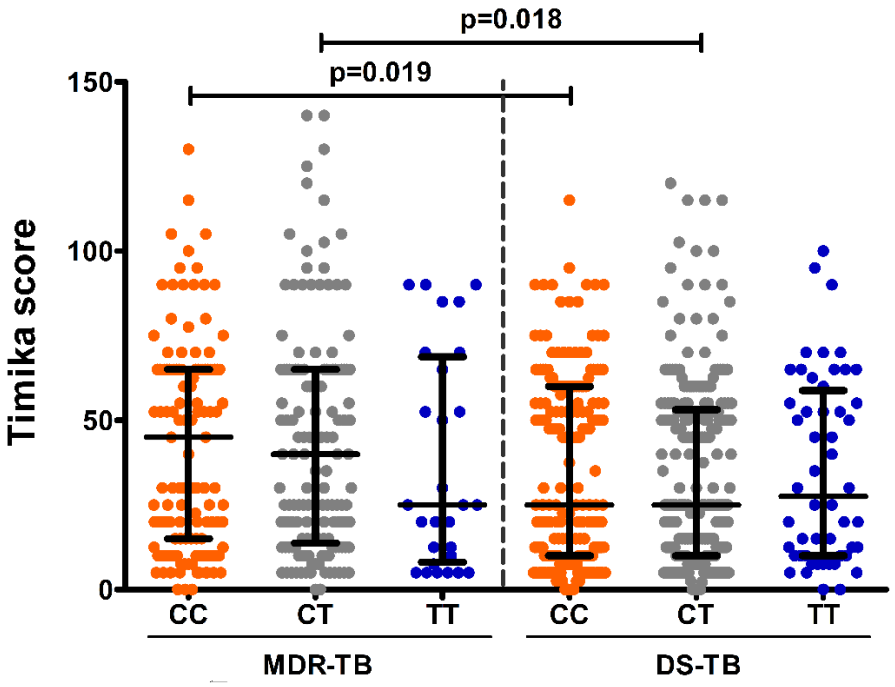


Figure 4-6. Differences in extent of lung damage on CXR by Timika score, stratified by LTA4H genotype. Data was for 300 MDR-TB patients with available CXR including 127 CC, 28 TT and 145 CT patients, and for 487 DS-TB including 193 CC, 52 TT and 242 CT. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

4.2.2.4 Symptom severity and disease severity

While there was no difference in individual TB signs and symptoms between the three genotypes, comparisons of symptom score and clinical score, the combinations of TB signs and symptoms, might be an integrated method to accurately estimate the impact of *LTA4H* genotype on TB presentations. Symptom scores were calculated for all 312 MDR-TB and 600 patients, while clinical scores were available for 300 MDR-TB and 486 DS-TB. There was no difference between median of symptom score and clinical score between genotypes in MDR (p=0.386 for symptom score; p=0.298 for clinical score) and in DS-TB (p=0.688 for symptom score; p= 0.994 for clinical score).

Patients were classified into severe symptoms when their symptom scores were equal or greater than five, and mild symptoms when symptom scores less than five. For clinical severity,

patients were considered to have severe disease when clinical score ≥ 6, and mild disease when clinical score <6 (106) (107). Among 312 MDR-TB, 8 (26.7%) TT, 52 (35.1%) CT and 44 (32.8%) CC had severe symptoms (p=0.660). In 300 MDR-TB patients with clinical severity information, 7 (25%) TT, 53 (36.6%) CT and 42 (33.1%) CC patients had severe disease. TT patients slightly reduced the risk of developing severe symptoms by 0.74 fold change (p=0.512) and severe disease by 0.67 fold change (p=0.407) when compared to CC genotype. After adjusting for duration of symptoms prior diagnosis, no genotype were associated with risk for severe symptoms and severe disease (***Table 4.4***).

In 600 DS-TB, 16 (24.6%) TT, 84 (28.8%) CT and 65 (26.7%) CC had severe symptoms (p=0.750). For 486 DS-TB patients with clinical severity information, 12 (23.1%) TT, 65 (26.9%) CT and 50 (26%) CC had severe disease. No association was observed between *LTA4H* genotype and symptom or disease severity.

Table 4.4. Associations between baseline severity and LTA4H genotype in MDR-TB patients by univariate and multivariate logistic regression

	Genotype	Severe	Univariate		Multivariate**	
		*N (%)**	*OR (95%CI)*	*P*	*aOR (95%CI)*	*P*
Symptom severity	TT	8 (26.7%)	0.74 (0.31-1.80)	0.512	0.75 (0.31-1.83)	0.528
	CT	52 (35.1%)	1.11 (0.68-1.82)	0.684	1.01 (1.00-1.01)	0.161
	CC	44 (32.8%)	1		1	
Clinical severity	TT	7 (25.0%)	0.67 (0.27-1.71)	0.407	0.69 (0.27-1.77)	0.440
	CT	53 (36.6%)	1.17 (0.71-1.92)	0.548	1.13 (0.68-1.87)	0.645
	CC	42 (33.1%)	1		1	

Data of symptom severity was for 312 MDR patients including 134 CC, 148 CT and 30TT patients.

Data of clinical severity was for 300 MDR-TB patients including 127 CC, 145 CT and 28 TT patients.

* Number of patients with severe symptoms and severe disease.

** Adjusting for duration of disease

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval.

4.2.3 Effect of *LTA4H* genotype on early treatment response

Effect of regimens on MDR-TB early bacterial clearance and treatment outcomes

MDR-TB patients were treated with two regimens: the 9 month regimen for 225 (72.1%) patients who were enrolled after July 2019 and eligible for this shorter regimen, and the 20 month regimen for 87 (27.9%) patients who did not receive the 9 month regimen (section 2.1.3.4 in chapter 2). The Kaplan Meier plot showed a small difference in time to first sputum culture conversion between the two regimens with p=0.96 in log-rank test (***Figure A.1*** in Appendix). At the end of treatment, 169 (77.2%) patients with 9 month regimen and 60 (75%) patients with 20 month regimen achieved favorable outcomes (including cured or treatment completed). The odds of having unfavorable outcomes (including failure, death and lost to follow-up) of 9 month regimen compared to 20 month regimen was 0.89 (95%CI: 0.49-1.61) in univariate logistic regression and 0.92 (95% CI: 0.50-1.71) after adjusting for baseline mean CT value.

Effect of mono-isoniazid resistance on DS-TB early bacterial clearance and treatment outcomes

Five hundred thirty eight isolates of DS-TB patients were sequenced to identify 122/538 (22.7%) isoniazid resistant cases and there was no significant difference in treatment response and treatment outcomes of patients with and without isoniazid resistant mutations (***Table A.1*** in Appendix).

Therefore, MDR-TB treatment regimens and isoniazid resistance among DS-TB were not included in our multivariate model for early bacterial clearance and treatment outcome analysis.

4.2.3.1 Sputum culture conversion at day 14, month 1, month 2 and month 3

Evaluation of early treatment response at 4 time points: day 14, month 1, month 2 and month 3 of MDR-TB treatment was performed within three *LTA4H* genotypes (***Table 4.5***). At each observed time point, patients who had at least two consecutive negative cultures were classified as “converted”, and patients with positive cultures as “not converted”. Patients who had

converted but then reverted to positive were also considered "not converted" if their sputum culture were positive at the observed time (section 2.2.10 in chapter 2). A total of 275 MDR-TB patients were included for sputum culture conversion analysis at day 14 and month 1, 268 patients (97.5%) at month 2 and 264 patients (96%) at month 3.

There was no significant difference between the three groups at any specified time point. With CC genotype as the reference, TT genotype had fluctuated effect on sputum culture conversion. The odds of no conversion of TT were 1.89 (95%CI: 0.23-15.78) at day 14, 0.53 (95%CI: 0.22-1.24) at month 1, 1.12 (95%CI: 0.48-2.62) at month 2 and 0.52 (95%CI: 0.16-1.62) at month 3. CT genotype had gradually increasing odds of no conversion along treatment, with odds ratio of 0.72 (95%CI: 0.28-1.82) at day 14, 1.06 (95%CI: 0.61-1.83) at month 1, 1.13 (95%CI: 0.68-1.88) at month 2 and 1.23 (95%CI: 0.69-2.19) at month 3. The effect of *LTA4H* did not significantly change after adjusting for important mediator of pretreatment bacterial load (***Table 4.5***).

Table 4.5. Associations between LTA4H genotype and risk of no culture conversion during MDR-TB treatment by univariate and multivariate logistic regression

Time point	Genotype	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* aOR (95%CI)	P
Day 14	TT	26 (96.3%)	1.89 (0.23-15.78)	0.556	1.95 (0.22-17.32)	0.550
	CT	118 (90.8%)	0.72 (0.28-1.82)	0.481	0.77 (0.28-2.14)	0.618
	CC	110 (93.2%)	1		1	
Month 1	TT	15 (55.6%)	0.53 (0.22-1.24)	0.143	0.43 (0.17-1.09)	0.077
	CT	93 (71.5%)	1.06 (0.61-1.83)	0.835	1.17 (0.63-2.19)	0.619
	CC	83 (70.3%)	1		1	
Month 2	TT	12 (41.6%)	1.12 (0.48-2.62)	0.789	1.02 (.041-2.51)	0.966
	CT	57 (44.5%)	1.13 (0.68-1.88)	0.646	1.33 (0.76-2.32)	0.316
	CC	47 (41.6%)	1		1	

Time point	Genotype	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* aOR (95%CI)	P
Month 3	TT	4 (14.8%)	0.52 (0.16-1.62)	0.257	0.50 (0.16-1.59)	0.242
	CT	37 (29.4%)	1.23 (0.69-2.19)	0.476	1.29 (0.71-2.36)	0.400
	CC	28 (25.2%)	1		1	

*: Adjusting for bacterial load accessed by mean CT value of GeneXpert.

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval.

In DS-TB, the effect of *LTA4H* genotype was also subtle (***Table A.2*** in Appendix). No association was found between risk of no sputum culture conversion and *LTA4H* genotype. The odds of no conversion of TT genotype was 0.90 (95%CI: 0.40-2.00) at day 14, 0.82 (95%CI: 0.46-1.46) at month 1 and 0.82 (95%CI: 0.40-1.70) at month 2.

4.2.3.2 Sputum culture conversion time in converted patients

In order to observe the overall influence of *LTA4H* genotypes on sputum culture conversion during treatment, I next compared the median time to the first sputum culture conversion in TB patients who achieved at least one conversion since the start to the end of treatment. The first sputum culture conversion was defined as the first of at least two consecutive negative cultures after patients started their MDR-TB treatment and at least one negative cultures in DS-TB patients.

Among 235 MDR-TB patients with conversion, TT patients had the lowest median time (40 [32-78]) compared to CT (61 [31-93]) and CC (60 [30-76]) (p overall=0.378). However, in 543 DS-TB patients with conversion, the time to first sputum culture conversion was shortest in CT genotype (35 [28-63] in CT vs 47[28-62] in TT vs 56 [30-62] in CC, p overall=0.641). ***Figure 4-7*** showed $\log_{10}$ of time to the first culture conversions of MDR and DS-TB patients, with no significant difference among *LTA4H* genotype. When stratified by *LTA4H* genotype,

MDR-TB patients with CC and CT genotype took longer time to convert than DS-TB patients (p=0.020 for CC; p<0.001 for CT), but not for TT genotype (p=0.420).

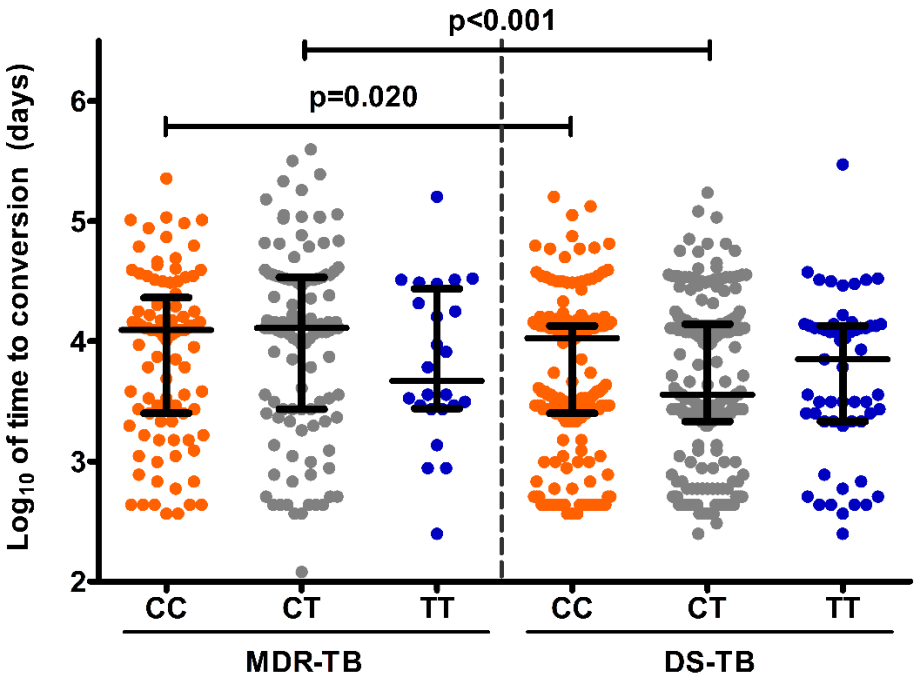


Figure 4-7. Log_{10} of time to sputum culture conversion in converted TB patients with different LTA4H genotype. Data were for 235 MDR-TB patients including 97 CC, 114 CT and 24 TT patients, and 543 DS-TB including 224 CC, 262 CT and 57 TT patients. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

4.2.3.3 Time to culture conversion using survival analysis

To explore whether any *LTA4H* genotype prolonged and prevented sputum culture conversion in account to all MDR-TB patients, a Kaplan Meier curve was plotted for 284 MDR-TB patients who had positive cultures at the start of treatment. Events were noted for any first sputum culture conversions, while censor included any deaths or lost to follow-up cases. Patients with CC and CT genotype exhibited similar bacterial clearance while TT genotype had irregularly faster bacterial clearance, mainly during 30 to 60 days of treatment (Figure 4-8). The p overall value of log rank test of Cox-regression analysis was 0.46, with p=0.218 for comparison between TT and CT, and p=0.303 for TT vs CC. However, the Cox analysis did not account for

the effect in different time points. Therefore, the effect (if any) of the *LTA4H* genotype on time to first culture conversion might be diminished when applying for the whole treatment time. To overcome this issue, restricted mean survival time analysis was performed to compare the median time to stay in culture positive from the start of treatment to a specified time point.

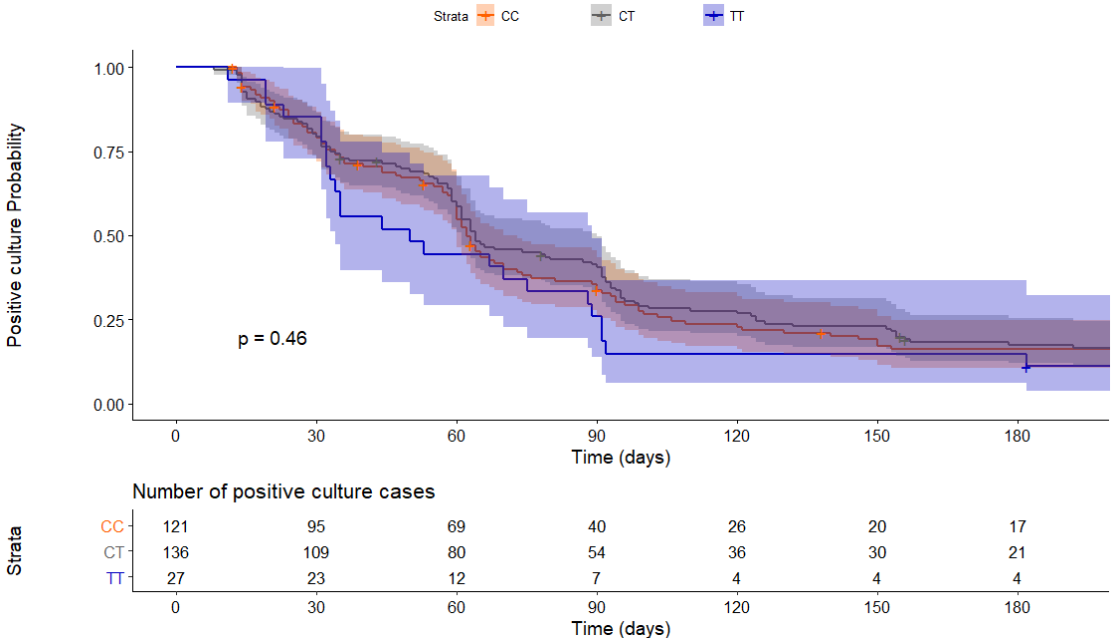


Figure 4-8 Kaplan Meier plot for sputum culture conversion of MDR-TB with three genotype of LTA4H. Data was for 284 MDR-TB patients with positive culture at baseline. Orange line represented 121 CC, grey line for 136 CT and blue line for 27 TT patients. P value was based on p overall of Log rank test.

By using restricted survival time analysis, I was able to measure the median time to stay culture positive and compare the areas under the curves of the two arms of study for a specific period of time. Figure 4-8 showed a similar treatment response pattern of CT and CC genotype while TT genotype slightly differed from the other two. This is consistent with the findings of Thuong *et al.* and Tobin *et al.* that TT genotype expresses different treatment response to dexamethasone compared to non-TT genotypes in TBM (85, 86). Hence, I calculated and tested the difference in restricted means of TT (as arm 1) and non-TT (CT and CC as arm 2). During MDR-TB treatment, 79.6% MDR-TB patients remained culture positive at the end of one

month, and 55.5% at two months, 23,8% at four months and 16.2% at six months. Therefore, I decided to truncate the time to observe the differences at four time points during treatment: one month, two months, four months and six months. For patients with MDR-TB, TT genotype had shorter time to stay in positive culture than non-TT, but the differences were not statistically significant The restricted mean positive time (RMPT) in days at the end of one month of treatment was 28 (95%CI: 26-30) in TT vs 28 [27-29] in CT and CC (p=0.703), at the end of two months was 45 [39-51] in TT vs 49 [47-51] in CT and CC (p=0.185), at the end of four months was 60 [47-73] in TT vs 71 [66-75] in CT and CC (p=0.155) and at the end of six months was 69 [49-89] in TT vs 82 [75-89] in CT and CC (p=0.214). There was no difference in RMPT in DS-TB patients (Table 4.6).

Table 4.6. Restricted mean positive time between TT and non-TT (CT and CC) genotype

	Diabetes status	**MDR-TB**	**P value****	**DS-TB**	**P value****
		RMPT (days)*		**RMPT (days)***	
1 month	TT	28 [26-30]	0.703	27 [26-29]	0.821
	CT and CC	28 [27-29]		27 [26-27]	
2 months	TT	45 [39-51]	0.185	43 [39-48]	0.751
	CT and CC	49 [47-51]		42 [41-44]	
4 months	TT	60 [47-73]	0.155	51 [44-59]	0.811
	CT and CC	71 [66-75]		50 [48-53]	
6 months	TT	69 [49-89]	0.214	55 [45-66]	0.665
	CT and CC	82 [75-89]		53 [50-56]	

*: RMPT: Restricted mean positive time. Value were presented as number and 95%CI (days)

**: p.value was based on RMST analysis for the difference in days of average time between TT and non-TT TB patients.

4.2.4 *LTA4H* genotype and treatment outcomes

Treatment outcome information was available for all 600 DS-TB patients and 299/312 MDR-TB patients. Among 13 MDR-TB patients who were still under treatment at the time of data analysis, two were TT, six CT and five CC genotype. Among 299 MDR-TB patients with treatment outcomes, 206 (68.9%) were cured, 23 (7.7%) completed the treatment, making a total of 229 (76.6%) had favorable outcomes. Unfavorable outcomes were found in 70 (23.4%) MDR-TB patients including 12 (4.0%) deaths, 22 (7.4%) failures and 36 (12.0%) lost to follow-up. TT patients had slightly higher cured rate of 75%, compared to 66.7% in CC and 69.7% in CT (p overall=0.660) and lower rate of lost to follow-up (3.6% in TT vs 14.0% in CC vs 12.0% in CT, p overall=0.351). Mortality rate was highest in CC genotype, with 6.2% in CC vs 3.6% in TT and 2.1% in CT, p overall=0.226 (***Table 4.7***)

In DS-TB, 507/600 (84.5%) patients had favorable outcomes, including 347 (59.5%) cured and 150 (25%) completed the treatment. Ninety three (15.5%) had unfavorable outcomes with 6 (1.0%) deaths, 66 (11.0%) failures and 21 (3.5%) lost to follow-up. Five (2.1%) CC patients died, compared to one (0.3%) CT and zero (0.0%) in TT (p=0.129) (***Table 4.7***).

Table 4.7. Treatment outcome of TB patients stratified by LTA4H genotype

Treatment outcome	MDR-TB			DS-TB		
	CC n=129	**CT n=142**	**TT n=28**	**CC n=243**	**CT n=292**	**TT n=65**
Cured	86 (66.7%)	99 (69.7%)	21 (75.0%)	149 (61.3%)	175 (59.9%)	33 (50.8%)
Completed	11 (8.5%)	9 (6.3%)	3 (10.7%)	53 (21.8%)	74 (25.3%)	23 (35.4%)
Failed	6 (4.7%)	14 (9.9%)	2 (7.1%)	27 (11.1%)	32 (11.0%)	7 (10.8%)
Died	8 (6.2%)	3 (2.1%)	1 (3.6%)	5 (2.1%)	1 (0.3%)	0 (0.0%)
Lost to follow up	18 (14.0%)	17 (12.0%)	1 (3.6%)	9 (3.7%)	10 (3.4%)	2 (3.1%)

Data was for 299 MDR-TB patients who finished treatment at the time of analysis and all 600 DS-TB patients. Value was presented as number (%).

In both MDR and DS-TB, no association was found between *LTA4H* genotype and all-cause mortality. With wild type genotype of CC as the reference, TT and CT had a small decrease in risk of mortality in MDR-TB (OR: 0.56, 95%CI: 0.07-4.67 for TT; OR: 0.33, 95%CI: 0.08-1.26 for CT). The odds of death in CT compared to CC in DS-TB was 0.16 (95%CI: 0.02-1.41) (***Table 4.8***).

LTA4H genotype also did not associate with TB treatment outcomes. The odds of having unfavorable outcomes was slightly lower in TT (OR: 0.51, 95%CI: 0.16-1.47 in MDR-TB; OR: 0.79, 95%CI: 0.36-1.73 in DS-TB), but the differences were not significant (p=0.237 in MDR, p=0.557 in DS-TB) (**Table 4.8**).

Table 4.8. Associations between LTA4H genotype and all cause mortality and unfavorable outcomes of TB patients by univariate logistic regression

	All-cause mortality			**Unfavorable outcomes**		
	N (%)	**OR (95%CI)**	***P value***	**N (%)**	**OR (95%CI)**	***P value***
TT_MDR	1 (3.6%)	0.56 (0.07-4.67)	0.592	4 (14.3%)	0.51 (0.16-1.57)	0.237
CT_MDR	3 (2.1%)	0.33 (0.08-1.26)	0.104	33 (23.9%)	0.95 (0.55-1.66)	0.869
CC_MDR	8 (6.2%)	1		32 (24.8%)	1	
TT_DS	0 (0.0%)	-	0.994	9 (13.8%)	0.79 (0.36-1.73)	0.557
CT_DS	1 (0.3%)	0.16 (0.02-1.41)	0.099	43 (14.7%)	0.85 (0.53-1.36)	0.497
CC_DS	5 (2.1%)	1		41 (16.9%)	1	

Data was for 299 MDR-TB patients and all 600 DS-TB patients.

OR: odds ratio; 95%CI: 95% confidence interval

4.2.5 *LTA4H* genotype and blood characteristics

Previous study in TBM revealed the association between cerebrospinal fluid cytokine levels and bacterial load (143). Hypo-inflammation, expressing with lower cytokine levels, were associated with death in TBM patients treated with dexamethasone (86). The TT genotype was associated with increased cerebrospinal fluid cytokines and improved survival with adjunctive

corticosteroid therapy (87). Those findings suggest that inflammatory response plays a crucial role in pathogenesis, and optimization of inflammatory response based on host genotype can improve treatment response in TBM. In order to understand whether this phenomenon in TBM also exists in pulmonary TB, especially in MDR-TB, I examined the associations between blood characteristics and TB severity, treatment response and outcomes and then compared the differences in circulating cytokine levels and other blood markers in MDR-TB patients with the three genotypes.

Elevated white blood cell (WBC) counts and platelets associated with high bacterial burden and high lung damage in both MDR (Table 4.9) and DS-TB (***Table 4.10***). Increase in neutrophil:lymphocyte ratio (NLR) associated with higher bacterial burden in DS-TB (OR: 1.21, 95% CI: 1.09-1.34), more lung damage for both MDR-TB (OR: 1.10, 95%CI: 1.02-1.20) and DS-TB (OR: 1.16, 95%CI: 1.05-1.27), more severe symptoms (OR: 1.23, 95%CI: 1.10-1.37 for MDR-TB; OR: 1.13, 95%CI: 1.05-1.23 for DS-TB) and more severe disease (OR: 1.25, 95%CI: 1.11-1.40 for MDR-TB; OR: 1.14; 95%CI: 1.05-1.24 for DS-TB). Under treatment, increase baseline NLR predicted a failure to sputum convert at one month (OR: 1.21, 95%CI: 1.05-1.40 for MDR-TB; OR: 1.07, 95%CI: 0.99-1.16 for DS-TB), mortality (OR: 1.17, 95%CI: 1.04-1.32 for MDR-TB; OR: 1.22, 95%CI: 1.08-1.37 for DS-TB) and unfavorable outcomes (OR: 1.09, 95%CI: 1.00-1.19 for MDR-TB; OR: 1.13, 95%CI: 1.04-1.23 for DS-TB).

Increased albumin level, albumin:globulin ratio (AG) and decreased globulin level were associated with higher bacterial burden in DS-TB, higher lung damage, more severe symptoms, severe diseases, higher mortality in both MDR and DS-TB, and higher unfavorable outcomes in MDR-TB (***Table 4.9*** and ***Table 4.10***).

A panel of eight cytokines, including TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IL-13, IL12p70, was measured in serum samples of all 312 MDR-TB and 316 DS-TB patients before treatment. Levels of TNF and IL-6 were detectable in most TB patients and were included in the analysis for association with baseline clinical presentations, severity, treatment response and outcome

(***Table 4.9*** and ***Table 4.10***). Elevated TNF-α level correlated with lower bacterial burden (OR: 0.95, 95%CI: 0.91-0.99 per 1pg/ml increase), higher mortalities (OR: 1.06, 95%CI: 1.01-1.10) and unfavorable outcomes (OR: 1.08; 95%CI: 1.02-1.14) in MDR-TB. In DS-TB, patients with elevated TNF-α had more severe symptoms (OR: 1.07, 95%CI: 1.02-1.12), severe disease (OR: 1.10, 95%CI: 1.04-1.16) and higher mortality rate (OR: 1.11, 95%CI: 1.02-1.21) (***Table 4.9***). Increasing IL-6 associated with high bacterial burden in DS-TB (OR: 1.05, 95%CI: 1.02-1.09) but did not associate with severity, treatment response and outcome in both MDR and DS-TB.

Table 4.9. Associations between blood characteristics and MDR-TB manifestations by univariate logistic regression and mortality

Characteristics	High bacterial burden		Severe disease		Mortality	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
WBC (G/L)	1.13 (1.04-1.24)	0.005	1.13 (1.04-1.23)	0.006	1.09 (0.91-1.31)	0.345
Neutrophil (%)	1.01 (0.99-1.04)	0.375	1.06 (1.03-1.09)	<0.001	1.12 (1.04-1.20)	0.002
Lymphocyte (%)	0.97 (0.95-1.00)	0.080	0.93 (0.90-0.96)	<0.001	0.86 (0.78-0.95)	0.003
Monocyte (%)	1.07 (0.97-1.18)	0.196	0.94 (0.85-1.05)	0.280	0.83 (0.64-1.09)	0.178
NLR*	0.99 (0.92-1.07)	0.818	1.25 (1.11-1.40)	<0.001	1.17 (1.04-1.32)	0.008
Platelet**	1.07 (1.04-1.09)	<0.001	1.01 (0.99-1.03)	0.169	0.97 (0.92-1.03)	0.316
Albumin (g/dl)	0.99 (0.61-1.62)	0.974	0.33 (0.19-0.58)	<0.001	0.07 (0.02-0.24)	<0.001
Globulin (g/dl)	1.39 (1.00-1.94)	0.047	1.56 (1.12-2.19)	0.009	1.70 (0.80-3.62)	0.171
A/G ratio***	0.92 (0.84-1.01)	0.084	0.82 (0.74-0.91)	<0.001	0.55 (0.39-0.77)	<0.001
TNF-α (pg/ml)	0.95 (0.91-0.99)	0.022	1.01 (0.98-1.03)	0.635	1.06 (1.01-1.10)	0.010
IL-6 (pg/ml)	1.00 (0.99-1.01)	0.519	1.00 (1.00-1.01)	0.594	1.01 (1.00-1.02)	0.133

Data of bacterial burden was for 309 MDR-TB with albumin, globulin and A/G ratio, 275 patients with WBC counts and 312 patients with TNF- α and IL-6 results. Data of severe disease was for 297 MDR-TB with albumin, globulin and A/G ratio, 264 patients with WBC counts and 288 patients with TNF- α and IL-6 results. Data of mortality was for 296 MDR-TB patients with albumin, globulin and A/G ratio, 262 patients with WBC counts and 299 patients with TNF- α and IL-6 results.

* Neutrophil:lymphocyte ratio

** Per 10G/ml increase of platelet count

** Per 0.1 increase of A/G ratio

Table 4.10. Relationships between blood characteristics and DS-TB manifestations and mortality by univariate logistic regression

Characteristics	High bacterial burden		Severe disease		Mortality	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
WBC (G/L)	1.24 (1.15-1.33)	<0.001	1.08 (1.01-1.15)	0.019	1.02 (0.80-1.32)	0.853
Neutrophil (%)	1.05 (1.03-1.07)	<0.001	1.04 (1.02-1.07)	<0.001	1.40 (1.17-1.67)	<0.001
Lymphocyte (%)	0.95 (0.93-0.98)	<0.001	0.96 (0.93-0.98)	0.002	0.68 (0.54-0.86)	0.001
Monocyte (%)	0.98 (0.02-1.05)	0.629	1.05 (0.97-1.13)	0.217	0.43 (0.26-0.72)	0.002
NLR*	1.21 (1.09-1.34)	<0.001	1.14 (1.05-1.24)	0.003	1.22 (1.08-1.37)	<0.001
Platelet**	1.04 (1.03-1.06)	<0.001	1.01 (1.00-1.03)	0.057	0.91 (0.84-1.00)	0.049
Albumin (g/dl)	0.33 (0.21-0.52)	<0.001	0.32 (0.20-0.52)	<0.001	0.14 (0.03-0.62)	0.010
Globulin (g/dl)	2.19 (1.65-2.92)	<0.001	1.35 (1.02-1.80)	0.036	1.77 (0.69-4.56)	0.239
A/G ratio***	0.75 (0.69-0.81)	<0.001	0.83 (0.75-0.91)	<0.001	0.63 (0.42-0.93)	0.022
TNF-α (pg/ml)	1.03 (0.98-1.08)	0.228	1.10 (1.04-1.16)	0.001	1.11 (1.02-1.21)	0.022
IL-6 (pg/ml)	1.05 (1.02-1.09)	0.002	1.00 (1.00-1.00)	0.903	1.00 (0.96-1.03)	0.889

Data of bacterial burden was for 598 DS-TB with albumin, globulin and A/G ratio, 536 patients with WBC counts and 309 patients with TNF- α and IL-6 results. Data of severe disease was for 484 DS-TB with albumin, globulin and A/G ratio, 429 patients with WBC counts and 271 patients with TNF- α and IL-6 results. Data of mortality was for 598 DS-TB patients with albumin, globulin and A/G ratio, 536 patients with WBC counts and 316 patients with TNF- α and IL-6 results.

* Neutrophil:lymphocyte ratio

** Per 10G/ml increase of platelet count

** Per 0.1 increase of A/G ratio

Baseline complete blood count, albumin, globulin and a panel of eight cytokines were compared between *LTA4H* genotypes in MDR-TB (***Table 4.11***). The median of platelet counts in TT patients (314G/l, IQR: 276-386) was lower than CT (380 [313-469]) and CC (351 [281-436]) (p=0.014). Albumin level was the highest in TT patients (4.23g/dl, IQR: 3.82-4.38), but the differences was not significant (p=0.361). Other blood characteristics were similar among *LTA4H* genotype (***Table 4.11***).

Table 4.11. Profile of white blood cell and biomarkers of MDR-TB and LTA4H genotype

	CC *N=134*	**CT** *N=148*	**TT** *N=30*	**P value**
WBC (G/L)	7.89 [6.53-9.94]	8.91 [7.10-10.9]	8.30 [6.65-11.4]	0.069
Neutrophil (G/L)	5.25 [3.80-6.95]	5.48 [4.39-7.71]	5.14 [4.19-7.72]	0.105
Neutrophil (%)	65.8 [57.3-72.6]	66.4 [59.4-71.4]	66.6 [61.9-71.8]	0.965
Lymphocyte (G/L)	1.72 [1.28-2.28]	1.90 [1.44-2.24]	1.80 [1.43-2.02]	0.601
Lymphocyte (%)	20.9 [15.0-27.9]	20.6 [16.6-26.9]	22.0 [16.9-26.1]	0.900
Monocyte (G/L)	8.80 [7.38-10.3]	8.90 [7.30-10.7]	8.60 [7.65-10.3]	0.974
Monocyte (%)	0.70 [0.55-0.97]	0.81 [0.60-1.00]	0.74 [0.53-1.11]	0.194
NLR	3.14 [2.11-4.76]	3.18 [2.26-4.31]	2.97 [2.42-4.17]	0.911
Platelet (G/L)	351 [281-436]	380 [313-469]	314 [276-386]	0.014
Albumin (g/dl)	4.07 [3.73-4.34]	4.06 [3.67-4.34]	4.23 [3.82-4.38]	0.361
Globulin	3.83 [3.49-4.38]	4.01 [3.49-4.53]	3.83 [3.17-4.22]	0.098
A/G ratio	1.04 [0.88-1.23]	1.02 [0.84-1.22]	1.10 [0.97-1.32]	0.167

Value were presented as median and [IQR]. Data was for 309 MDR-TB patients with available results of albumin, globulin and A/G ratio, and 275 patients with WBC counts and differentiation results. P overall value of Wilcoxon test was reported.

Among 312 MDR-TB patients, TNF-α levels were detectable in all 30 (100%) TT, 143 (96.6%) CT and 128 (95.5%) CC patients (p=0.665). No significant differences between the three genotypes were observed in 301 MDR-TB patients with detectable TNF-α level (p=0.389). CC genotype had lower detected IL-6 levels (124 patients (92.5%)) compared to CT (146 (98.6%)) and TT (29 (96.7%)) (p=0.030). No difference was found between the levels of IFN-γ, IL-2, IL-13, I-12p70 and *LTA4H* genotype (***Table 4.12***). TT patients had slightly higher levels of IL-1β (10.2pg/ml [3.53-41.4] in TT; 5.52pg/ml [2.67-7.80] in CT; 4.34pg/ml [2.15-7.22], p=0.089) and more detectable levels of IL-10 (13 patients (43.3%) in TT; 48 (32.4%) in CT; 44 (32.8%) in CC, p=0.497).

Table 4.12. Pro-inflammatory and anti-inflammatory cytokines in MDR-TB stratified by LTA4H genotype

	CC *N=134*	CT *N=148*	TT *N=30*	P value
TNF-α detected	128 (95.5%)	143 (96.6%)	30 (100%)	0.665
TNF-α (pg/ml)	4.40 [2.64-5.98]	4.33 [2.75-7.89]	3.57 [2.41-5.61]	0.389
IFN-γ detected	52 (38.8%)	53 (35.8%)	7 (23.3%)	0.279
IFN-γ (pg/ml)	13.0 [5.72-25.9]	11.8 [6.19-33.4]	12.2 [7.72-33.6]	0.877
IL-2 detected	2 (1.49%)	3 (2.03%)	2 (6.67%)	0.238
IL-2 (pg/ml)	45.9 [23.4-68.3]	20.6 [10.5-40.5]	5.03 [3.92-6.14]	0.898
IL-6 detected	124 (92.5%)	146 (98.6%)	29 (96.7%)	0.030
IL-6 (pg/ml)	5.81 [3.81-11.1]	6.42 [3.32-11.5]	5.92 [2.27-16.9]	0.997
IL-13 detected	19 (14.2%)	26 (17.6%)	5 (16.7%)	0.746
IL-13 (pg/ml)	112 [86.7-134]	112 [68.7-151]	174 [161-186]	0.146
IL-1β detected	42 (31.3%)	47 (31.8%)	12 (40.0%)	0.642
IL-1β (pg/ml)	4.34 [2.15-7.22]	5.52 [2.67-7.80]	10.2 [3.53-41.4]	0.089
IL-10 detected	44 (32.8%)	48 (32.4%)	13 (43.3%)	0.497
IL-10 (pg/ml)	2.12 [1.27-3.00]	2.11 [1.46-3.58]	2.47 [1.63-4.34]	0.785
IL-12p70 detected	11 (8.21%)	13 (8.78%)	0 (0.00%)	0.263
IL-12p70 (pg/ml)	133 [49.1-621]	207 [75.5-366]	-	0.931

Value was presented as number (%) for category variables and median [IQR] for continuous variables. Data was for 312 MDR-TB patients. P overall value of Wilcoxon test was reported for continuous variables, and p overall value of chi square test was reported for category variables.

4.3 DISCUSSION

Previous studies of host-genotype-specific therapy in TBM has revealed that SNP at rs17525495 of *LTA4H* genotype predicted response to dexamethasone therapy and associated with cytokine expression in TBM patients (85) (86) (87) (137) (142). It provided deeper understandings about pathogenesis and mechanism of host genotype in responding to

adjunctive corticosteroid therapy in TBM. The current study was conducted to explore whether and how this genotype influences treatment outcomes of pulmonary TB, especially the difficult-to-treat MDR-TB.

No association between *LTA4H* genotype and TB clinical presentations at the time of diagnosis was found for both MDR and DS-TB. *LTA4H* genotype did not significantly influence bacterial burden by analyzing GeneXpert levels and mean CT value. Lung damage, assessed by CXR, was more profound in MDR-TB than DS-TB. Among MDR-TB patients, TT genotype had lesser cavities and lower Timika score than other genotypes, but the difference was not significant. MDR-TB patients with TT genotypes had similar Timika score with DS-TB patients. Moreover, lesser TT patients experienced weight loss, making their mean BMI higher than CT and CC patients. Although a trend of protective effect of TT genotype was observed in TB presentations in MDR-TB, this genotype did not significantly decreased the risk of developing severe symptoms and diseases. Delay in treatment is a major host factor for increasing TB severity (144) (145) and also in this cohort (unpresented data). After adjusting for duration of disease before diagnosis, no significant association was found between *LTA4H* genotype and disease severity.

During follow-up, some pulmonary TB patients stopped producing sputa at some time points, which might be a sign of good treatment response. However, in MDR-TB, some patients re-produced positive culture sputa at later time points. The exclusion of a small number of patients (none at month 1, seven/275 patients at month 2 and 11/275 patients at month 3) or imputing negative culture results for those patients would equally cause subtle bias (if any) to the analysis. Therefore, I decided to exclude MDR-TB patients who did not have sputa for at least two months before the observed time point in the proportion culture conversion analysis.

During treatment, *LTA4H* genotype could not predict treatment response and outcomes, even in the early time points at 14 days, one, two and four months. In 235 MDR-TB and 543 DS-TB patients with sputum culture conversion, subtle changes in time to the first sputum culture

conversion were observed. TT patients took the shortest time to convert in MDR-TB (p=0.378). In DS-TB, the heterozygote CT had the shortest time to convert (p=0.641). Among 299 MDR-TB and 600 DS-TB patients with outcome information, mortality rates were lower in TT and CT compared to CC genotype. TT genotype also had the lowest unfavorable outcomes, but the differences were not significant. This is inconsistent to the findings of Tobin *et al*. that TT genotype had the lowest survival rate without dexamethasone treatment (137). With dexamethasone treatment, survival rates improved in the high inflammatory TT genotype in TBM patients (85). In our cohort, although effect of *LTA4H* genotype was obscured in both MDR and DS-TB, TT genotype seemed to associate with milder disease and better response to MDR-TB treatment. The impact of *LTA4H* genotype in DS-TB is lesser than in MDR-TB, possibly because of the effective chemotherapy in DS-TB.

TBM is the most devastating form of TB, which expressed high inflammatory response. Both serum and cerebrospinal fluid cytokine levels of TBM patients were much higher than control (146). *LTA4H* TT genotype is associated with more inflammation with higher cerebrospinal fluid cytokine levels, and therefore responds better to dexamethasone in TBM patients without HIV co-infection (86) (87) (142). Pulmonary TB patients also had higher pro-inflammatory cytokines than latent TB and healthy control (147). Cytokine levels of TNF-α, IFN-γ, IL-17A, IL-17F correlated with bacterial burdens and were elevated in patients who did not have sputum culture conversion at two months of treatment (147). In this study, circulating cytokines and other blood characteristics were measured to estimate the effect of *LTA4H* genotype on inflammatory response in pulmonary TB patients. Increases in WBC, neutrophil, NLR, globulin and decreases in lymphocyte, albumin, A/G were associated with advanced pulmonary TB and could predict mortality in MDR and DS-TB, which is in concordance with findings of previous studies (147-149). In MDR-TB, patients with increased TNF-α had lower bacterial burden at the start of treatment but higher mortality during treatment. Elevated TNF- α was associated with severe symptoms, severe disease and mortality in DS-TB. These results highlighted that

host hyper-inflammatory response affected TB presentations and complicated treatment outcomes. However, I did not find any association between *LTA4H* genotype with WBC counts, albumin, globulin and cytokines level, except that TT patients had lower platelet counts and the number of detectable IL-6 was different between the three genotypes. Six of eight cytokines in this study had more than 50% samples with levels under detection range of the assay (***Table 4.12***). In TBM, cerebrospinal fluid cytokine levels were measurable in all samples and strongly associated with *LTA4H* genotypes (87). While circulating cytokine concentrations might be substantially different with those at local sites of infection (150), it is impractical to measure cytokine concentrations in the lungs of pulmonary TB patients. In this study, no significant association was found between this genotype and inflammatory response expressed by circulating cytokine concentrations and other blood markers. One possible explanation for this obscured impact was that the effect of this host genotype was diminished when evaluating circulating cytokine concentrations instead of their concentrations at the lung. However, the effect of *LTA4H* genotype was also limited in TB presentations and treatment outcome of pulmonary TB. In a study of Tram *et al*, TBM patients had higher neutrophil counts and NLR than active pulmonary TB and healthy control (151). It suggested pulmonary TB induces weaker inflammatory response than TBM, and the effect of host genotype might be modest in chronic disease with less inflammation like pulmonary TB.

4.4 CONCLUSION

Although *LTA4H* genotype is a predictor for treatment response to dexamethasone and mortality in TBM, this host genotype is not a significant factor in pulmonary TB. Modest effect of *LTA4H* genotype on pulmonary TB presentations, inflammatory phenotypes, treatment response and outcomes of pulmonary MDR-TB and DS-TB indicated that this host genotype might not be the target for adjunctive therapy for pulmonary TB patients. In order to improve outcomes for pulmonary MDR-TB patients, other host factors should be explored.

APPENDIX

Table A.1 Univariate and multivariate logistic regression to evaluate the association between isoniazid resistant status and treatment response among rifampicin susceptible TB patients.

Time point	Isoniazid resistant status	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* aOR (95%CI)	P
Day 14	Resistance	101 (83.5%)	1.01 (0.58-1.74)	0.972	1.06 (0.59-1.90)	0.845
	Susceptibility	335 (83.3%)	1		1	
Month 1	Resistance	73 (60.3%)	1.15 (0.76-1.74)	0.511	1.17 (0.76-1.81)	0.466
	Susceptibility	229 (57.0%)	1		1	
Month 2	Resistance	36 (29.8%)	1.37 (0.87-2.16)	0.169	1.42 (0.90-2.26)	0.134
	Susceptibility	94 (23.6%)	1		1	
Outcome**	Resistance	16 (13.1%)	0.80 (0.44-1.44)	0.458	0.75 (0.41-1.37)	0.352
	Susceptibility	66 (15.9%)	1		1	

Data for sputum conversion analysis and treatment outcome was for 538 DS-TB patients with WGS information.

*: Adjusting for bacterial load accessed by mean CT value of GeneXpert.

**: Treatment outcome was presented as number (%) of unfavorable outcomes, including death, failure and lost to follow-up

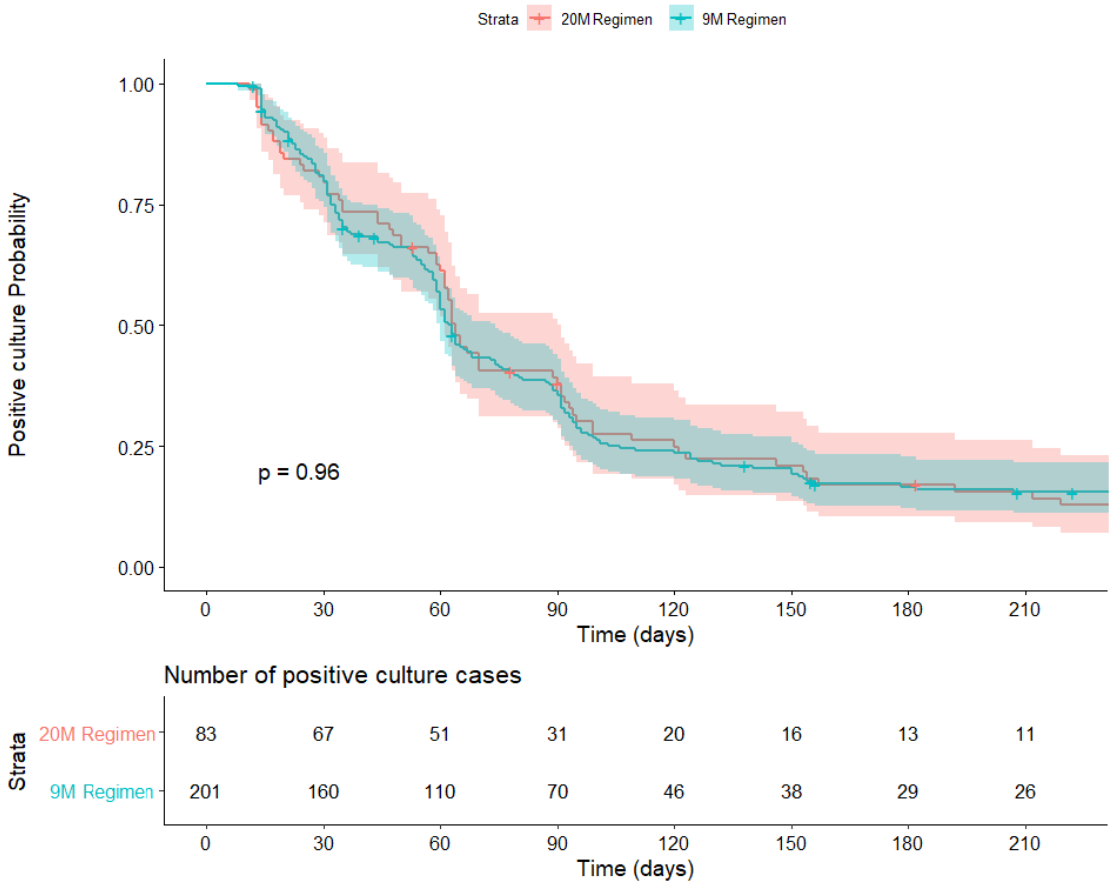


Figure A.1 Kaplan Meier plot for sputum culture conversion in respect of two MDR-TB treatment regimens. Data was for 284 MDR-TB patients with positive culture at baseline. Pink line represented 83 MDR-TB patients receiving 20 month regimen and green for 201 patients with 9 month regimen. P value of log-rank test was reported.

Table A.2 Associations between LTA4H genotype and DS-TB treatment response by univariate and multivariate logistic regression

Time point	Genotype	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* aOR (95%CI)	P
Day 14	TT	49 (84.5%)	0.90 (0.40-2.00)	0.793	0.86 (0.37-1.96)	0.715
	CT	224 (83.0%)	0.80 (0.49-1.31)	0.378	0.86 (0.51-1.43)	0.555
	CC	200 (85.8%)	1		1	
Month 1	TT	32 (55.2%)	0.82 (0.46-1.46)	0.496	0.80 (0.44-1.46)	0.463
	CT	151 (55.9%)	0.84 (0.5901.20)	0.346	0.88 (0.60-1.27)	0.483
	CC	140 (60.1%)	1		1	
Month 2	TT	11 (19.3%)	0.82 (0.40-1.70)	0.600	0.83 (0.39-1.72)	0.609
	CT	72 (27.0%)	1.27 (0.84-1.92)	0.252	1.35 (0.89-2.06)	0.157
	CC	52 (22.5%)	1		1	

*: Adjusting for bacterial load accessed by mean CT value of GeneXpert.

Chapter 5

DIABETES MELLITUS

AND PULMONARY TUBERCULOSIS

5.1 INTRODUCTION

Diabetes mellitus (DM) is a global non-infectious pandemic. According to the International Diabetes Federation, from 2009 to 2019, the global DM prevalence increased from 285 million people to 463 million people, accounting for 9.3% of the adult population (152). Sedentary lifestyles, unhealthy diets, and longer lifespan have contributed to the rise of DM. Once considered a disease of the rich, DM is now affecting all continents, with rapid increase in low and middle income countries (153), where tuberculosis (TB) is also prevalent. Among the countries with the greatest numbers of DM cases are countries with high burdens of TB (11).

DM triples the risk of developing active TB (154). DM and pre-diabetes (pre-DM) are found to be highly prevalent in TB patients (155) (156) (157) and in those with latent TB infection (158). Hyperglycemia increases the risk of infections, including TB, due to impaired innate and adaptive immune response (153) (159). Obesity is a major risk factor for developing type 2 DM, in which abundant adipose tissue not only increases insulin resistance but also promotes systemic inflammation by excessive production of pro-inflammatory cytokines and chemokines (160). DM-TB patients have more symptoms and lung cavities (77) and persistent inflammation prior and during TB treatment (161). DM was found to worsen TB treatment outcomes with higher odds of death, failure, and relapse (162). DM patients needed more time to reach the peak concentration of rifampicin, and had lower isoniazid maximum concentrations (163). In reverse, TB, as a chronic infectious disease, can induce stress-hyperglycemia (164), which sometimes can be reversible with TB treatment (165).

Although DM is among the most common comorbidities in MDR-TB (80) (81) (130) (166) (167), there are very few data on the relationship between DM and the MDR-TB treatment outcome. Previous studies showed DM doubled the risk of having MDR-TB (168) and was associated with MDR-TB among patients with no previous history of TB (75, 77). During MDR-TB treatment, DM prolonged the time to sputum conversion in 52 MDR/XDR-TB patients in Tbilisi, Georgia (128) and worsened the treatment outcomes with higher rates of

death and failure in 1,407 MDR-TB patients in South Korea (81). Other studies in Georgia (77), China (71) , Vietnam (166) and Mexico (167) did not find a difference in treatment outcomes among MDR-TB patients with and without DM. In a meta-analysis of 13 studies of MDR-TB treatment outcomes, Samuels et al showed a pooled relative risk of 0.97 for unfavorable outcomes in MDR-TB patients with DM (169). Most of the studies on the impacts of DM on MDR-TB were retrospective, except one prospective study with 52 MDR/XDR-TB patients (128). Consequently, whether this important comorbidity exaggerates clinical manifestations of MDR pulmonary TB and worsens treatment outcomes are still unanswered. Understanding the impact of DM, pre-DM on MDR-TB and the biological mechanism for this effect is crucial to improve management of this "hard-to-treat" form of TB. To evaluate the effect of DM on TB, especially on MDR-TB, two prospective studies were conducted to investigate the influence of DM on MDR and DS-TB in HCMC, Vietnam.

I hypothesized that DM worsened the treatment response of MDR, mostly in the early stage of treatment, when the chemotherapy needed time to eliminate the bacteria. In this study, DM was actively screened for all MDR-TB to determine 1) the impacts of DM on TB manifestation, severity and bacterial burden at the time of MDR-TB diagnosis, 2) the association between DM and treatment response of MDR-pulmonary TB patients, 3) the association between DM and treatment outcome of MDR-TB, and 4) the effect of DM on biomarkers of white blood cell counts, A/G ratio and cytokines prior MDR-TB treatment. I also compared the effect of DM on MDR-TB and DM on DS-TB to investigate whether DM had stronger influence on MDR-TB on treatment response and outcomes.

5.2 RESULTS

Prevalence of DM and medications to treat DM

A total of 312 new MDR/RR pulmonary TB patients and 598 DS TB patients were tested for glycated hemoglobin (HbA1C) and fasting plasma glucose (FPG) at study enrolment. MDR/RR TB was defined as either rifampicin resistance detected by GeneXpert or both isoniazid and rifampicin resistance detected by LPA (Hain test) or phenotypic DST. Among 312 MDR-TB patients, 288 patients were initially diagnosed by GeneXpert, 10 by DST and 14 by Hain test. Then, GeneXpert was performed for 10 patients diagnosed by DST and 14 by Hain test to have information for semi-quantitative level of GeneXpert of all 312 MDR-TB patients. DS-TB was defined as rifampicin resistance not detected in GeneXpert. Among 598 DS-TB patients, 10 patients had AFB positive but GeneXpert negative in raw sputum samples. I re-tested with GeneXpert in the culture samples and all had *M.tb* detected but rifampicin resistance not detected.

The diabetes prevalence was 23.7% (74/312) in MDR-TB and 21.6% (129/598) in DS-TB. The prevalence of DM increased from 10.6% in MDR-TB and 10.9% in DS-TB in patients from 30 to 40 years of age to 36.3% and 33.3% in patients from 40-65 years of age, respectively. Among DM-TB patients, 25 (33.8%) MDR-TB and 41 (31.8%) DS-TB patients were newly diagnosed with diabetes. Median time of DM in those who were previously diagnosed with DM was 4 years [IQR: 1-8] in MDR-TB and 3 years [IQR: 1-6] in DS-TB. Another 104 (32.7%) MDR and 250 (41.8%) DS-TB patients had pre-DM. When using the criteria of just either FPG or HbA1C for DM diagnosis, the rate of DM reached 30.1% in MDR-TB and 30.4% in DS-TB.

Before the start of TB treatment, anti-diabetic drugs, including insulin, metformin and sulfonylurea, were used in 42/74 (56.7%) MDR and 73/129 (53.6%) DS with DM. Insulin was used in 16/74 (21.6%) MDR-TB and 28/129 (21.7%) DS-TB with DM. Metformin was used in 26/74 (35.1%) MDR-TB patients and 45/129 (34.9%) DS-TB patients. At the time of MDR-TB treatment initiation, median and interquartile range (IQR) of FPG (in mmol/l) in patients

using metformin was 15.3 [11.5-18.5] vs 11.3 [8.62-16.1] in patients not using metformin (p=0.081). In DS-TB patients, median and IQR of FPG in metformin group was 9.99 [6.00-14.0] vs 11.2 [8.51-15.6] in non-metformin (p=0.050).

HbA1C represents the average of blood glucose in 8 to 12 weeks. I further classified DM patients whose HbA1C < 7% as having "good glycemic control", and those with HbA1C ≥ 7% as "poor glycemic control". Sixty-four/74 (86.5%) MDR-TB and 112/129 (86.8%) DS-TB had poor glycemic control at the time of TB diagnosis.

Characteristics of DM and non-DM TB patients

Baseline characteristics and clinical manifestations of patients with and without DM were described in ***Table 5.1*** for MDR-TB and ***Table 5.2*** for DS-TB. P trend was reported for the comparisons to observe the ordered relationship of DM status (non-DM, pre-DM, DM) on TB characteristics. Of 312 MDR-TB patients, DM patients were older (49.5 [44.0-55.8] years in DM vs 42.0 [34.8-51.2] in pre-DM vs 35.5 [28.0-46.0] in non-DM, p<0.001), heavier (BMI: 20.9 [19.0-22.7] in DM vs 18.2 [16.5-20.3] in pre-DM vs 18.7 [17.0-20.5] in non-DM, p<0.001) and more likely to have anemia (46.8% in DM vs 45.8% in pre-DM vs 30.8% in non-DM, p=0.002). Consistent with MDR-TB, DS-TB patients with DM were also older (p<0.001), heavier (p<0.001), and more likely to have anemia (p=0.025) (***Table 5.2***).

For both MDR and DS-TB, the proportions of HIV co-infection, smoking, and duration of disease before receiving treatment were similar between DM and non-DM (***Table 5.1*** and ***Table 5.2***). Patients with DM were more likely to have history of regularly drinking alcohol than pre-DM and non-DM in DS-TB (p=0.011), but not in MDR-TB (p=0.650). The prevalence of patients with a history of previous TB episodes in patients with DM and non-DM was not significantly different for both MDR-TB (p=0.716) and DS-TB (p=0.927). DM and non-DM patients had similar individual TB-related symptoms, except DS-TB patients with DM experienced more weight loss (p=0.004) than patients without DM (***Table 5.2***).

Table 5.1. Characteristics and clinical manifestations of 312 MDR-TB patients stratified by DM status

	MDR-TB			
	Non-DM	**Pre-DM**	**DM**	**P trend**
	N=134	***N=104***	***N=74***	
Age (years)	35.5 [28.0-46.0]	42.0 [34.8-51.2]	49.5 [44.0-55.8]	<0.001
Male	98 (73.1%)	85 (81.7%)	53 (71.6%)	0.949
HIV co-infection	10 (7.52%)	7 (6.73%)	4 (5.41%)	0.566
Drinking alcohol	47 (35.1%)	39 (37.5%)	23 (31.1%)	0.650
Regular smoker	48 (35.8%)	40 (38.5%)	24 (32.4%)	0.715
History of previous TB	75 (56.0%)	60 (57.7%)	39 (52.7%)	0.716
Symptoms duration (days)	20.0 [12.0-30.0]	15.0 [11.2-30.0]	30.0 [10.0-30.0]	0.741
Cough	125 (93.3%)	99 (95.2%)	71 (95.9%)	0.391
Sputum	121 (90.3%)	89 (85.6%)	68 (91.9%)	0.916
Weight Loss	58 (43.6%)	51 (49.0%)	40 (54.1%)	0.143
Low grade fever	68 (50.7%)	53 (51.0%)	36 (48.6%)	0.797
Haemoptysis	19 (14.2%)	12 (11.5%)	7 (9.46%)	0.307
Chest pain	47 (35.1%)	36 (34.6%)	25 (33.8%)	0.854
Malaise	84 (62.7%)	61 (58.7%)	49 (66.2%)	0.735
Dyspnea	38 (28.4%)	39 (37.5%)	23 (31.1%)	0.518
BMI (kg/m^2)	18.7 [17.0-20.5]	18.2 [16.5-20.3]	20.9 [19.0-22.7]	<0.001
BMI group				<0.012
Normal	64 (47.8%)	36 (34.6%)	43 (58.1%)	
Overweight	5 (3.73%)	14 (13.5%)	18 (24.3%)	
Underweight	65 (48.5%)	54 (51.9%)	13 (17.6%)	
Cavity	50 (39.4%)	48 (48.0%)	35 (47.9%)	0.193
Timika score	25.0 [11.2-62.5]	47.5 [15.0-65.0]	50.0 [15.0-65.0]	0.093
GeneXpert				0.006

	MDR-TB			
	Non-DM	**Pre-DM**	**DM**	**P trend**
	N=134	***N=104***	***N=74***	
Not Detected	12 (9.0%)	5 (4.8%)	1 (1.4%)	
Very Low	16 (11.9%)	16 (15.4%)	5 (6.8%)	
Low	24 (17.9%)	25 (24.0%)	10 (13.5%)	
Medium	57 (42.5%)	38 (36.5%)	36 (48.6%)	
High	25 (18.7%)	20 (19.2%)	22 (29.7%)	
Mean CT*	21.0 [18.0-26.8]	21.9 [18.2-27.9]	19.1 [16.6-22.0]	0.032
Anemia	36 (30.8%)	44 (45.8%)	29 (46.8%)	0.002

*: bacterial load was accessed by mean CT value of GeneXpert

Value was presented as number (%) for category variables and median [IQR] for continuous variables. P value was based on test for trend.

Table 5.2. Characteristics and clinical manifestations of 598 DS-TB patients stratified by DM status

	DS-TB			
	Non-DM	**Pre-DM**	**DM**	**P trend**
	N=219	***N=250***	***N=129***	
Age (years)	32.0 [25.0-44.0]	46.0 [33.0-54.0]	50.0 [45.0-55.0]	<0.001
Male	159 (72.6%)	213 (85.2%)	104 (80.6%)	0.024
HIV co-infection	6 (2.74%)	11 (4.40%)	1 (0.78%)	0.481
Drinking alcohol	49 (22.5%)	80 (32.0%)	44 (34.1%)	0.011
Regular smoker	53 (24.2%)	56 (22.4%)	24 (18.6%)	0.238
History of previous TB	27 (12.3%)	34 (13.6%)	15 (11.6%)	0.927
Symptoms duration (days)	15.0 [12.5-30.0]	17.0 [11.2-30.0]	20.0 [13.8-30.0]	0.566
Cough	207 (94.5%)	236 (94.4%)	123 (95.3%)	0.774
Sputum	180 (82.2%)	219 (87.6%)	111 (86.0%)	0.232
Weight Loss	81 (37.0%)	121 (48.4%)	67 (51.9%)	0.004

	DS-TB			
	Non-DM	**Pre-DM**	**DM**	**P trend**
	N=219	***N=250***	***N=129***	
Low grade fever	106 (48.4%)	127 (50.8%)	73 (56.6%)	0.154
Haemoptysis	19 (8.68%)	26 (10.4%)	11 (8.53%)	0.936
Chest pain	64 (29.2%)	90 (36.0%)	40 (31.0%)	0.544
Malaise	98 (44.7%)	140 (56.0%)	60 (46.5%)	0.454
Dyspnea	29 (13.2%)	49 (19.6%)	18 (14.0%)	0.608
BMI (kg/m^2)	18.8 [17.4-20.9]	18.9 [17.3-21.1]	21.3 [19.5-22.7]	<0.001
BMI group				<0.001
Normal	100 (45.7%)	111 (44.4%)	76 (58.9%)	
Overweight	19 (8.68%)	31 (12.4%)	29 (22.5%)	
Underweight	100 (45.7%)	108 (43.2%)	24 (18.6%)	
Cavity	68 (40.2%)	80 (37.9%)	50 (46.7%)	0.371
Timika score	23.8 [7.50-52.5]	27.5 [12.5-60.0]	45.0 [10.0-58.8]	0.042
GeneXpert				
Not Detected	6 (2.7%)	3 (1.2%)	1 (0.8%)	
Very Low	25 (11.4%)	21 (8.4%)	8 (6.3%)	
Low	47 (21.5%)	54 (21.6%)	28 (21.9%)	
Medium	96 (43.8%)	95 (38.0%)	56 (43.8%)	
High	45 (20.5%)	77 (30.8%)	35 (27.3%)	
Mean CT*	20.8 [17.5-25.6]	20.1 [16.1-24.0]	20.0 [16.7-23.6]	0.043
Anemia	67 (34.4%)	98 (43.4%)	53 (46.5%)	0.025

*: bacterial load was accessed by mean CT value of GeneXpert

Value was presented as number (%) for category variables and median [IQR] for continuous variables. P value was based on test for trend.

5.2.1 Effects of DM and pre-DM on clinical presentations

5.2.1.1 TB signs and symptoms

There were no significant differences in presenting individual symptoms between DM and non-DM in both MDR and DS-TB, except more weight loss in DS-TB (***Table 5.1*** and ***Table 5.2***). I performed univariate logistic regression to explore the association between DM, pre-DM on MDR-TB signs and symptoms (Figure 5-1). MDR-TB patients with DM presented with similar odds of having cough (OR: 1.72 [0.45-6.55]), haemoptysis (OR: 0.63 [0.25-1.57]), fever (OR: 0.91 [0.51-1.60]), weight loss (OR: 1.52 [0.86-2.69]), night sweats (OR: 1.63 [0.91-2.90] and chest pain (OR: 0.94 [0.52-1.72]). Both DM and pre-DM patients had a tendency to lose weight (OR: 1.52 [0.86-2.69] for DM, OR: 1.24 [0.74-2.08] for pre-DM) but not statistically significant (Figure 5-1).

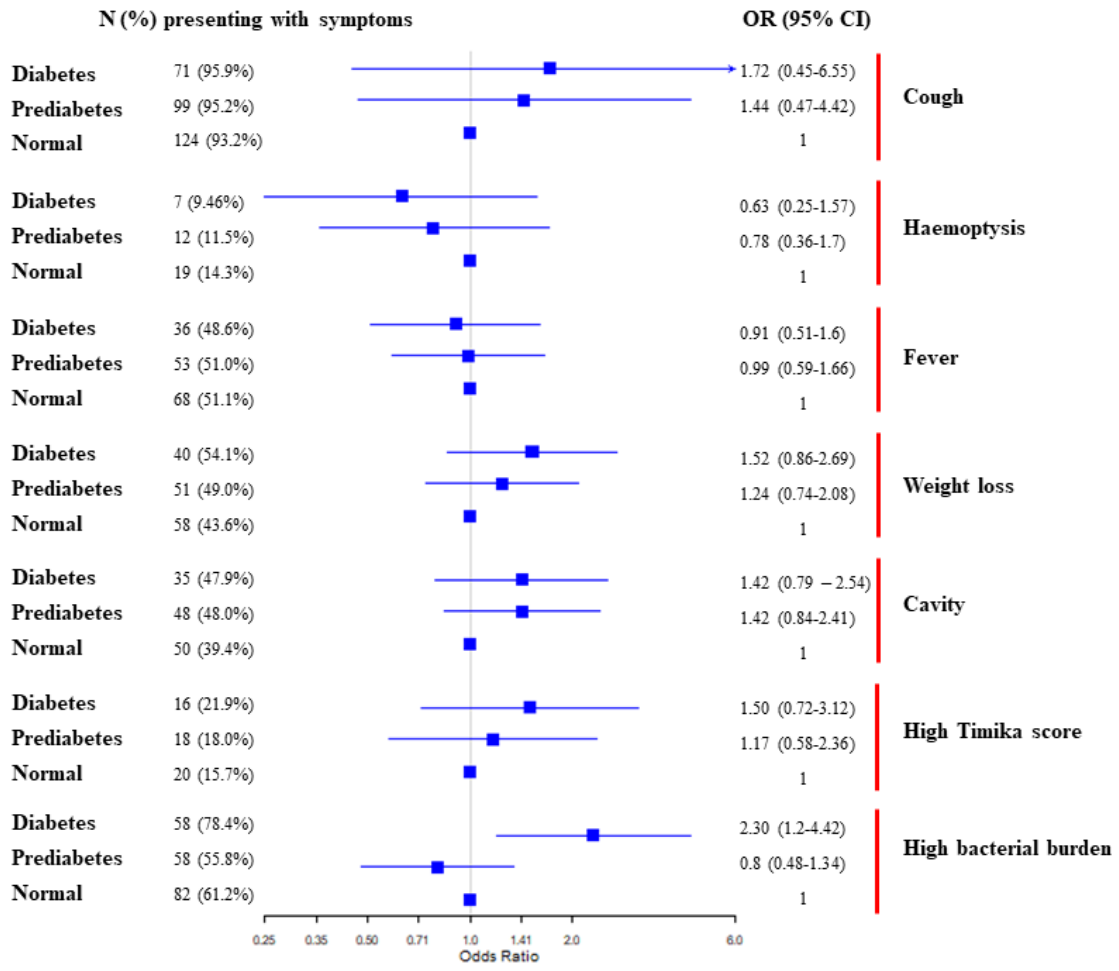


Figure 5-1. Effect of diabetes on MDR-TB signs and symptoms by univariate logistic regression. Data of TB symptoms and bacterial burden was for 312 MDR-TB patients. Data of cavity and Timika score was for 300 MDR-TB patients with CXR. OR: odds ratio; 95%CI: 95% confidence interval.

In DS-TB, DM patients were more likely to lose weight (OR: 1.84, 95%CI: 1.18-2.86) (Figure 5-2) and did not significantly differ for other individual symptoms (cough: OR: 1.19 [0.44-3.25]; haemoptysis: OR: 0.98 [0.45-2.13]; fever: 1.39 [0.9-2.15]; night sweats: OR: 1.30 [0.84-2.01]; chest pain: OR: 0.92 [0.60-1.40]).

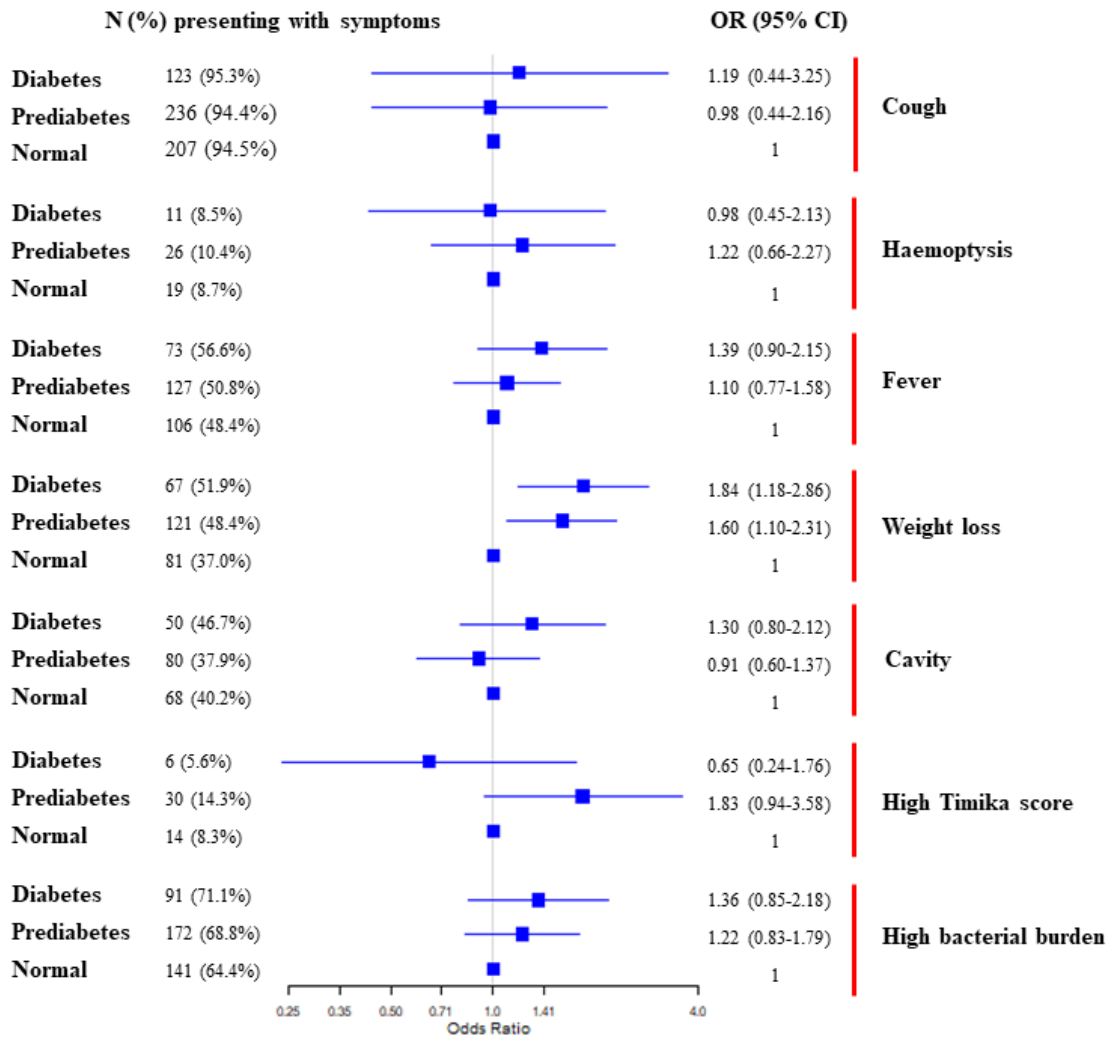


Figure 5-2. Forest plot of univariate logistic regression of effect of DM on DS-TB signs and symptoms. Data of TB symptoms was for 598 DS-TB patients. Data of cavity and Timika score was for 485 DS-TB patients with CXR. Data of bacterial burden was for 597 DS-TB patients. OR: odds ratio; 95%CI: 95% confidence interval.

5.2.1.2 *M.tb* bacterial burden

Bacterial burden by GeneXpert levels

I evaluated the *M.tb* bacterial burden using the automated GeneXpert assay. The 5-level GeneXpert results were grouped into binary variable with high bacterial burden, which included high and medium *M.tb* detected, and low burden including low, very low and *M.tb* not detected (section 2.2.9 in chapter 2).

At the time of MDR-TB diagnosis, DM patients were more likely to have positive sputum culture (OR: 10.7 [1.39-82.11]) and higher bacterial burden (OR: 2.30 [1.2-4.42]). After adjustment for duration of disease before treatment, DM still significantly increased the odds of having high or medium levels of GeneXpert (aOR: 2.32 [1.2-4.49]). In DS-TB patients, DM did not increase risks of having high bacterial burden by GeneXpert (OR: 1.36 [0.85-2.18]).

Bacterial burden by CT value

To further assess the effect of DM on bacterial burden, I compared the quantitative mean CT value of GeneXpert (section 2.2.9 in chapter 2). In MDR-TB patients, mean CT value of DM patients was 19.1 [16.6-22.0] and significantly lower than that of non-DM patients (21.0 [18.0-26.8]) (p=0.006) (Figure 5-3). There was no difference in mean CT value between pre-DM and non-DM patients (21.9 [18.2-27.9] vs 21.0 [18.0-26.8], p=0.459). In DS-TB, there was a decreasing trend in mean CT value (20.8 [17.5-25.6] in non-DM, 20.1 [16.1-24.0] in pre-DM, 20.0 [16.7-23.6] in DM, p trend=0.043), but the difference between DM and non-DM did not reach significance (p=0.065). Also, there was no significant difference in mean CT value of DM patients with MDR and DS-TB (p=0.445), and non-DM patients with MDR and DS-TB (p=0.318).

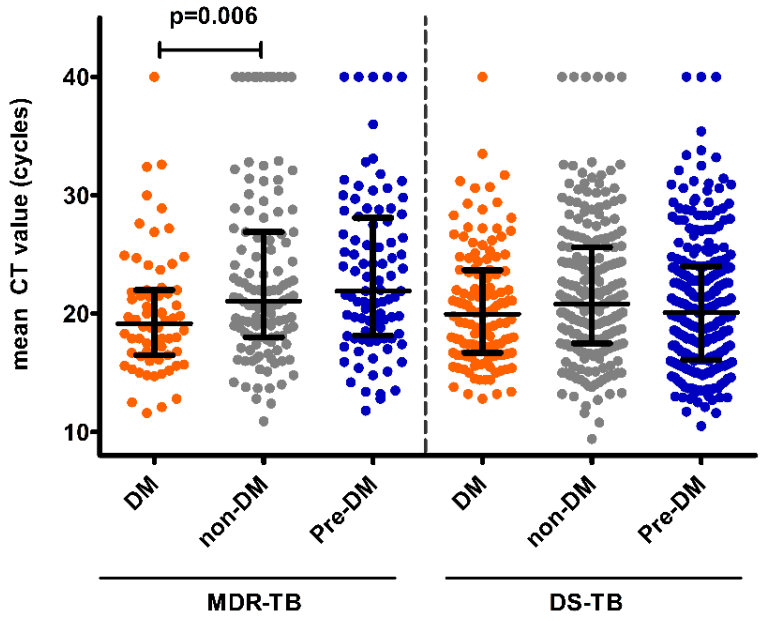


Figure 5-3. Associations between CT value of GeneXpert at baseline and DM status in MDR and DS-TB patients. Data was for 280 MDR-TB patients with available mean CT values including 122 non-DM, 92 pre-DM and 66 DM patients, and for 596 DS-TB including 219 non-DM, 249 pre-DM and 128 DM patients. P value was based on Mann-Whitney test.

Glycemic control and bacterial burden

I investigated whether the effect of DM on bacterial load came from the level of glycemic control. Poor glycemic control DM in MDR-TB patients increased 3.42 times (95%CI: 1.6-7.31, p=0.002) the risk of having high or medium GeneXpert level (Figure 5-4), and 2.32 (95%CI: 1.2-4.49, p=0.012) after adjusting for duration of disease. In contrast, MDR-TB patients with good glycemic control DM (HbA1C<7%) and pre-DM did not significantly increase the risk compared to non-DM patients (OR: 0.42, 95%CI: 0.11-1.57, p=0.198 in good glycemic control; OR: 0.80, 95%CI: 0.48-1.34, p=0.399 in pre-DM). Each percentage increase in HbA1C increased the odds of having high bacterial burden by 1.23 (95%CI: 1.08-1.39, p=0.001). In DS-TB, DM patients with poor glycemic control slightly increased the odds of having high bacterial burden (OR: 1.43, 95%CI: 0.87-2.35, p=0.162), and increase in 1% of HbA1C lead to increase 1.07 odds of having high burden in GeneXpert (95%CI: 0.99-1.16, p=0.087) (Figure 5-4).

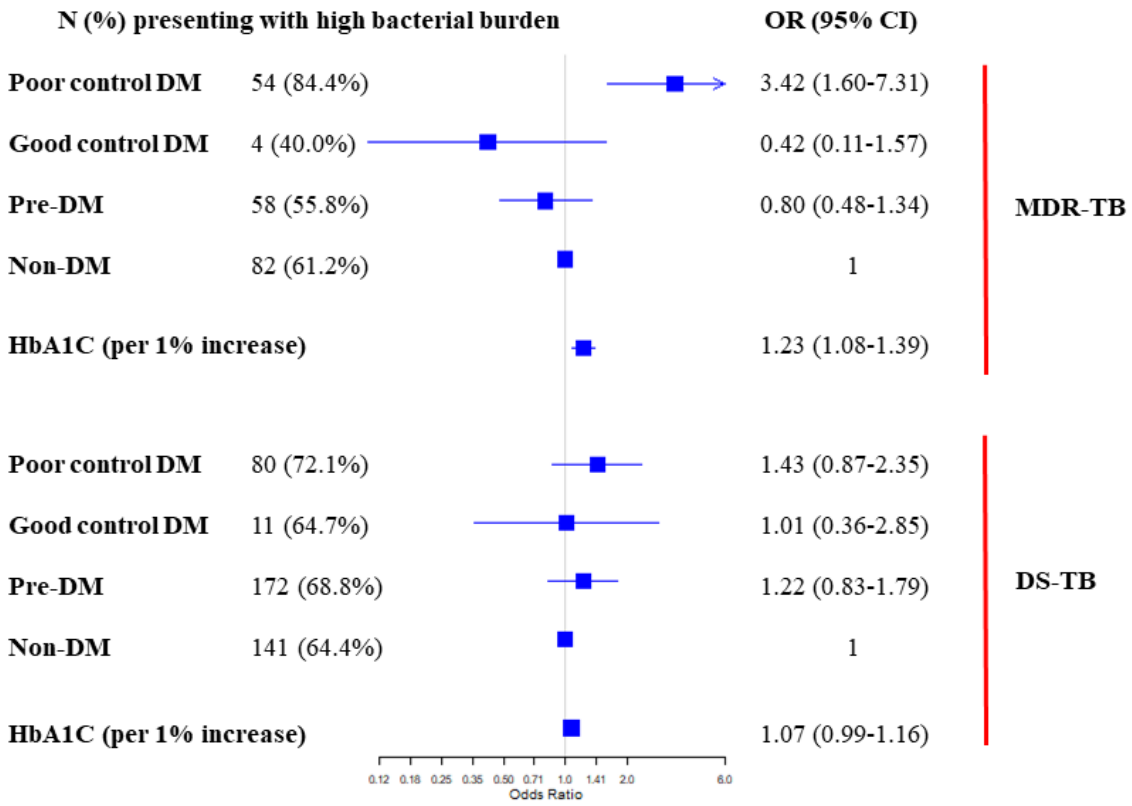


Figure 5-4. Forest plot of effect of glycemic control and HbA1C on *M.tb* bacterial burden. Data was for 312 MDR-TB patients including 134 non-DM, 104 pre-DM, 10 good glycemic control DM and 64 poor glycemic control DM patients, and for 597 DS-TB including 219 non-DM, 250 pre-DM, 17 good glycemic control DM and 111 poor glycemic control DM patients

5.2.1.3 Chest X-ray cavity and severity Timika score

Among 300 MDR-TB patients with CXR available, DM and pre-DM patients had a tendency to have lung cavities on chest Xray (CXR) but the difference was not significantly different (OR: 1.42 [0.79 – 2.54] for DM; OR: 1.42 [0.84-2.41] for pre-DM). After adjustment for age and duration of disease prior treatment, DM and pre-DM did not significantly increase the risk of having cavities (p=0.205 for DM, p=0.185 for pre-DM). Timika score was used to assess the extent of lung damage and the presence of cavity on CXR (section 2.2.1 in chapter 2). Patients with Timika scores equal or greater than 71 were considered to have high lung damage, and patients with Timika scores less than 71 as low lung damage. Median Timika score of DM (50.0, IQR: [15-65]) and pre-DM patients (47.5, IQR: [15-65]) were higher than non-DM (25,

IQR: [11.2-62.5]) but did not reach statistical significance (p trend = 0.093) (***Table 5.1***). In the univariate logistic regression, DM did not significantly increase the risk of having high lung damage (OR: 1.5 (0.72-3.12) in DM, OR: 1.17 (0.58-2.36) in pre-DM). Consistent with MDR-TB, DS-TB patients with DM did not increase risks of having lung cavities (OR: 1.30 [0.80-2.12] and high lung damage (OR: 0.65 [0.24-1.76]).

5.2.1.4 Symptom severity and disease severity

Severity and DM status

In order to comprehensively assess the influence of DM on TB presentations, I integrated seven TB symptoms into a symptom score, and then combined TB symptoms score, extent of lung damage by Timika score and bacterial burden by GeneXpert into a clinical score (section 2.2.2 in chapter 2). I compared the symptom scores and clinical scores TB patients with ordered DM status to explore the trend of those scores. The median symptom scores and clinical score were not higher in MDR-TB patients with DM, but significantly higher in DS-TB patients with DM and pre-DM (p=0.003 and p=0.005, respectively).

Using the cut off of five for symptom severity and six for clinical severity, both DM and pre-DM MDR-TB patients were more likely to have severe disease (***Table 5.3***). I further explored whether DM was associated with more severe disease in MDR-TB by using univariate and multivariate logistic regression analysis. Among 312 MDR-TB patients, 28 (37.8%) DM patients had severe symptoms, compared to 38 (28.4%) in non-DM patients. DM slightly increased the risk of having severe symptom and severe disease, but not significantly, in univariate logistic regression model. After adjusting for age and duration of disease, DM increased the risk of having severe symptoms by 2.13 (95%CI: 1.06-4.27, p=0.033) and of having severe disease by 2.26 (95%CI: 1.11-4.59, p=0.024).

Table 5.3. Associations between severity and DM status in MDR-TB patients by univariate and multivariate logistic regression

	DM status	Severe	Univariate		Multivariate**	
		*N (%)**	*OR (95%CI)*	*P*	*aOR (95%CI)*	*P*
Symptom severity	DM	28 (37.8%)	1.54 (0.84-2.81)	0.161	2.13 (1.06-4.27)	0.033
	Pre-DM	38 (36.5%)	1.45 (0.84-2.52)	0.180	1.64 (0.92-2.91)	0.095
	Non-DM	38 (28.4%)	1		1	
Clinical severity	DM	29 (39.7%)	1.67 (0.91-3.06)	0.099	2.26 (1.11-4.59)	0.024
	Pre-DM	37 (37.0%)	1.48 (0.85-2.6)	0.167	1.63 (0.90-2.94)	0.106
	Non-DM	36 (28.3%)	1		1	

Data of symptom severity was for 312 MDR patients including 134 non-DM, 104 pre-DM and 74 DM patients. Data of clinical severity was for 300 MDR-TB patients including 127 non-DM, 100 pre-DM and 73 DM patients.

* Number of patients with severe symptoms and severe disease.

** Adjusting for age and duration of disease

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval.

The same effect was also observed in DS-TB, with OR of DM for severe symptoms expanded from 1.76 (95%CI: 1.06-2.92, p=0.029) in univariate model to 2.24 (95%CI: 1.26-3.96, p=0.006) in multivariate model adjusted for age and duration of disease, and for severe disease from 1.54 (95%CI: 0.87-2.73, p=0.139) to 1.74 (95%CI: 0.91-3.30, p=0.09), respectively. Pre-DM also significantly increased risk of having severe symptoms by 2.17 times (95%CI: 1.42-3.32) and severe disease by 1.92 times (95%CI: 1.19-3.09) (***Table 5.4***).

Table 5.4. Associations between severity and DM status of DS-TB patients by univariate and multivariate logistic regression

	DM status	Severe	Univariate		Multivariate**	
		*N (%)**	*OR (95%CI)*	*P*	*aOR (95%CI)*	*P*
Symptom severity	DM	38 (29.5%)	1.76 (1.06-2.92)	0.029	2.24 (1.26-3.96)	0.006
	Pre-DM	85 (34.0%)	2.17 (1.42-3.32)	<0.001	2.50 (1.58-3.96)	<0.001

	DM status	Severe *N (%)*[*]	Univariate *OR (95%CI)*	*P*	Multivariate[**] *aOR (95%CI)*	*P*
	Non-DM	42 (19.2%)	1		1	
Clinical severity	DM	29 (27.4%)	1.54 (0.87-2.73)	0.139	1.74 (0.9103.30)	0.091
	Pre-DM	67 (31.9%)	1.92 (1.19-3.09)	0.008	2.05 (1.23-3.43)	0.006
	Non-DM	33 (19.6%)	1		1	

Data of symptom severity was for 598 DS-TB patients including 219 non-DM, 250 pre-DM and 129 DM patients. Data of clinical severity was for 484 patients including 168 non-DM, 210 pre-DM and 106 DM patients.

[*] Number of patients with severe symptoms and severe disease.

[**] Adjusting for age and duration of disease

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval.

Severity and glycemic control of DM patients

Poor glycemic control did not significantly expose MDR-TB patients to greater risks of having severe symptoms and severe disease. However, after adjusting for age and duration of disease, MDR-TB patients with good glycemic control DM slightly increased the risk of severe symptoms (aOR: 1.53, 95%CI: 0.36-6.58) and severe disease (aOR: 1.76, 95%CI: 0.39-7.90), while DM patients with poor glycemic control was strongly associated with more severe symptoms (aOR: 2.23, 95%CI: 1.09-4.57) and severe disease (aOR: 2.33, 95%CI: 1.13-4.83).

In DS-TB, after adjusting for age and duration of disease, both good and poor glycemic control DM significantly increased the risk of severe symptoms (aOR: 1.96, 95%CI: 1.08-3.55 for poor glycemic control; aOR: 5.40, 95%CI: 1.86-15.67 for good glycemic control). However, the risk of severe disease was significantly higher in good glycemic control DM (OR: 3.62, 95%CI: 1.02-12.78) but not in poor glycemic control DM (OR: 1.58, 95%CI: 0.81-3.07) in multivariate logistic regression model.

Effect of HbA1C on TB severity

To deepen our understanding on the effect of glycemic control on symptom and disease severity, I assessed the association between HbA1C and TB severity. Firstly, I plotted generalized additive model of HbA1C on symptom and clinical severity to explore their non-linear effects with the cut off at 8.5% (**Figure 5-5**). Then, I performed univariate and multivariate logistic regression with linear patterns for HbA1C ≤8.5% and index variable for HbA1C>8.5%.

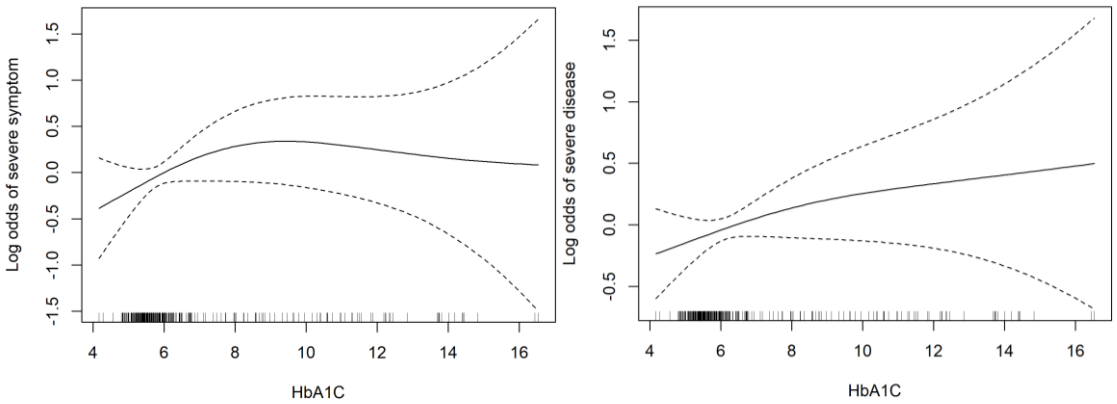


Figure 5-5. Non-linear effect of HbA1C on symptom and clinical severity of MDR-TB patients

For patients with HbA1C≤8.5%, each 1% increase of HbA1C increased the odds of having severe symptoms by 1.32 times (95%CI: 1.01-1.72) and after adjusting for age and duration of disease by 1.43 times 95%CI: 1.07-1.92 per 1% increase for HbA1C ≤8.5%). HbA1C also correlated with severe clinical presentations of MDR-TB in multivariate model (OR: 1.33, 95%CI: 1.02-1.73) (Table 5.5).

Table 5.5. Associations between HbA1C and symptom and disease severity of MDR-TB patients

	HbA1C	Univariate		Multivariate*	
		OR (95%CI)	P	aOR (95%CI)	P
Symptom severity	HbA1C≤8.5%	1.32 (1.01-1.72)	0.041	1.43 (1.07-1.92)	0.016
	HbA1C>8.5%	0.87 (0.70-1.10)	0.251	0.90 (0.71-1.15)	0.402
Clinical severity	HbA1C≤8.5%	1.29 (1.01-1.64)	0.038	1.33 (1.02-1.73)	0.033
	HbA1C>8.5%	0.86 (0.70-1.07)	0.176	0.85 (0.69-1.06)	0.150

* Adjusting for age and duration of disease. Data was for 305 MDR-TB patients with HbA1C and symptom severity, and 294 patients with HbA1C and clinical severity. OR: odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval.

5.2.2 Effect of diabetes on early treatment response

There was no significant difference in the treatment response and treatment outcome between two regimens of MDR-TB (section 4.2.3 in chapter 4). Therefore, I did not adjust for treatment regimen in the treatment response and outcome analysis.

Among DS-TB, isoniazid resistance did not associate with poorer treatment response and outcome, hence, was not included in our multivariate logistic analysis.

5.2.2.1 Sputum culture conversion at day 14, month 1, month 2 and month 3

The association of DM on culture conversion at day 14, month 1, month 2 and month 3 of anti-tuberculosis treatment were analyzed to assess its influence on early treatment response. Patients with at least two consecutive negative cultures at each defined time point were considered as "converted", and patients with positive culture, including reversion after conversion, or not having two consecutive negative cultures were considered as "not converted". Patients with reversion to positive cultures at the observed time points were considered as "not converted". Patients who died or lost to follow-up before observed time point and did not have culture conversion were considered as "not converted".

In MDR-TB, after 14 days of treatment, 97 (88.2%) non-DM, 74 (91.2%) pre-DM and 67 (97.1%) DM patients still had positive cultures. The influence of DM on sputum culture conversion was statistically significant. The odds of not converting in DM compared to non-DM patients gradually decreased from 4.61 (95%CI: 1.01-21.09) at day 14 to 2.69 (95%CI: 1.34-5.38) at month 1, at month 2 2.85 (95%CI: 1.54-5.27) and 2.04 (95%CI: 1.04-4.02) at month 3.

The directed acyclic graph showed interaction between DM and treatment response with potential confounding factors and mediators (Figure 5-6). BMI and age both influenced the

possibility of having DM and treatment response, and therefore, were confounding factors for the total effect of DM on treatment response. The two-way interaction between BMI and DM indicated that BMI was both confounding factor and mediator. Bacterial load increased in patients with DM, and could have impact on treatment response, hence, was an important mediator. Therefore, the effects of DM was adjusted for patients' age, BMI, and bacterial load, but not for treatment regimens. In this multivariate logistic regression model, DM significantly increased the rate of persistent sputum culture positive. The odds of not converting by day 14 was 8.62 times (95%CI: 1.32-56.18), that of month 1 was 2.85 (95%CI: 1.16-7.03) and month 2: 2.60 (95%CI: 1.21-5.57) (***Table 5.6***).

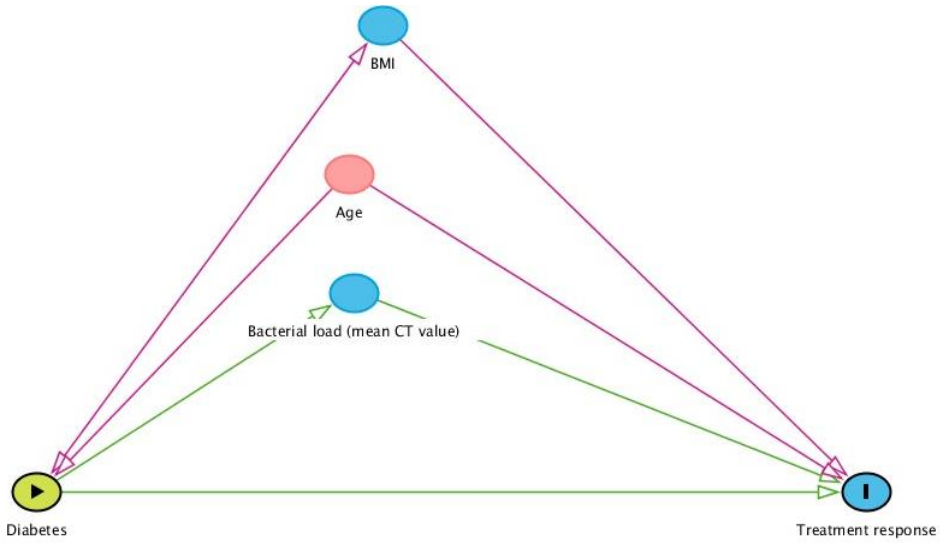


Figure 5-6. Directed acyclic graph of effect of diabetes on TB treatment response. The green line represents the causal path, and the pink for biasing path. The green circle (diabetes) represents exposure, blue (treatment response, BMI) as outcome, and pink (age) as confounding factor. Both confounding factors (BMI and patients' age) and important mediator (bacterial load) were included in multivariate logistic regression.

Table 5.6 Univariate and multivariate logistic regression for risk of no culture conversion during MDR-TB treatment

Time point	DM status	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* OR (95%CI)	P
Day 14	DM	71 (97.3%)	4.61 (1.01-21.09)	0.049	8.62 (1.32-56.18)	0.024
	Pre-DM	80 (93.0%)	1.73 (0.63-4.576)	0.286	3.07 (0.84-11.21)	0.090
	Non-DM	100 (88.5%)	1		1	
Month 1	DM	59 (80.8%)	2.69 (1.34-5.38)	0.005	2.85 (1.16-7.03)	0.022
	Pre-DM	60 (69.8%)	1.47 (0.81-2.67)	0.204	1.84 (0.91-3.73)	0.090
	Non-DM	69 (61.1%)	1		1	
Month 2	DM	41 (57.7%)	2.85 (1.54-5.27)	<0.001	2.60 (1.21-5.57)	0.014
	Pre-DM	36 (43.4%)	1.60 (0.89-2.86)	0.120	2.00 (1.04 -3.86)	0.038
	Non-DM	36 (32.4%)	1		1	
Month 3	DM	24 (33.8%)	2.04 (1.04-4.02)	0.039	2.19 (0.97-4.92)	0.059
	Pre-DM	22 (26.8%)	1.47 (0.75-2.88)	0.267	1.51 (0.74-3.10)	0.259
	Non-DM	22 (20.0%)	1		1	

* Adjusting for mean CT value of GeneXpert, age and BMI

In DS-TB, the effect of DM was also significant at day 14 (OR: 2.30, 95%CI: 1.15-4.58). Afterwards, the odds of DM for not converting quickly decreased over time (month 1: OR: 1.62, 95%CI: 1.02-2.56; month 2: OR: 1.26, 95%CI: 0.75-2.11). After adjusting for age and mean CT value, the effect of DM in DS-TB was diminished and not statistically significant (***Table 5.7***).

Table 5.7. Early treatment response in DS-TB with and without DM

Time point	DM status	No conversion N (%)	Univariate OR (95%CI)	P	Multivariate* OR (95%CI)	P
Day 14	DM	111 (90.2%)	2.30 (1.15-4.58)	0.018	1.64 (0.75-3.59)	0.218
	Prediabetes	199 (84.7%)	1.37 (0.84-2.26)	0.210	1.12 (0.64-1.95)	0.689
	Normal	161 (80.1%)	1		1	
Month 1	DM	78 (63.4%)	1.62(1.02-2.56)	0.041	1.12 (0.66-1.90)	0.680
	Prediabetes	139 (59.1%)	1.35 (0.92-0.97)	0.121	1.065(0.69-1.61)	0.809
	Normal	104 (51.7%)	1		1	
Month 2	DM	34 (27.6%)	1.26 (0.75-2.11)	0.375	0.96 (0.53-1.71)	0.882
	Prediabetes	54 (23.3%)	1.00 (0.64-1.57)	0.992	0.82 (0.50-1.33)	0.416
	Normal	46 (23.2%)	1		1	

* Adjusting for mean CT value of GeneXpert and patients' age

5.2.2.2 Sputum culture conversion time in converted patients

Among 235 (83%) MDR-TB patients who had at least one conversion since the start to the end of treatment, the median time to the first conversion increased from 50 days [IQR: 28-67] in non-DM patients to 60 days [IQR: 32-90] in pre-DM and 68 days [IQR: 49-95] in DM (p trend =0.001). The median time in 541 (94.3%) DS-TB patients with conversion were 34 [IQR: 20-62], 48 [IQR: 29-62] and 59 [IQR: 31-63] days in non DM, pre-DM and DM patients, respectively (p trend=0.004). I plotted the $\log_{10}$ of days to culture conversion to reveal that MDR-TB patients took longer time to convert than DS-TB patients (p<0.001) and DM prolonged culture conversion in both MDR-TB (p<0.001) and in DS-TB (p=0.005) (Figure 5-7)

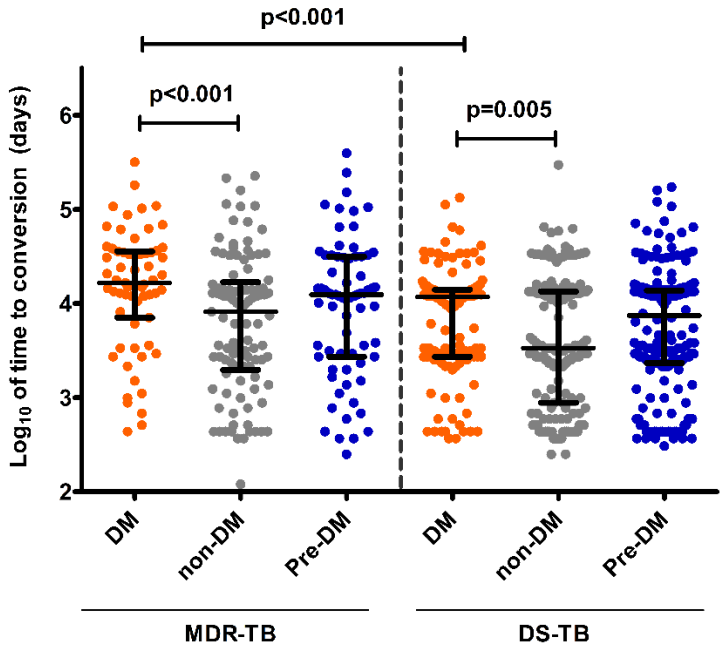


Figure 5-7. Differences in log_{10} of time to sputum culture conversion of MDR and DS-TB stratified by DM status. Data are for 235 MDR-TB patients including 63 diabetes (DM), 71 pre-DM (Pre-DM) and 101 non-diabetes (non-DM), and 541 DS-TB including 118 DM, 228 Pre-DM and 195 non-DM patients. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

5.2.2.3 Time to culture conversion using survival analysis

The Kaplan Meier plots showed the time to the first culture conversion of all 284 culture positive MDR-TB patients at the start of treatment (Figure 5-8) and 573 culture positive DS-TB patients (Figure 5-9). Events were noted when they had culture sputum conversion during treatment with at least two consecutive negative cultures. Patients who died or lost to follow-up before conversion were treated as censor. In this time to event analysis, I focused on the time to first culture conversion.

In MDR-TB, patients with DM prolonged the time to conversion, but not significantly in the log rank test (p=0.119 for DM and p=0.071 for pre-DM, with non-DM as reference). In DS-TB, DM patients also slightly prolonged the time to conversion with p=0.29 in log rank test. However, Cox-regression analysis did not account for the different impact on different time point, and the plot showed that DM might have strong influence during early time of treatment.

Therefore, restricted mean survival time analysis was performed with different truncated time to observe the influence of DM on different time of treatment.

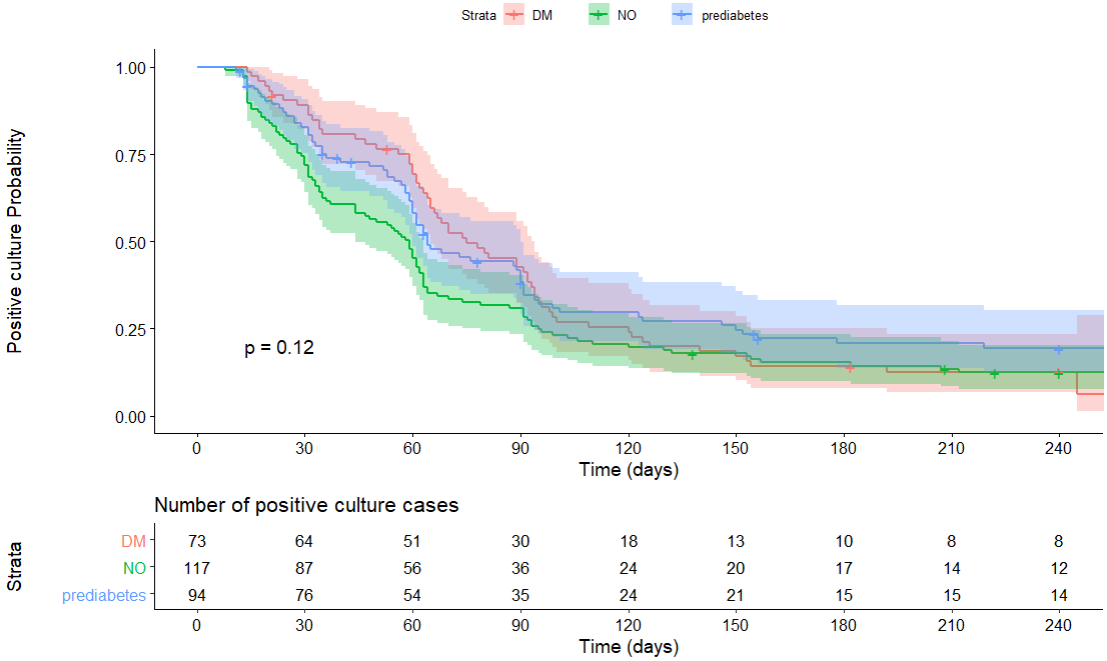


Figure 5-8. Kaplan Meier plot for sputum culture conversion of MDR-TB patients, stratified by DM status. Data was for 282 MDR-TB patients with positive culture at baseline. Pink line represents 73 DM, green for 117 non-DM and blue for 94 pre-DM. P value was based on p overall of Log rank test.

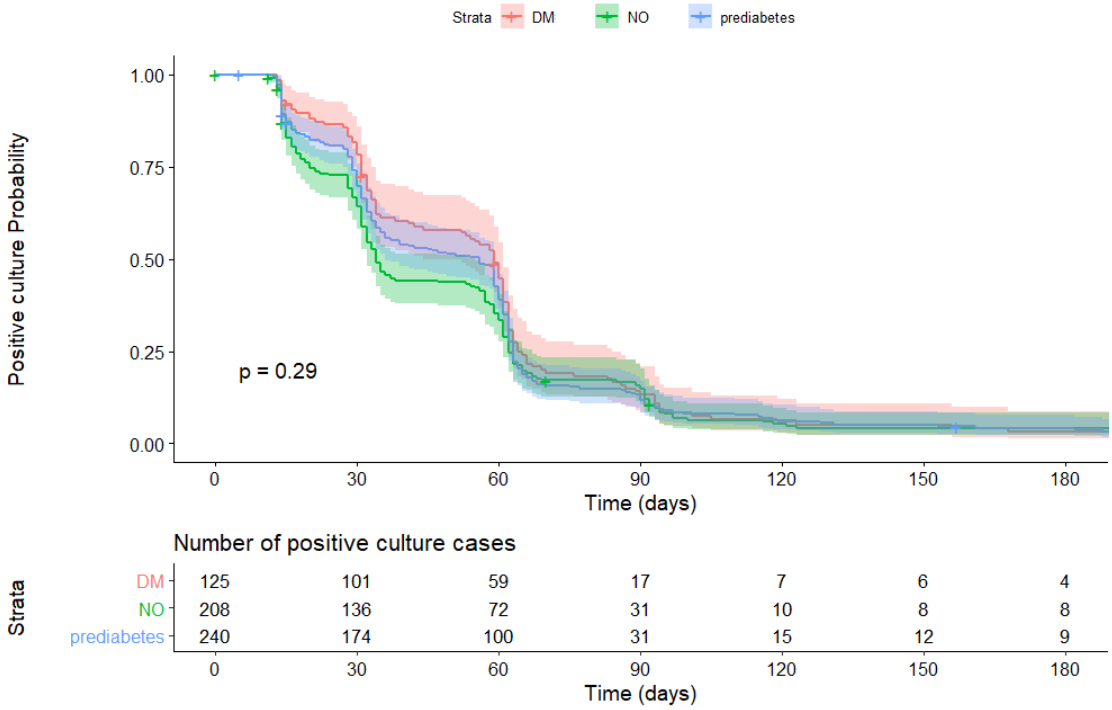


Figure 5-9. Kaplan Meier plot for sputum culture conversion of DS-TB patients during treatment, stratified by DM status. Data was for 573 DS-TB patients with positive culture at baseline. Pink line represents 125 DM, green for 208 non-DM and blue for 240 pre-DM. P value was based on p overall of Log rank test.

Restricted mean survival time analysis was used to estimate the median time of TB patients to stay culture positive from the start of treatment (time 0) to a specified time point (restricted interval) and then compare the difference between areas under the curves of the two arms from time 0 to that time point. As the effect of pre-DM on treatment response was intermediate between DM and non-DM, I decided to exclude pre-DM in the restricted mean survival time analysis and tested the difference in restricted means of DM (arm 1) and non-DM patients (arm 2). I truncated the time at four time points: one month, two months, four months and six months to observe whether DM influenced on culture conversion of TB patients differently during treatment course, as during treatment, 79.6% MDR-TB patients remained positive culture at the end of one month, and 55.5% at two months, 23.8% at four months and 16.2% at six months. The differences in average time of staying in positive cultures in DM and non-DM TB patients were statistically significant (***Table 5.8***). DM and pre-DM patients had culture positive longer

than non-DM in the first one, two and four months in both MDR and DS-TB patients. In MDR-TB, RMPT of the first two months of treatment of DM and pre-DM patients were 53 [50-56] days vs 45 [41-48] days in non-DM patients (p=0.001). The effect of DM on treatment response at 4 months was lessen but still significant in both MDR (p=0.005) and DS-TB (p=0.033). There was no statistically significant difference between DM and non-DM patients at six months in DS-TB (p=0.084) while DM still slightly prolonged the time of positivity in MDR-TB (p=0.058).

Table 5.8 Restricted Mean Positive Time in MDR and DS-TB

	Diabetes status	MDR-TB RMPT (days)*	P value**	DS-TB RMPT (days)*	P value**
1 month	DM	29 [28-30]	0.011	28 [27-29]	0.001
	Non-DM	27 [26-28]		26 [25-27]	
2 months	DM	53 [50-56]	0.001	46 [42-49]	0.002
	Non-DM	45 [41-48]		40 [37-42]	
4 months	DM	77 [69-85]	0.005	55 [49-60]	0.033
	Non-DM	62 [55-69]		47 [43-52]	
6 months	DM	87 [75-99]	0.058	57 [51-64]	0.084
	Non-DM	72 [62-82]		50 [45-55]	

*: RMPT: Restricted mean positive time. Value were presented as number and 95%CI (days)

**: p.value was based on RMST analysis for the difference in days of average time between DM and non-DM TB patients.

5.2.3 DM and treatment outcome

Treatment outcomes for MDR and DS-TB followed the WHO definitions (section 2.3.2 in chapter 2). Of 312 MDR-TB patients, 13 patients were still under treatment, including five with DM, three with pre-DM and five with non-DM. Among 299 MDR-TB patients with treatment outcome information, 229 (76.6%) MDR-TB patients had favorable outcomes including 206 (68.9%) cured and 23 (7.7%) treatment completed for those who completed their therapy

without any evidence of failure but did not meet the criteria for being cured. Seventy (23.4%) MDR-TB patients had unfavorable outcomes including 12 (4.0%) deaths, 22 (7.4%) failures and 36 (12.0%) lost to follow-up. When stratified by DM status, four (5.8%) DM and five (4.9%) pre-DM patients died, compared to three (2.3%) non-DM. In reverse, six (8.7%) DM patients were lost to follow-up compared to 15 (14.9%) pre-DM and 15 (11.6%) non-DM (***Table 5.9***). DM patients had higher rate of failure (11.6% in DM, 4.0% in pre-DM and 7.8% in non-DM) but the difference was not significant (p=0.169).

All 598 DS-TB patients had treatment outcome at the time of analysis with 355 (59.4%) cured, 150 (25.1%) treatment completed, 6 (1.0%) died, 66 (11.0%) failed the treatment and 21 (3.5%) lost to follow-up.

Table 5.9. Treatment outcome of TB patients with and without DM

Treatment outcome	MDR-TB			DS-TB		
	Non-DM n=129	**Pre-DM n=101**	**DM N=69**	**Non-DM n=219**	**Pre-DM n=250**	**DM N=129**
Cured*	95 (73.6%)	67 (66.3%)	44 (63.8%)	126 (57.5%)	151 (60.4%)	78 (60.5%)
Completed**	6 (4.7 %)	10 (9.9%)	7 (10.1%)	58 (26.5%)	62 (24.8%)	30 (23.3%)
Failed	10 (7.8%)	4 (4.0%)	8 (11.6%)	21 (9.6%)	28 (11.2%)	17 (13.2%)
Died	3 (2.3%)	5 (4.9%)	4 (5.8%)	1 (0.5%)	2 (0.8%)	3 (2.3%)
Lost to follow up	15 (11.6%)	15 (14.9%)	6 (8.7%)	13 (5.9%)	7 (2.8%)	1 (0.8%)

Data was for 299 MDR-TB patients who finished treatment at the time of analysis and all 600 DS-TB patients.

Value was presented as number (%).

* For MDR-TB, cured was defined as treatment completed and at least three (for 20 month regimen) or two (for 9 month regimen) consecutive negative cultures taken at least 30 days apart after the intensive phase.

**Treatment completed in MDR-TB referred to any patients who completed their treatment without any evidence of failure but no (not enough) records of at least three (for 20 month regimen) or two (for 9 month regimen) consecutive negative cultures taken at least 30 days apart after the intensive phase.

DM increased risk of death by 2.58 times in MDR-TB (95%CI: 0.56-11.9, p=0.223) and 5.19 times in DS-TB (95%CI: 0.53-50.43, p=0.156). After adjustment for mean CT value, age and BMI, the odds of death were 2.81 (95%CI: 0.52-15.16, p=0.229) in MDR-TB and 4.58 (95%CI: 0.26-81.92, p=0.301) in DS-TB. No death was reported in DM patients treated with metformin in both MDR and DS-TB. MDR-TB DM patients without using metformin (treated with insulin/sulfonylurea instead) increased the odds of death by 4.10 (95%CI: 0.87-21.5) in MDR-TB and by 8.07 (95%CI: 0.83-78.74) in DS-TB.

DM did not significantly increase the odds of unfavorable outcomes in both MDR and DS-TB (***Table 5.10***). In MDR-TB, 18 (26.1%) DM patients experienced unfavorable outcomes including 4 (5.8%) deaths, 8 (11.6%) failures and 6 (8.7%) lost to follow-up while 28 (21.7%) non-DM patients had unfavorable outcomes with 3 (2.3%) deaths, 10 (7.8%) failures and 15 (11.6%) lost to follow-up. The odds of developing unfavorable outcomes in DM and pre-DM were insignificant (OR: 1.27 (0.64-2.52) in DM; OR: 1.12 (0.60-2.09) in pre-DM).

In DS-TB, DM and pre-DM did not increase risk of developing unfavorable outcomes (OR: 1.02 (0.53-1.75) in DM, OR: 0.91 (0.55-1.51) in pre-DM).

Table 5.10. Associations between DM status and all cause mortality and unfavorable outcomes of MDR and DS-TB patients by univariate logistic regression

	All-cause mortality			Unfavorable outcomes		
	N (%)	**OR (95%CI)**	**P value**	**N (%)**	**OR (95%CI)**	**P value**
DM_MDR	4 (5.8%)	2.58 (0.56-11.9)	0.223	18 (26.1%)	1.27 (0.64-2.52)	0.487
Pre-DM_MDR	5 (4.9%)	2.16 (0.50-9.28)	0.298	24 (23.8%)	1.12 (0.60-2.09)	0.711
Non-DM_MDR	3 (2.3%)	1		28 (21.7%)	1	
DM_DS	3 (2.3%)	5.19 (0.53-50.43)	0.156	21 (16.3%)	1.02 (0.53-1.75)	0.942
Pre-DM_DS	2 (0.8%)	1.76 (0.16-19.52)	0.646	37 (14.8%)	0.91 (0.55-1.51)	0.723
Non-DM_DS	1 (0.5%)	1		35 (16.0%)	1	

5.2.4 DM and blood characteristics

Complete blood count, albumin, globulin and a panel of eight cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-13) were measured at baseline of MDR-TB patients and compared to observe the trend between DM, pre-DM and non-DM patients. White blood cell count (WBC) was higher in DM (median: 9.26G/L, IQR: 7.42-12.2), pre-DM (8.43 [6.89-10.8]) than non-DM (7.92 [6.74-9.79]) (p<0.001) (*Table 5.11*). Both absolute neutrophil count, proportion of neutrophil and neutrophil:lymphocyte ratio were higher in DM (Figure 5-10). Absolute neutrophil count gradually increased from 5.0 G/L [3.8-6.6] in non-DM to 5.7 [4.2-7.3] in pre-DM and 6.8 [4.2-9.0] in DM (p<0.001). Absolute lymphocyte count similar among DM, pre-DM and non-DM patients (p=0.806), but the proportion of lymphocyte in DM was significantly lower than non-DM (22.4% [17.9-29.2] in non-DM vs 21.0% [14.5-26.3] in DM, p=0.005). Albumin level were the lowest in DM (p trend<0.001) while levels of globulin were similar between the three groups (p=0.307). Twelve/74 (16.2%) DM patients and 21/104 (20.2%) pre-DM patients had hypoalbuminemia (albumin < 3.5g/dl) compared to nine/131 (6.9%) non- DM (p=0.009). A/G ratio was lower in DM and pre-DM compared to non-DM (p=0.006) (Figure 5-10).

Table 5.11. Profile of WBC and biomarkers of MDR-TB, stratified by DM status

	Non-DM ***N=131***	**Pre-DM** ***N=104***	**DM** ***N=74***	**P trend**
WBC (G/L)	7.9 [6.7-9.8]	8.43 [6.9-10.8]	9.3 [7.4-12.2]	0.002
Neutrophil (G/L)	5.0 [3.8-6.6]	5.7 [4.2-7.3]	6.8 [4.2-9.0]	<0.001
Neutrophil (%)	63.9 [55.5-70.3]	68.0 [60.7-72.9]	66.9 [62.5-74.5]	0.002
Lymphocyte (G/L)	1.8 [1.5-2.3]	1.7 [1.2-2.3]	1.9 [1.4-2.2]	0.778
Lymphocyte (%)	22.4 [17.9-29.2]	20.0 [14.3-25.5]	21.0 [14.5-26.3]	0.005
Monocyte (G/L)	0.7 [0.6-1.0]	0.7 [0.6-1.0]	0.8 [0.6-1.0]	0.137
Monocyte (%)	8.9 [7.6-11.0]	8.7 [7.6-10.3]	8.6 [7.0-10.5]	0.237
NLR*	2.7 [2.0-4.0]	3.4 [2.5-4.9]	3.2 [2.4-5.0]	0.002

	Non-DM *N=131*	Pre-DM *N=104*	DM *N=74*	P trend
Albumin (g/dl)	4.2 [3.9-4.4]	4.0 [3.6-4.3]	3.9 [3.6-4.3]	<0.001
Globulin	3.8 [3.4-4.3]	4.1 [3.6-4.5]	3.9 [3.5-4.4]	0.307
A/G ratio	1.1 [0.9-1.3]	1.0 [0.8-1.2]	1.0 [0.8-1.2]	0.006

Value was presented as median [IQR] for continuous variables. Data was for 309 patients with available results of albumin, globulin and A/G ratio, and 275 patients with WBC results. P value was based on trend test.

*NLR: neutrophil lymphocyte ratio

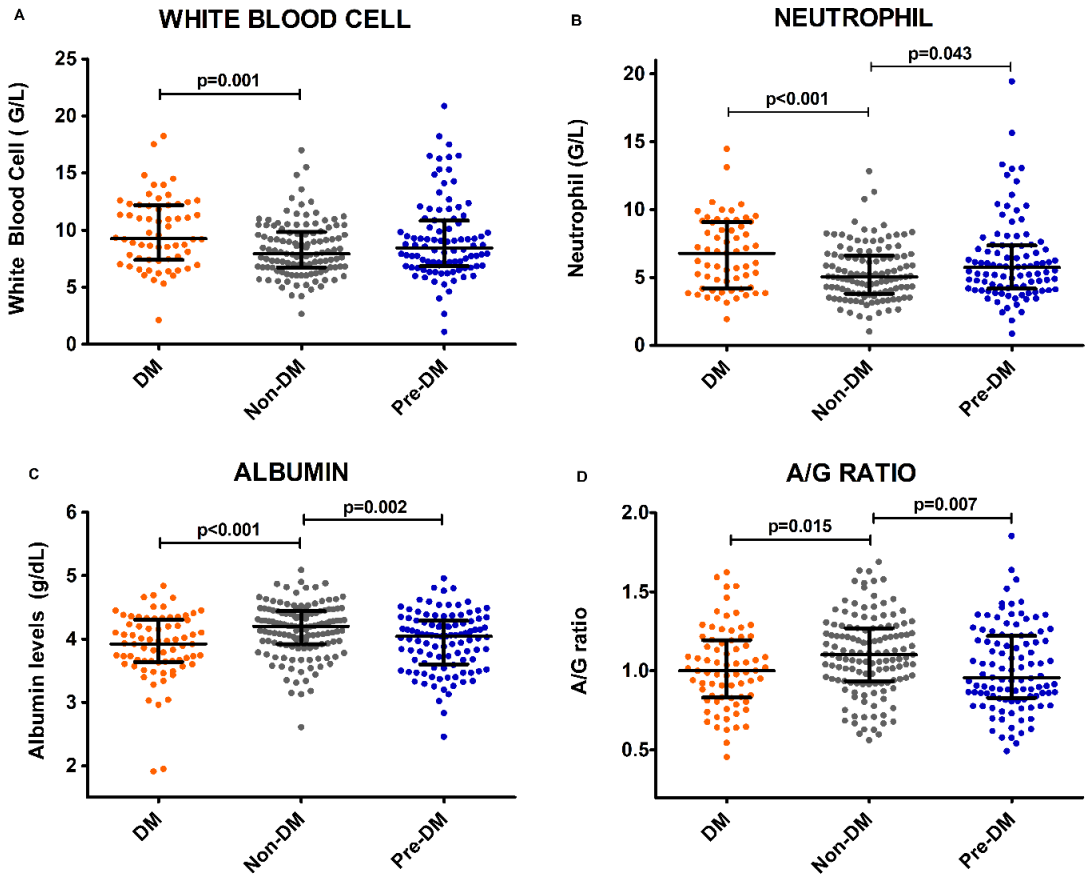


Figure 5-10. Associations between diabetes status and baseline white blood cell (A), absolute neutrophil count (B), albumin (C) and albumin/globulin ratio (D) in MDR-TB patients. Data was for 275 patients with complete blood count results (A and B), and 309 patients with albumin and globulin results (C and D). P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

In the panel of eight cytokines, TNF-α and IL-6 were detected in most of patients, but more than half of the IFN-γ, IL-1β, IL-13 and IL-10 concentrations were lower than the detection limit. Only 7/312 (2.2%) samples had detectable IL-2 and 24/312 (7.7%) detectable IL-12p70. Among 301/312 (96.5%) patients with detected TNF-α, concentration of this cytokine in DM and pre-DM patients was higher than non-DM patients (p=0.009). DM and pre-DM patients had higher level of IL-6 (p=0.001), and all DM patients had their IL-6 detected, compared to 93.3% in non-DM and 96.2% in pre-DM (p=0.021). Figure 5-11 shown the differences of $\log_{10}$ of TNF- α and IL-6 in MDR-TB patients with DM, pre-DM and non-DM. DM had lower concentration of IL-12p70 in DM than non-DM (p=0.090) but with few detected samples (5.4% in DM, 5.8% in pre-DM, 10.4% in non-DM, p=0.150). MDR-TB patients had very low IL-2, IL-10, IL-13 concentrations and there were no significant differences between DM and non-DM in the concentrations of those cytokines (***Table 5.12***).

Table 5.12. Pro-inflammatory and anti-inflammatory cytokines in MDR-TB patients with and without DM

	Non-DM ***N=134***	**Pre-DM** ***N=104***	**DM** ***N=74***	**P trend**
TNF-α detected*	129 (96.3%)	99 (95.2%)	73 (98.6%)	0.467
TNF-α (pg/ml)	3.81 [2.44-5.80]	4.74 [2.66-7.74]	4.65 [3.19-7.22]	0.009
IFN-γ detected*	49 (36.6%)	38 (36.5%)	25 (33.8%)	0.715
IFN-γ (pg/ml)	10.9 [5.47-28.4]	10.7 [6.25-21.1]	21.4 [9.66-62.1]	0.038
IL-2 detected*	4 (3.0%)	2 (1.9%)	1 (1.4%)	0.426
IL-2 (pg/ml)	13.9 [5.53-30.5]	1.87 [1.40-2.34]	90.8	0.701
IL-6 detected*	125 (93.3%)	100 (96.2%)	74 (100%)	0.021
IL-6 (pg/ml)	4.99 [3.09-9.06]	6.82 [3.73-12.9]	7.75 [3.95-16.7]	0.001
IL-13 detected*	27 (20.1%)	15 (14.4%)	8 (10.8%)	0.068
IL-13 (pg/ml)	122 [81.1-169]	122 [90.4-145]	82.5 [49.3-130]	0.255
IL-1β detected*	44 (32.8%)	34 (32.7%)	23 (31.1%)	0.810

	Non-DM *N=134*	Pre-DM *N=104*	DM *N=74*	P trend
IL-1β (pg/ml)	4.45 [2.72-5.95]	5.90 [2.70-14.3]	4.92 [2.19-11.0]	0.360
IL-10 detected*	43 (32.1%)	36 (34.6%)	26 (35.1%)	0.630
IL-10 (pg/ml)	1.92 [1.19-2.95]	2.50 [1.47-4.42]	2.18 [1.50-2.97]	0.528
IL-12p70 detected*	14 (10.4%)	6 (5.8%)	4 (5.4%)	0.150
IL-12p70 (pg/ml)	342 [110-628]	26.4 [9.86-65.2]	125 [104-573]	0.090

Value was presented as number (%) for category variables and median [IQR] for continuous variables. Data was for 312 MDR-TB patients, and p value was based on trend test.

* number and % of patients with detected level of corresponding cytokines.

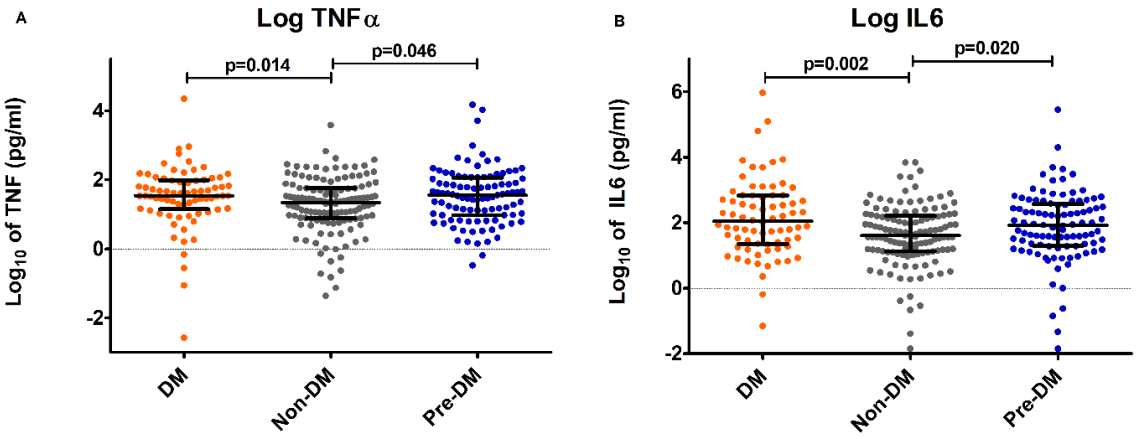


Figure 5-11. Associations between DM status and log_{10} of TNFα (A) and IL6 (B) in 312 MDR-TB patients at the start of treatment. P value was based on Mann-Whitney test for 2 groups with significant differences between non-DM vs DM and non-DM vs pre-DM in both MDR and DS-TB. Long bars represented median and short bars represented interquartile ranges.

5.3 DISCUSSION

High prevalences of DM comorbidity were found among MDR-TB (23.7%) and DS-TB (21.6%) in HCMC. Those prevalences were double that of DM in HCMC in 2010 (10.8% in men and 11.7% in women) (127), and more than three times higher than the prevalence of urban adult in a north province of Vietnam (6.7%) in 2011-2013 (170). In this study, I screened DM in TB patients by both HbA1C and FPG to accurately diagnose DM (171). High proportions of pre-DM were also detected in MDR-TB patients (32.7%) and DS-TB (41.8%), making a total

of 56.4% of MDR-TB and 63.4% DS-TB patients had either DM or pre-DM conditions. A cross-sectional study in 2016 in Ha Noi, northern of Vietnam found a lower prevalence of 13.7% DM and 29% pre-DM among TB patients older than 15 years when screening by FPG (118). One of the reasons for this discrepancy might be due to higher prevalence of DM in general population in HCMC than other areas in Vietnam (126). When using a less stringent criteria of either FPG or HbA1C to diagnose, the DM rate in MDR-TB in our study reached 30.1%, consistent with our retrospective study in HCMC from 2011 to 2015 (30.2%) (166). With the high prevalence and nearly a third of DM patients had not been diagnosed previously, our findings indicate the importance of this comorbidity and the need to systematically screen all TB patients with DM.

To evaluate the influence of DM on MDR-TB manifestation, I analyzed individual TB-related symptoms, CXR (cavities and Timika score), bacterial burden by GeneXpert, symptom severity and disease severity. In this study, no association was found between DM and individual symptoms including cough, hemoptysis, fever, anorexia and weight loss. DM slightly increased the odds of having severe symptoms and severe disease. However, after adjusting for age and duration of disease, DM strongly correlated with severe symptoms (aOR: 2.13, 95%CI: 1.06-4.27) and severe disease (aOR: 2.26, 95%CI: 1.11-4.59). Our results were consistent with findings of Alisjahbana *et al* that reported similar symptoms among 737 Indonesian pulmonary TB patients with and without DM, but DM patients had more severe disease defined by Karnofsky index and symptom score (107). Among MDR-TB, poor glycemic control DM patients increased risks of severe symptom and disease compared to non-DM, but good glycemic control DM patients did not. Increase in HbA1C, representing the average blood glucose levels over two to three months, exposed MDR-TB at higher risk of severe symptoms and disease. Our new findings indicate that the effect of DM on MDR-TB severity is strongly associated with raised HbA1C and poor glycemic control.

There was evidence that DM was associated with increased bacterial burden in MDR-TB. DM patients had lower mean CT value of GeneXpert (p=0.01) and higher risk of having high bacterial burden than in non-DM. Poor glycemic control DM patients had higher odds of having high and medium GeneXpert level than non-DM, even after adjustment for duration of disease. Increase in HbA1C was associated with high and medium GeneXpert levels in MDR-TB. No significant difference in mean CT value was found between MDR and DS-TB stratified by DM status, which indicated that bacterial load did not differ due to the resistance of *M.tb.* In an animal model, Podell also found higher colony-forming units of *M.tb* in lungs of diabetic Guinea pigs compared to non-DM Guinea pigs on day 30 of infection (172). However, clinical studies using AFB smear have showed inconsistent results (77) (107) (106). As AFB smear grading strongly relies on the microscopic readers' skills, semi-quantitative levels and mean CT value of GeneXpert were more accurate to assess bacterial burden. Therefore, I concluded that before treatment DM increased sputum bacterial burden in MDR-TB.

Our main research question was whether DM influenced treatment response and treatment outcome of MDR-TB patients. I found that effect of DM was more prominent in the first 2 months of treatment, and reduced over time in MDR-TB in culture conversion logistic regression analysis. Our findings were also supported by the results of restricted mean survival time analysis. Furthermore, among patients with culture conversion, DM patients took longer time to convert in both MDR and DS-TB (Figure 5-7). Associations between DM and the risk of remaining culture positive in MDR-TB were highest at 14 days, then decreased but still significant after 1 month and 2 months of treatment. After adjusting for bacterial load, age and BMI, the effect of DM was still significant in early treatment but showed a decreasing impact over time. In DS-TB, the effect of DM was most profound in day 14 and quickly neutralized at month 2. After adjusted for BMI, mean CT value and patients age, the effect of DM was not significant, even in day 14. An explanation for our observation was that DM did adversely affect the treatment response until the killing effect of anti-tuberculosis drugs reached plateau.

As rifampicin and isoniazid quickly killed *M.tb* within days (173), the impact of DM was diminished much faster in DS-TB than in MDR-TB. Therefore, the effect of DM was lessened in DS than in MDR-TB.

With prolonged treatment in MDR-TB (nine to 20 months) and DS-TB (six to eight months), DM had little effect on final treatment outcome with modest increase in all-cause mortality and failure. Although I did not find the association between DM and MDR-TB mortality, no deaths were reported in DM patients who were treated with metformin, suggesting that metformin might help to prevent death of all cause in TB patients. This is consistent with findings of Danger *et al* that DM patients treated with metformin may have reduced mortality during TB treatment, compared to DM patients without metformin (133).

Drop-out rates were lower in TB patients with DM, leading to neutralize the rate of poor outcomes. One of the reasons for low drop-out rate might be due to that fact that DM patients were emphasized the importance of treatment adherence in both DTUs and DM clinics. It might be a sign that effective patient-doctor communication can improve treatment adherence and hence, treatment outcome. However, one limitation of our study was that I did not record the treatment adherence of patients, therefore, could not adjust for this factor in our treatment outcome analysis. Furthermore, drug adverse reactions were scanty in our study and I could not explore the effect of DM on drug adverse reactions during TB treatment.

To better understand the relationship between DM and MDR-TB, I measured WBC counts and differentiation at baseline. DM patients had higher absolute WBC count (p trend <0.001), neutrophil count (p<0.001) and neutrophil:lymphocyte ratio (p=0.002). WBC counts, absolute neutrophil counts and proportion of neutrophil are indicators of inflammation. In previous study, Carole Chedid *et al* found high WBC counts and low lymphocyte proportions at baseline predicted unsuccessful treatment outcome (149). In addition, neutrophil:lymphocyte ratio were higher in pulmonary TB patients compared to healthy control, and higher in advanced than in mild to moderate pulmonary TB (148). Our results suggested that DM increased inflammation

in MDR-TB, demonstrated by elevated WBC, absolute neutrophil counts, neutrophil:lymphocyte ratio and lower proportion of lymphocyte than non-DM.

Further evidences of the inflammation of DM on MDR-TB were higher level of pro-inflammatory cytokines TNF-α, IFN-γ and IL-6. Although pro-inflammatory cytokines are necessary for host to defense the invasion of pathogen, higher TNF-α, IFN-γ, IL-17A and IL-1β were associated with more severe disease and slower treatment response in pulmonary TB (147). I concluded that superimposed inflammation of DM on MDR-TB dampened the host ability to control bacterial infection, leading to higher bacterial burden at presentation and prolong bacterial clearance during treatment.

Although DM patients were heavier than non-DM patients, their albumin level and A/G ratio were significantly lower (Figure 5-10). Previous studies also found hypoalbuminemia to be a predictor of in-hospital deaths due to TB in Brazil (174) and of treatment failure in MDR-TB patients in Korea (175). The effect of DM on treatment outcomes might be partially mediated by albumin and A/G ratio.

This is the first prospective study to investigate the effect of DM on MDR-TB and its potential biological mechanisms (Figure 5-12). I also was able to compare the effect of DM on MDR-TB and DS-TB to show that DM, a disease that increased inflammation, had more effect on MDR-TB than DS-TB. In this study, I used strict criteria to diagnose DM by using of both HbA1C and FPG. One strength of our study was complete data on sputum culture that enabled us to observe the descending effect of DM on treatment response using survival analysis, and on final treatment outcome. Although DM did not significantly increase risk of poor treatment outcome, it did influence early treatment response, prolong the time to culture conversion both in MDR and DS-TB.

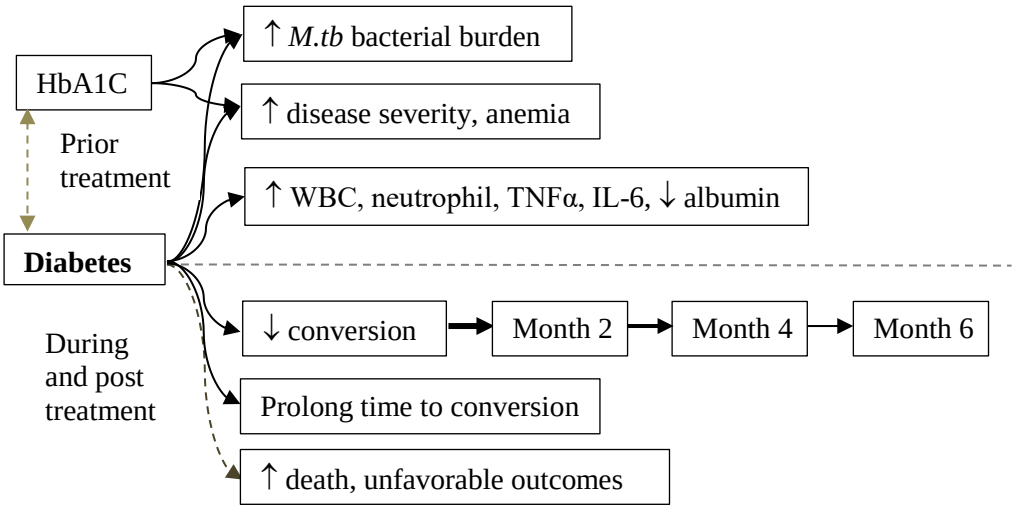


Figure 5-12 Diagram of DM effect on MDR-TB before and after treatment.

Solid arrow represents statistically significant association and dashed arrow for insignificant association. Two-way arrow represents the relationship between HbA1C and diabetes. The decreasing thickness of arrow represents the decreasing impact of DM on sputum culture conversion at month 2, month 4 and month 6 during treatment.

5.4 CONCLUSION

The convergence of DM and TB in low-middle income countries is a major public health problem, but there are limited data on the influence of DM on MDR-TB. This study has provided more insights of detrimental effects of DM, a major co-morbidity, on MDR-TB presentations, severity, and treatment response. DM increased inflammation in MDR-TB patients and was associated with higher bacterial burden and more severe disease. This effect was strongly associated with HbA1C and glycemic control level. During treatment, DM delayed sputum culture conversion, but did not prevent cure. In the search to shorten TB treatment, DM should be considered as a potential host factor that slows treatment responses, especially in patients with MDR-TB.

Chapter 6

VITAMIN D AND PULMONARY TUBERCULOSIS

6.1 INTRODUCTION

Vitamin D, originally used to treat rickets and osteomalacia, is known recently to have much wider effects on human health. Vitamin D acts as a regulator of the immune system with vitamin D receptors expressed on the surfaces of many immune cells including monocytes, macrophages, dendritic cells, neutrophils (88). In tuberculosis (TB), vitamin D_3 enhances anti-mycobacterial activities (3). Stimulation of toll-like receptors upregulates vitamin D receptors in human macrophages. This further activates the transcription of antimicrobial peptides and cathelicidin antimicrobial peptides and induces the killing of *Mycobacterium tuberculosis* (*M.tb*) within macrophages (176).

Vitamin D is mainly synthesized from 7-dehydrocholesterol in the skin into cholecalciferol (vitamin D_3) after exposure to ultraviolet (UV) B. People who live far from the equator are at risk of vitamin D deficiency, especially during winter time. A minor source of vitamin D is from diet. Vitamin D is hydroxylated by the enzyme CYP2R1 and CYP27A1 in the liver to produce the most common circulating form 25(OH)vitamin D. Then, in the kidney, 25(OH)vitamin D is hydroxylated by 1-α hydrolase (CYP27B1) into its active form 1,25(OH)2 vitamin D (calcitriol) (177).

With an increasing practice of protecting skin from UV light, vitamin D deficiency is prevalent in all age groups worldwide, even in countries near the equator (178). Vitamin D concentration in TB patients were significantly lower than healthy controls (179), making vitamin D deficiency a common condition in TB (179) (180) (181). Ho Chi Minh City (HCMC), Vietnam, locating at 10º45'N and 106º40'E, is a tropical region with sunny weather for most of the time. A study in 2009 revealed that prevalences of vitamin D deficiency in men and women were 1% and 3% respectively (182). In the same year, the prevalences of vitamin D deficiency among TB patients in HCMC were found higher than healthy control, 4.4% vs 1.8% in men and 5.7% vs 1.9% in women, respectively (183). Vitamin D deficiency was linked with more severe

disease in pulmonary TB (184) (90). This condition also increased risk of progressing from latent TB infection to active disease (185) (186).

As early as 1849, vitamin D, in the form of cod liver oil, was reported to successfully treat pulmonary TB (4). High doses of vitamin D quickly became the treatment of choice for many forms of TB until the late 1940s when antibiotics were discovered. Then, the role of vitamin D in treating TB rapidly vanished (5). However, in the more recent search for adjunctive host directed therapy to shorten treatment and improve outcomes for TB, vitamin D has been re-ignited as a potential candidate. In 2003-2005, Wejse *et al.* conducted a randomized clinical trial (RCT) of adjunctive vitamin D_3 in 365 TB patients and found no difference in sputum smear conversion, 12-month mortality and clinical severity score (92). Later, in 2007-2008, an RCT conducted by Martineau *et al.* showed vitamin D supplementation insignificantly reduced time to sputum culture conversion in 126 TB patients, 60% of whom had vitamin D deficiency. However, the reduction became significant in patients with *tt* genotype of the *Taq*I vitamin D receptor polymorphism (93). A study in 2009 in Pakistan showed TB patients with vitamin D deficiency benefitted from adjunctive vitamin D therapy in improving TB severity score and sputum smear clearance compared to placebo arm (90). In an RCT in Bangladesh with 288 TB patients, vitamin D_3 increased proportion of negative sputum culture at week 4 and decrease TB score at week 10 (94). An observational study of Rathored *et al.* also showed vitamin D concentration significantly correlated smear conversion time in MDR-TB, but not in DS-TB (91). An individual participant data meta-analysis of eight RCTs revealed that vitamin D supplementation did not hasten sputum culture conversion in drug susceptible (DS) TB, but accelerated culture conversion in multidrug resistant (MDR)TB patients (95).

Whilst the result of adjunctive vitamin D therapy is neutral for DS-TB, there may still be a role for vitamin D supplementation for MDR-TB. Furthermore, participants in previous RCTs received treatment regardless of their vitamin D status. It raises a question whether vitamin D supplementation benefits only deficient patients. Therefore, understanding the influences of

vitamin D concentration as well as vitamin D deficiency on clinical presentations and treatment response of both MDR and DS-TB are important. Exploring the relationship between vitamin D and cytokine expression might provide understanding on the mechanism of vitamin D on TB treatment response.

I hypothesised that vitamin D, through its impact on host immune response, influenced disease severity and treatment response of pulmonary MDR-TB, and had lesser effect on treatment response of DS-TB. Two prospective studies were conducted in Ho Chi Minh City, Vietnam to assess 1) the impacts of vitamin D on MDR and DS pulmonary TB manifestation, severity and bacterial burden before treatment, 2) the association between vitamin D and early bacterial clearance of MDR and DS-TB patients, 3) the association between vitamin D and treatment outcomes of MDR and DS-TB patients, and 4) the relationships between vitamin D and blood cytokines in MDR-TB and DS-TB patients before treatment.

6.2 RESULTS

Two prospective studies were conducted in HCMC to recruit 312 MDR-TB and 600 DS-TB patients in HCMC, as previously described in chapter 4 (section 4.2). Vitamin D2 and D3 levels in plasma samples were measured in 309 of 312 patients in our MDR-TB cohort. The other three MDR-TB patients were missing baseline plasma samples for vitamin D measurement, therefore, were excluded for analysis. An equivalent number of DS-TB were included to evaluate the effect of vitamin D deficiency. Among 600 DS-TB patients, I chose to test 301 DS-TB patients who had more severe symptoms and diseases in order to clearly observe the effect of vitamin D on TB presentations. There were differences in baseline characteristics between the selected group and unselected group as a consequence of more severe disease in selected group, but most of baseline characteristics between the selected group and the whole DS-TB cohort were similar (***Table B*** in Appendix). Therefore, a total of 309 MDR-TB and 301 DS-TB patients were tested for 25-hydroxy vitamin D_2 and D_3 in their plasma samples collected prior to TB treatment.

25(OH) vitamin D_2 levels were detectable in one MDR-TB and one DS-TB patients while 25(OH) vitamin D_3 levels were detectable in all patients. Total concentration of 25(OH) vitamin D_2 and D_3 was described as vitamin D level in this study. The median concentrations of vitamin D were 29.3 ng/ml (interquartile range (IQR): [22.9-35.0]) for MDR-TB, and 25.5 ng/ml (IQR: [20.0-32.6]) for DS-TB ($p<0.001$). Patients were diagnosed with vitamin D deficiency when their total vitamin D concentration was less than 20ng/ml. Among TB patients with vitamin D deficiency, the median vitamin D concentration in MDR-TB (16ng/ml, IQR: [13.8-18.2]) was insignificantly higher than that of DS-TB (14.4ng/ml, IQR: [12.3-18.1]) ($p=0.158$). The frequencies of vitamin D deficiency were 17.5% (54/309) in MDR-TB and 24.9% (75/301) in DS-TB ($p=0.031$).

Culture of samples collected within 14 days of treatments were positive for 281 (90.9%) MDR-TB and 286 (95.0%) DS-TB, who were included for culture conversion analysis. Patients of whom sputa collected within 14 days of treatment showed negative culture results were excluded from culture conversion analysis.

Final treatment outcome was available for all 301 DS-TB and 298 MDR-TB patients. Among 11 MDR-TB patients who were still under treatment at the time of analysis, two had vitamin D deficiency.

6.2.1 Characteristics of MDR/RR-TB and DS-TB by vitamin D status

Table 6.1 described the baseline vitamin D levels in MDR and DS-TB patients. There was no correlation between patients' age and vitamin D concentration in MDR-TB, but in DS-TB, increasing age associated with higher vitamin D concentration (the correlation coefficient $r=0.14$, 95% confident interval (CI): 0.07-0.22, $p<0.001$). Female patients had significantly lower median concentrations of vitamin D in both MDR (25.0 ng/ml, IQR: [21.2-29.8]) and DS-TB (22.2 [17.9-25.0]) than men (30.6 [23.7-36.5] in MDR, 27.4 [20.8-33.6] in DS-TB, both $p < 0.001$). TB patients who smoked regularly also had higher vitamin D levels ($p=0.008$

in MDR, p=0.001 in DS-TB). There was no difference in vitamin D levels between patients with and without HIV co-infection, and anemia (***Table 6.1***).

Table 6.1. Vitamin D concentrations in pulmonary TB patients

	MDR-TB		DS-TB	
	Vitamin D concentration (ng/ml)	**P value**	**Vitamin D concentration (ng/ml)**	**P value**
Age (years)*	0.06 (-0.03 to 0.15)	0.171	0.14 (0.07-0.22)	<0.001
Gender: Male	30.6 [23.7-36.5]	<0.001	27.4 [20.8-33.6]	<0.001
Female	25.0 [21.2-29.8]		22.2 [17.9-25.0]	
HIV: Positive	25.1 [17.3-34.5]	0.287	23.9 [19.3-31.0]	0.777
Negative	29.3 [23.5-35.0]		25.5 [20.1-32.6]	
Alcohol: Yes	31.4 [24.8-36.9]	0.034	26.4 [21.2-34.7]	0.068
No	28.4 [22.1-34.7]		24.6 [19.7-31.3]	
Regular smoker: Yes	31.4 [24.0-38.4]	0.008	28.9 [23.8-35.6]	0.001
No	28.4 [22.3-34.3]		24.3 [19.3-31.3]	
Previous TB: Yes	29.2 [23.3-35.2]	0.948	25.7 [20.6-32.7]	0.745
No	29.3 [22.6-35.0]		25.1 [19.8-32.4]	
Anemia: Yes	29.1 [21.1-35.0]	0.126	25.4 [19.8-32.6]	0.548
No	30.0 [24.5-36.7]		26.2 [20.6-32.6]	
BMI**	0.23 (-0.11 to 0.58)	0.182	0.26 (-0.07 to 0.59)	0.123

Data was for 309 MDR-TB and 301 DS-TB patients. P value was based on Mann-Whitney test for category variables and linear regression for continuous variables.

*Co-efficient (95% CI) per every 1 year increase of age on vitamin D concentration was based on linear regression.

**Co-efficient (95% CI) per every 1kg/m^2 increase of BMI on vitamin D concentration was based on linear regression.

To evaluate the effect of vitamin D deficiency on TB presentations, comparisons of vitamin D status and baseline characteristics of 309 MDR-TB and 301 DS-TB are shown in ***Table 6.2***. Vitamin D deficiency was more prevalent among female than male patients. Female gender

increased the risk of having vitamin D deficiency by 1.63 fold changes (95%CI: 0.86-3.12, p=0.137) in MDR-TB, and by 1.95 fold changes (95%CI: 1.08-3.50, p=0.026) in DS-TB. Vitamin D deficient patients were significantly lighter than sufficient patients in DS-TB, but not in MDR-TB. No significant difference was observed in median age, alcohol consumption, history of previous episode of TB, frequency of anemia and time of disease before diagnosis between pulmonary TB patients with vitamin D deficiency and sufficiency.

Table 6.2. Baseline characteristics of MDR and DS pulmonary TB stratified by vitamin D status

	MDR-TB			**DS-TB**		
	Sufficiency ***N=255***	**Deficiency** ***N=54***	**P value**	**Sufficiency** ***N=226***	**Deficiency** ***N=75***	**P value**
Age (years)	42.0 [32.0-51.0]	40.5 [31.0-48.0]	0.351	46.0 [33.0-53.0]	43.0 [27.0-52.0]	0.078
Male	199 (78.0%)	37 (68.5%)	0.187	182 (80.5%)	51 (68.0%)	0.037
HIV co-infection	14 (5.49%)	7 (13.0%)	0.068	9 (3.98%)	4 (5.33%)	0.743
Alcohol drinker	94 (36.9%)	14 (25.9%)	0.169	65 (28.8%)	16 (21.3%)	0.268
Regular smoker	93 (36.5%)	17 (31.5%)	0.590	53 (23.5%)	9 (12.0%)	0.050
Previous TB	141 (55.3%)	32 (59.3%)	0.702	40 (17.7%)	10 (13.3%)	0.483
Symptom duration	15.5 [10.0-30.0]	30.0 [15.0-60.0]	0.057	20.0 [10.0-30.0]	20.5 [14.0-33.0]	0.648
Cough	240 (94.1%)	52 (96.3%)	0.746	211 (93.4%)	72 (96.0%)	0.576
Sputum	227 (89.0%)	48 (88.9%)	1.000	189 (83.6%)	69 (92.0%)	0.109
Weight loss	118 (46.5%)	29 (53.7%)	0.413	134 (59.3%)	51 (68.0%)	0.228
Low grade fever	125 (49.0%)	29 (53.7%)	0.634	127 (56.2%)	46 (61.3%)	0.519
Haemoptysis	32 (12.5%)	4 (7.41%)	0.403	23 (10.2%)	4 (5.33%)	0.299
Chest pain	88 (34.5%)	18 (33.3%)	0.994	74 (32.7%)	32 (42.7%)	0.156
Malaise	158 (62.0%)	33 (61.1%)	1.000	115 (50.9%)	46 (61.3%)	0.150
Dyspnea	80 (31.4%)	18 (33.3%)	0.904	41 (18.1%)	20 (26.7%)	0.154
Symptom score	3.0 [2.0-5.0]	4.0 [2.0-6.0]	0.435	4.0 [2.0-5.0]	4.0 [2.5-6.0]	0.041

	MDR-TB			DS-TB		
	Sufficiency ***N=255***	**Deficiency** ***N=54***	**P value**	**Sufficiency** ***N=226***	**Deficiency** ***N=75***	**P value**
Clinical score	4.0 [3.0-6.0]	5.0 [3.0-6.3]	0.145	4.0 [3.0-6.0]	5.0 [3.0-7.0]	0.008
BMI, kg/m^2	19.1 [17.1-21.1]	18.8 [16.8-20.2]	0.147	19.8 [17.6-22.0]	18.2 [16.8-20.4]	0.003
Cavity on CXR	104 (42.1%)	28 (53.8%)	0.163	86 (43.7%)	34 (50.7%)	0.387
Lung proportion*	20.0 [10.0-27.5]	25.0 [18.8-50.0]	0.001	15.0 [7.50-25.0]	20.0 [10.0-42.5]	0.011
Timika score	30.0 [12.5-65.0]	60.0 [20.0-90.0]	0.008	30.0 [10.0-55.0]	50.0 [12.5-65.0]	0.014
Mean CT value	20.9 [17.8-26.3]	21.0 [18.7-25.9]	0.726	20.6 [17.2-24.4]	18.7 [16.0-21.8]	0.011
Anemia	85 (37.4%)	23 (51.1%)	0.122	83 (44.4%)	31 (52.5%)	0.344

Value are presented as number (%) for category variables and median [IQR] for continuous variables. P value was based on χ^2 test for category variables and Mann-Whitney test for continuous variables.

* Proportion of lung damage on chest Xray

6.2.1.1 TB signs and symptoms

Univariate logistic regression was performed to assess the impact of vitamin D on signs and symptoms of pulmonary MDR-TB and DS-TB (**Figure 6-1**). Every increase of 5ng/ml of vitamin D decreased risk of fever (OR: 0.88, 95%CI: 0.78-0.99), high Timika score of chest Xray (CXR) (OR: 0.78, 95%CI: 0.65-0.94) and severe disease (OR: 0.87, 95%CI: 0.76-0.99) in MDR-TB patients. In DS-TB, increased vitamin D significantly decreased Timika score (OR: 0.74, 95%CI: 0.58-0.95) and severe symptoms (OR: 0.86, 95%CI: 0.76-0.98) (**Figure 6-1**).

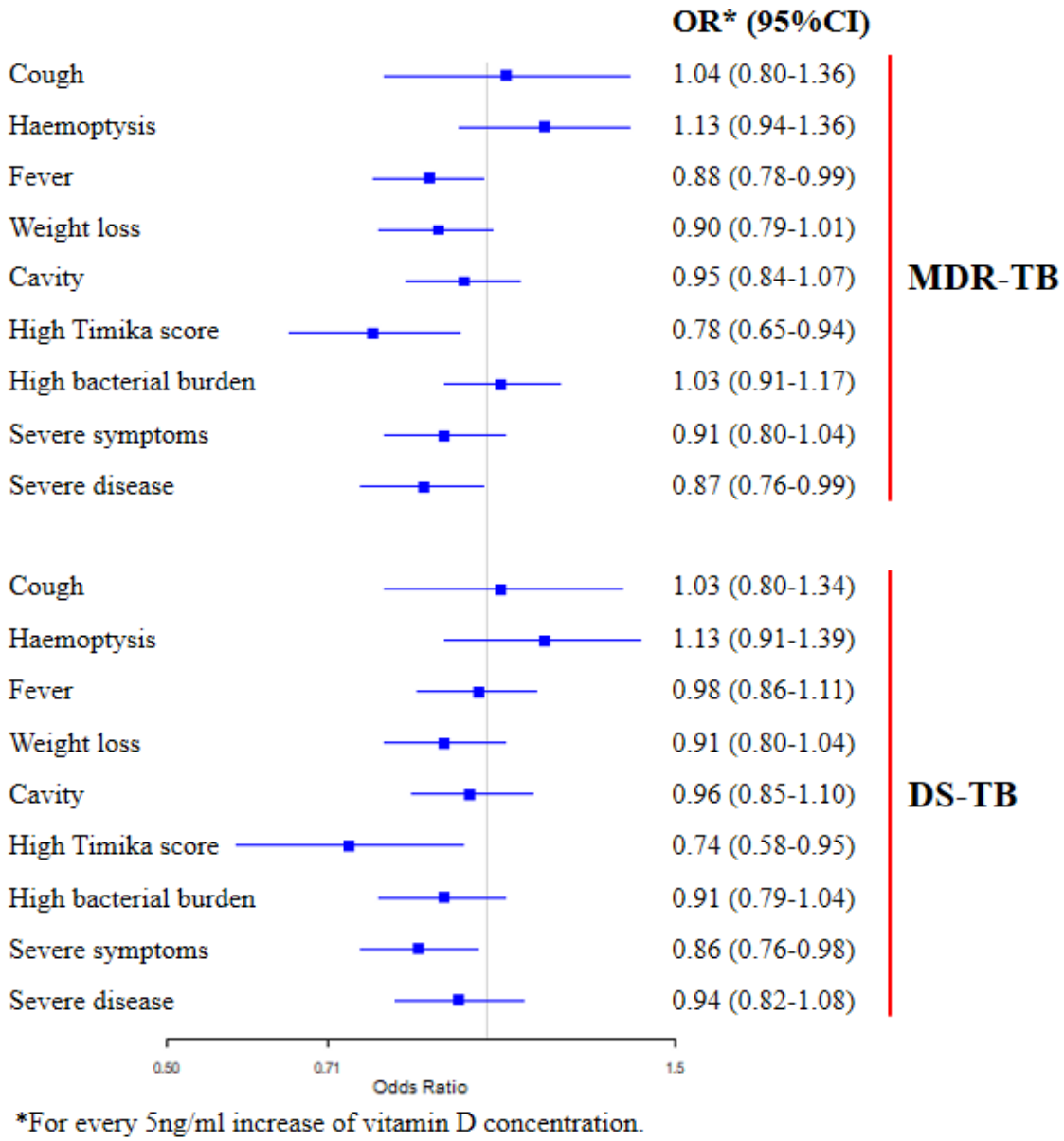


Figure 6-1. Forrest plot of effect of vitamin D on MDR and DS-TB signs and symptoms by univariate logistic regression. Data of TB symptoms was for 309 MDR and 301 DS-TB patients. Data of cavity and Timika score was for 299 MDR and 264 DS-TB patients. Data of bacterial burden was for 309 MDR and 300 DS-TB patients.

OR: odds ratio; 95%CI: 95% confidence interval.

6.2.1.2 *M.tb* bacterial burden

In order to evaluate the effect of vitamin D on bacterial burden, GeneXpert results were classified into binary variables of high and low bacterial burdens (section 2.2.9 in chapter 2), and univariate and multivariate logistic regression were performed. Vitamin D levels did not

associate with bacterial burden in MDR-TB (OR: 1.03, 95%CI: 0.91-1.17 for every increase of 5ng/ml) and DS-TB (OR: 0.91, 95%CI: 0.79-1.04). After adjustment for duration of disease and patients' age, the influence of vitamin D on bacterial burden remained insignificant in MDR (aOR:1.02, 95%CI: 0.90-1.16) and DS-TB (aOR: 0.92, 95%CI: 0.90-1.05).

Quantitative mean CT value of GeneXpert (section 2.2.9 in chapter 2) was used to estimate the correlation of vitamin D concentration on bacterial load. Increasing vitamin D levels did not correlate with bacterial load in MDR (r=-0.012, 95%CI: -0.099 to 0.074, p=0.783) and DS-TB (r=0.051, 95%CI: -0.015 to 0.117, p=0.127). Mean CT value was not different between the two groups of vitamin D status among MDR-TB (p=0.726), but was significantly lower in DS-TB patients with vitamin D deficiency than those with vitamin D sufficiency (p=0.011) (***Table 6.2***).

6.2.1.3 Chest X-ray cavity and severity Timika score

To evaluate the effect of vitamin D on lung damage, linear regression was used to understand the correlation between vitamin D and Timika score, the combination of proportion of lung damage and the presence of cavity on CXR. Among 299 MDR-TB and 264 DS-TB patients with CXRs available, increasing vitamin D levels decreased Timika score in both MDR (r= -0.536, 95%CI: -0.916 to -0.157, p=0.006) and in DS-TB (r= -0.431, 95%CI: -0.801 to -0.061 p=0.023) (Figure 6-2).

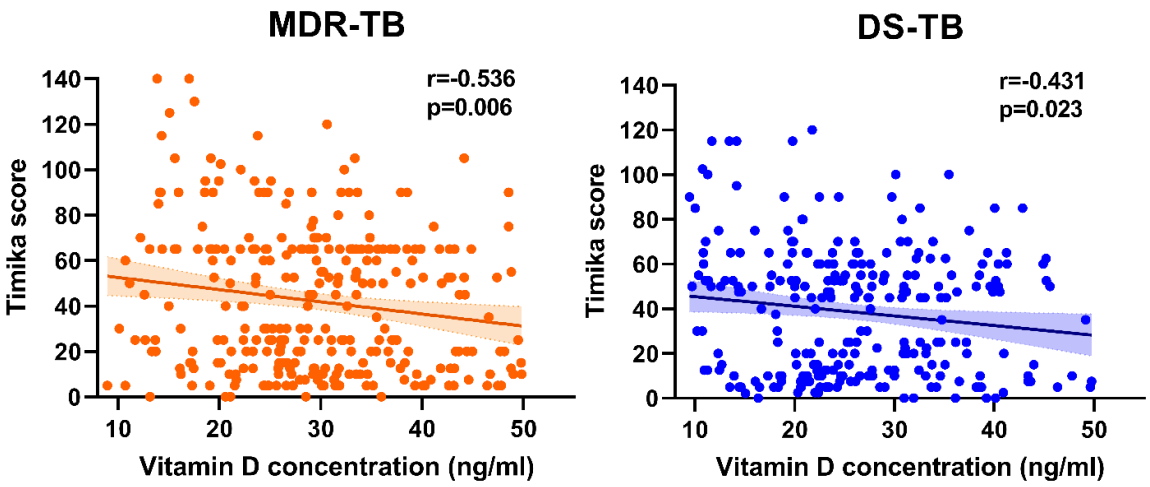


Figure 6-2. Correlations between Timika score of CXR and vitamin D concentration in MDR and DS-TB. Data was for 299 MDR-TB and 264 DS-TB patients. P value and r slope were based on linear regression.

To further understand how vitamin D deficiency influenced extent of lung damage, Mann-Whitney U test was used to compare the proportion of lung damage and Timika score between two groups of vitamin D status. Vitamin D deficient patients had significantly higher proportion of lung damage than sufficient patients with MDR (p=0.001) and DS-TB (p=0.011) (Table 6.2). Figure 6-3 illustrated that Timika score were also higher in patients with vitamin D deficiency than sufficiency in both MDR and DS-TB. Medians of Timika score of vitamin D deficiency was significantly higher than those of vitamin D sufficiency in both MDR (p=0.008) and DS-TB (p=0.014) (Table 6.2).

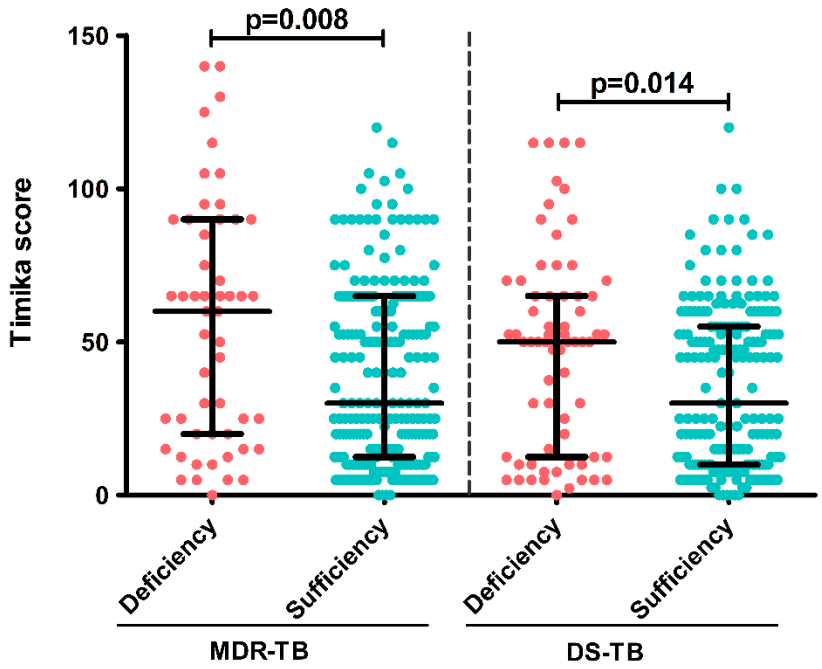


Figure 6-3. Timika's score of MDR and DS-TB. Data was for 299 MDR-TB and 264 DS-TB patients. P value was based on Mann-Whitney test. Long bars represented median values and short bars for interquartile ranges.

Patients were classified into high lung damage (Timika score ≥ 71) or low lung damage (Timika score < 71). Increase in vitamin D concentration decreased the risk of high lung damage for both MDR and DS-TB (**Figure 6-1**). In MDR-TB, 30.8% patients with vitamin D deficiency had high lung damage, compared to 11.3% in sufficiency group (p=0.001). The proportion of high lung damage in DS-TB was 19.4% in vitamin D deficiency and 6.6% in sufficiency (p=0.005). Vitamin D deficiency had 3.48 fold increase in the risk of high lung damage in MDR-TB (95%CI: 1.71-7.06), and 1.88 fold increase in DS-TB (95%CI: 1.01-3.49) . After adjusting for patients' age and duration of disease, vitamin D deficiency significantly increased the risk of high lung damage (aOR: 3.69, 95%CI: 1.78-7.66 in MDR-TB; aOR: 3.68, 95%CI: 1.55-8.74 in DS-TB).

6.2.1.4 Effect of vitamin D deficiency on symptom and disease severity

Linear regression was performed for integrated TB symptoms and clinical scores (section 2.2.2. in chapter 2) to estimate the total impact of vitamin D on TB presentations. Vitamin D levels showed a trend of negative correlation with symptom score in MDR (r=-0.021, 95%CI: -0.045 to 0.003, p=0.079) and DS-TB (r=-0.016, 95%CI: -0.037 to 0.006, p=0.164). Negative correlations were observed between vitamin D levels and clinical score for both MDR (r= -0.028, 95%CI: -0.053 to -0.002, p=0.034) and DS-TB (r= -0.022, 95%CI: -0.048 to -0.003, p=0.083) (***Figure 6-4***).

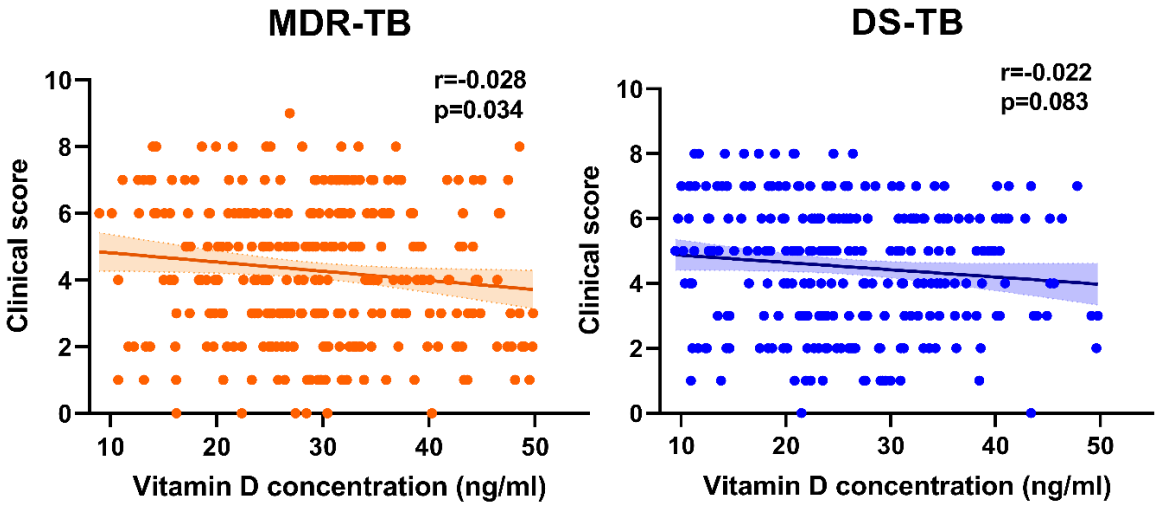


Figure 6-4. Correlations between vitamin D concentrations and clinical score in pulmonary TB. Data was for 299 MDR and 234 DS-TB patients. P value and correlation co-efficient r were based on linear regression.

The median symptom and clinical scores of MDR-TB patients with vitamin D deficiency were slightly higher than patients with sufficiency, while DS-TB patients with vitamin D deficiency had significantly higher symptom and clinical scores than patients with sufficiency (***Table 6.2***). Patients were categorized into mild symptoms when their symptoms score were less than five, and mild disease when their clinical score were less than six. Patients with symptom score equal or greater than five and clinical score equal or greater than six were considered to have severe symptoms and severe disease. For every 5ng/ml increase of vitamin D, patients decreased risk

of severe symptom in DS (OR: 0.86, 95%CI: 0.76-0.98) and severe disease in MDR (OR: 0.87, 95%CI: 0.76-0.99) (***Table 6.3***). However, after adjustment for patients' age and duration of symptoms before diagnosis, levels of vitamin D did not significantly associate with severe symptoms and severe disease in both MDR and DS-TB.

Table 6.3. Associations between vitamin D and baseline severity of MDR and DS-TB patients by univariate and multivariate logistic regression

		Univariate		Multivariate**	
		OR* (95%CI)	***P***	***aOR (95%CI)***	***P***
MDR-TB	**Severe symptoms**	0.91 (0.80-1.04)	0.152	0.92 (0.81-1.05)	0.196
	Severe disease	0.87 (0.76-0.99)	0.040	0.89 (0.78-1.02)	0.087
DS -TB	**Severe symptoms**	0.86 (0.76-0.98)	0.030	0.88 (0.77-1.00)	0.053
	Severe disease	0.94 (0.82-1.08)	0.375	0.92 (0.80-1.06)	0.244

Data of symptom severity was for 309 MDR and 301 DS-TB. Data of clinical severity was for 308 MDR and 263 DS-TB patients.

* For every 5ng/ml increase of vitamin D concentration

**Adjusted for patients'age and duration of symptom before diagnosis

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confident interval

Vitamin D deficient patients did not significantly differ from sufficient patients in both symptom and disease severity in MDR-TB. However, in DS-TB, patients with vitamin D deficiency had significantly more severe symptoms (p=0.015) and disease (p=0.044) than those with sufficiency. Vitamin D deficiency significantly increased risk of severe symptoms (univariate analysis: OR: 1.99, 95%CI: 1.17-3.38; multivariate analysis: aOR: 1.92, 95%CI: 1.12-3.28, adjusting for patients' age and duration of symptom before diagnosis) and severe disease in DS-TB patients (univariate analysis: OR: 1.86, 95%CI: 1.06-3.28; multivariate analysis: aOR: 1.87, 95%CI: 1.08-3.22).

6.2.2 Effect of vitamin D deficiency on treatment response

6.2.2.1 Sputum culture conversion at day 14, month 1, month 2 and month 3

I speculated that the effect of vitamin D on treatment response was more profound in the early time of treatment. Therefore, evaluation of vitamin D effect on treatment response was done at four time points for MDR-TB: day 14, month 1, month 2 and month 3; and three time points for DS-TB: day 14, month 1 and month 2. At each observed time point, patients were considered as "converted" when they had at least two consecutive negative, and "not converted" when they did not fulfill the criteria for converted. Patients with reversion to positive were considered "not converted". Two hundred eighty one MDR-TB and 286 DS-TB patients with positive sputum cultures at baseline were included in treatment response analysis. Univariate and multivariate logistic regression was performed to evaluate the association between vitamin D deficiency and proportion of sputum culture conversion at specified time points.

Figure 6-5 of directed acyclic graph illustrated the interaction between vitamin D deficiency and treatment response with potential confounding factors and mediators. Gender influenced vitamin D status, but not treatment response, and was not included in multivariate analysis. Nutritional status could have effect on both vitamin D status and BMI of patients. BMI and patients' age associated with vitamin D status and treatment response, therefore, were confounding factors in the multivariate model of effect of vitamin D on treatment response. Bacterial load was an important mediator, and was adjusted in this model.

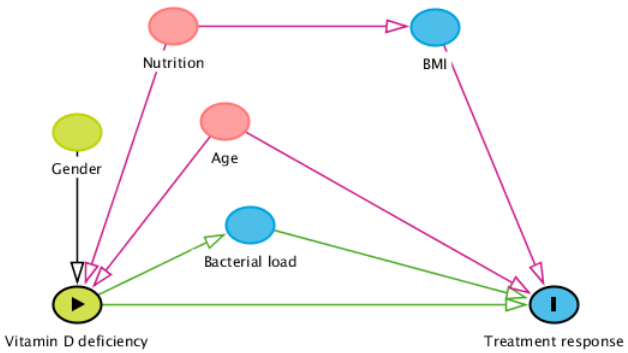


Figure 6-5. Directed acyclic graph of vitamin D deficiency and TB treatment response

Among MDR-TB, increase in vitamin D levels did not significantly associate with risk of not converting at day 14, month 1, 2 and 3 of treatment (***Table 6.4***). The odds ratio of not converting for every increase of 5ng/ml of vitamin D was 0.94 (95% CI: 0.74-1.19) at day 14, 0.92 (95%CI: 0.80-1.06) at month 1, 0.96 (95%CI: 0.84-1.09) at month 2 and 0.95 (95%CI: 0.81-1.10) at month 3. Increase in vitamin D levels in DS-TB decreased the risk of sputum culture no conversion at month 3 (OR 0.83, 95%CI: 0.69-0.99). However, after adjusting for patients' age, BMI and mean CT value before treatment, the association became insignificant (p = 0.090) (***Table 6.5***).

Table 6.4. Univariate and multivariate logistic analysis for associations between vitamin D levels and non conversion of sputum culture during MDR-TB treatment

Time point	Univariate		Multivariate**	
	OR* (95%CI)	P	aOR (95%CI)	P
Day 14	0.94 (0.74-1.19)	0.601	0.92 (0.70-1.220	0.560
Month 1	0.92 (0.80-1.06)	0.256	0.93 (0.79-1.10)	0.406
Month 2	0.96 (0.84-1.09)	0.527	0.98 (0.85-1.14)	0.805
Month 3	0.95 (0.81-1.10)	0.482	0.97 (0.83-1.14)	0.725

* For every 5ng/ml increase of vitamin D concentration.

**Adjusted for patients' age, BMI and mean CT value

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confident interval.

Table 6.5. Associations between vitamin D levels and risk of not converting during treatment in DS-TB by univariate and multivariate analysis

Time point	Univariate		Multivariate**	
	OR* (95%CI)	P	aOR (95%CI)	P
Day 14	1.16 (0.97-1.39)	0.112	1.23 (1.00-1.52)	0.055
Month 1	1.09 (0.96-1.24)	0.203	1.11 (0.96-1.29)	0.150
Month 2	1.00 (0.87-1.16)	0.953	1.01 (0.87-1.18)	0.856
Month 3	0.83 (0.69-0.99)	0.043	0.85 (0.71-1.02)	0.090

* For every 5ng/ml increase of vitamin D concentration

**Adjusted for patients' age, BMI and mean CT value

OR: Odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confident interval

Similarly, in MDR-TB, vitamin D deficiency slightly increased risk of no culture conversion at day 14 (OR: 1.91, 95%CI: 0.43-8.51), month 1 (OR: 2.25, 95%CI: 1.00-5.08), month 2 (OR: 1.77, 95%CI: 0.91-3.44) and month 3 (OR: 1.67, 95%CI: 0.82-3.38). After adjusting for patients' age, BMI and mean CT value, the adjusted odds ratio of not converting was 1.33 (95%CI: 0.28-6.27) at day 14, 1.45 (95%CI: 0.79-2.58) at month 1, 1.92 (95%CI: 1.12-3.28) at month 2 and 1.87 (95%CI: 1.08-3.22) at month 3. In DS-TB, vitamin D deficiency slightly decreased the risk of no culture sputum conversion of DS-TB at day 14 in univariate analysis (OR: 0.55, 95%CI: 0.28-1.09). The association was significant after adjusting for patients' age, BMI and mean CT value of GeneXpert (aOR: 0.39, 95%CI: 0.18-0.83). However, the effect of vitamin D was quickly diminished at month 1 (OR: 0.73, 95%CI: 0.43-1.26). After two months of treatment, vitamin D deficiency insignificantly associated with increased risk of no culture conversion (OR: 1.26, 95%CI: 0.69-2.32).

6.2.2.2 Effect of vitamin D on time to culture conversion

The time to culture conversion reflected the effectiveness of treatment. Survival analysis was used to observe the impact of vitamin D on treatment response. However, interpretation of multiple levels of vitamin D on time to culture conversion was complicated. Therefore, the time to sputum culture conversion was compared between two groups of vitamin D status. Kaplan Meier curves were plotted for 281 MDR-TB patients (***Figure 6-6***) and 286 DS-TB patients (***Figure 6-8***). Events were noted when they had culture sputum conversion during treatment with at least two consecutive negative cultures for MDR-TB and one negative culture for DS-TB. Patients who died or lost to follow-up before culture conversion were treated as censor. Survival analysis was used to compare the time to the first culture conversion, regardless of reversion, between two vitamin D groups.

Under Cox regression analysis, vitamin D deficiency significantly prolonged the positive culture time of MDR-TB patients during treatment (p=0.017) (***Figure 6-6***). In DS-TB, patients with vitamin D deficiency slightly reduced the positive culture time in the first 60 days, and no difference was observed thereafter (p=0.82) (***Figure 6-8***).

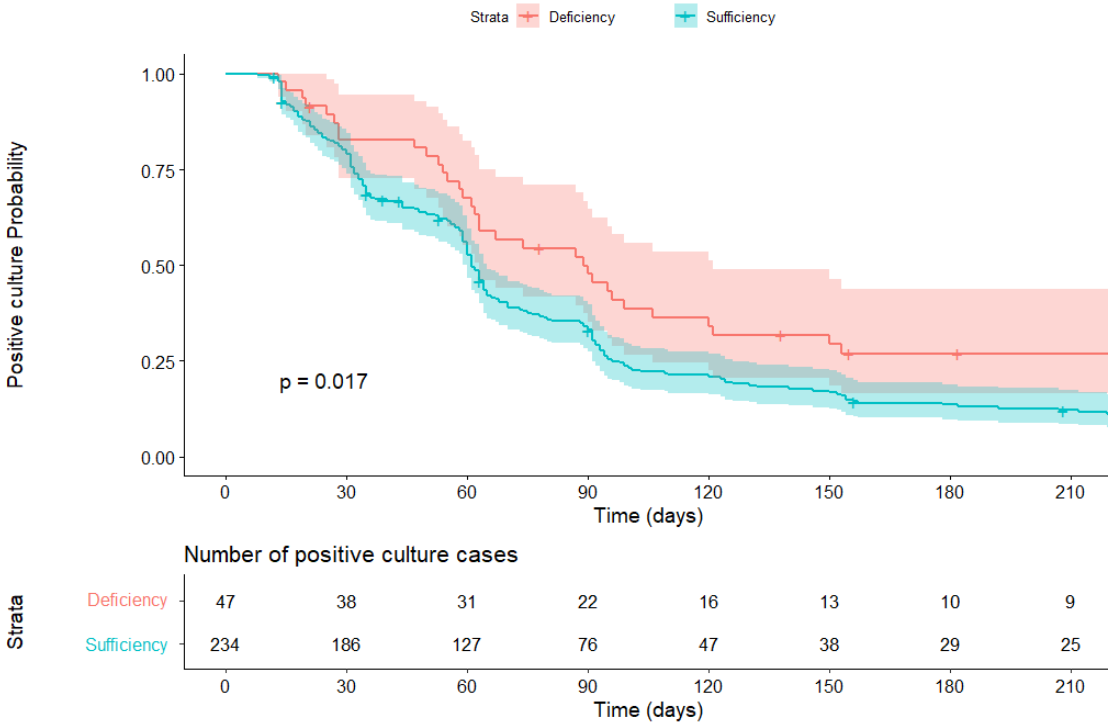


Figure 6-6. Kaplan Meier curve for sputum culture conversion of MDR-TB, stratified by vitamin D status. Data was for 281 MDR-TB patients with positive sputum culture at baseline. Red line represented 47 patients with vitamin D deficiency, and green line for 234 vitamin D sufficiency patients. P value of log rank test was reported.

The level of vitamin D which influences TB treatment response remains unknown. The cut off value used to define vitamin D deficiency in this study and other previous studies originated from definition for skeletal health of the US Endocrine society (114). I investigated whether pulmonary MDR-TB treatment responded to a higher level of vitamin D level. Hence, I divided the sufficient groups into two subgroups of "insufficiency" (vitamin D from 20 to 30ng/ml) and "normal" (vitamin D ≥ 30ng/ml). ***Figure 6-7*** illustrated the treatment response of MDR-TB patients with vitamin D deficiency, insufficiency and normal. Kaplan Meier curves showed that effect of insufficiency was similar to normal vitamin D patients, while the treatment response

pattern of "deficiency" differed from the other two. Therefore, the cut off at 20ng/ml to classify two groups of vitamin D status was appropriate in this cohort.

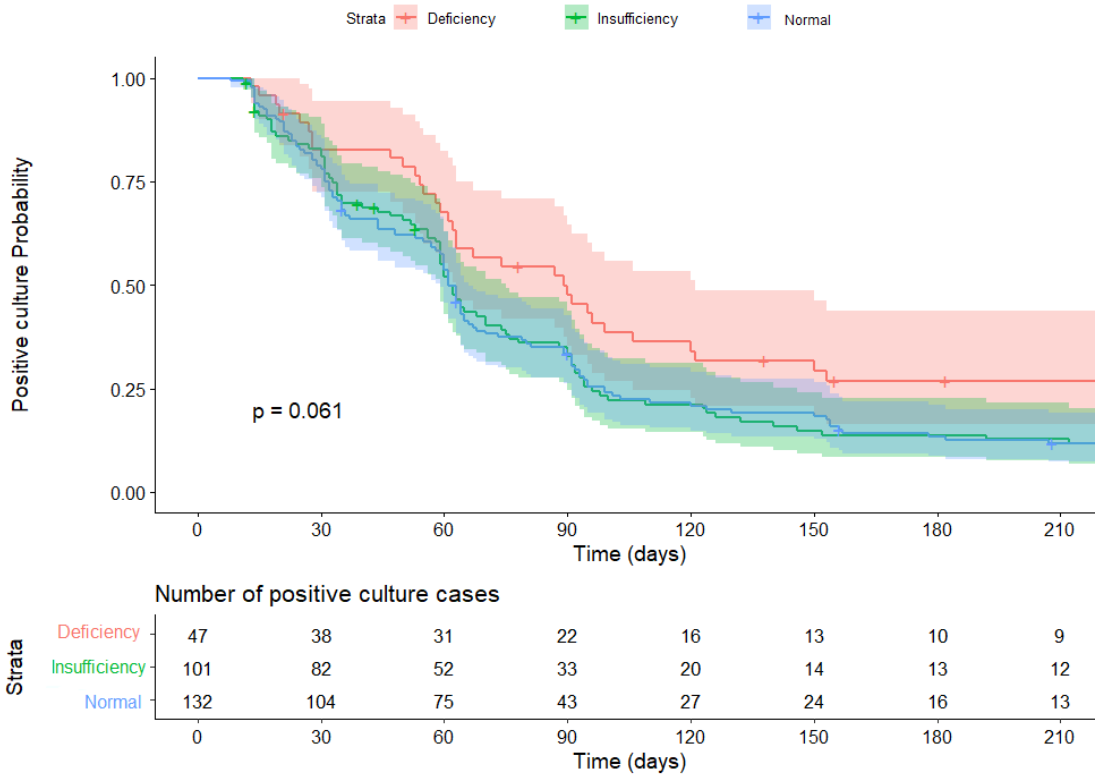


Figure 6-7. Kaplan Meier curve with three groups of vitamin D status and sputum culture conversion of MDR-TB. Data was for 281 MDR-TB patients with positive sputum culture at baseline. Red line represented 47 patients with vitamin D deficiency, and green line for 101 insufficiency and blue line for 132 normal. P value of log rank test was reported.

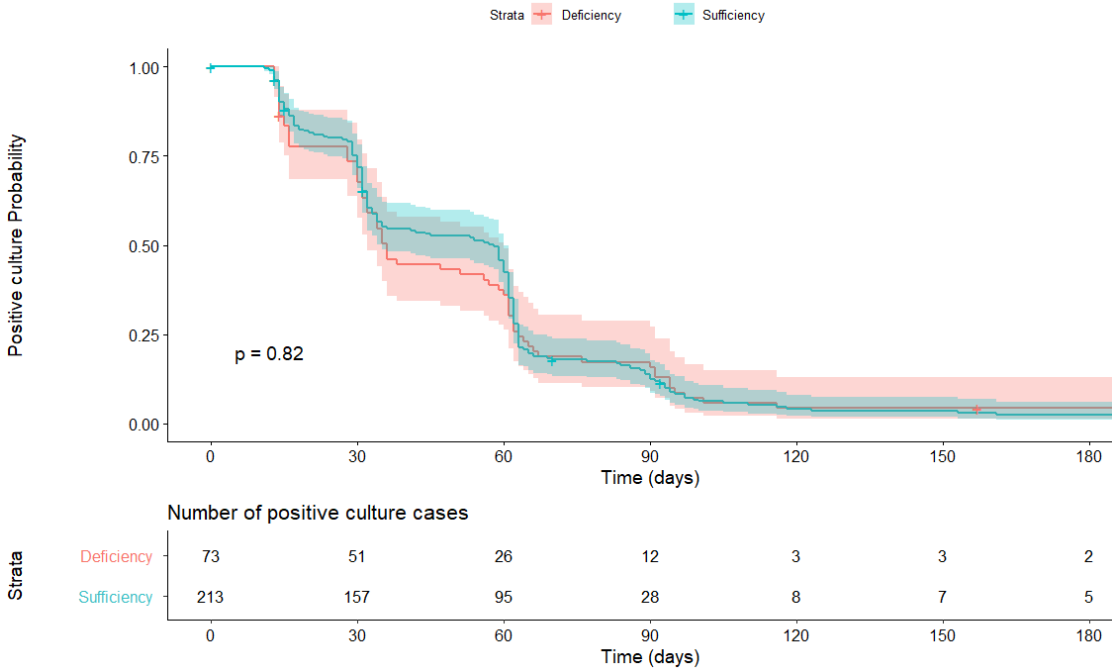


Figure 6-8. Kaplan Meier curve for DS-TB patients with vitamin D deficiency and sufficiency. Data was for 286 DS-TB patients with positive cultures at baseline. Red line represented 73 patients with vitamin D deficiency, and green line for 213 vitamin D sufficiency. P value of log rank test was reported.

The Kaplan Meier plots of MDR and DS-TB patients showed heterogeneous effects of vitamin D deficiency with different time points. While Cox regression analysis provided the overall estimation for effect of vitamin D deficiency on sputum culture conversion, vitamin D status imposed different impacts at different time. Therefore, in order to closely observe the effect of vitamin D at different time point, restricted mean survival time analysis was used to compare the median time to stay in positive culture from the start of treatment to a specified time point. During MDR-TB treatment, 79.6% MDR-TB patients remained culture positive at the end of one month, and 55.5% at two months, 23,8% at four months and 16.2% at six months. Therefore, I decided to truncate the time to observe the differences at four time points during treatment: one month, two months, four months and six months. MDR-TB patients with and without vitamin D deficiency had similar median positive time at the first month of treatment. However, vitamin D deficiency started to significantly prolong the positive time at month 2

(p=0.042), then month 4 (p=0.014) and month 6 (p=0.017). Although DS-TB patients with vitamin D deficiency had shorter median positive time than those without vitamin D deficiency, no significant difference was observed for all time points (month 1: p=0.484; month 2: p=0.227; month 3: p=0.502; month 4: p=0.675) (Table 6.6).

Table 6.6. Restricted mean positive time between vitamin D deficiency and sufficiency of MDR and DS-TB patients

	Vitamin D status	MDR-TB RMPT (days)*	P value**	DS-TB RMPT (days)*	P value**
1 month	Deficiency	29 [28-30]	0.206	26 [25-28]	0.484
	Sufficiency	28 [27-28]		27 [26-28]	
2 months	Deficiency	52 [48-57]	0.042	40 [36-45]	0.227
	Sufficiency	47 [45-50]		43 [41-46]	
4 months	Deficiency	81 [71-92]	0.014	48 [41-55]	0.502
	Sufficiency	67 [62-71]		51 [47-55]	
6 months	Deficiency	99 [82-116]	0.017	51 [42-60]	0.675
	Sufficiency	76 [69-83]		53 [48-58]	

*: RMPT: Restricted mean positive time. Value were presented as number and 95%CI (days)

**: p.value was based on RMST analysis for the difference in days of average time between vitamin D deficient and sufficient patients.

6.2.3 Vitamin D deficiency and TB treatment outcome

Treatment outcome information was available for 298 MDR-TB and 301 DS-TB patients. Eleven MDR-TB patients were still under treatment at the time of analysis, including two vitamin D deficient and nine sufficient patients. Treatment success was achieved in 228 (76.5%) MDR-TB patients, including 205 (68.8%) cured and 23 (7.7%) treatment completed without any signs of failure. Among 70 (23.5%) MDR-TB patients with unfavorable outcomes, 12 (4.0%) died, 22 (7.4%) failed the treatment and 36 (12.1%) lost to follow-up. In 301 DS-TB

patients, 158 (52.5%) patients were cured, 85 (28.2%) completed the treatment, five (1.7%) died, 38 (12.6%) failed and 15 (5.0%) lost to follow-up.

Increase in vitamin D level slightly decreased risk of mortality (OR: 0.74, 95%CI: 0.53-1.04) and unfavorable outcomes (OR: 0.89, 95%CI: 0.77-1.03) in MDR-TB patients. The effect of vitamin D on treatment outcomes of DS-TB was modest (OR: 0.98, 95%CI: 0.61-1.59 for mortality, OR: 1.07, 95%CI: 0.92-1.25 for unfavorable outcomes) (***Table 6.7***).

Table 6.7. Associations between vitamin D concentration and treatment outcome of MDR and DS-TB by univariate logistic regression

	All-cause mortality		Unfavorable outcomes	
	OR* (95%CI)	**P**	**OR* (95%CI)**	**P**
MDR-TB	0.74 (0.53-1.04)	0.086	0.89 (0.77-1.03)	0.108
DS-TB	0.98 (0.61-1.59)	0.940	1.07 (0.92-1.25)	0.385

* For every 5ng/ml increase of vitamin D concentration

Data was for 298 MDR-TB patients who had treatment outcome at the time of analysis and all 301 DS-TB patients.

OR: Odds ratio; 95%CI: 95% confident interval

There was no significant difference in treatment outcomes between patients with and without vitamin D deficiency in both MDR and DS-TB. In MDR-TB, vitamin D deficiency patients had insignificantly higher failure rate (9.6% in deficiency vs 6.9% in sufficiency) and mortality rate (7.7% in deficiency vs 3.3% in sufficiency). Vitamin D deficiency insignificantly increased risk of death by 2.48 times (95%CI: 0.72-8.56) and risk of unfavorable outcomes by 1.58 times (95%CI: 0.82-3.06). The association remains insignificant after adjustment for patient's age, BMI and bacterial load at baseline.

In DS-TB, although patients with vitamin D deficiency had lower rate of failure (8.0% in deficiency vs 14.2% in sufficiency, p=0.234), mortality rate in deficient group (2.7%) was higher than sufficient group (1.3%) (p=0.601). Vitamin D deficiency insignificantly increased

risk of death by 2.04 (95%CI: 0.33-12.43) fold changes and decreased risk of unfavorable outcomes by 0.65 (0.32-1.34) fold changes.

6.2.4 Vitamin D deficiency and blood characteristics

Vitamin D has been known as an immuno-modulator that influences innate immunity and cytokine expression. To understand the mechanism of vitamin D on TB presentation and treatment outcome, correlation between vitamin D levels and blood characteristics and cytokine was assessed.

Table 6.8 showed the correlation between vitamin D levels and white blood cell (WBC), albumin, globulin and albumin:globulin (AG) ratio. In MDR-TB, increase in vitamin D decreased neutrophil proportion (r=-0.248, p<0.001) and elevated lymphocyte proportion (r=0.204, p<0.001) (***Figure 6-9***). Vitamin D concentration positively correlated with albumin level (r=0.013, p<0.001) and negatively correlated with globulin level (r=-0.015, p<0.001) (***Figure 6-10***). In DS-TB, increase in vitamin D concentration correlated with decrease in neutrophil and increases in albumin and A/G ratio (***Table 6.8***).

Table 6.8. Correlation between vitamin D concentration and white blood cells and biomarkers in pulmonary TB

	MDR-TB		**DS-TB**	
	r (95%CI)	***P value***	***r (95%CI)***	***P value***
WBC (G/L)	-0.034 (-0.072 to 0.004)	0.080	-0.008 (-0.054 to 0.037)	0.726
Neutrophil (G/L)	-0.045 (-0.978 to -0.012)	0.007	-0.023 (-0.064 to 0.018)	0.272
Neutrophil (%)	-0.248 (-0.374 to -0.123)	<0.001	-0.167 (-0.303 to -0.031)	0.016
Lymphocyte (G/L)	0.011 (0.002 to 0.020)	0.016	0.012 (0.002 to 0.021)	0.016
Lymphocyte (%)	0.204 (0.099 to 0.311)	<0.001	0.110 (-0.004 to 0.224)	0.057
Monocyte (G/L)	-0.003 (-0.008 to 0.001)	0.154	-0.002 (-0.007 to 0.003)	0.456
Monocyte (%)	0.001 (-0.030 to 0.032)	0.940	-0.003 (-0.039 to 0.034)	0.888
NLR	-0.090 (-0.130 to -0.048)	<0.001	-0.037 (0.081 to 0.007)	0.103

	MDR-TB		DS-TB	
	r (95%CI)	***P value***	***r (95%CI)***	***P value***
Albumin (g/dl)	0.013 (0.008 to 0.019)	<0.001	0.009 (0.004 to 0.015)	<0.001
Globulin	-0.015 (-0.023 to -0.006)	0.001	-0.009 (-0.018 to 0.001)	0.067
A/G ratio	0.007 (0.004 to 0.010)	<0.001	0.004 (0.001 to 0.007)	0.019
Calci (mg/ml)	0.004 (0.002 to 0.006)	<0.001	0.002 (-0.0002 to 0.004)	0.085

Data was for 309 MDR-TB patients with available results of albumin, globulin and A/G ratio, and 272 patients with WBC counts and differentiation results. P value and correlation co-efficient r of linear regression were reported.

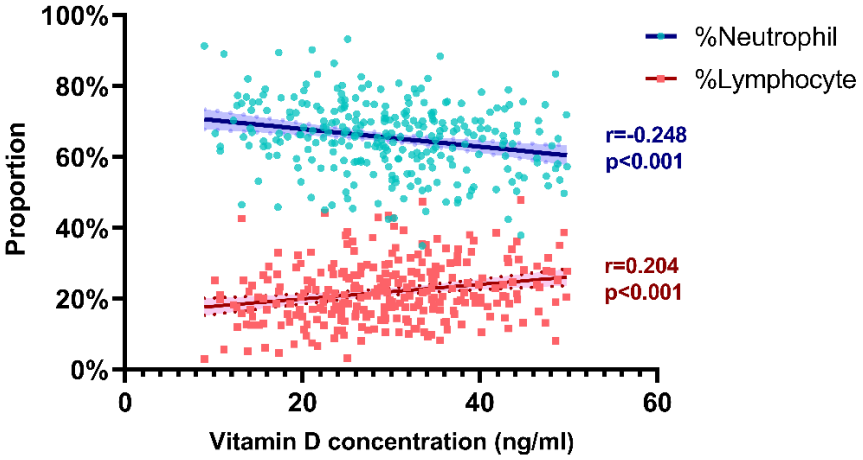


Figure 6-9. Correlations between vitamin D concentrations and neutrophil and lymphocyte proportion in MDR-TB. Data was for 272 patients MDR-TB patients. Green dots represented neutrophil proportion. Pink dots represented lymphocyte proportion. Lines and shades represents the linear equations and 95%CI. P value and correlation co-efficients (r) were based on linear regression.

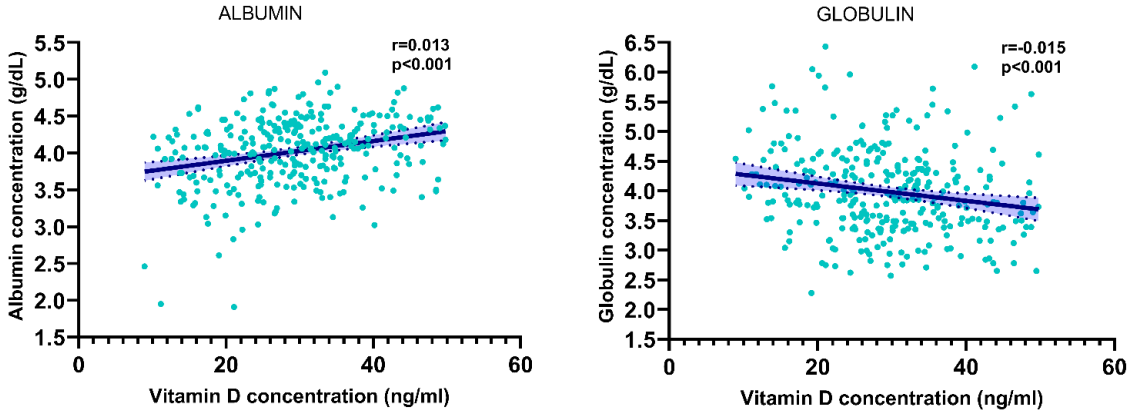


Figure 6-10. Correlations between vitamin D concentrations and levels of albumin and globulin in MDR-TB. Data was for 309 MDR-TB patients. Lines and shades represents the linear equations and 95%CI. P value and correlation co-efficients (r) were based on linear regression.

A panel of eight cytokines, including TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IL-13, IL12p70, was measured in serum samples of all 306 MDR-TB and 299 DS-TB patients before treatment. Linear regression was performed to evaluate the correlations between vitamin D concentration and cytokines levels which had more than detectable 50% of samples (TNF-α and IL6). For cytokines that had less than 50% of samples detected (IFN-γ, IL-2, IL-10, IL-13, IL12p70), logistic regression was used to estimate the association between vitamin D concentration and the detection of cytokine in circulating serum of MDR and DS-TB patients. For IL-1β, logistic regression was performed for MDR-TB patients and linear regression for DS-TB (***Table 6.9***). Vitamin D levels negatively correlated with TNF- α (r=-0.130, p=0.005) in MDR-TB but not in DS-TB. No significant correlation was found between IL-6 and vitamin D level in both MDR and DS-TB. Vitamin D levels did not associate with detection of IFN-γ, IL-1β ,IL-2, IL-10, IL-13, IL-12p70 in MDR-TB, but increase in vitamin D significantly decreased the detections of IFN-γ, IL-2 and IL-12p70 in DS-TB (***Table 6.9***).

Table 6.9. Cytokine levels and vitamin D concentration in pulmonary TB patients

	MDR-TB			DS-TB		
	N(%)*	OR (95% CI)	P value	N(%)*	OR (95% CI)	P value
TNF-α (pg/ml)**	301 (96.5%)	-0.130 (-0.222 to -0.039)	0.005	310 (98.1%)	-0.139 (-0.305 to 0.027)	0.100
IL-6 (pg/ml)**	299 (95.8%)	-0.199 (-0.563 to 0.165)	0.284	315 (99.7%)	-0.252 (-1.032 to 0.528)	0.526
IL-1β (pg/ml)§	101 (32.4%)	0.90 (0.79-1.03)	0.125	173 (54.7%)	-0.063 (-0.176 to 0.050)	0.275
IFN-γ detected***	112 (35.9%)	0.93 (0.82-1.05)	0.229	106 (33.5%)	0.77 (0.67-0.89)	<0.001
IL-2 detected***	7 (2.2%)	0.87 (0.58-1.32)	0.512	41 (13.0%)	0.71 (0.57-0.88)	0.002
IL-13 detected***	50 (16.0%)	0.94 (0.80-1.11)	0.487	64 (20.3%)	0.88 (0.75-1.03)	0.121
IL-10 detected***	105 (33.7%)	0.93 (0.82-1.05)	0.247	52 (16.5%)	1.00 (0.85-1.18)	0.990
IL-12p70 detected***	24 (7.7%)	0.96 (0.77-1.20)	0.734	89 (28.2%)	0.78 (0.67-0.91)	0.001

Data was for 306 MDR and 299 DS-TB.

OR: odds ratio; 95% CI: 95% confident interval

* Number (%) of samples with detectable levels of cytokine.

** Co-efficient (95%CI) and p value of linear regression were reported for TNF-α and IL-6.

*** OR (95%CI) and p value of logistic regression were reported for IFN-γ, IL-2, IL-10, IL-13, IL12p70, for every 5ngl/ml increase of vitamin D concentration.

§ OR (95%) was reported for IL-1β of MDR-TB, and co-efficient r was reported for IL-1β of DS-TB.

In MDR-TB, patients with vitamin D deficiency had significantly higher levels of IFN- γ (deficiency: 37.0 pg/ml [IQR: 11.9-65.1] vs sufficiency: 10.9 pg/ml [IQR: 5.47-21.4], p=0.001), IL-6 (deficiency: 8.95 pg/ml [4.74-17.5] vs sufficiency: 5.67 pg/ml [3.32-10.9], p=0.006). The differences in IFN- γ and IL-6 between vitamin D deficiency and sufficiency were still significant after Bonferroni correction. TNF-α levels in vitamin D deficient patients was insignificantly higher than sufficient patients (p=0.077). DS-TB patients with vitamin D defficiency had higher levels of IL-6 (p=0.029), however the difference became insignificant

after Bonferroni correction. More DS-TB patients with vitamin D deficiency had detected levels of IFN-γ (p=0.001) and IL-2 (p=0.008).

6.3 DISCUSSION

Vitamin D deficiency is a common comorbidity in patients with TB. The frequencies of vitamin D deficiency in MDR-TB (15.7% in men, 23.3% in women) and DS-TB (21.9% in men, 35.3% in women) in our study were much higher than those of previous study done by Ho-Pham *et al.* in 2009 in HCMC (4.4% in men, 5.7% in women) (183). An explanation for the high frequencies in our study was the widespread lifestyle of avoiding exposure to sunlight in this urban city, especially in women. Comparing with other studies, our cohort had lower prevalence of vitamin D deficiency than those in Korea (88.3%) (187), China (74.2%) (188), Pakistan (57%) (189), Uganda (44.2%) (190) and Malawi (39%) (180). One possible reason for the discrepancy in prevalences was the differences in climate and locations in respect to the equator, leading to different levels of exposure to UVB.

To evaluate the influence of vitamin D on pulmonary TB manifestation before treatment, I analyzed the association between vitamin D level and individual TB-related symptoms, extent of lesion of CXR, bacterial burden by GeneXpert results, symptom severity and disease severity. There was no significant association between vitamin D and individual TB-related symptoms. However, increase in vitamin D concentration was significantly associated with decreased clinical score in MDR-TB. Vitamin D deficiency also associated with increased risk of more severe symptoms and severe disease in MDR-TB and DS-TB, although the association in MDR-TB was not significant. Our findings suggested that vitamin D influenced severity of pulmonary TB, and vitamin D deficiency was associated with more severe disease.

In addition, vitamin D levels negatively correlated with extent of lung damage on CXR, assessed by Timika score. Vitamin D deficiency was associated with significantly increased proportion of lung damage and Timika score in both MDR and DS-TB. Our findings was consistent with the SUCCINCT study that lower level of vitamin D was associated with more

severe chest radiographic involvement at baseline (90). It indicated that low vitamin D concentration was strongly associated with increased lung damage in pulmonary TB.

I also found an association between vitamin D deficiency and high bacterial burden in DS-TB but not in MDR-TB. DS-TB patients with vitamin D deficiency had significantly lower mean CT value than patients without deficiency. However, no association was found between vitamin D deficiency and bacterial load in MDR-TB. A study of Yuvaraj *et al.* in 65 AFB positive pulmonary TB patients revealed a negative correlation between vitamin D levels and smear grades (184) while other studies showed no association (90) (180). While those studies used AFB smear grades, our study assessed the mean CT value of semi-automated GeneXpert, which provided more accurate results.

The main focus of this study was to investigate the impact of vitamin D on treatment response and outcome of pulmonary TB, especially MDR-TB. Increase in vitamin D level did not obviously influence treatment response or outcomes from pulmonary TB. However, vitamin D deficiency significantly prolonged the time to the first sputum culture conversion in both Cox regression analysis and restricted mean survival time analysis in MDR-TB, but not in DS-TB. While the effect of vitamin D deficiency on increasing clinical severity, CXR and bacterial burden in DS-TB was apparent, its detrimental impact on treatment response and outcomes of DS-TB was less clear. In the pre-antibiotic era, vitamin D, in many forms from cod liver oil to exposure to sun light, and later high doses of vitamin D supplementation, was reported to be safe and effective in treating TB (5). After chemotherapy was discovered, the role of vitamin D supplementation was reassessed with controversial results. Many RCTs using vitamin D supplementation failed to prove its beneficial effect in reducing time to sputum culture conversion (93) (191) (192) (193), and smear conversion (90) (92). Although Ganmaa *et al.* did not detect significant difference in time to sputum culture conversion after 8 weeks of treatment in their RCT with 390 TB patients, their subgroup analysis showed 5/13 MDR-TB patients receiving 4 doses of 35,000IU vitamin D_3 had culture negative at 8 weeks compared to 0 of 8

patients in the placebo arm (p=0.11) (191). Yet the small number of MDR-TB patients in Ganmaa *et al.* was insufficient to conclude the impact of vitamin D supplementation on MDR-TB treatment. By contrast, Mily *et al.* found significant clinical improvement and higher rate of negative sputum culture at week 4 and week 8 in 62 TB patients receiving 5,000IU of vitamin D_3. However, in this study, sputum smear conversion between patients treated with vitamin D_3 did not differ from that of placebo (94). Other study in Pakistan also found higher rate of negative smear at week 12 of treatment in 60 patients with 4 doses of 100,000IU intramuscular vitamin D (194). Meta-analysis using individual participant data from eight RCTs showed that vitamin D did not shorten the time to sputum culture conversion in pulmonary TB patients, especially those with DS-TB (aHR: 1.02, 95%CI: 0.88-1.19). In a subgroup of 37 MDR-TB patients from four RCTs, vitamin D supplementation did improve sputum culture conversion (aHR: 13.44, 95%CI: 2.96-60.90) (95). The results from the meta-analysis and our findings suggested that the impact of vitamin D deficiency on treatment response and outcomes of DS-TB was obscured by the effective chemotherapy. This may partly explain why vitamin D was reported as a cure for TB before the discovery of chemotherapy, but was shown to have little effect in many recent RCTs and in meta-analysis. In our cohort, vitamin D deficiency significantly prolonged sputum culture conversion of MDR-TB, although did not significantly worsen treatment outcome. It suggests that in MDR-TB, where drugs are less effective, the host immunity may be more important, and the impact of vitamin D deficiency on delaying sputum culture conversion can be more easily observed.

Increase in vitamin D levels slightly decreased mortality and unfavorable outcomes in MDR-TB. The mortality rate in TB patients with vitamin D deficiency was higher than patients without deficiency (MDR-TB: 7.7% vs 3.3%; DS-TB: 2.7% vs 1.3%). However, both univariate and multivariate logistic regression analysis did not demonstrate any significant effect of vitamin D on mortality. Similarly, Wejse *et al.* did not detect effect of vitamin D supplementation on 12-month mortality of TB patients (92). The failure rate in patients with

vitamin D deficiency was higher in MDR-TB, but lower in DS-TB. No significant association was found between this condition and unfavorable outcomes for both MDR and DS-TB, which was consistent with previous studies. The longitudinal study of Sloan *et al.* found that vitamin D status at baseline did not have significant relationship with treatment response and final treatment outcome of DS-TB (180). Another study in China did not detect the difference in vitamin D levels between TB patients with favorable and unfavorable treatment outcome (188). To clarify the effect of vitamin D on pulmonary TB, associations between vitamin D and WBC counts, albumin, globulin and a panel of cytokines before treatment were evaluated. Among MDR-TB patients, elevated vitamin D decreased neutrophil proportion and increased lymphocyte proportion, which led to significantly increased NLR. Our analysis in chapter 4 (section 4.2.5) also showed increased NLR associated with more lung damage and more severe disease in both MDR and DS-TB. Low proportion of lymphocyte before treatment was a proven predictor of unfavorable treatment outcome (149), while high NLR was a marker of inflammation and associated with more advanced disease of pulmonary TB (148). Vitamin D also strongly correlated with albumin level, globulin and AG ratio, which strongly associated with severe disease and mortality of MDR-TB (section 4.2.5). Moreover, high levels of pro-inflammatory IFN- γ and IL-6 reflected increased inflammation in MDR-TB patients with vitamin D deficiency. Levels of TNF- α in vitamin D deficient patients were also higher, but not significantly. It indicated that vitamin D deficiency in MDR-TB was associated with more inflammation and disease extension, which might explain the detrimental effect of vitamin D deficiency on response to MDR-TB treatment. In DS-TB, vitamin D levels correlated with proportion of neutrophil, lymphocyte counts, levels of albumin, and AG ratio. Vitamin D was also associated with decreased detection of IFN-γ, IL-2 and IL-12p70. A tendency of increased NLR, IFN-γ and IL-2 was also noticed in DS-TB patients with vitamin D deficiency. In this cohort, median concentration of vitamin D in DS-TB was lower than that in MDR-TB, and the frequency of vitamin D deficiency in DS-TB was higher than in MDR-TB. However, the effect

of vitamin D on DS-TB was lesser than on MDR-TB. It suggested that vitamin D deficiency induced a weaker inflammatory response in DS-TB than in MDR-TB, and therefore, modest effect on treatment response in DS-TB. Our findings might also provide new insight into discrepancy results of vitamin D supplementation in treating DS and MDR-TB.

Our study was the first to comprehensively evaluate the impact of vitamin D in MDR pulmonary TB, and compare the effect of vitamin D on MDR and DS-TB. ***Figure 6-11*** summarized the effect of vitamin D deficiency on MDR and DS pulmonary TB. Although I was unable to detect the effect of vitamin D deficiency on treatment response and outcome of DS-TB, vitamin D deficiency had clear impact on clinical presentations, severity and inflammation response of both MDR and DS-TB. Vitamin D deficiency had a tendency to decrease the proportion of culture conversion after 1 and 2 month of MDR-TB treatment. Survival analysis showed a significant delay in time to sputum culture conversion among MDR-TB patients with vitamin D deficiency.

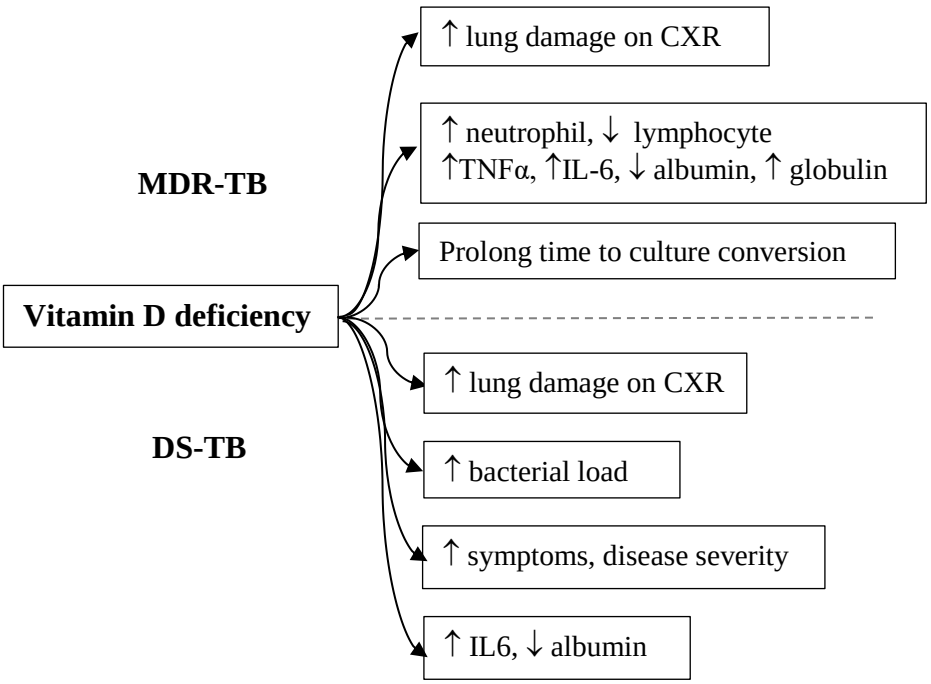


Figure 6-11. Diagram of effect of vitamin D deficiency on pulmonary TB.

6.4 CONCLUSION

This study clarified the influence of vitamin D and emphasized the effect of vitamin D deficiency on pulmonary TB. Vitamin D deficiency was associated with increased inflammation and associated with more severe pulmonary TB. However, vitamin D deficiency did not significantly influence treatment responses and outcome from DS-TB. Vitamin D deficiency was associated with delayed culture conversion during MDR-TB treatment. Further study is required to evaluate whether vitamin D deficiency should be screened and corrected by supplementation in order to augment MDR-TB treatment response.

APPENDIX

Table B. Differences between all DS-TB patients and those who were included for vitamin D measurement

	All patients ***N=600***	**Tested patients** ***N=301***	**P value**
Age (years)	43.5 [31.0-52.0]	45.0 [31.0-53.0]	0.361
Male	478 (79.7%)	233 (77.4%)	0.486
HIV co-infection	18 (3.00%)	13 (4.32%)	0.406
Drinking alcohol	174 (29.0%)	81 (26.9%)	0.263
Regular smoker	134 (22.3%)	62 (20.6%)	0.610
History of previous TB	76 (12.7%)	50 (16.6%)	0.131
Symptoms duration (days)	18.0 [12.0-30.0]	20.0 [12.0-30.0]	0.298
BMI	19.4 [17.6-21.6]	19.5 [17.5-21.8]	0.880
Cavity on CXR	198 (40.5%)	120 (45.5%)	0.216
Diabetes mellitus	129 (21.6%)	81 (26.9%)	0.084
GeneXpert levels:			0.944
MTB Not Detected	10 (1.67%)	3 (1.00%)	
MTB Detected Very Low	54 (9.02%)	24 (8.00%)	
MTB Detected Low	129 (21.5%)	65 (21.7%)	
MTB Detected Medium	249 (41.6%)	128 (42.7%)	
MTB Detected High	157 (26.2%)	80 (26.7%)	
Symptom score	3.00 [2.00-5.00]	4.00 [2.00-5.00]	<0.001
Clinical score	4.00 [2.00-5.00]	5.00 [3.00-6.00]	<0.001
Severe symptom	165 (27.5%)	115 (38.2%)	0.001
Severe disease	148 (24.7%)	103 (34.2%)	0.003
Treatment outcome			0.280
Completed	150 (25.0%)	85 (28.2%)	
Cured	357 (59.5%)	158 (52.5%)	

	All patients *N=600*	Tested patients *N=301*	P value
Died	6 (1.00%)	5 (1.66%)	
Failed	66 (11.0%)	38 (12.6%)	
Lost to follow-up	21 (3.50%)	15 (4.98%)	

P value of Wilcoxon test was reported for continuous variables, and p value of chi square test was reported for category variables.

Chapter 7

GENERAL DISCUSSION

New treatment for tuberculosis (TB), especially for MDR-TB, has been rigorously sought to improve outcomes of this widespread and fatal infectious disease. Host-directed therapy is an emerging approach for many diseases, with a focus on personalizing treatment to optimize outcomes. Understanding of the host determinants of treatment response and outcomes of MDR-pulmonary TB can provide foundation for identifying candidate(s) for targeted therapy. In my thesis, I explored three important host factors of leukotriene A4 hydrolase (*LTA4H*) genotype, diabetes mellitus (DM) and vitamin D to understand their effects on presentation, inflammatory response and treatment outcomes of pulmonary TB (PTB), particularly MDR-TB.

In both cohorts of MDR and DS-PTB, host immunity, expressed as white blood cell counts, neutrophil proportion, lymphocyte proportion, neutrophil:lymphocyte ratio (NLR), globulin and albumin:globulin ratio, was associated with bacterial burden, disease severity prior to treatment, and mortality. Inflammation also played a crucial role in pathogenesis of PTB, as increased TNF-α was significantly associated with disease severity and mortality. It suggests that host immune and inflammatory responses influence PTB progression and treatment outcomes. Corticosteroids, a potent anti-inflammatory agent, have been studied as an adjunctive therapy for PTB since 1959, but systematic reviews have shown no effect on the rate of sputum conversion (195). However, in a randomized clinical trial (RCT) of 178 advanced smear positive PTB patients with persistent high-grade fever and weight loss after two weeks of anti-tuberculosis drugs, prednisolone shortened the time of hospitalization, fever, weight gain and radiological recovery, but not smear conversion rate (196). Inflammation is a host response to protect against infectious pathogen. Only those with hyper-inflammatory reaction might benefit from anti-inflammatory adjunctive therapy. Even in a highly inflammatory disease like TB meningitis (TBM), dexamethasone was probably only beneficial to the TT genotype of *LTA4H*, the group with high inflammatory response (87). However, I found no association between

LTA4H genotype and blood inflammatory parameters including circulating cytokines. Consistently, *LTA4H* genotype did not associate with TB manifestation, severity, treatment response and outcome of both MDR and DS-TB patients. The fact that TBM is a more acute disease and has higher inflammation (151) and much higher mortality rate than PTB (mortality rate: 36.5% in TBM (135), 4.0% in MDR-PTB ; 1.0% in DS-PTB) might be the reason why the effect of host *LTA4H* genotype was clearly observed in TBM but not in PTB. The data presented in this thesis suggest that *LTA4H* genotype is not a potential candidate to identify the group of PTB patients, even in MDR-TB, to benefit from anti-inflammatory adjunctive therapy. The quest of identifying host biomarker for adjunctive therapy in MDR-PTB remains unsolved and needs further investigation.

Vitamin D has long been studied as a main treatment and then an adjunctive therapy for TB. I studied the effect of vitamin D on presentation and outcomes of both MDR and DS-PTB. Vitamin D correlated with inflammatory response and tissue damage (extent of lung damage) in PTB before treatment. Patients with vitamin D deficiency had more severe symptoms and disease in DS-TB but not in MDR-TB. However, under treatment, vitamin D deficiency was associated with significantly prolonged sputum culture conversion in MDR-TB, but not DS-TB. It suggests that vitamin D, as an immune-modulator, may influence the course of TB before treatment, but vitamin D deficiency is not a strong enough host factor to influence the effective treatment of DS-TB. However, in MDR-TB, when chemotherapy was less effective, effect of this host factor was more pronounced, with prolonged sputum culture conversion in vitamin D deficiency patients. My findings could help to partially explain why vitamin D supplementation did not fasten sputum conversion time of PTB patients in many RCTs (90, 92, 93, 95, 191) although it was once used as a main therapy for TB in the pre-antibiotic era (4). In an individual participant data meta-analysis of 1,850 participants in eight RCTs, vitamin D supplementation speeded up sputum conversion in MDR, but not in DS-PTB. However, there were only 55/1,350 (4.0%) MDR-TB patients in this analysis, and two RCTs with 500 patients did not test for drug

susceptibility (95). I argue that without anti-tuberculosis drugs, vitamin D supplementation could reverse the tissue damage and enhance bacterial clearance, and took a certain role in treating TB. In the less effectively treated form of PTB, MDR-TB, vitamin D deficiency was not significantly associated with mortality. Considering the mortality rate of 4% in MDR-TB patients, the effect of vitamin D (if any) on mortality was hard to detect. I suspect that its effect would be clearer in subgroup of MDR-TB patients who had much higher mortality rate. In my retrospective and prospective studies, HIV co-infection was the only co-morbidity that was significantly associated with death during treatment. Compared to those without HIV infection, MDR-TB patients with HIV co-infection had much higher mortality (24.4% for HIV vs 8.1% for non-HIV in retrospective study, 10.5% for HIV vs 4.0% for non-HIV in prospective study). Moreover, MDR-TB patients with HIV co-infection had a higher rate of vitamin D deficiency (33.3%) than those without HIV co-infection (16.3%). However, due to a low prevalence of HIV in my cohort (6.7%, 21/312), I did not further evaluate the effect of vitamin D on this subgroup. The findings in my thesis together with results of the meta-analysis study suggest a termination for vitamin D trials in DS-TB with highly effective treatment, yet there might still be the role of vitamin D in a more devastating group of MDR-TB: MDR-TB with HIV co-infection. To go forward, prospective study for the role of vitamin D in MDR-TB should be conducted in areas with high HIV co-infection such as Sub-Sahara Africa, where more than half TB patients had HIV co-infection (11).

Among three observed host factors, DM had the most obvious association with inflammation and severe disease in PTB, and its effect on treatment response was more profound on MDR than DS-PTB. Patients with DM expressed much higher levels of white blood cell counts, neutrophil, NLR, albumin:globulin ratio and pro-inflammatory cytokines (TNF-α, IFN-γ and IL-6). Hyperglycemia has been known to promote inflammatory response by elevating pro-inflammatory cytokines (197). The negative impacts of hyperglycemia were observed in both

MDR and DS-TB before treatment. Levels of glycemic control was associated with bacterial burden and disease severity prior to treatment in MDR-TB. During treatment, DM patients took longer time to convert in MDR, and a weaker effect of DM was also observed in DS-TB. Moreover, compared to non-DM patients, MDR-TB with DM had significantly higher rate of obesity, a condition that upregulates pro-inflammatory cytokines, chemokines and adipokines, leading to chronic inflammation and worsening hyperglycemia (160). The combination of hyperglycemia and obesity might be parts of the causes for hyper-inflammatory response found in MDR-TB patients with DM. MDR-TB patients with DM might be the key group to need anti-inflammatory adjunctive therapy. However, corticosteroids can be harder to use safely in DM patients due to risks of increasing insulin resistance and disrupting glycemic control. Metformin, besides its glycemic lowering effect, has been considered as an anti-inflammatory agent (198). Although DM patients had insignificantly higher rate of mortality in both MDR and DS-TB, all those with metformin survived. Metformin was also associated with increasing rate of 2-month sputum culture conversion and reducing the risk of mortality in PTB with DM (199). The effect of metformin on reducing inflammation and mortality of MDR-TB patients with DM should be further evaluated in RCT.

DM was the most common co-morbidity for MDR-TB in my retrospective and prospective studies, a third of whom were not aware of their DM before being screened in the study. Another third of pulmonary TB patients had pre-DM. DM not only placed patients at increased risk for more severe PTB and delayed response to treatment of MDR-TB, but also had posed a greater risk for its complications on cardiovascular, microvascular and renal systems. Early diagnosis and treatment of DM might reduce not only the risk of delayed response to treatment of MDR-PTB, but also benefit patients' overall health. Given a high proportion of DM and pre-DM among PTB, DM screening, which is simple, should be implemented in a routine settings for TB patients.

On the other hand, surprisingly, patients with DM had lower rate of lost to follow-up. It is important to conduct quantitative as well as qualitative studies to investigate this phenomena. Understanding the reasons why DM patients had better compliance to treatment would help to reduce the rate of lost to follow-up in non-DM patients.

The characteristics of host and pathogen, and their interaction define the nature of infectious disease. In the complex picture of MDR-TB, my work has just unveiled a part of host determinants of treatment outcomes. Still other parts of the picture need to be explored and put together. Arachidonate 5-lipoxygenase genotype was also evaluated in a smaller population of MDR-TB cohort, but was not associated with clinical presentation and treatment response. Another study has also been conducted to compare the levels of vitamin D in serum and in bronchoalveolar lavage fluid of PTB patients. The TB group in Oxford University Clinical Research Unit (OUCRU) is trying to solve the puzzle of *Mycobacterium tuberculosis* and the interaction between host and this pathogen in different stages of infection and treatment. I am still following-up patients to extend our understanding to the long-term outcomes, including early relapse or re-infection of PTB patients. I hope that my thesis and the work of my whole TB group in OUCRU would contribute to improve treatment outcomes of this hard-to-treat infectious disease.

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