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The Impact of Tuberculosis on Pulmonary Health in Maputo, Mozambique

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ABSTRACT

Background

Pulmonary tuberculosis (PTB) is curable but is still a major health problem. PTB is associated to chronic lung impairment even after microbiological cure. The type, severity and risk factor for lung impairment (LI) are poorly described. The prevalence of LI and reference equations have not been established in Mozambican healthy population.

Methods

A cohort of PTB patients was followed for 52 weeks after TB diagnosis (2014 to 2016), spirometry and 6-Minute Walk Test was assessed at weeks 8, 26 and 52 of follow-up, in Mavalane, Maputo. Saint George Respiratory Questionnaire (SGRQ) was evaluated during treatment. In 2017, the prevalence of lung impairment and 6-Minute Walk Test were evaluated in healthy volunteers from the same neighbourhood.

Results

The proportion of LI is 73,3% on week 8, declining to 67,7% at week 26 and 61,3% (26% of moderate-severe) at week 52 in PTB patients. On week 52 the mean Vital Capacity (FVC) is 2.65l (66.7% of predicted) in participants with LI versus 3.68l (90% of predicted) in participants without LI. All study participants suffered from pulmonary restriction (except one). Female sex (RRR = 5), higher CD4 in HIV positives (RRR: 7.33) were significantly associated with LI. The increase of haemoglobin was protective (RRR = 0.61). The PTB patients travelled a mean distance of 442 meters on week 52 and a mean total score of 5.58 in SGRQ.

The proportion of LI in the Healthy volunteers was 20%, with 19,35% of restriction (one case of obstruction) mean FVC of 3.27l (89.4% of predicted) and FEV1 of 2.7l (93.80% of predicted).

Conclusion

Pulmonary restriction occurs in a fifth of healthy volunteer, it develops early during TB disease or treatment affecting more than half of the PTB patients. There is a need of more studies on lung outcome in PTB and to establish reference equation in healthy volunteers.

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ABBREVIATIONS

Abbreviation	**Explanation**
6MWD	6-Minute Walk Distance
6MWT	6-Minute Walk Test
AFO	Air Flow Obstruction
BMI	Body Mass Index
CI	Confidence Interval
CISPOC	Centro de Investigação e Treino em Saúde da Polana Caniço
CNBS	Comité Nacional de Bioética para Saúde
COPD	Chronic Obstructive Pulmonary Disease
CPA	Chronic Pulmonary Aspergillosis
CRF	Case Report Forms
CRP	C Reactive Protein
DALYS	Disability Adjusted Life Years
DOTS	Directly observed treatment, short-course
DS TB	Drug Susceptible TB
DST	Drug Sensibility Testing
E	Ethambutol
ECG	Electrocardiogram
FAST	Find cases Actively, Separate temporarily and Treat effectively
FDC	Fixed Doses Combination
FEV1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GLI	Global Lung Initiative
H	Isoniazid
HBC	High Burden Countries
HF	Health Facilities
HIV	Human immunodeficiency Virus
IgG	Immunoglobulin G
INS	Instituto Nacional de Saúde

IQR	Interquartile Range
LLN	Lower Limit of Normality
LMIC	Lower- and Middle-Income Countries
LMU	Ludwig-Maximilians-Universität Münich
LPA	Line Probe Assay
MDR	Multi Drug Resistant
MDR TB	Multi Drug Resistant TB
MMPs	Matrix Metalloproteinases
MoH	Ministry of Health
MTB	Mycobacterium tuberculosis
MZ	Mozambique
NTP	National TB Programme
OR	Odds-Ratio
P	Pyrazinamide
PLHIV	People Living With HIV
PTB	Pulmonary TB
PTBLD	Post TB Lung Disease
QA	Quality Assurance
QC	Quality Control
R	Rifampicin
RR	Risk Ratio
RRR	Relative Risk Ratio
RSD	Residual Standard Deviation
SA	South Africa
SOP	Standard Operating Procedure
SPO2	Peripheral Capillary Oxygen Saturation
STB	Susceptible TB
STR	Short Standardized Regimen
TB	Tuberculosis
WHO	World Health Organization

1. INTRODUCTION

Tuberculosis (TB) and its resistant forms continues being important causes of morbidity and mortality worldwide although curable, with around 10 million prevalent cases each year and around 1.3 million death in 2017 [1]. In low middle income countries (LMIC) the TB epidemic is driven by HIV, undernutrition, diabetes, smoking and alcohol consumption [1]. Between 1995 and 2015, an estimated 66 million lives were saved through the implementation of Directly Observed Treatment, Short-course (DOTS) by the National TB Programs in many countries worldwide. Early TB diagnosis and initiation of anti-microbial treatment are key elements of the DOTS strategy [1].

Many of those treated TB patients, however, undergo pulmonary anatomical changes secondary to illness, such as parenchyma structural damage including bronchovascular distortion, bronchiectasis, emphysematous and fibrotic band formations, which are leading to a reduction in overall lung compliance. These anatomical changes result in radiographic and functional changes that may remain after microbiological cure [2-5]. Finally, chronic lung damage can lead to oxygenation problems and consecutive pulmonary hypertension which further results in cardiac damage and heart failure.

The current end TB strategy focus on reduction of TB associated mortality, TB incidence and also social and economic burden to individual and households. Patients are not followed after cure, this results in weak evidence on chronic lung disease post TB. Further, the type and severity grades of post TB lung disease as well as associated risk factors are not well described. Consequently, there are no treatment suggestion or guidelines for patients with chronic pulmonary symptoms and function impairments existent. Although the number of studies on post TB lung disease have been increasing since 2010, only three TB treatment guidelines acknowledge post TB lung disease [6]. In LMIC the capacity (Spirometry) and management of chronic lung conditions is deficient as programs are not designed for long time follow up of TB patients and focus only on the microbiology cure.

Weak evidence exists about the main diseases and conditions after TB treatment. Pulmonary tuberculosis (PTB) is one important risk factor for chronic lung disease due to residual lung damage [6]. In recent years there is increased evidence that in LMIC [7] COPD is often associated with TB, in contrast to high income countries (HIC) where smoking is the main risk factor. Chronic

Pulmonary Lung disease (COPD) is globally the third most important cause of death [6]. Based on the global burden of disease projections, TB and COPD are among the 10 leading causes of death and disability for low- and middle-income countries at the beginning of the 21st century (sixth and eight, respectively) [8, 9].

In order to address the existing knowledge gap a prospective cohort facility based study was conducted in Maputo Mozambique, a LMIC with the highest TB rates in the world (551 per 100 000 inhabitants) [1]. In this study PTB patients were followed for one year after their TB diagnosis, and pulmonary lung function (spirometry and 6-minute walk test), clinical, socio-economical and environment risk factors were longitudinally assessed.

Additionally, a healthy volunteers group control was established in order to enable comparisons of the spirometry (Lung impairment, patterns) and 6-minute walk test results of (previous) TB patients with results from the healthy population.

2. LITERATURE REVIEW

I. Global TB Burden

TB is the ninth cause of death and the leading cause of death due to a single agent worldwide ranking above HIV [1]. It is estimated that 1.7 billion people (23% of world population) are infected with TB and at risk of developing active disease during their lifetime, People Living with HIV (PLHIV) are at a higher risk with estimated risk of 10% per year, compared to 5-10% lifetime in HIV negative [10].

In 2017, 10 million (9-11.1 million) people: 5.8 million man, 3.2 million woman and 1.0 million children developed TB and 1.6 million died (300 000 with HIV) [1]. The 30 High burden countries (HBC) accounted for 87% of all estimated incident cases worldwide. Two thirds of the cases happened in 8 countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). 9% of all TB cases worldwide were PLHIV, with 72% of them living in Africa [1]. Sub-Saharan Africa and Southeast Asia presented incidence rates above 100 cases per a 100 000 population per year as in Figure 2-1.

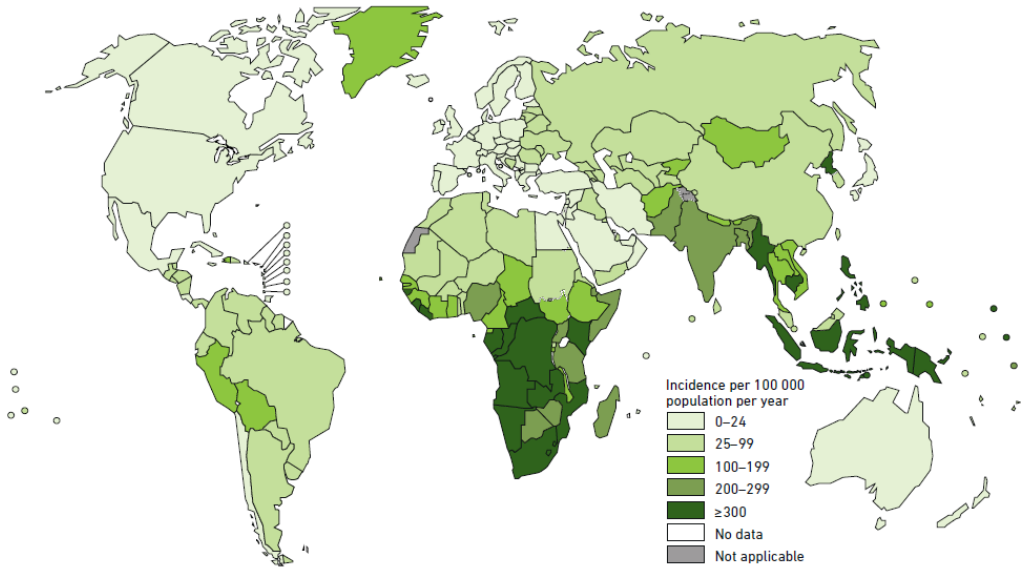


Figure 2-1 Estimated TB incidence rates in 2017

Source: World TB Report 2018 [1]

The rising numbers of drug resistant TB cases constitute a global public health crisis, with 558 000 (483 000 – 639 000) people diagnosed with Rifampicin resistant TB (RR-TB), 82% had Multi-Drug Resistant TB (MDR) with additional resistance to Isoniazid (INH) in 2017 [1].

In 2017, 6.7 million (6.4 new or relapse episodes) TB cases were notified to National TB Programmes (NTPs) worldwide. The coverage of drug resistance testing was 24% for new TB patients and 70% for previously treated patients. In addition, 60% of notified cases had a documented HIV result. There is a gap of 3.4 million between the number of notification and the best estimated number of cases (6.6 million vs 10 million) [1] as result of underdiagnosing and underreporting.

Most death from TB can be prevented by early diagnosis and initiation of an appropriate treatment regimen. In 2017, the global TB treatment coverage was 64% (58-72) with a treatment success rate of 82% cases globally. Thus, it is estimated that 66 million TB cases have successfully treated from 1995 to 2015 [1].

Despite decreasing TB estimated incidence rates globally, TB related morbidity and mortality shows slow reductions in countries of Sub Saharan Africa [1] and in some cases case notification are increasing due to the efforts to find the missing case including availability resistance testing.

II. TB and HIV burden in Mozambique

Mozambique has a triple burden of disease with high rates of TB, TB/HIV and MDR TB being listed in the three World Health Organization (WHO) lists: 30 high burden countries (HBC) for Absolute TB cases burden, HIV/TB co-infection burden and MDR-TB burden (Figure 2-2).

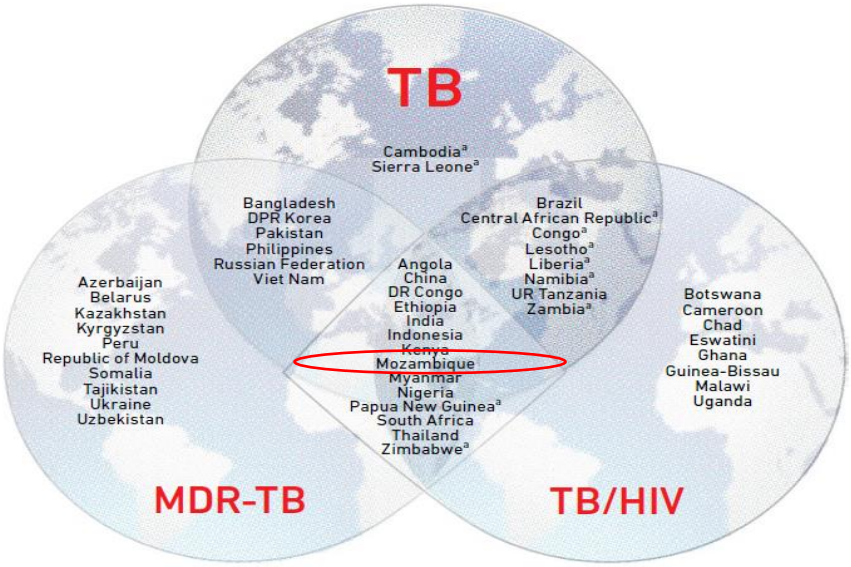


Figure 2-2 List of WHO high burden countries – Mozambique highlighted

Source: World TB Report 2018 [1]

In 2017, 86 515 TB cases were notified in Mozambique. Mozambique (551/100 000 inhabitants) together with Democratic People's Republic of Korea, The Philippines and South Africa are the few countries with estimated incidence rates above 500 TB cases per 100 000 Inhabitants. The mortality is estimated at 163/100 000 (116-217) inhabitants, implying a case fatality ratio of 0.31 (0.17-0.46) [1].

TB treatment coverage is 52% with a reported treatment success of 90% among new and relapse cases registered in 2016 [1].

Despite continuous increase in the notification rates from 2004 to 2016 (Figure 2-3), Mozambique is notifying less than half of its cases due to gaps and weakness in the health system, underdiagnosis, underreporting and inadequate access to health services. WHO estimated 163 000 cases versus 86 515 notified by the NTP in 2017 [1]. The missed cases occur in vulnerable groups, children and PLHIV resulting into high mortality and continuous transmission the community.

HIV prevalence rate at ages 15-49 years countrywide was 13.2% (11.9-14.4), 15.4% in females and 10.1% in males in 2015. Southern provinces Maputo city, Maputo province and Gaza present 16.9%, 22.9% and 24.4% respectively [11]. The TB notification of 529/100 000 population in Maputo city is close to WHO estimation for Mozambique (551/100 000), all southern provinces have higher TB notification (Figure 2-4) that is a reflex of high HIV prevalence and better diagnostic coverage.

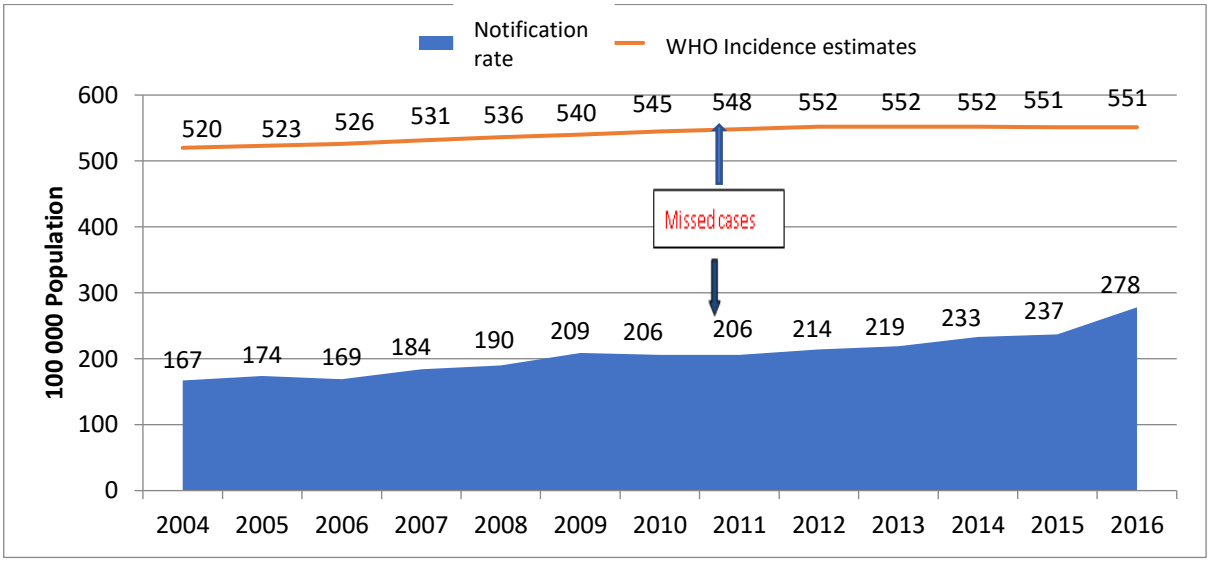


Figure 2-3 Annual notification rates per 100 000 population compared to WHO estimations 2004-2016

Source: NTP 2016 report [12] and WHO global TB Report [1]

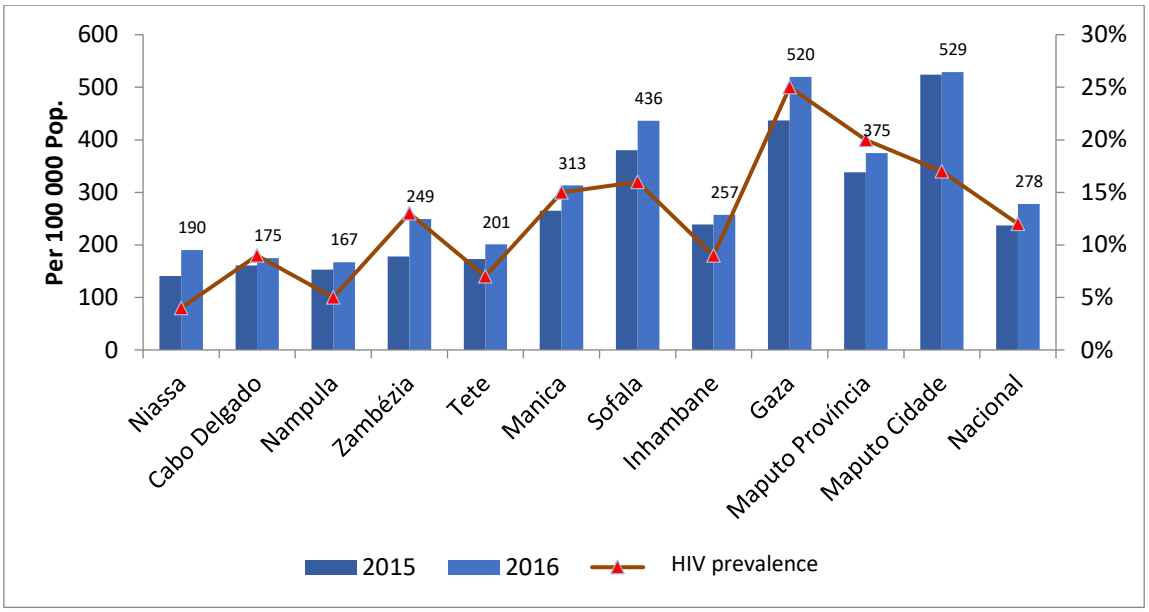


Figure 2-4 TB cases notification rates per 100 000 population per province

Source: NTP 2016 report [12]

In Mozambique, HIV infection is still an important cause of morbidity and mortality[1] with 130 000 new infections (110 000 adults aged 15 and over and 18 000 children aged 0-14[2]) and 70 000 deaths reported in 2017 [13] . According to population-based surveys conducted in Mozambique, HIV/AIDS prevalence increased from 11.5% to 13.2% between 2009 and 2015 in adults aged 15-49 years (not statically significant). Women are the most affected by HIV/AIDS, with an infection rate of 15.4% - 20.5% among women in urban areas and 12.6% among women in rural areas. In men, the infection rate is 10.1%, with 12.3% in urban areas, and 8.6% in rural areas. HIV infection is directly related to age, peaking between the ages of 35-39 years in both sexes, with prevalence of 17.5% in men and 23.4% in women [14].

The HIV epidemic in Mozambique is widespread with a higher infection rate in the southern region, followed by the central and the northern regions of the country. In 2017, an estimated 2.1 million people were living with HIV, and 170 000 of these were children aged 0-14 years [13]. The province of Gaza, which is located in the southern region, has the highest HIV infection rate at 24.4%, followed by Maputo Province, further south, at 22.9%, and Maputo City, at 16.9%. The province of Tete, in the central region, has the lowest infection rate at 5.2%, followed by Nampula Province at 5.7%, and Niassa Province, at 7.8%. The HIV infection rate is highest in urban areas, at 16.8%, compared to rural areas at 11% [14].

[1] Means the number of people infected by other diseases and the number of deaths due other disease, comparing to people tested.
[2] www.unaids.org 2017

In Mozambique, antiretroviral therapy (ART) was introduced in 2003, with massive scale-up efforts in all regions of the country starting in 2005. Estimations show that in 2017, 1 156 101 individuals in the national health system were on ART, 1 069 593 were adults and 86 508 were children between 0-14 years of age. ART coverage at the national level is 54% in adults and 51% in children [13]. In 2017, viral suppression (HIV viral load <1000 copies / ml) in the individuals on ART was estimated to be 65%—69% in women and 48% in men [14].

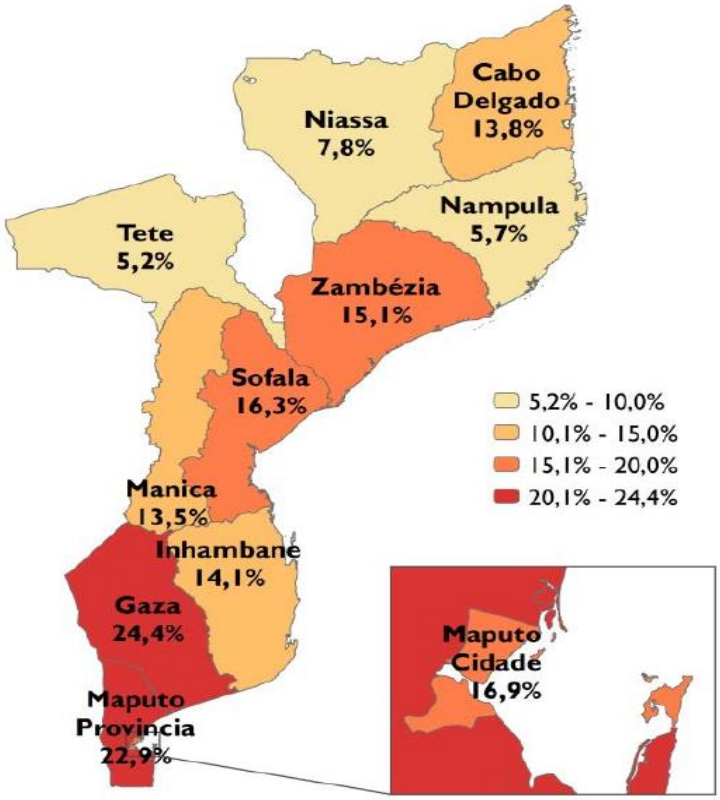


Figure 2-5 Proportion of man e woman 15-49 years old that are HIV positive

Source: IMASIDA [14]

III. TB Diagnostic and Treatment in Mozambique

All patients attending to a Health Facility (HF) should be screened for TB irrespective of the reason for the consultation. Priority groups for TB screening includes pregnant woman, children between 0-14 years old, PLHIV, chronic or immunosuppressed patients, individuals with close contact with TB patients and risk group population (health care professionals, miners, prisoners and among others). In Mozambique HF are implementing the Find cases Actively, Separate temporarily and Treat effectively (FAST) strategy and training and placing cough officers to increase the number of patients screened for TB. Community health workers are also engaged to increase the number

of contact screening at the community level by symptom screening and referral for testing and treatment initiation at the HF.

Any potential delay in TB diagnostic by the patient or the health system may increase the risk for severe disease, lung impairment and mortality in TB patients. A study report from Sofala Province (central region of the country) indicated that the median total delay for TB diagnostic (patient delay and health system delay) was 150 days (IQR 91-240) with a median of 61 days (IQR 28-113) related to the patient delay and 62 days (IQR 37-120) related to the health system delay [15]. Farming, visiting a traditional healer, low knowledge on TB and coexistence of a chronic disease were associated to increased patient delay [15].

Smear microscopy is still the most used screening test in presumptive TB cases in HF in Mozambique. HF with a GeneXpert device are recommended to perform initial diagnosis by running both Xpert MTB/RIF and the smear microscopy. Mozambique has three reference laboratories: the TB national reference lab in Maputo (Maputo city - southern region), Beira reference lab (Sofala province - Central region) and Nampula reference lab (Nampula province – northern region). For advanced TB laboratory assays such as line probe assay (LPA), culture and Drug Sensibility Testing (DST), the biological samples are referred to these three regional laboratories. There are still some challenges with sample referring system that are related to delivery of viable samples and maintaining the cold chain during the sample shipment-transportation from the HF to the reference laboratories. Despite the increased availability of diagnostic tools, the majority of cases are not bacteriologically confirmed (clinical or radiological diagnosis).

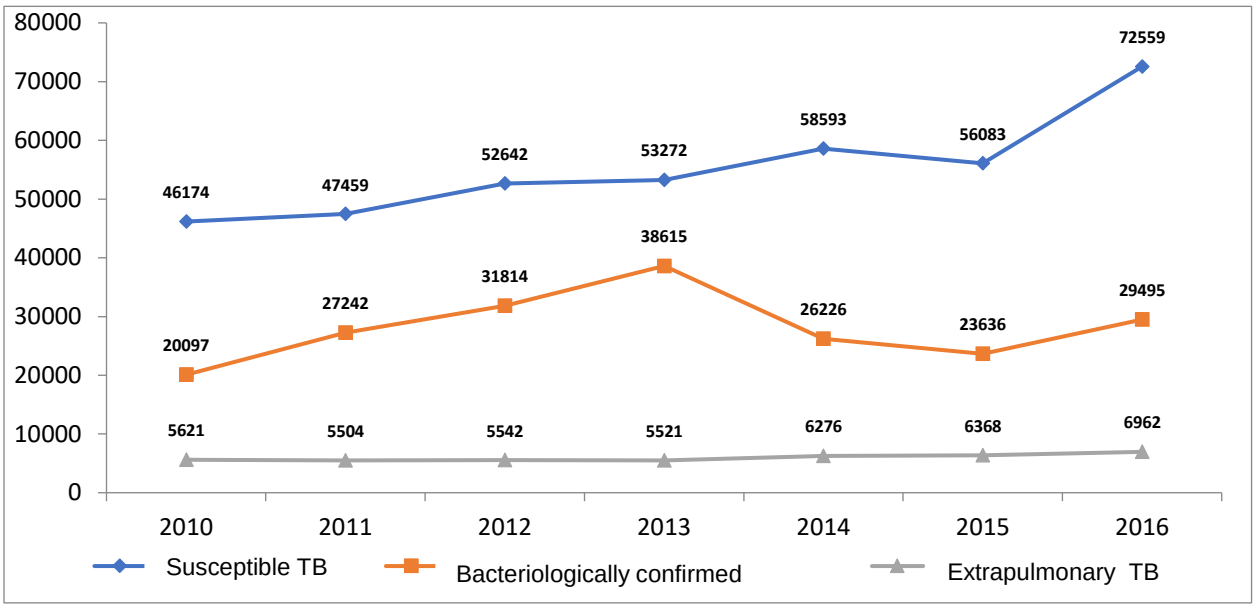


Figure 2-6 Susceptible TB case notified, Cases bacteriologically confirmed and extrapulmonary TB cases 2010-2016

Source: NTP 2016 report [12]

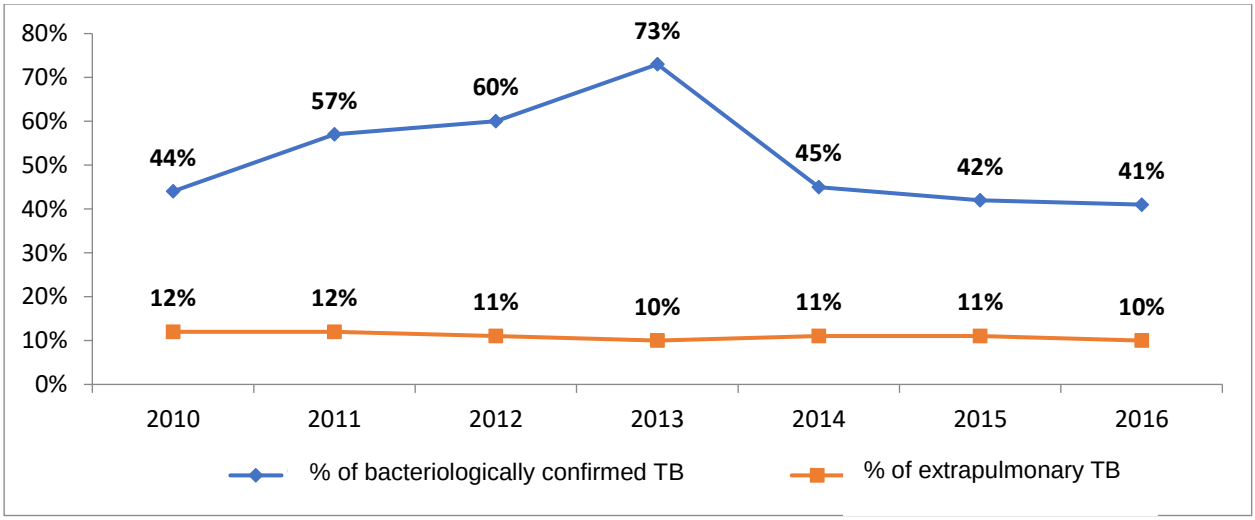


Figure 2-7 Susceptible TB case notified, Proportion of cases bacteriologically confirmed and extrapulmonary TB cases 2010-2016.

Source: NTP report 2016 [12]

The DOTS program was stablished in the early 80s in Mozambique. Currently, all public HFs have trained staff to provide TB treatment.

The susceptible TB (STB) cases are treated with rifampicin based fixed dose combination (FDC) during intensive phase Rifampicin-R, Isoniazid-H, Pyrazinamide-Z and Ethambutol-E are given for 2 month and during maintenance phase R and H are prescribed for additional 4 months.

Table 2-1 FDC drugs available for susceptible TB Treatment in Mozambique

Formulation	Drugs and dosage (mg)	Treatment period (Months)
4 FDC	RHZE (150, 75, 400, 275)	Intensive (2 months)
2 FDC adults	RH (150, 75)	Maintenance (4 months)
3 FDC paediatrics (dispersible pills)	RHZ (75, 50, 150)[3]	Intensive (2 months)
2 FDC paediatrics (dispersible pills)	RH (75, 50)	Maintenance (4 months)

The follow-up of patients with STB include clinical follow-up (weight gain, signs and symptoms improvement and adverse event monitoring) a bacteriological follow-up (smear) at months 2 and 5. The follow-up does not include monitoring of lung function and pulmonary rehabilitation during or after the TB treatment completion. TB treatment success for STB is recommend to be equal or above 85% by WHO, Mozambique had 95% in 2017 [1].

For MDR TB there are two regimens: 20 months regimen and the short-standardized regimen (STR) (9-11 months), Patients with resistance to quinolones or injectables, exposure to patients with resistance to quinolones or injectables, exposure to second line drugs more than a month, pregnancy, intolerance to a short regimen drug, extrapulmonary TB and extensive disease are non-eligible to the STR and therefore are treated with the 20 months TB regimen.

The treatment and testing are free of charge. However, there are always indirect costs incurred by the patients in seeking and staying in care for TB in Mozambique.

[3] Associated to Ethambutol 100 mg in intensive phase

Table 2-2 Standardized MDR-TB treatment regimens in Mozambique

Standardized 20 months regimen		
Treatment period (route of administration)	**Duration**	**Drugs**
Intensive (Injectables and oral)	6-8 months (Depends on culture conversion)	Capreomycin (Cm), Moxifloxacin (Mfx), Cycloserin (Cs), Protionamide (Pro), Ethambutol (E), Pyrazinamide(Z)
Maintenance (Oral)	12 months	Moxifloxacin (Mfx), Cycloserin (Cs), Protionamide (Pro), Ethambutol (E), Pyrazinamide (Z)
Standardized short regimen (9-11 months)		
Treatment period (route of administration)	**Duration**	**Drugs**
Intensive (Injectables and oral)	4-6 months (Depends on culture conversion)	Capreomycin (Cm), Moxifloxacin* (Mfx) Protionamide (Pto), Clofazimine (Cfz), Pyrazinamide (Z), Isoniazida**(Hh), Ethambutol (E)
Maintenance (Oral)	5 months	Moxifloxacin (Mfx), Clofazimine (Cfz), Pyrazinamide (Z), Ethambutol (E)

In case of resistance to other TB drugs an individualized treatment is assigned by a national therapeutic TB Committee of Ministry of Health (MoH).

Follow-up of patients with MDR-TB treatment includes bacteriology confirmation. Laboratory follow up includes LPA, cultures and DST, and safety tests (hemogram, creatinine, sodium, alanine aminotransferase-ALT, aspartate aminotransferase-AST, thyroid-stimulating hormone-TSH, glycemia, pregnancy). The clinical follow-up includes weight gain, signs and symptoms improvement, adverse event monitoring that includes electrocardiogram (ECG) and audiometry as applicable and chest x-ray. The follow-up does not include lung function or pulmonary rehabilitation during or after TB Treatment period.

In Mozambique, less than half of the TB patients who started MDR treatment in 2015 achieved treatment success (treatment success rate of 48%) [1]. Both STB and MDR-TB are only followed

under the routine TB clinics in HF until treatment completion or cure. The specialized services for the management of complicated cases and lung sequel are scarce in the country.

IV. Post TB lung disease burden

Treatment success in TB is defined as cured (smear or culture conversion) or treatment completed. These are the main objective of the WHO End TB strategy and national TB programs for TB control. After successful treatment completion there is no further follow-up of patients recommended [16]. However more than half of TB patients remain with symptoms or lung impairment after successful TB treatment completion [17-19].

Post TB lung disease (PTLD) is not a well-defined entity. Several conditions are classified here: radiological abnormalities, respiratory symptoms, pulmonary function impairment (includes airflow obstruction, restriction and the combination), post-TB aspergillosis, bronchiectasis, haemoptysis, bronchial stenosis, obstructive disease and TB sequelae [6].

In the pre-antibiotic era high rates of obstructive airways disease were observed in patient exiting the sanatorium [20]. In one study it could be shown that PTB patients usually develops maximum loss of lung function within six months of the diagnosis of tuberculosis and it stabilizes 18 months after completion of TB treatment [21].

A study in Tanzania showed abnormal lung function in 74% of PTB patients at week 20 of treatment (42% of obstructive, 19% of mixed and 13% of restrictive). The predictor for abnormal lung function were recurrent TB (adjusted odds ratio 2.8 CI: 1.247-6.0106), HIV negative status (1.7, 1.055-2.583), age more than 40 years (1.7, 1.080-2.804) and male sex (1.7, 1,123-2.614) [7].

In another study from Tanzania has shown that recurrent TB was the most important risk factor associated to a decrease in Forced Expiratory Flow (FEF) 25-75 as well as % of predicted FEV1 [22].

In South Africa a study with 33 MDR patients successfully treated for MDR TB in Limpopo, only 2 (6%) had normal pulmonary function tests (PFT), 14 (42%) had restrictive disease, 13 (39%) mixed disease and 4 (12%) had obstructive disease with a median FVC of 61% of predicted and FEV1 of 56% of predicted [23].

Black South African working in golden mines (n = 27 660) with 1 episode of TB in 2 137, 2 episodes of TB in 366, 3 episodes of TB in 93 and 4 or more episodes of TB in 17, presented

chronic airflow impairment (FEV <80% of predicted) 18.4%, 27.1% and 35.2% respectively and an average decrease of FEV1 of about 180 ml, 362 ml, 462 ml and 964 ml respectively [21].

Another study conducted in South East Asia, 200 PTB Indonesian patients had shown that at least 27% residual moderate to severe pulmonary function impairment (FEV % < 60) at month six of TB treatment and 57% of the patients yet had respiratory symptoms despite achieving successful treatment and all reported a good quality of life [17]. The travelled distance on 6MWT was 479 meters (IQR 460- 529) in controls vs 470 meters (IQR 418-515) after month 6 (p= 0.02) and Saint George Respiratory Questionnaire (SGRQ), was zero (IQR 0-2.9) in controls vs 4.9 (1.7-8.8) in TB patients at month 6 [17].

An observational case control study, conducted in India 50-65 years old patients with TB Sequelae (30 males/ 30 females) were compared to healthy control matched by gender. All healthy volunteers had a VO2max > 21 ml/kg/minute while in the TB Sequelae group only 60% of man and 36% of woman had the VO2max > 21ml/kg/minute during the 6MWT [3].

As part of the South Korean COPD prevalence survey, the prevalence of airflow obstruction (AFO) was compared between participants with and without radiologic signs of TB. In this study, 1.384 subject were included in this analysis with a median age of 51 years old. 167 (12,1%) had radiologic changes related to TB, among these 44 (26,3%) showed AFO and only 8,6% of participant without radiologic changes showed AFO. The Adjusted OR for this difference was 3.12 (95% CI 2,01, 4.67) [24].

In Texas, United States of America (USA) a case control study compared pulmonary TB patient (cases) to latent TB infected patients (comparison subjects). In this study, 59% of cases had lung impairment at week 20 (restrictive 31%, obstructive 15% and mixed 13%) while for the controls only 20% had lung impairment (restrictive 16%, obstructive 3% and mixed 1%). Severe pulmonary dysfunction was more between the cases 10% with FVC lower than 50% vs controls 0,48%. TB patients were 5 times more likely to have lung impairment (OR, 5.37; 95% CI, 2.98 to 9.68) [18].

During the same period, another cohort (n=123) showed different results. In this study, 9% had severe impairment (< 50% predicted FVC), 22% of subject had a restrictive pattern, 16% an obstructive pattern and mixed pattern was observed in 14%. This study did not show any evidence that the delay in diagnostic and treatment is associated with pulmonary impairment and suggested that impairment when present may be permanent [2].

Another study in the same setting estimated that 177 TB patients lost a total of 1189 disability adjusted life years (DALYS), with 247 DALYS lost from years of life lost (16 deaths), and 915 DALYS lost from acute (from TB diagnosis to treatment) and chronic (after TB treatment) disability. Years lived with disability constituted the majority of TB burden (75%) and 73% being years lived with chronic disability [25].

The population based (n=5 571), multicenter, cross sectional study Platino (Proyeto Latinoamericano de Investigaticion en Obstrucion Pulmonar) performed in Latin America using a post-bronchodilator spirometry enrolled 5 571 subjects with age above 40 years old. Individuals from five Latin America countries showed a prevalence of AFO of 30.7% among those with history of TB and 13.9% among those without history [9].

In Minas Gerais, Brazil pulmonary function test in 50 patients with TB Sequelae found 34% of Mixed ventilatory disorder, 12% of obstructive and 9% of restrictive [26].

A cases series of MDR TB patients in Rio de Janeiro, Brazil showed among 18 patients found obstructive pattern in 39%, restrictive pattern in 22% and mixed pattern in 17% of patients. The patient also reported a mild reduced functional capacity (evaluated by 6MWT) and quality of life (assessed by Airways Respiratory Questionnaire 20) [27].

In Lima, Perú, patients with cured DS-TB and MDR-TB patients and unexposed participants (comparison group) were evaluated to assess the lung impairment. There was minimal lung impairment among patients treated for DS-TB when compared with the control group. However, the MDR cured patient had lower lung volumes with an adjust mean differences -370 ml (95% CI – 611, -97), and with an adjusted OR 4.89 (95% CI 1.27- 18.78) [28].

In a worldwide study (19 sites from Africa, Asia, Europe and North America) performed in adults 40 years old or above, multicenter, cross sectional, general population-based Burden of Obstructive Lung Disease (BOLD), self-reported history of TB was associated with airflow obstruction adjusted odds ratio 2.51 (95% CI 1.83-3.42) and spirometry restriction 2.13 (95% CI 1.43 – 3.19). The airflow obstruction association was more preeminent in LMIC, Cape Town (n=846) the OR was 3.49 (2.19-5.57) and for restriction 1.47 (0.95-2.27) [29].

Successfully treated TB patient also present a higher mortality when compared with the general population [30, 31]. Miller *et al.* found an excess of 7.6 deaths per 1000 person years (8.8 vs 1.2;

p<.001) when comparing all-cause mortality between posttreatment TB patients vs a latent TB comparison group in USA.

There is a knowledge gap in this area, from a total of 212 only three international guidelines referred to TB sequelae but did not address diagnosis and management of the case. Since 2010 the number of studies in this area have been increase although Post TB chronic lung disease [6].

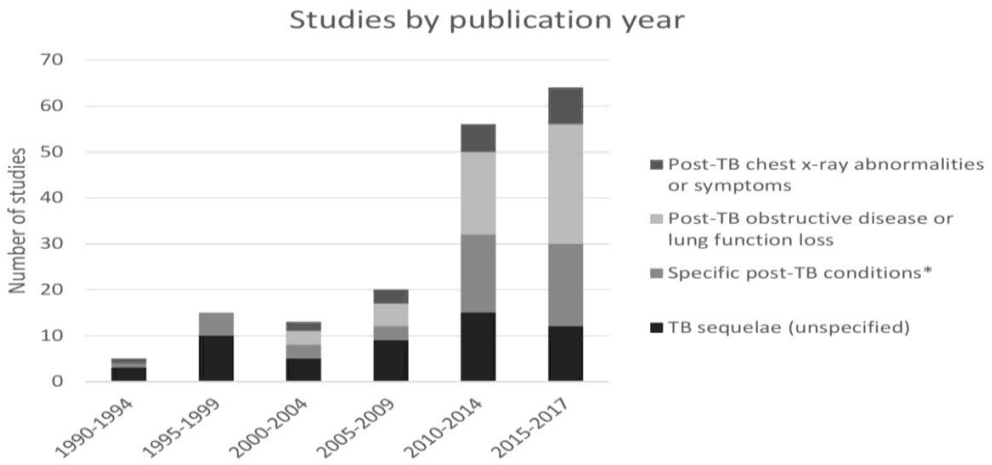


Figure 2-8 Periodical distribution of research articles on post-tuberculosis (TB) chronic lung disorders.

*Aspergillosis, bronchiectasis, haemoptysis, bronchial stenosis, pulmonary hypertension, emphysema, fibrosis, asthma.

Source: Van Kampen *et al.* [6].

Chronic obstructive pulmonary disease (COPD) is closely linked to TB in LMIC and affects 65 million people worldwide and is responsible for 3 million deaths annually (291 per 100 000) [19, 28] was the 3rd cause (Figure 2-7) of death in 2016 [1].

COPD includes several conditions causing chronic lung disease culminating in AFO. COPD is on the raise globally and remains a neglected global epidemic. COPD is actually the third most common specific cause of death with 5% of the death. Despite the high prevalence in HIC, 90% of the death occurs in the LMIC [19, 28]. The main risk factors for COPD are: Tobacco smoking, Indoor air pollution (such as biomass fuel used for cooking and heating), Outdoor air pollution and Occupational dusts and chemicals (vapors, irritants, and fumes), but many studies [9, 24, 29] have shown that TB is a risk factor for COPD in LMIC instead.

COPD is an important comorbidity in TB Patients, coming in 2nd only to diabetes mellitus[32], both diseases evolve with destruction of the Extracellular matrix (ECM) of the lung parenchyma and imbalance of the matrix metalloproteinases (MMPs).

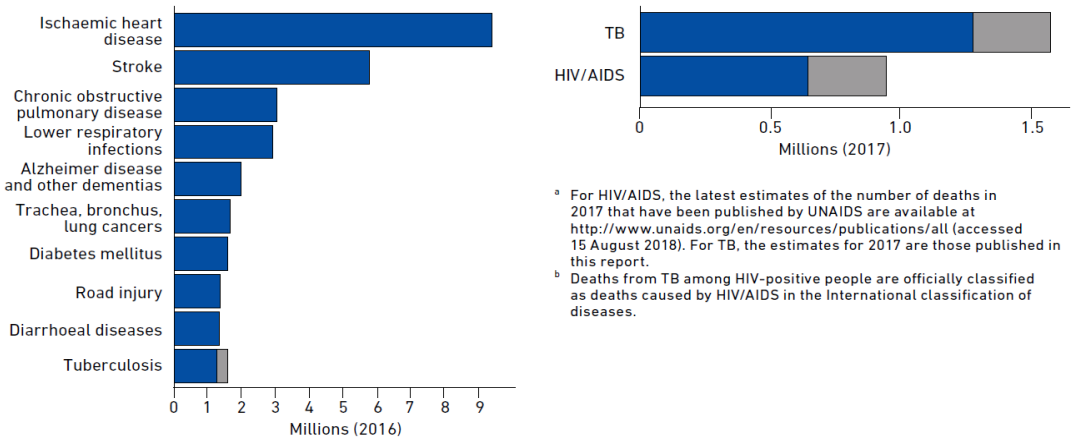


Figure 2-9 Top cause of death worldwide & estimate number of deaths for HIV and TB

Source: World TB Report [1]

PTLD diagnosis is not standardized, the assessment in different studies included lung function test and exercise capacity (Spirometry and 6MWT), quality of life (SGRQ or Airways respiratory questionnaire 20), sign and symptoms.

V. The mechanism and risk factor for Post TB Lung Disease

The healing process the lung undergoes during and after treatment, can cause excessive fibroses and stiffening of the lung parenchyma associated to the incapacity of inhaling completely leading restrictive spirometry, in contrast in case airflow obstruction there is incapacity of completely exhaling associated with air ways narrowing from excessive inflammation (figure 2-8) [33].

In the case of obstructive pattern, the main mechanisms are

- Cavitation: distorts and obliterates airways.
- Bronchiectasis and bronchiolises: Destruction of muscular and elastic components of the bronchial wall, predisposing patients to recurrent episodes of purulent sputum production, haemoptysis and pneumonia.

In contrast restriction is related to:

- Abnormal remodelation (fibroses and pleural thickening) the lung. The MMP are responsible for the lung remodelation, inflammation leads to the destruction of extracellular matrix. The MPPs have different roles during mycobacterial infection. MMP 9 contains the

infection leading the formation of a granuloma while MMP1 produced by type II pneumocyte is related to alveolar destruction and cavitation [34].

There is immense heterogeneity in the type of lesion and ventilatory defect in TB patients. This might be related to the host, pathogen and the diverse immunological event following.

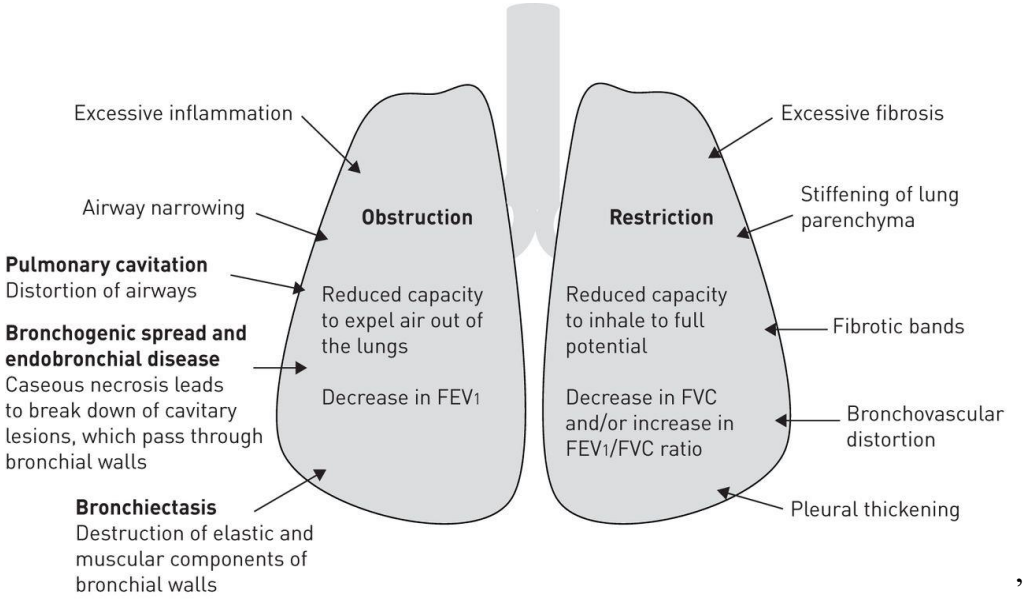


Figure 2-10 Mechanism and radiographic features associated with Airflow Obstruction and restrictive ventilatory defect in patients with a history of TB.

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity.

Source: Ravimohan *et al.* [33]

As shown above in the summary of existing literature, several predictors and risk factors for PTLD have been poorly and inconsistently described like demographics (age, sex and Body Mass Index - BMI), socioeconomic status (education, occupation and monthly income), radiological features (extension of lesion and cavitary disease), clinical history (previous episodes of TB, delay to treatment start, childhood respiratory tract infection and clinical improvement after treatment start), microbiological (smear quantitative result, Culture time to positivity), behavioural (smoking, drinking), environmental (air pollution, indoor air pollution and biomass fuel exposure) and comorbidities (diabetes and HIV infection) [35, 36].

In India, smoking, education, duration of disease prior to diagnosis and number of previous TB treatment we found as significant risk factors for pulmonary impairment [36].

In LMIC although smoking is less prevalent when compared to industrialized countries, patients are exposed to multiple lung insulting factor like occupational exposure or indoor air pollution (dust, fumes and gases), childhood lower respiratory tract illness and air pollution.

3. RATIONALE AND OBJECTIVES

I. Output 1

The active lung infection (TB disease) with *Mycobacterium tuberculosis* (MTB), progresses with parenchymal structure damage, which includes bronchovascular distortion, fibrosis, bronchiectasis and pleural thickening in many patients [33]. These anatomical changes may result in chronic pulmonary symptoms and a long-term reduction of overall lung compliance, including ventilatory defects, in up to 70% of successfully treated TB cases [7, 18, 33, 37, 38]. The common clinical signs and symptoms shared by most former patients with post TB sequelae are chronic cough with and without phlegm, wheezing, breathlessness as well as physical activity limitations [17, 39-41]. The clinical assessment usually leads to abnormalities in spirometry, associated to a significant morbidity and mortality burden in a high proportion of post-TB patients [6, 30]. Even in developed countries such as the USA, previous TB diagnosis was a risk factor for loss in longevity [31, 42]. Several studies, including some from the African continent, provided data suggesting that tuberculosis and chronic airflow obstruction (COPD) may be interrelated [19, 22, 43, 44]. Both conditions, TB and COPD, are ranked among the ten leading causes of death and disability for low- and middle-income countries [9].

Ravimohan *et al.*, described several, probably genetically defined, immunological and inflammatory pathways, which may result in the destruction of lung tissue and consecutive function impairment [33]. However, clinical, behavioural or microbiological risk factors for the development of pulmonary sequelae were, so far, not systematically assessed. Additionally, demographic, clinical (time to diagnosis and comorbidities), occupational (silica dust) and environmental (smoking and biomass smoke) factors are described as risk factors for lung impairment [2].

The cardiopulmonary morbidity of people with pulmonary TB and the relevant risk factors can be evaluated using measures such as pulmonary/physiological function test, quality of life questionnaires as well as by clinical assessment of treatment response and complications [7]. Risk factors can be assessed using questionnaires, clinical investigations as well as blood tests.

In order to assess and quantify TB related cardiopulmonary morbidity and to investigate risk factors for poor cardiopulmonary TB outcome, several physiological tests (ECG, 6MWT, spirometry) might be combined. This are easy to implement but usually not broadly available in most developing countries.

The primary study objective is:

- To describe the evolution of long-term pulmonary function impairment in Mozambican pulmonary TB patients by assessing lung function with spirometry and 6 MWT at different time points after TB diagnosis and treatment initiation.

The secondary objectives are:

- Determine the type and severity of pulmonary function abnormalities;
- Describe the association between loss of lung function with radiology images at the beginning of TB treatment;
- Describe the influence of different risk factors: demographic (sex, age), clinical and laboratorial (BMI, HIV infection, haemoglobin, C reactive protein and time to culture conversion), Behavioural (Smoking and drinking habits), environmental (indoor air pollution);
- Describe Microbiological treatment outcome in patients with and without pulmonary impairment;
- Describe the functional capacity of TB patients in the 6-MWT including the distance walked, fatigue and dyspnoea scores.

II. Output 2

The evaluation of a spirometry test results or a 6MWT is based on the comparison of the actual result of a subject to the predicted results based on normal ranges established by equations from "normal" populations similar characteristic to the patient (anthropometric where relevant ethnic). In our literature search, we did not find information about spirometry and 6MWT reference values for the Mozambican population or prediction equations directly applicable to this population. Some neighbourhood countries like South Africa and Tanzania have established prediction equations for their populations. However due to the small sample size of these studies (ideally more than 300 subjects should be recruited into such studies [45]) and due to the diversity of African populations we know there are some limitations for applying these values (e.g., from South African or Tanzania) directly to a Mozambican population.

Therefore, this study will describe the lung volumes measured by spirometry and the walking distance measured by 6MWT in Mozambican population. The values obtained from healthy volunteers will enable us to quantify the amount of lung impairment and functional capacity lost due to TB disease comparing the parameters from healthy volunteers to TB patients (see output 1) living in a similar environment and exposed to the same environmental hazards.

The primary objective of the output II is:

- To describe the normal lung function capacity (measured by spirometry) and functional capacity (measured by 6MWT) in healthy volunteers living in Mavalane area, Maputo, Mozambique.

The secondary objectives are:

- To compare the lung function values of healthy subjects with the lung outcome of the TB cohort (output 1).
- To determine the Saint George Respiratory Questionnaire (SGRQ) scores in healthy volunteers.

4. METHODS AND MATERIALS

I. Study Setting

The Mavalane health area is located in a periurban area of Maputo city, with a total population of about 891 314 inhabitants in 2017 [46]. Mavalane is greatly overcrowded (Maputo city populational density is 3670.6 inhabitant per Km^2) [46]. Poor sanitation, poor waste disposal, indoor air pollution and poor health education in Mavalane are promoting TB infection and the development of TB. About 65% of the Mozambican population lives under the poverty line with less than USD1.90 per day and with a national average of 43% of stunting in children under 5 years old [47].

Maputo city had a HIV prevalence of 16,9% in 2015 [11] which further increases the TB mortality and morbidity. HIV is the most important risk factor for the development of active TB in sub-Saharan Africa. In Maputo city 6 760 all forms of TB were notified in 2016 corresponding to 529/100 000 [12].

The TB Research Unit of Instituto Nacional de Saúde (INS) is located at the premises of the Mavalane Health Center. The National TB Program (NTP) diagnosis and treatment clinic is also collocated in the same place. The Mavalane Health Center offers outpatient and first contact personal care health services including MDR TB treatment. On average, the HF reports around 15-20 smear positive TB patients and the same amount of smear negative and extrapulmonary TB patients that are diagnosed and treated for TB in each month at Mavalane health center. A four modules Xpert MTB/ RIF device was established at the study clinic. HIV counselling and HIV rapid tests, hepatitis B rapid tests (HBsAg), urine dip stick and LAM analyses were also be performed in the TB study clinic. Blood samples were sent for analyses to the nearby study laboratory at Polana Caniço Research and Training Center (CISPOC), a research center of INS. All sputum culture related methods were performed at the National TB Reference Laboratory of INS.

TB Treatment was provided and supervised by the National TB program clinic located in the HF. Each study participant treated for TB was registered at the Mavalane Health Center.

II. Output I: Lung impairment in TB patients

a. Study design

From 14th of June 2014 to 28th of May 2015, TB presumptive or TB confirmed patients referred from peripheral health centers (PHC) to Mavalane Health Center for TB diagnosis or treatment were approached for participation in this study. All consenting patients were screened for active

TB. Additionally, TB presumptive patient identified at Mavalane HC were asked to participate in the study. TB diagnosis was established or confirmed by Xpert MTB/RIF assay.

Patient with a confirmed pulmonary TB were consecutively enrolled in study cohort and prospectively followed for 52 weeks after diagnosis. TB treatment to all study participants was administered by the Mavalane NTP treatment clinic and according to the National TB program guidelines. All patients, who tested positive for HIV were referred for comprehensive HIV care, treatment and further care according to national guidelines.

The study was embedded in a joint project of CISPOC, Maputo, Mozambique and the Division of Infectious Diseases and Tropical Medicine at Ludwig-Maximilian University (LMU), Munich, Germany. This collaboration aimed at setting up and further develop a clinical TB Research unit in Mozambique. This study also aimed to establish clinical, laboratory capacity, enrol and follow-up pulmonary TB patients following principle of Good Clinical Practices (GCP) regulated for TB treatment trials. The study was registered under ClinicalTrials.gov: NCT02156882.

In this context, the TB cohort study was used as a pilot to further build capacity of the local staff and collect basic information on the clinical, microbiological and TB outcomes characteristics of Mozambican TB patients in Maputo city. Therefore, no sample size calculation was performed prior to study start.

b. Eligibility criteria

i. Inclusion criteria:

- >/= 18 years old of age.
- Able and willing to give informed consent to study participation, including HIV-testing
- Positive TB result by Xpert MTB/RIF performed at the study clinic

ii. Exclusion criteria:

- TB treatment in the last 6 months
- Abandoned TB treatment at any time point in the past
- Suffering from a condition likely to lead to uncooperative behaviour e.g. psychiatric illness or alcoholism.
- Karnofsky score below 50% [48]

c. Clinical study procedures

i. screening visits (visit 1)

Study related procedures were performed only after the patient has provided informed consent. First, a clinical examination and vital signs were performed by the study doctor or delegated medical staff in all patients, followed by capturing of basic demographic data.

From each participant (including those who were already referred to the study clinic with a positive TB screening result) one spot sputum sample was collected either for confirmation of a previously positive TB test or for TB screening with Xpert MTB/RIF assay. The analysis of one sputum sample in all participants ensured that the baseline qualitative and quantitative readouts were established by one consistent standard. In patients with a negative TB result for Xpert MTB/RIF assay, screening was repeated using a morning sputum sample, analysed in the study clinic on next day (see Figure 4-1)

All study participants were referred to the National TB Programme for further treatment and/or evaluation. Those patients with confirmed TB diagnosis (positive Xpert MTB/RIF test) at screening (visit 1), got another study appointment for enrolment into the study and baseline (visit 2) and further follow up. In all participants with a negative TB testing result, the study ended after patient referral to the NTP at the end of screening visit(s) [visit 1].

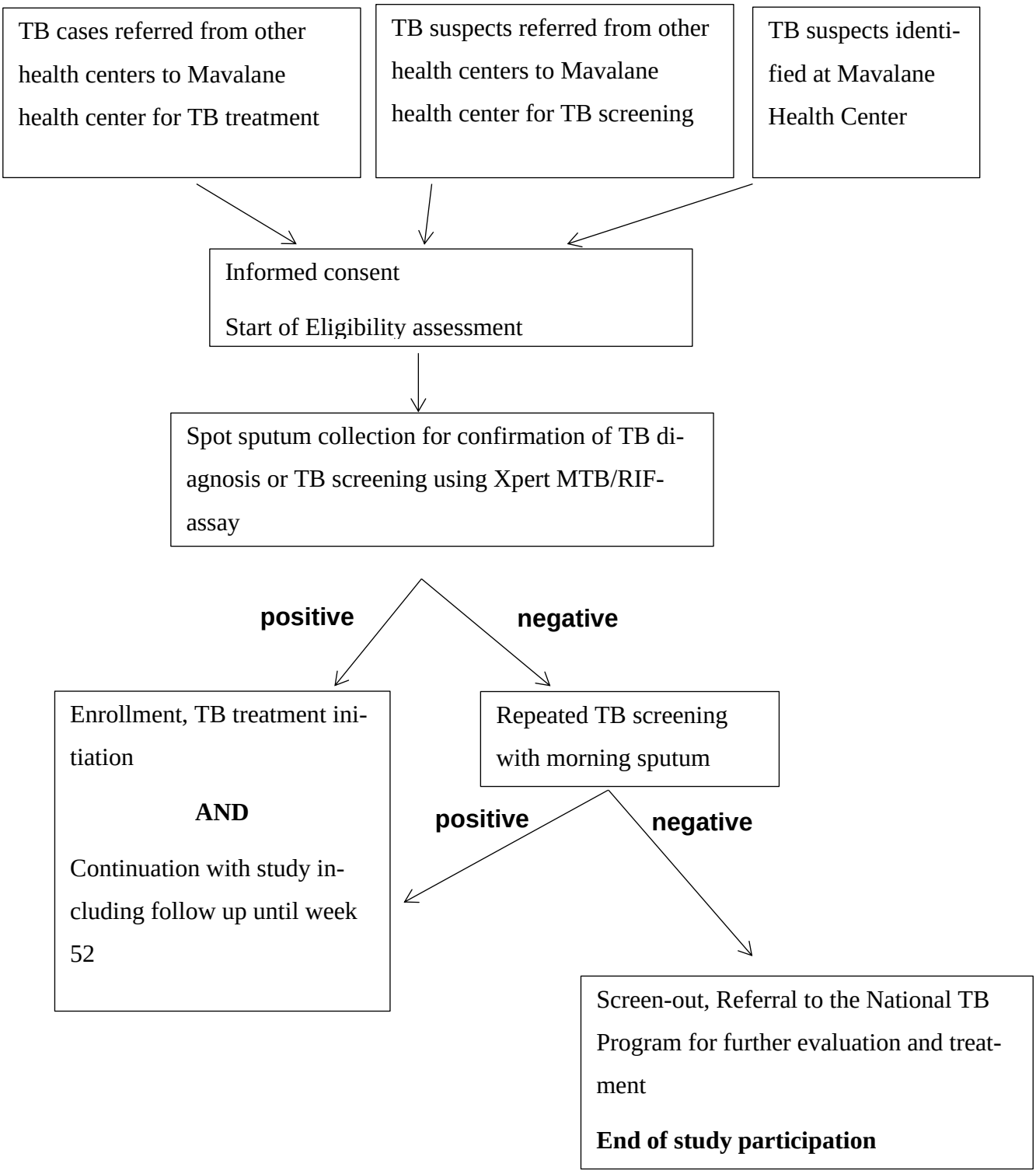


Figure 4-1 Study work flow and screening algorithm (diagram)

Source: Adapted from the MaTuTU protocol

ii. Baseline (visit 2)

Those patients with confirmed TB diagnosis (positive Xpert MTB/RIF assay) at screening (visit 1) were eligible for enrolment into the study. For the baseline visit (visit 2), all patients were asked to bring the first sputum they produced in the morning (morning sputum) and to provide a 2nd sample (spot sputum) at the study clinic.

A study questionnaire which comprises questions on patients´ socioeconomic data, clinical symptoms, co-morbidities and patients' history was completed for all study participants. Each patient had a chest x-ray if medically justified and if there was no chest x-ray of this patient available which was not older than 7 days.

In addition, one blood sample for HIV-testing including CD4 count (where applicable), full blood picture, serum biochemistry and rapid hepatitis B testing were collected. Those patients who were diagnosed with HIV were referred to the HIV-clinic for further treatment and care. All HBsAg-positive patients identified received counselling on measures to prevent or reduce the risk of hepatitis B virus- transmission to their household contacts and sexual partners. All patients were advised how to avoid potential liver damage (e.g. by alcohol). In addition, HBs-Ag-positive patients were referred to a specialized service of gastroenterology at Maputo Central Hospital for further care and treatment based on the standard of care available at the site. HIV/HBV co-infected individuals were referred for co-infection treatment with TDF based ART which is part of WHO recommendations. Finally, TB treatment will be started on the day of the baseline visit (visit 2).

iii. Follow up visits (Visit 3 to 9)

Follow up visits were performed according to the schedule of events. On each follow up visit, a medical questionnaire recorded for all patients as well as a physical examination by the study doctor or a medical officer. Further, clinical treatment data including side effects and TB symptoms were collected. All participants were asked to bring an early morning sputum sample to each follow up visits as well as to produce a spot sputum sample the study site. In case, a patient should not bring a morning sputum sample to the clinic, a second spot sample was collected at the clinic.

At visits at week 8, 26, a comprehensive pulmonary function assessment was performed in all study patients to establish the physiological capacity of the lung of TB patients at those time points. This assessment includes ECG, spirometry and 6-minutes walking test (6MWT).

iv. visit week 52 (visit 10)

At this study visit, a medical questionnaire with focus on TB symptoms, TB treatment, relapse and treatment failure and a short socioeconomic questionnaire was applied to the patients. If a study participant was found to suffer from respiratory symptoms a sputum sample was collected to exclude TB relapse. Symptomatic patients were be referred to the health system for evaluation and follow up. Additionally, at this visit the last cardiopulmonary assessment was performed including the reversibility test in patient with an obstructive abnormality on spirometry.

d. Laboratory study procedures performed in the study clinic/at the Mavalane health center

For screening procedures, a fresh sputum sample about 0.7ml was used for Xpert MTB/RIF testing performed by study staff at the TB research clinic. The rest of the sputum sample was kept at 8°C and sent to the TB Reference Laboratory later. HIV rapid testing, was performed in the TB Research Unit following the national testing algorithm and after counselling of the study participants.

e. Laboratory procedures performed at National TB Reference Laboratory

All TB testing related procedures were performed in the National TB Reference Laboratory of the INS under the supervision of one of the study PIs and according to GLP. For each procedure an SOP will be in place and the respective staff received an appropriate training on procedures.

i. Sputum smear microscopy in study laboratory

Decontaminated and concentrated sputum samples was used for smear microscopy. After Ziehl-Neelsen staining, the slides were examined with the 100x objective using oil-immersion. Representative areas of the slides are read until either 300 fields are read or mycobacteria were found. The smear were scored using the WHO/IUATLD [49].

ii. Culture on solid Lowenstein Jensen medium

Decontaminated and concentrated sputum samples were used for culturing of mycobacteria. LJ media were examined weekly for a total of 56 days. The week of first culture growth was reported. Colonies suspicious for mycobacteria are examined by Ziehl-Neelsen staining.

iii. MGIT liquid culture

Decontaminated and concentrated sputum samples were used for TB culture. Positive MGIT-culture was confirmed by Ziehl-Neelsen staining. For *M.tb* positive cultures the duration of incubation until positive result was recorded.

iv. Speciation of mycobacteria by GenoType® Mycobacterium MTB/CM

Identification of the mycobacteria species were performed from positive solid and/or liquid cultures. The GenoType® Mycobacterium CM is a DNA-STRIP® technology that gives the possibility to diagnose patients with a positive culture result (from solid or liquid culture) for mycobacterial growth. If the patient was therefore suspected to suffer from a tuberculosis or mycobacteriosis the identification of the particular species helped to decide on an appropriate treatment.

The GenoType® Mycobacterium CM permits the simultaneous molecular genetic identification of the M. tuberculosis complex and 24 of the most common NTM species.

v. Drug resistance testing using BACTEC® 960 / MGIT liquid culture

Tuberculosis phenotypic resistance testing was performed for first line drugs (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin) using Bactec liquid culture system. All procedures were performed following published standards and/or the instructions of the manufacturer.

f. Safety laboratory procedures

i. HIV-Testing

All patients were screened for HIV according to the National Guidelines in Mozambique. In addition, CD4 cell count was performed in all HIV positive patients. Those patients who were diagnosed with HIV were referred to the HIV-clinic for further treatment and care. For CD4 count, analyses the Alere Pima™ was used.

ii. Blood safety tests

Safety blood tests (Lactate dehydrogenase-LDH, Alkaline phosphatase-ALP, albumin, bilirubin, AST, ALT, creatinine) were performed in the research laboratory based at CISPOC using commercial assays and analysers.

Table 4-1 Study procedures

Study Phase	**Screening**	**Base-line**	**Follow up after start of TB treatment**							
Study Visit	Screening Visit(s) [Visit 1]	Baseline visit [Visit 2]	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
Study Time Point	Day -3 to -1	Day 0 (day of TB treatment initiation)	Week 1 (+/- 2 days	Week 2 (+/- 2 days)	Week 4 (+/- 2 days)	Week 8 (+/- 2 days)	Week 12 (+/- 2 days)	Week 17 (+/- 2 days)	Week 26 (+/- 2 days)	Week 52 (+/- 2 days)
Informed Consent	X									
Spot Sputum	X	X	X	X	X	X	X	X	X	
Morning Sputum	(X)	X	X	X	X	X	X	X	X	
Medical Question-naire		X		X	X	X	X	X	X	X
Clinical Examination	X		X	X	X	X	X	X	X	
HIV- test (CD_4)*		X								
Haematol-ogy Chemis-try C Reactive Protein Hep-B-test*		X				X			X	
Chest x-ray		X							X	
Spirometry, ECG and 6-minutes walking test						X			X	X
Socio eco-nomical question-naire		X							X	X
St-George Respiratory Question-naire		X				X			X	X
TB Treat-ment adher-ence		X	X	X	X	X	X	X	X	

** Performed only at baseline visit*

g. Lung function assessment

i. Spirometry and reversibility test

Spirometry is a physiological test that measures how an individual inhales or exhales volumes of air as a function of the time [50]. When lung health is evaluated this test should be viewed as blood pressure is viewed during cardiovascular health is assessment. There are three basic measures in spirometry: volume, time and flow.

In this test different lung volumes and capacities can be evaluated (see Table 4-2 and Figure 4-2).

From standard normative values, we can conclude about the nature and extend of pulmonary ventilation disorders. Sick subject values are compared to standard reference values from healthy population or to predict values from equations based on race, gender, age and height.

Table 4-2 Spirometric measures

Parameter	Explanation
Forced vital capacity (FVC)	The maximum amount of air that can be exhaled when blowing out as fast as possible
Forced expiratory volume in 1 s (FEV1)	The amount of air that can be exhaled when blowing out as fast as possible in the first second
Vital capacity (VC),	The maximum amount of air that can be exhaled when blowing out as fast as possible
FEV1/FVC ratio	the ratio of FEV1 to FVC
Peak expiratory flow (PEF)	The maximal flow that can be exhaled when blowing out at a steady rate
Forced expiratory flow, also known as mid-expiratory flow	the rates at 25%, 50% and 75% FVC are given
Inspiratory vital capacity (IVC)	the maximum amount of air that can be inhaled after a full expiration

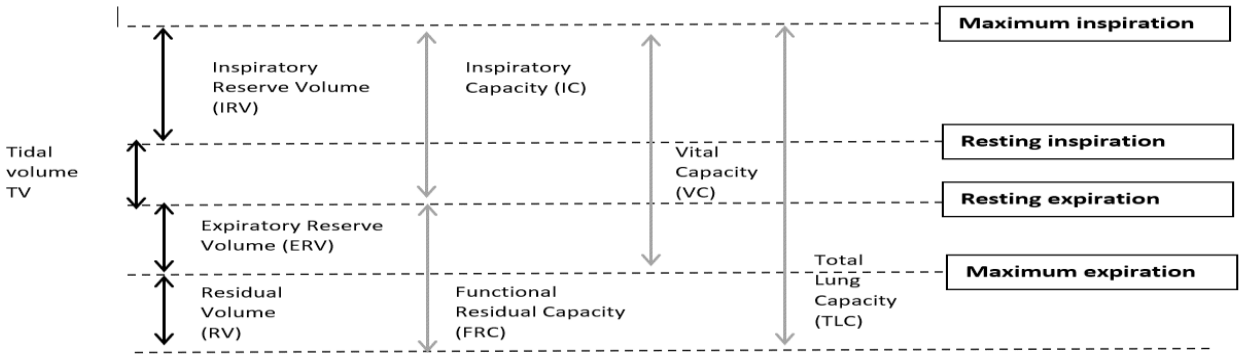


Figure 4-2 Standard lung volumes and capacities from a spirometer trace

The solid black and grey arrows indicate lung volumes and capacities respectively

The Reversibility test consists in the use of a bronchodilator to evaluate if the obstruction is fixed or reversible. In COPD the obstruction is not reversible while in Asthma it is.
Usually, for description of lung function abnormalities FEV1 and FVC are used [51]. Also, in this study, data analysis is mainly based on the values for FEV1, FVC and the FEV1/FVC ratio of a specific subject.

ii. Device and test procedures

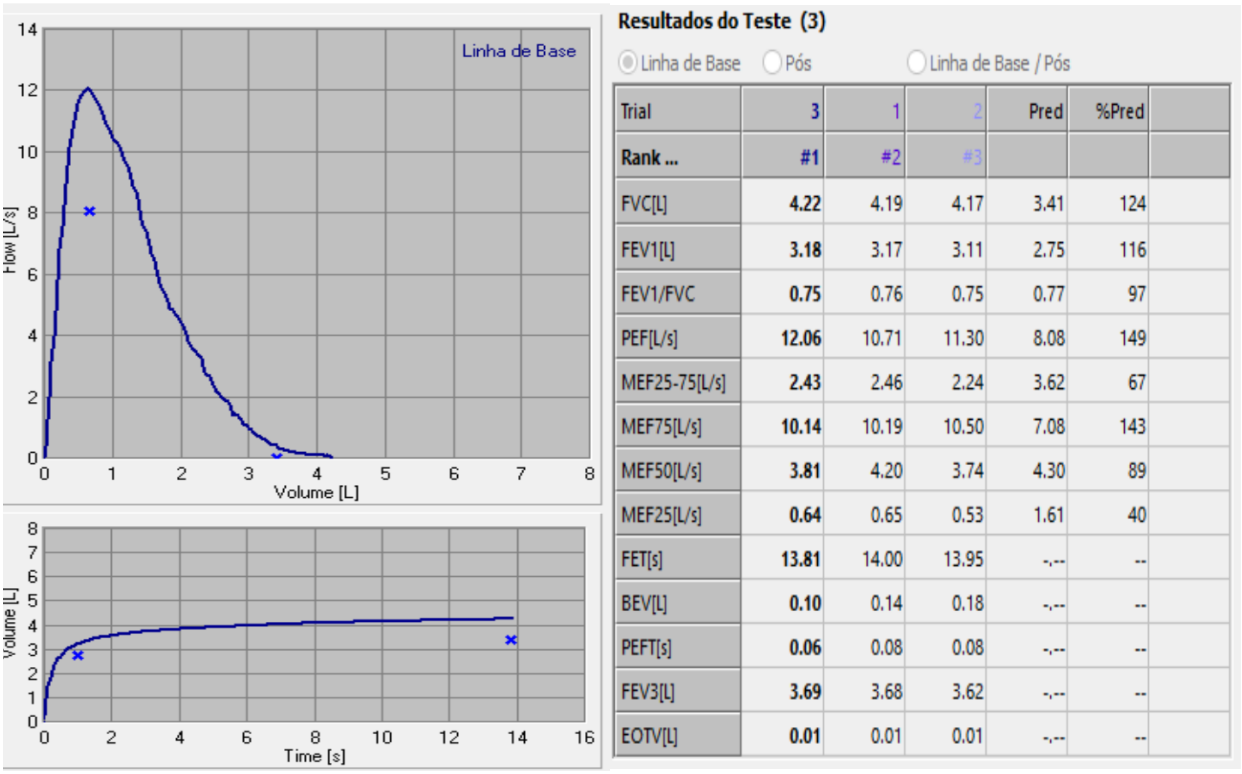
Weight and height were measured using a mobile scale and stadiometer. Spirometry was be performed using a handheld spirometer (EasyOne®ndd Medical Technologies, Inc), with individual-use filtered one-way single-use mouthpieces (Spirrettes® and Medical Technologies, Inc)
(Please see Spirometry and reversibility SOP in appendix 9). This device do not require regular calibration checks as it uses ultrasonic reading technology with high accuracy is used [52].

Figure 4-3 Easyone® cradle, spirometer and spirette.

Source: ndd Medizintechnik AG Technoparkstrasse 1 CH-8005 Zurich

The FVC expiratory manoeuvre used in our patients was performed in three phases : 1)Maximal inspiration, 2) a “blast” of exhalation and 3) complete exhalation to the end of the test [50]. All individuals performed a minimum of 3 and maximum of 8 curves. Test were performed with patient seated, wearing a nose clip with a disposable mouth piece.
Readings for FVC and FEV1 were assessed for repeatability and reliability by the investigating physician or nurse and compared with the assessment performed by two clinical researchers - the site Principal Investigator (PI) and PI located at LMU. Study PIs received a spirometry training provided by a consultant pulmonologist from LMU, who further trained the clinical staff at the study site. To ensure data completeness and quality, regular monitoring of study procedures, including spirometry. All data collected were performed by study PIs and clinical monitors from

LMU. ATS/ERS guidelines were followed for the measurement of ventilatory parameters and for quality control [23]



Trial	3	1	2	Pred	%Pred	
Rank ...	#1	#2	#3			
FVC[L]	4.22	4.19	4.17	3.41	124	
FEV1[L]	3.18	3.17	3.11	2.75	116	
FEV1/FVC	0.75	0.76	0.75	0.77	97	
PEF[L/s]	12.06	10.71	11.30	8.08	149	
MEF25-75[L/s]	2.43	2.46	2.24	3.62	67	
MEF75[L/s]	10.14	10.19	10.50	7.08	143	
MEF50[L/s]	3.81	4.20	3.74	4.30	89	
MEF25[L/s]	0.64	0.65	0.53	1.61	40	
FET[s]	13.81	14.00	13.95	-.--	--	
BEV[L]	0.10	0.14	0.18	-.--	--	
PEFT[s]	0.06	0.08	0.08	-.--	--	
FEV3[L]	3.69	3.68	3.62	-.--	--	
EOTV[L]	0.01	0.01	0.01	-.--	--	

Figure 4-4 Output from a spirometry test from EasyOne®ndd: A - flow volume curve, B – Time volume curve and Spirometry parameters

iii. Interpretation and classification of spirometry results

Both European Respiratory Society (ERS) and American Thoracic Society (ATS) recommends using the Lower limit of Normal (LLN) for analysis and interpretation of FEV1 and FVC although the percent of predicted was a previous most used methodology.

Fixed ratios, were associated to false negative in younger populations and false positives in the elderly for example FEV1/FVC decreases with increase of age and height. This means more than 20% of individuals are misclassified (false positives) [53].

Table 4-3 Quality for spirometry curves Quality for spirometry curves

Source: Walter *et al.* [54]

Grade	Quality criteria for FVC and FEV
A	>3 acceptable tests with repeatability within 0.150 L for age 2–6, 0.100 L, or 10% of highest value, whichever is greater
B	>2 acceptable tests with repeatability within 0.150 L for age 2–6, 0.100 L, or 10% of highest value, whichever is greater
C	>2 acceptable tests with repeatability within 0.200 L for age 2–6, 0.150 L, or 10% of highest value, whichever is greater
D	>2 acceptable tests with repeatability within 0.250 L for age 2–6, 0.200 L, or 10% of highest value, whichever is greater
E	One acceptable test
F	No acceptable tests

Interpretation of spirometry results require a comparison with reference normal values, which consider height, sex, age and sex/ethnicity to predict individual predicted lung function. Selected reference values need to be appropriate for the population being studied. The Global Lung Initiative (GLI) established global equations based on 74 000 individuals from 26 countries ages between 3-95 years. Those reference values are applicable to Whites (applicable to Hispanics and Europeans), African-Americans, North East Asians and South East Asians. Africans are not included in data used. Therefore, the recommendation is to use a composite equation for African population (also for other individuals not included in 4 main group or mixed).

The selection of reference equations follows certain criteria: matching age- range, anthropometric, race/ethnic, socioeconomic and environmental characteristics between the subject being evaluated and the reference population from which the equations derived [55]. In this study spirometry, prediction equations for South-African populations [56] were applied as reference standard to calculate predicted values for FVC, FEV1 and residual z-scores. There are no South African equations for FEV1/FVC ratio, therefore the European Coal and Steel community equation was used with a correction factor of 10%. The type of ventilatory impairment was defined as follows:

- **Normal (No lung impairment):** FEV1/FVC > LLN and FVC > LLN
- **Restriction:** FEV1/FVC > LLN and FVC < LLN
- **Obstruction:** FEV1/FVC < LLN and FVC > LLN
- **Mixed:** FEV1/FVC < LLN and FVC < LLN

Table 4-4 Prediction equations for South African Black

Source: Adapted from Guideline for office spirometry in adults 2012 [56]

Sex	Parameter	Prediction equation	RSD	LLN
Male	FVC (l)	4.8H-0.02A-3.08	0.89	Predicted value – (1.64 x RSD)
	FEV1(l)	2.9H-0.027-0.54	0.75	
Female	FVC (l)	4.5H-0.023A-3.04	0.67	
	FEV1(l)	3.4H-0.028A-1.87	0.64	

RSD = Residual Standard Deviation, LLN = Lower Limit of Normality (Lower 5th percentile), H = Height in meters, A = Age in years

Table 4-5 European Community for Steel and Coal prediction equations (valid for ages 18 - 70 years)

Source: Adapted from Guideline for office spirometry in adults 2012 [56]

Sex	Parameter	Prediction equation	RSD	LLN
Male	FVC1/VC	-0.1A + 87.21	11.8	Predicted value – (1.64 x RSD)
Female	FVC1/VC	-0.19A + 89.10	10.7	

RSD = Residual Standard Deviation, LLN = Lower Limit of Normality (Lower 5th percentile), H = Height in meters, A = Age in years

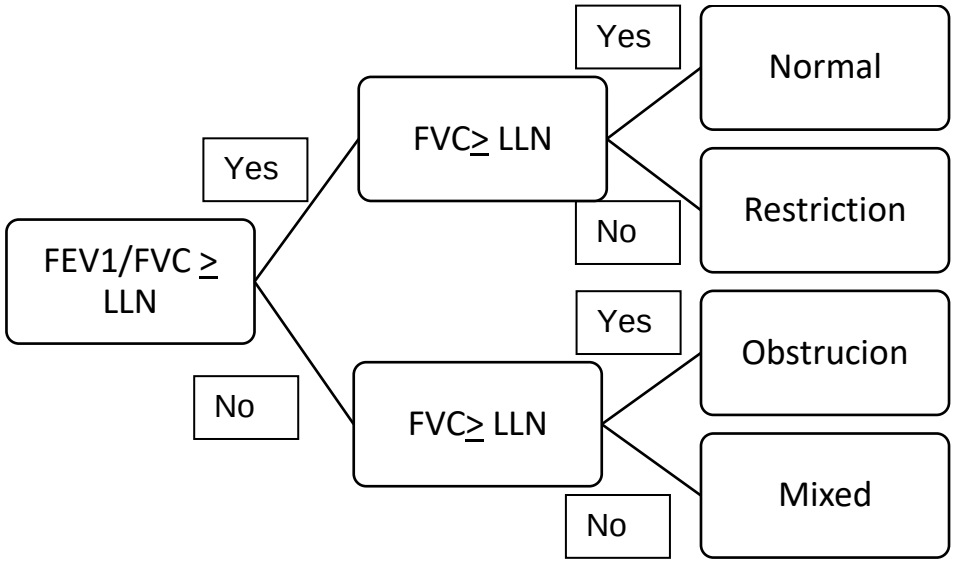


Figure 4-5 Algorithm for characterization of spirometry ventilatory defect based on the Lower Limit of Normality (LLN)

The abnormal ventilatory function values were graded for severity (Table 4-6 Table 4-7) according to FVC for pulmonary restriction, FEV1 for pulmonary obstruction and FVC for mixed impairment as FVC was the most affected parameter. Therefore, mild restriction or obstruction is defined as FVC or FEV1 greater or equal to 85% of LLN, moderate restriction or obstruction is defined as

FVC or FEV1 between 85% and 55% of the LLN and severe restriction or obstruction is defined as FVC or FEV1 below 55% of LLN accordingly [57].

Table 4-6 Severity grading of spirometry results – if restrictive or mixed – based on % of LLN [57]

FVC	**Restriction**
≥ 85% LLN	Mild
< 85% LLN and ≥ 55% LLN	Moderate
< 55% LL	Severe

Table 4-7 Severity grading of spirometry results – if obstructive – based on % of LLN [57]

FEV	**Restriction**
≥ 85% LLN	Mild
< 85% LLN and ≥ 55% LLN	Moderate
< 55% LL	Severe

h. Functional capacity assessment

i. 6 Minute Walk Test

The 6-minute walking test (6MWT) is a self-paced test of the walking capacity. This a valid and reliable measure of exercise capacity in people with lung disease. The main outcomes of this test are: 6-minute walking distance (6MWD), peripheral capillary oxygen saturation (SPo2), heart and respiratory rate, dyspnoea and fatigue.

It is a simple and inexpensive functional test. Functional walk tests either alone or in combination with other exercise tests have been validated in people with chronic cardiac and pulmonary disease, chronic obstructive pulmonary diseases and asthma, older adults with chronic heart failure, and in healthy, elderly adults [3]. Lower 6MWD is strongly associated with increased risk of Mortality and hospitalization in patient with chronic respiratory patients [58].

The 6MWT is an example of a functional walk test that is practical and simple and only requires the ability to walk. The distance that a patient can walk on a flat surface in 6 minutes may be used as a generic one-time measure of functional status or as an outcome measure from a rehabilitation program [3].

Few studies have conducted this functional test in TB patients and have shown there was a significant difference in physical functional capacity between a group of normal older people and those

with TB sequelae [3]. After 6 months of treatment, the majority of participants had impaired functional or Quality of Life scores compared to the average healthy control [17]. TB patients were request to walk as far as possible along a 30 meters straight track in a balcony, the distance was recorded in meters. Standardized instructions and encouragement were given during the test. Patient reported fatigue was reported was measured at the beginning and at the end of the test suing the Borg scale (Please see 6MWT SOP in appendix 10). This test was applied according to ATS/ERS guidelines [59].

Predicted walking distance in 6MWT are determined by reference equations. Those equations are affected by many factors like: sex, age, height, weight, race/ethnicity percentage of peak heart rate, and methodology used [58]. There is a lot of variation in the predicted 6MWD produced by different equations, local population generated and verified equations should be applied when possible. We were unable to identify equations generated for the Mozambican population.

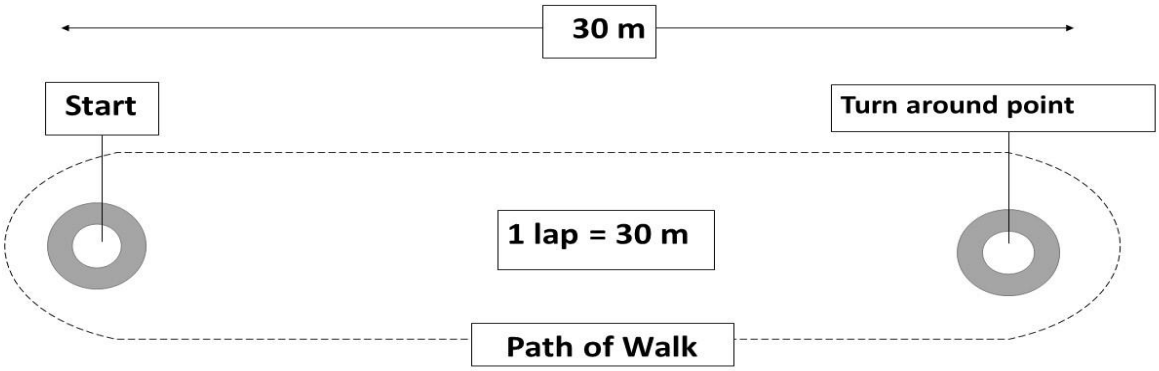


Figure 4-6 6-Minutes Walk Test track used - 30 meters

ii. The Saint George Respiratory Questionnaire (SGRQ)

The Saint George Respiratory Questionnaire (SGRQ) is a disease-specific instrument designed to measure impact on overall health, daily life, and perceived well-being in patients with obstructive airways disease. The questionnaire is composed by 50 items divided into 2 parts (3 components) Part I: Symptoms component (frequency and severity) and Part II: Activities that cause or are limited by breathlessness; impact components (social functioning, psychological disturbances resulting from respiratory disease) [60].

The questionnaire scores range from 0 to 100, with higher scores indicating more limitations. In the study we used the Portuguese version of the questionnaire.

iii. Ethical considerations

The study protocol was approved by Comité Nacional de Bioética para Saúde (CNBS), in Mozambique (letter reference 274/CNBS/13) and the Ethics Commission of the Medical Faculty at LMU, in Germany before initiation of study. Each participant received a written information about the study. The consent was also be explained in the presence of an impartial literate witness, if the patient was illiterate. The informed consent sheet was provided in Portuguese language. The person providing study information should ensure that the potential study participant understood the study procedures and also why the research is performed.

The researchers of this study were committed to the recommendations guiding physicians in biomedical research involving human subjects issued by the World Medical Association Declaration of Helsinki, as last updated in 2013 in Fortaleza, Brazil [61].

All patient information was treated in a strictly confidential manner and anonymized by unique study Identification (ID) number. All files were stored at a secure, locked place to which only authorized study staff will had access.

Patients and their community directly benefited from 'being found' with TB – a disease with significant attendant morbidity and mortality. The risks related to study participation were considered low. The sampling procedures for sputum investigation and HIV-testing were part of standard care. Additional blood draws comprised a sample volume which did not pose any harm to the patients.

Study patients were not be paid for their participation in the study. However, a financial compensation for time and travel costs of 50,00 meticais (approximately 1,00 Euro) was provided to each participant in each study visit.

iv. Data collection and management

Data collection procedures in the study clinic and laboratories were described in SOPs or in Manual of Procedures.

The data management aspects of the study were defined in a separate Clinical Data Management Plan. Paper Case Report Forms (CRFs), as well as a web-based data management system (Open Clinica R) for on-site double data entry and data cleaning, was developed and supervised by the LMU data manager. All study documentation was stored on site for at least 3 years after publication.

Study activities related to data generation, recording and management processes were continuously monitored through the Quality control (QC)/ Quality Assurance (QA) office at Cispoc. LMU staff

performed monitoring activities during their visits to the site, which checked both the procedures, CRFs and data points in the clinical data management system.

v. Data analysis

Data was analysed using Stata 15.0 Stata Corp, Texas, USA. The primary outcome was lung function as measured by spirometry at 52 weeks after initiation of TB treatment.

Descriptive statistics was used to describe study population and characteristics. Persons' Chi square test and Fisher exact test were used to compare proportions were adequate. The Wilcoxon Rank Sum, was used to compare medians. FVC and FEV1 Z-scores for TB patients, healthy volunteers group and for the standard South African equations were used to construct kernel density plots.

For the risk factor analyses three categories were considered: no impairment (standard category), mild impairment and moderate/severe impairment. For these categories a multinomial regression was performed to evaluate the association the association with different factor described in the literature. Like in a logistic regression we obtained Risk Ratios (RR) from the model for mild impairment and moderate/severe impairment categories, describing in this manner the influence of different risk factors [62].

III. Output II: Healthy volunteers lung function

a. Study design and population

A cross sectional study design was conducted in the INS TB Research Unit of INS in Maputo city, Mozambique between April 20th, and December 6th, 2017. In order to ensure that the healthy controls came from the same neighbourhood and environment like our TB cohort (output 1) we enrolled two healthy volunteers that came from the same household/neighbourhood per each study patient enrolled in the main MaTuTu study. Prior, we calculated that about 150-180 subjects should be enrolled to ensure that data can be used to generate standard normal values.

Two healthy household or neighbourhood volunteers (one male and one female) were invited to take part in this study. These control subjects were screened for TB disease and respiratory symptoms. The participants benefited from lung function assessment and those found with abnormal function were referred to the nearest health center for care and treatment, therefore in our study healthy volunteers refers to the absence or acute or respiratory disease.

b. Eligibility criteria

i. Inclusion criteria:

- >/= 18 years old
- Able and willing to give informed consent for study participation

ii. Exclusion criteria:

- Positive TB symptoms screening or acute/chronic respiratory disease
- Suffering from a condition likely to lead to uncooperative behaviour e.g. psychiatric illness or alcoholism
- Any contra-indication to perform spirometry or 6MWT

c. Study Activities

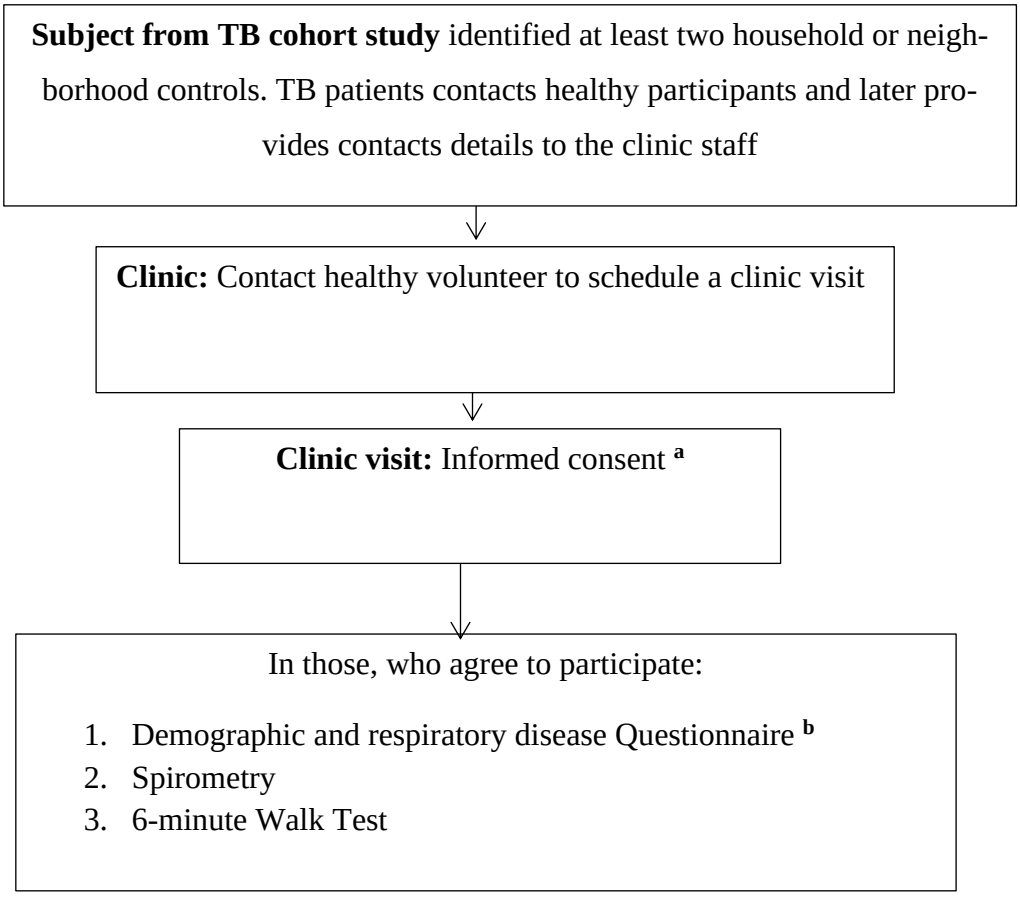


Figure 4-7 Study work flow and screening algorithm (diagram)

[a] If participants did not consent to study procedures was not included;

[b] Participants with contraindication for spirometry did not proceed perform the procedure;

- **Socio-demographic questionnaire:** individual data like (date of birth, gender, ethnicity, race), respiratory symptoms and disease history to exclude any previous acute or chronic lung disease (TB and chest symptoms, asthma and TB history).
- **Lung function test:** Weight and height was measured using a mobile scale and stadiometer. Spirometer was performed using a handheld spirometer (EasyOne®ndd Medical Technologies, Inc), with individual-use filtered one-way single-use mouthpieces (Spirrettes® and Medical Technologies, Inc) (Please see Spirometry and reversibility SOP in appendix 9)

- **6MWT:** Self-paced walking capacity was evaluated in a 30 meters course, heart rate, respiratory rate, blood pressure, capillary oxygen saturation was measured before and after the walk, the 6 minutes walked distance was the main outcome.
  (Detailed description of the procedures in page 30 and 35)

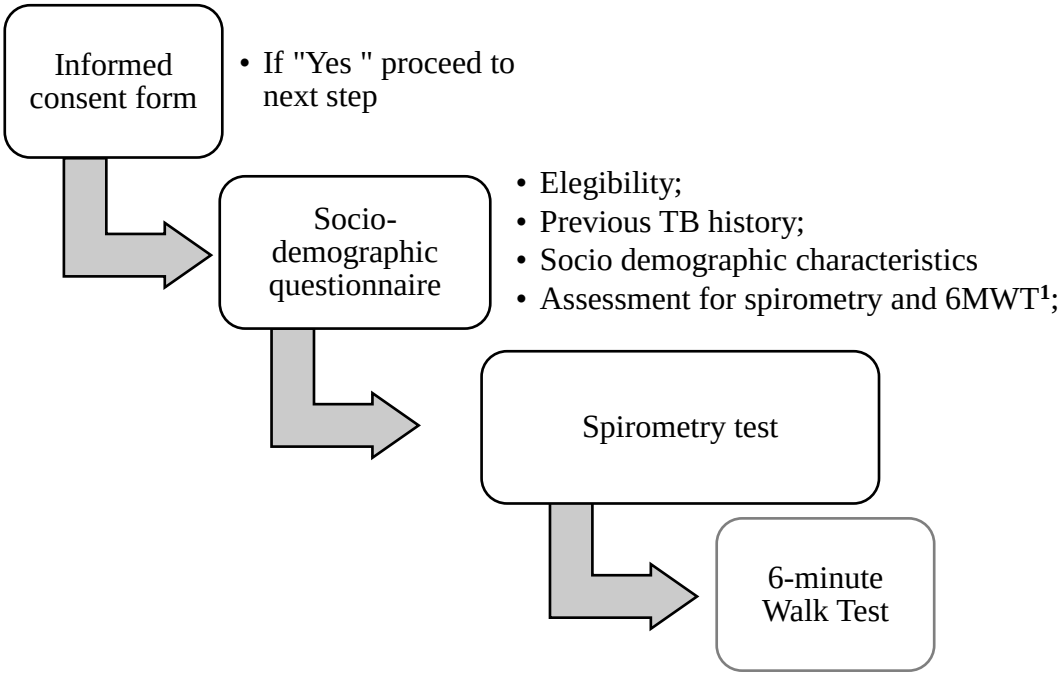


Figure 4-8 Study procedures

[1] do not proceed to step 4 if any contraindication

d. The establishment of reference equations in a population

Many LMIC did not establish spirometry reference equations for their population. The equations are generally established from healthy populations with similar anthropometric and race/ethnicity. These countries rely on foreign equations with adjusting factors, therefore uncertainty derives from this strategy resulting in misdiagnosis [63].

Knudsen *et al.* [63] applied Mokoetle's[64] and louw's[65] for black South African man equations to Tanzanian population resulting in overestimation of FEV1. This can lead to false positives. These studies used workers cohorts, the healthy worker effect is one of the explanations for the finding higher lung volumes, other factor explanation can be associated to altitude and socioeconomic status.

e. Ethical considerations

The study protocol was approved by National Ethics (CNBS) in Mozambique (letter of reference 449/CNBS/2016) and the Ethics Commission of the Medical Faculty at LMU, in Germany before initiation of study.

Each participant received written information about the study. In addition, study staff provided an oral study description in the presence of an impartial literate witness if the patient was illiterate. The informed consent sheet was provided in Portuguese language.

Study visit was performed after being scheduled via phone with potential study subjects. The study was committed to follow the recommendations guiding physicians in biomedical research involving human subjects issued by the World Medical Association Declaration of Helsinki 2013 [61].

All subjects' information was treated in a strictly confidential manner and anonymized by unique study ID number. All files were stored at a secure and locked place to which only authorized study staff had access.

The risks related to study participation was considered low. Spirometry is a non-invasive procedure, although requiring some effort by the patient there are minimal risk related to it and study team is training on how to react if any of those happens (see Appendix 8 – MaTuTU Standard Operating Procedure - Spirometry and Reversibility Test). Study patients were not paid for their participation in the study. However, a financial compensation for time and travel costs of 100,00 meticais (approximately 1,10 Euro) was provided to each participant.

f. Data collection, management and analysis

Data was primary collected in the study clinic using paper CRFs. Double data entry into Epi Info™ 7.0 version 3.0 CDC, Atlanta, GA, USA, then QC and QA was performed and queries were generated and managed by CISPOC data manager. All study documentation was stored on site for at least 3 years after publication.

The data was analysed using Stata 15.0 Stata Corp, Texas, USA. Descriptive statistics was used to describe study participants characteristics. FVC, FEV and the ratio was described in absolute values, percentage of predicted and Z-scores. For the comparison of proportions fisher exact tests or person's chi square were used where appropriate, Wilcoxon Rank Sum was used to compare medians.

5. RESULTS

I. Output I

a. Description of study population

81 presumptive TB patients were screened for study participation, 12 were not enrolled for not meeting all legibility criteria. From the 69 enrolled participants four died before week 8, 65 participants performed week 8 visit, five were not able to produce valid spirometry test.

From the 65 participant that attended week 26, 3 were not able to produce valid spirometry test. One participant was lost to follow-up after week 26, from the 64 participants attending to the week 52 visit 2 were no able to produce valid spirometry tests. Sixty-two study participants had a valid spirometry result at week 52 visit and consequently were included in the final analysis (Figure 5-1).

The Majority of study participant were males 67.7% (42/62). Participants median age was 29.5 years (IQR 25-40), with 74.2 % (46/62) of participants ages below 40 years old. 62.3% (39/62) were coinfected with HIV, 46.2% (18/39) had severe immunodepression with a CD4 count below 200 cells/µl. In Four participants this was their second episode of TB, four participants had Rifampicin resistance on the Xpert MTB/RIF.

At the baseline visit the median haemoglobin in males was 11.7 g/dl (IQR 10.2-12.6) and 9.9 g/dl (IQR 9.45-11) in females, 83.9% of the participants had anaemia. C reactive Protein was increased (Median 76.8 mg/dl IQR 44-106.95) and only normal in one participant.

The median BMI was 19.23 Kg/m^2, 93.6% (58/62) of participant had culture conversion at week 26, the overall affected lung median was 15% (IQR (15-20).

Only male participants had history of smoking, 37% (23/62) of study participants had ever smoked, and about half of the study population (53.3%, 32/69) reported to regular consumption of alcohol critical amounts (Table 5-1).

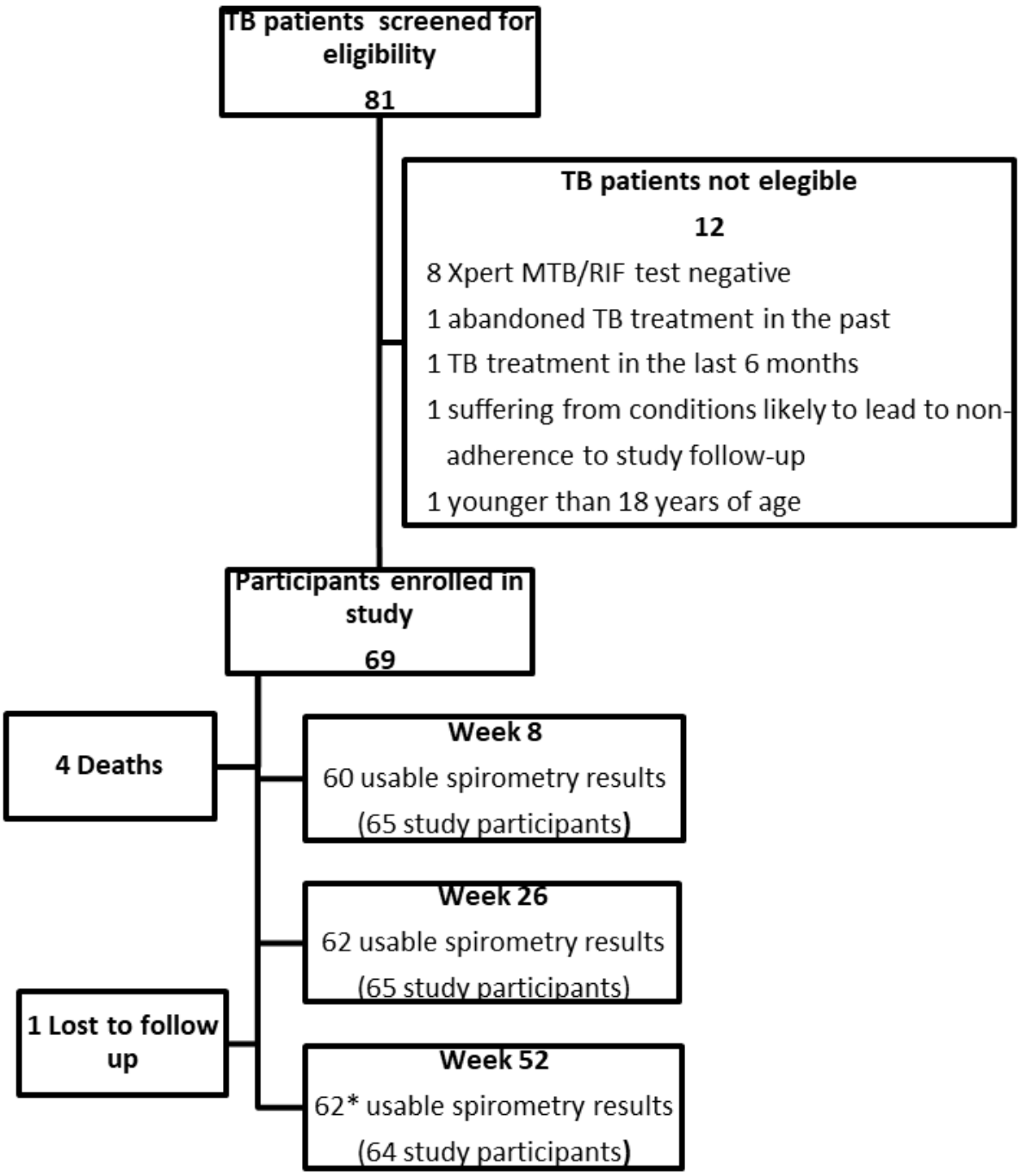


Figure 5-11 Study flow diagram

* Participants included in the final analyses.

Participants no able to produce acceptable curves week 8 = 5, week 26 = 2 and week 52 = 2'

Source: Adapted from figure 1. Khosa *et al*, "Development of chronic lung impairment in Mozambican TB patients and associated risks"

Table 5-1 Baseline characteristics of TB cohort participants, included in final analysis

Characteristic	TB patients in final analysis (n=62)
Sex	
Male, % (n/N)	67.74 (42/62)
Age	
Median (IQR)	29.5 (25, 40)
< 40 years old, % (n/N)	74.19 (46/62)
HIV-status	
Positive, % (n/N)	62.90 (39/62)
CD4-cells < 200/µl, % (n/N)	46.15 (18/39)
CD4-cells 200-499/µl, % (n/N)	38.46 (15/39)
CD4-cells ≥ 500/µl, % (n/N)	15.38 (6/39)
History of TB	
Yes, % (n/N)	6.45 (4/62)
RIF resistance (Xpert MTB/RIF)	
Present, % (n/N)	6.45 (4/62)
Haemoglobin	
Median male, g/dl (IQR)	11.70 (10.2, 12.6)
Median female, g/dl (IQR)	9.90 (9.45, 11.00)
Anaemia**	
No, % (n/N)	16.13 (10/62)
Mild, % (n/N)	37.10 (23/62)
Moderate, % (n/N)	41.94 (26/62)
Severe, % (n/N)	4.84 (3/62)
C-reactive protein (CRP)*	
Median, mg/dl (IQR)	76.78 (44.0, 106.95)
<50 mg/dl, % (n/N)	31.03 (18/58)
51-100 mg/dl, % (n/N)	39.66 (23/58)
>100 mg/dl, % (n/N)	29.31 (17/58)
BMI*	
Median (IQR)	19.23 (17.63, 20.51)
<18.5, % (n/N)	40.98 (25/61)
Culture conversion	
Until week 8	56.45 (35/62)
Until week 26	93.55 (58/62)
Overall affected lung**	
Median % (IQR)	15 (15, 20)
Smoking	
Ever smoked, % (n/N)	37.10 (23/62)
Pack Years*/****	
None	50.00 (20/40)
<10	32.50 (13/40)
≥10	17.50 (7/40)
Alcohol consumption*	
Ever alcohol, % (n/N)	77.42 (48/62)
Amount of alcohol, median, g/week (IQR)	80.0 (40, 240)
Critical alcohol consumption**, % (n/N)	53.33 (32/60)

*Available observations for CRP for 58 participants (normal range for CRP: 0.0 mg/dl-10 mg/dl, only one participant had a CRP value below 10mg/dl at baseline), available observations for BMI for 61 participants, available information for Pack Years for 60 participants, available information for amount of alcohol consumption for 60 participants.

**definitions:

- Anaemia, definition according to WHO, non-anaemia: 12mg/dl or higher (women) or 13 mg/dl or higher (men), mild: 11.0-11.9 g/dl (women) and 11.0-12.9 g/dl (men), moderate: 8.0g/dl-10.9g/dl (both sexes), severe: <8.0g/dl (both sexes) [66].
- X-ray scoring system for affected lung, according to Ralph *et al*. [22].
- Pack Years: numbers of years a person smoked 1 pack (20 cigarettes) per day, ≥10 pack years means that the respective study participant smoked an equivalent of one pack of cigarettes every day for 10 years (e.g. 1 pack of cigarettes for 10 years, or 2 packs of cigarettes for 5 years, or ½ packs of cigarettes for 20 years) at baseline study visit.
- Critical alcohol consumption: in men (women) more than 60g (30g) alcohol per occasion of alcohol drinking and/or more than 150g (80g) alcohol per week, according to the International Alliance for Responsible Drinking for general population [67].

Source: Adapted from table 1, Khosa *et al* "Development of chronic lung impairment in Mozambican TB patients and associated risks"

b. Spirometry results

The spirometry results (FVC, FEV1 and FEV1/FVC-ratio) on the different study visits are summarized separately for those with and without lung impairment in Table 5-2. The proportion of LI is 73.33% at week 8, declining to 67.74% at week 26 and 61.29% at week 52. The FVC and FEV1 means (in litres) and percentages of predicted also show increasing trends during the follow up. There is no statistical difference between mean FVC at week 8 versus week 26 (P = 0.36) and week 26 versus week 52 (p = 0.23), but there is a significant statistical difference between week 8 versus week 52 (p = 0.046). There is no statistical difference for the FEV1 mean between all study visits: week 8 and week 26, p = 0.46, week 26 and week 52, p = 0.34, and week 8 and week 52, p=0.12.

On week 52 the mean FVC is 2.65 l (66.7% of predicted) in participants with LI versus 3.68 l (90% of predicted) in participants without LI. On the same week the FEV1 mean is 2.10 l (65.8% of predicted) in participants with LI versus 2.97 l (90.2% of predicted) in participants without lung impairment. The mean FEV1/FVC was approximately 80% for all groups in all study visits.

We calculated the z-scores for FVC and FEV1 for all participants and study visits. As per definition, a z-score of -1.64 and below was considered as abnormal. In Figure 5-2, the z-scores for FVC and FEV1 at different study visits (week 8, 26 and 52) are shown for the 44 participants (a and b), who had abnormal FVC and FEV1 at week 8 and for the 16 participants with normal FVC and FEV1 at week 8. Both lung volumes (FVC, FEV1) were improving in the majority of participants

with abnormalities at week 8, but only few improve to normalization until week 52. Almost all women (red lines) with abnormal volumes at week 8 do not reach the normalization at week 52.

Three participants with normal FVC at week 8 evolve with abnormal FVC until week 52, and four participants with normal FEV1 at week 8 develop abnormal FEV1 until week 52 (Figure 5-2 b and c).

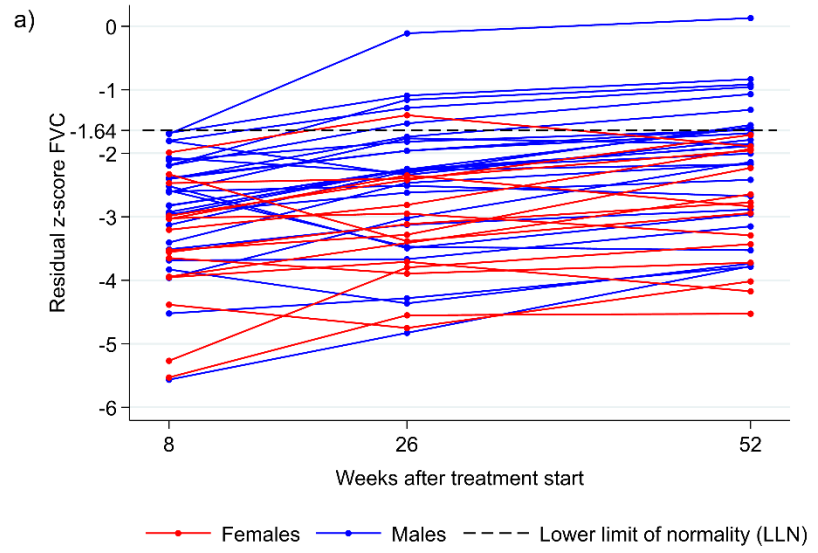
Table 5-2 Spirometry results of study participants at week 8, 26 and 52 after TB treatment initiation, included in final analysis (N=62)

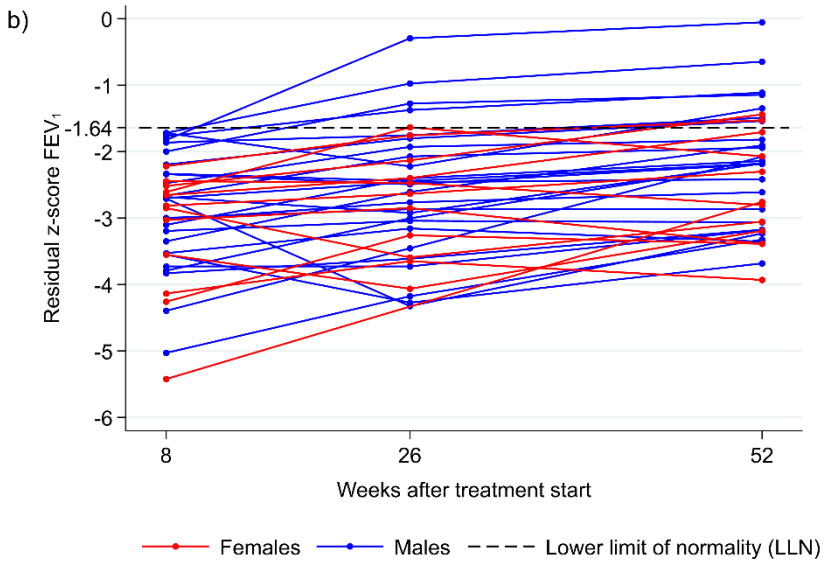
	Week 8*			Week 26			Week 52		
	All (N=60)	**LI (N=44)**	**No LI (N=16)**	**All (N=62)**	**LI (N=42)**	**No LI (N=20)**	**All (N=62)**	**LI (N=38)**	**No LI (N=24)**
% of N	100.00	73.33	26.67	100.00	67.67	32.26	100.00	61.33	38.67
FVC in l (SD)	2.77 (0.78)	2.48 (0.61)	3.55 (0.66)	2.90 (0.78)	2.56 (0.60)	3.62 (0.62)	3.05 (0.78)	2.65 (0.59)	3.68 (0.60)
FVC in % of pred. (SD)	68.37 (15.85)	61.75 (11.50)	87.80 (9.63)	71.70 (15.63)	63.84 (11.09)	89.10 (8.40)	75.51 (15.08)	66.72 (10.78)	90.04 (8.43)
FVC z-score (SD)*	-2.52 (1.30)	-3.07 (0.98)	-1.04 (0.80)	-2.25 (1.24)	-2.90 (0.89)	-0.93 (0.70)	-1.99 (1.21)	-2.71 (0.85)	-0.85 (0.70)
FEV1 in l (SD)	2.25 (0.70)	2.01 (0.57)	2.88 (0.64)	2.32 (0.69)	2.01 (0.52)	2.97 (0.55)	2.44 (0.70)	2.10 (0.53)	2.97 (0.60)
FEV1 in % of pred. (SD)	68.91 (17.28)	62.28 (13.58)	88.36 (11.28)	71.25 (17.80)	62.40 (13.08)	90.81 (8.97)	74.98 (16.72)	65.78 (12.80)	90.18 (9.95)
FEV1 z-score (SD)*	-2.26 (1.28)	-2.76 (1.04)	-0.92 (0.87)	-2.10 (1.27)	-2.74 (0.91)	-0.78 (0.78)	-1.85 (1.21)	-2.52 (0.90)	-0.79 (0.81)
Ratio (FEV1/FVC)	81.09 (9.67)	81.17 (10.49)	80.86 (7.22)	79.39 (8.65)	78.22 (9.60)	81.86 (5.66)	79.44 (7.95)	78.91 (9.27)	80.27 (5.29)
FEV1/FVC z-score (SD)*	1.07 (1.35)	1.07 (1.50)	1.09 (0.88)	0.81 (1.18)	0.63 (1.33)	1.20 (0.70)	0.82 (1.09)	0.72 (1.29)	1.00 (0.66)

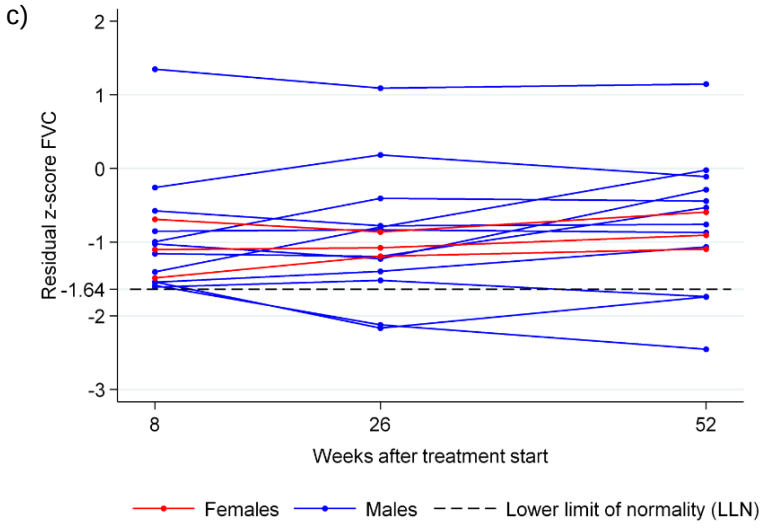
SD= standard deviation, pred= predicted, LI= lung impairment, %= percentage, n= number of subjects in (sub-) group. There is a statistical evidence for a difference between the mean FVC at week 8 and week 52, p= 0.046, and no statistical difference between the mean FVC at week 8 and week 26, p=0.36, and the mean FVC at week 26 and

week 52, p=0.23. There is no statistical difference for mean FEV1 between different study visits: week 8 and week 26, p = 0.46, week 26 and week 52, p = 0.34, and week 8 and week 52, p=0.12.

Source: Adapted from table 2, Khosa *et al.* "Development of chronic lung impairment in Mozambican TB patients and associated risks"


a)
Residual z-score FVC
0
-1
-1.64
-2
-3
-4
-5
-6
8
26
52
Weeks after treatment start
Females
Males
Lower limit of normality (LLN)


b)
Residual z-score FEV1
0
-1
-1.64
-2
-3
-4
-5
-6
8
26
52
Weeks after treatment start
Females
Males
Lower limit of normality (LLN)


c)
Residual z-score FVC
2
1
0
-1
-1.64
-2
-3
8
26
52
Weeks after treatment start
Females
Males
Lower limit of normality (LLN)

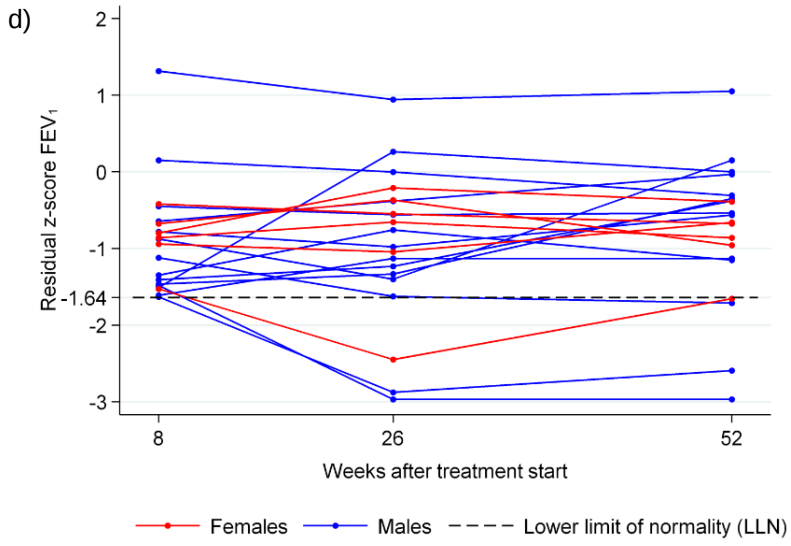


Figure 5-2 Trends of Z-scores over time for FVC (a) and FEV1 (b) of those participants with lung impairment at week 8 (n=48) and for FVC (c) and FEV1 (d) of those participants without lung impairment at week 8 (n=16).

Change in residual z-scores for FVC (a) and FEV1 (b) over time in study participants with lung impairment (residual z-score <-1.64) at week 8 and study participants without LI (residual z-score > 1.64) at week 8. Residual z-scores (adjusted for sex, height and age) were calculated based on South African reference standard (and prediction equations) [56].

Source: Adapted from figure 2 and supplementary figure 2. Khosa *et al.* "Development of chronic lung impairment in Mozambican TB patients and associated risks"

c. Type and severity of lung impairment

Except one participant, who had obstructive ventilation impairment at week 52. All other study participants suffered from pulmonary restriction and no participants with mixed ventilatory impairment during the study (Figure 5-3). The overall proportion of study participants with any lung impairment declined from week 8 to week 52, together with the proportion of participants with at least moderate or severe pulmonary restriction which decreased from 46% at week 8 to 26% at week 52.

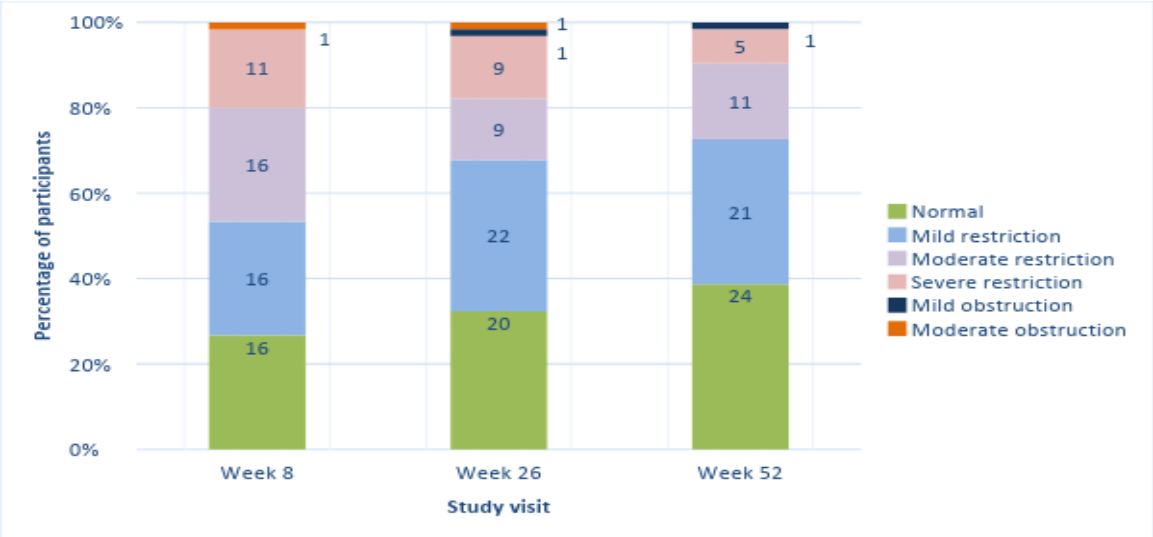


Figure 5-3 Proportions of types and severity grades of lung impairment at different study visits

Week 8: N=60, 16 (26.67%) normal, 16 (26.67%) mild restriction, 16 (26.67%) moderate restriction, 11 (18.33%) severe restriction and 1 (1.67%) moderate obstruction. Week 26: N=62, 20 (32.36%) normal, 22 (35.48%) mild restriction, 9 (14.52%) moderate restriction, 9 (14.52%) severe restriction, 1 (1.61) mild obstruction and 1 (1.61%) moderate obstruction. Week 52: N=62, 24 (38.71%) normal, 21 (33.87%) mild restriction, 11 (17.74%) moderate restriction, 5 (8.06%) severe restriction, and 1 (1.61) mild obstruction.
Source: Adapted from figure 3, Khosa *et al.* "Development of chronic lung impairment in Mozambican TB patients and associated risks"

d. Risk factors for lung impairment

Several risk factors were analysed for associations with LI at week 52. Female sex had a risk of developing moderate or severe LI five times (RRR = 5, p = 0.03) more than their male counterparts. HIV coinfection was not associated with LI, but between HIV coinfected patients a CD4 count above 200 cells/ µl were more likely to develop mild LI (RRR: 7.33, p = 0.02).

The increase of one g/dl of haemoglobin reduced the relative risk for LI at week 52 by 31% (RRR = 0.69, p = 0.03) for mild lung impairment and by 37% (RRR = 0.61 p = 0.01) for moderate-severe LI. Surprisingly "ever smoking" was associated with reduced risk of 72% (RRR = 0.28, p = 0.02) for mild LI and 63% (RRR = 0.37, p = 0.04). Although not statistically significant smoking more than 10 pack years increased 10 times de risk for mild LI (RRR = 10, p = 0.11) and 7.5 times for moderate/ severe impairment (RRR = 7.5, p = 0.09).

Age above 40 years old, C- reactive protein above 100 mg/dl were protective factor but not statistically significant, from the other side low BMI, a delayed culture conversion and critical alcohol consumption increased the risk for abnormal lung function at week 52 and not statistically significant.

Table 5-3 Risk factor for lung impairment

Baseline*	Comparator*	Severity grade of lung impairment	Risk Ratio	95% CI of RR	p-value
Male 44 (63.77%)	Female 25 (36.23%)	Mild	2.86	0.72, 11.37	0.14
		moderate/severe	5.00	1.17, 21.39	0.03
Age < 40 years 53 (76.81%)	Age ≥ 40 years 16 (23.19%)	Mild	0.71	0.19, 2.70	0.62
		moderate/severe	0.81	0.19, 3.40	0.77
BMI ≥ 18.5 38 (55.07%)	BMI < 18.5 30 (43.48%)	Mild	1.56	0.47, 5.19	0.47
		moderate/severe	1.46	0.39, 5.40	0.57
HIV negative 23 (33.33%)	HIV positive 46 (66.67%)	Mild	1.05	0.32, 3.48	0.94
		moderate/severe	1.00	0.27, 3.69	1.00
CD4 < 200/µl 20 (28.99%)	CD4 ≥ 200/µl 26 (37.68%)	Mild	7.33	1.38, 38.88	0.02
		moderate/severe	2.00	0.39, 10.31	0.41
Culture conversion until week 8 35 (50.72%)	No culture conversion until week 8 30 (43.48%)	Mild	1.39	0.43, 4.51	0.58
		moderate/severe	1.67	0.46, 6.01	0.43
Culture conversion until week 26 61 (88.40%)	No culture conversion until week 26 4 (5.80%)	Mild	3.63	0.35, 37.86	0.28
		moderate/severe**	-	-	-
Never smoked (Males only) (No women smoking) 20 (45.45%)	Ever smoked (Males only) (No women smoking) 24 (54.55%)	Mild	0.22	0.05, 0.95	0.04
		moderate/severe	0.90	0.16, 4.92	0.90
Never smoked (Males) (adjusted for PY)	Ever smoked (Males) (adjusted for PY)	Mild	0.28	0.09, 0.84	0.02
		moderate/severe	0.37	0.15, 0.95	0.04
< 10 Pack Years*** 13 (54.17%)	≥ 10 Pack Years*** 9 (37.50%)	Mild	10.00	0.58, 171.2	0.11
		moderate/severe	7.50	0.72, 78.36	0.09
No critical alcohol consumption**** 37 (53.62%)	Critical alcohol consumption**** 15 (21.74%)	Mild	1.00	0.22, 4.50	1.00
		moderate/severe	1.67	0.34, 8.10	0.53
C-reactive protein (mg/dl) <100 46 (66.67%)	C-reactive protein (mg/dl) ≥100 19 (27.53%)	Mild	0.71	0.18, 2.76	0.63
		moderate/severe	0.97	0.24, 3.90	0.97

Haemoglobin, 1 g/dl increase at baseline	Mild	0.69	0.49, 0.96	0.03
	moderate/severe	0.63	0.43, 0.91	0.01
Overall affected lung, each 1% increase	Mild	0.98	0.92, 1.05	0.58
	moderate/severe	1.02	0.95, 1.09	0.65

Risk factor analysis using a multinomial regression model, comparing subjects with no impairment at week 52 (n= 24) with those who have mild impairment (n=22) or moderate/severe impairment (n=16) at week 52. The absolute numbers and proportions of subjects in each individual stratum (baseline, comparator, mild lung impairment and moderate/severe lung impairment) can be found in appendix 7.

*available observations for CRP in 58 participants, available observations for BMI in 61 participants, available information for Pack Years in 60 participants, available information for amount of alcohol consumption for 60 participants.

**no subject with moderate/severe lung impairment with no culture conversion at week 26

***Pack Years (PY): for definition of Pack Years, refer to legend to table 1

****critical alcohol consumption: for definition of critical alcohol consumption, refer to legend to table 1

Source: Adapted from table 3, Khosa *et al*. "Development of chronic lung impairment in Mozambican TB patients and associated risks"

e. Functional capacity and quality of life

The 6MWT parameters (Peripheral capillary oxygen saturation – Spo2, fatigue and dyspnoea) after the walk show very small variation during the study visits. Spo2 was above 98%, reported fatigue below 2 and reported dyspnoea below 1 in all study visits in 75% of the walks performed. During the walk test in all the visit no patients interrupted the test or adverse event were registered, the tolerance for this test was very good from week 8 and improved along the study.

Table 5-4 6-Minute Walk Test parameters after walk at week 8, 26 and 52 after TB treatment initiation, included in final analysis (N=62)

Parameters after walk	**Week 8**	**Week 26**	**Week 52**
Median - Spo2 (IQR)	98 (97, 99)	98 (98, 99)	99 (98, 99)
Median fatigue (IQR)*	2 (1,3)	1 (1,2)	1 (0,1)
Median Dyspnoea (IQR)*	1 (1,2)	1 (0.5,1)	0 (0,1)
Median 6-Minute walk distance in meters (IQR)	420 (385.25, 456.25)	429 (396, 466.25)	442 (399.75, 485.50)

Spo2 – Median capillary oxygen saturation, IQR = Interquartile Range, *fatigue and dyspnoea were self-reported according to the Modified Borg Dyspnoea and Fatigue Scale (Scale 0-10) by patients after the walk (See Appendix 10 – MaTuTU Standard Operating Procedure - 6 Minute Walk Test)

On week 8 the median distance travelled by participants was 420 meters (IQR 385.25 -456.25), the distance increased to 429 meters (IQR 396-466.25) on week 26 and 442 meters (IQR 399.75-485.50) on week 52.

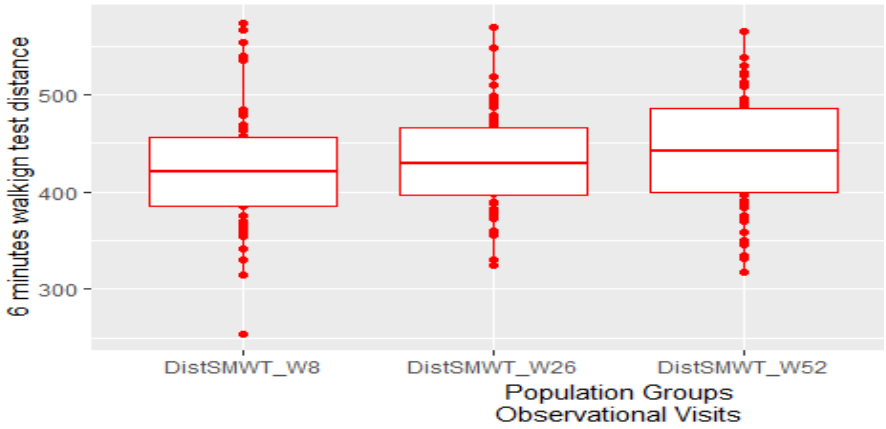


Figure 5-4 6MWT distance Boxplot at different study visits

The SGRQ total scores show a mean of 43.7 (SD: 19.2) at the baseline visit. On week 8 the total scores decrease to 21.1 (SD: 13.2) and on week 26 the score is 5.9 (SD: 6.4). The 3 components scores of the SGRQ are very similar in the visits. Due to the late implementation of the questionnaire not all participant responded to the SGRQ at baseline and week 8 visits.

5-5 Saint George Respiratory Questionnaire (SGRQ) score (Symptoms, activity, impact and total) at different study visits

Study visit	Variable	Mean	SD
Baseline (n= 26)	**Symptoms score**	46.74	16.37
	Activity score	42.36	24.12
	Impact score	43.54	19.94
	Total score	43.70	19.21
Week 8 (n =42)	**Symptoms score**	20.39	14.23
	Activity score	24.16	20.58
	Impact score	19.61	13.22
	Total score	21.16	13.18
Week 26 (n=62)	**Symptoms score**	7.44	7.73
	Activity score	5.38	9.70
	Impact score	5.85	6.99
	Total score	5.85	6.41

SD = Standard deviation. Portuguese version of SGRQ used, scale 0 (best) – 100 (worse)

II. Output II

a. Description of study population

In this cross-sectional study, lung function and demographic data were collected from 155 participants. In this study, 40.64% were male, mean BMI was 25.6 Kg/m^2, 53.55% of the participants had normal BMI (between 18,5 – 24.9 kg/m^2). The mean age was 35.2 years with 67.74% with less than 40 years, 87.74% never smoked, 41.29% were single, 45,16% had education between grade 6-10, 26.45% reported being positive for HIV and 30.32% were never tested for HIV. At least 75.48% reported the use of charcoal to cook.

Table 5-6 Characteristics of the healthy volunteers population

Characteristics	**Male, n=63** (40.64%)	**Female, n=92** (59.35%)	**All, n=155**
Age (years)			
Mean (SD)	33.83 (10.74)	36.13 (11.12)	35.20 (10.99)
Height (meters)			
Mean (SD)	1.67 (0.08)	1.60 (0.06)	1.63 (0.08)
Weight (kgs)			
Mean (SD)	65.52 (9.80)	69.24 (15.68)	67.73 (13.69)
BMI (kg/m^2)			
Mean (SD)	23.43 (3.70)	26.95 (5.60)	25.52 (5.20)
Age group			
<30 years	27 (42.86%)	30 (32.61%)	57 (36.77%)
30-40 years	20 (31.75%)	28 (30.43%)	48 (30.97%)
>=40 years	16 (25.39%)	34 (39.96%)	50 (32.26%)
BMI categories			
Underweight	2 (3.17%)	2 (2.17%)	4 (2.58%)
Normal	45 (71.43%)	38 (41.30%)	83 (53.55%)
Pre-Obesity	11 (17.46%)	28 (30.43%)	38 (24.52%)
Obese	5 (7.94%)	24 (26.09%)	30 (19.35%)
Smoking			
Never Smoked	49 (77.78%)	87 (94.57%)	136 (87.74)
Past Smoker	8 (12.70%)	5 (5.43%)	13 (8.39%)
Current Smoker	6 (9.52%)	0 (0%)	6 (3.87%)

Marital Status			
Single	25 (39.68%)	39 (42.39%)	64 (41.29%)
Married	11 (17.46%)	11 (11.96%)	22 (14.19%)
Living with spouse/partner	25 (39.68%)	37 (40.22%)	62 (40.00%)
Widowed	2 (3.17%)	5 (5.43%)	7 (4.52%)
Education			
Illiterate	0 (0%)	2 (2.17%)	2 (1.29%)
Grades 1-5	5 (7.94%)	24 (26.09%)	29 (18.71%)
Grades 6-10	27 (42.86%)	43 (46.74%)	70 (45.16%)
Grades 11-12	17 (26.98%)	20 (21.74%)	37 (23.87%)
Vocational	8 (12.70%)	2 (2.17%)	10 (6.45%)
University	6 (9.52%)	1 (1.09%)	7 (4.52%)
Self-reported HIV status			
Unknow/never tested	26 (41.27)	21 (22.83)	47 (30,32%)
Negative	27 (42.86)	40 (43.48)	67 (43,23)
Positive	10 (15.87)	31 (33,69)	41 (26,45)
Worked in mines			
No	61 (96.83%)	92 (100%)	153 (98.71%)
Yes	2 (3.17%)	0 (0%)	2 (1.29%)
Cooking Material (More than one options possible)			
Electricity	26 (41.27%)	30 (32.61%)	56 (36.13%)
Gas	25 (39.68%)	32 (34.78%)	57 (36.77%)
Charcoal	48 (76.19%)	69 (75.00%)	117 (75.48%)
Wood	6 (9.52%)	14 (15.22%)	20 (12.90%)

SD= standard deviation, Continuous risk factors have the mean values and range, stratified for sex as well as for the combined sample. We consider only samples having a valid spirometry outcome with an observed FVC and FEV1 for the risk factor summary, which is only 155 out of 212 observed individuals. For categorical risk factors the counts segregated by sex is reported; for the combined sample, we report the proportion belonging to each of the observed categories in addition to the count. Cooking material does not have mutually exclusive categories. HIV status is missing for 47 samples out of the considered 155 samples for statistical analysis.

Source: Adapted Ivanova & Khosa *et al*. "Lung function testing and predictive equations in adult population in Maputo, Mozambique"

b. Spirometry results

In Table 5-7 spirometry results (FVC, FEV1 and FEV1/FVC ratio) are summarized segregated by sex. Male participants presented higher volumes, mean FVC of 3.77l (90.8% of predicted) and FEV1 of 3.12l (91.2% of predicted) compared to females with FVC of 2.94l (88.6% of predicted) and FEV1 of 2.43l (95.5% of predicted). The mean FEV1/FVC ratio was 81% in both groups.

Table 5-7 Spirometry results of healthy volunteers controls (N=155)

	Male, n=63	Female, n=92	All, n=155
FVC in l	3.77	2.93	3.27
(SD)	(0.69)	(0.47)	(0.70)
FVC in % of pred.	90.82	88.38	89.37
(SD)	(11.43)	(12.04)	(11.82)
FEV1 in l	3.12	2.43	2.71
(SD)	(0.67)	(0.42)	(0.63)
FEV1 in % of pred.	91.28	95.53	93.80
(SD)	(13.69)	(13.02)	(13.41)
FEV1/FVC Ratio	82.48	82.93	82.75
(SD)	(6.42)	6.62)	6.52)

SD= standard deviation, pred= predicted, %= percentage, n= number of subjects in (sub-) group.
Predicted values were calculated based on South African reference standard (and prediction equations) [56].

Source: Adapted Ivanova & Khosa *et al*. "Lung function testing and predictive equations in adult population in Maputo, Mozambique"

c. Type and severity of lung impairment

In our healthy volunteers control population, 20% (31/155) of the participants had abnormal lung function test. Restriction was the most predominant type of impairment 19,35% (30/155) overall, followed by obstruction in one female participant (0.65% overall) and no cases of mixed ventilatory impairment. In this study 23.91% (22/92) of females had restriction compared to 12,70% (8/63) of males. In general, women were more likely to suffer from lung impairment compared to men. The severity of impairment is similar in both sexes.

Table 5-8 Proportions of types of Lung impairment

Type of impairment	Male, n=63	Female, n=92	All, n=155
Normal (%)	55 (87.30%)	69 (75.00%)	124 (80.00%)
Restriction (%)	8 (12.70%)	22 (23.91%)	30 (19.35%)
Obstruction (%)	0 (0.00%)	1 (1.09%)	1 (0.65%)

Source: Adapted Ivanova & Khosa *et al*. "Lung function testing and predictive equations in adult population in Maputo, Mozambique"

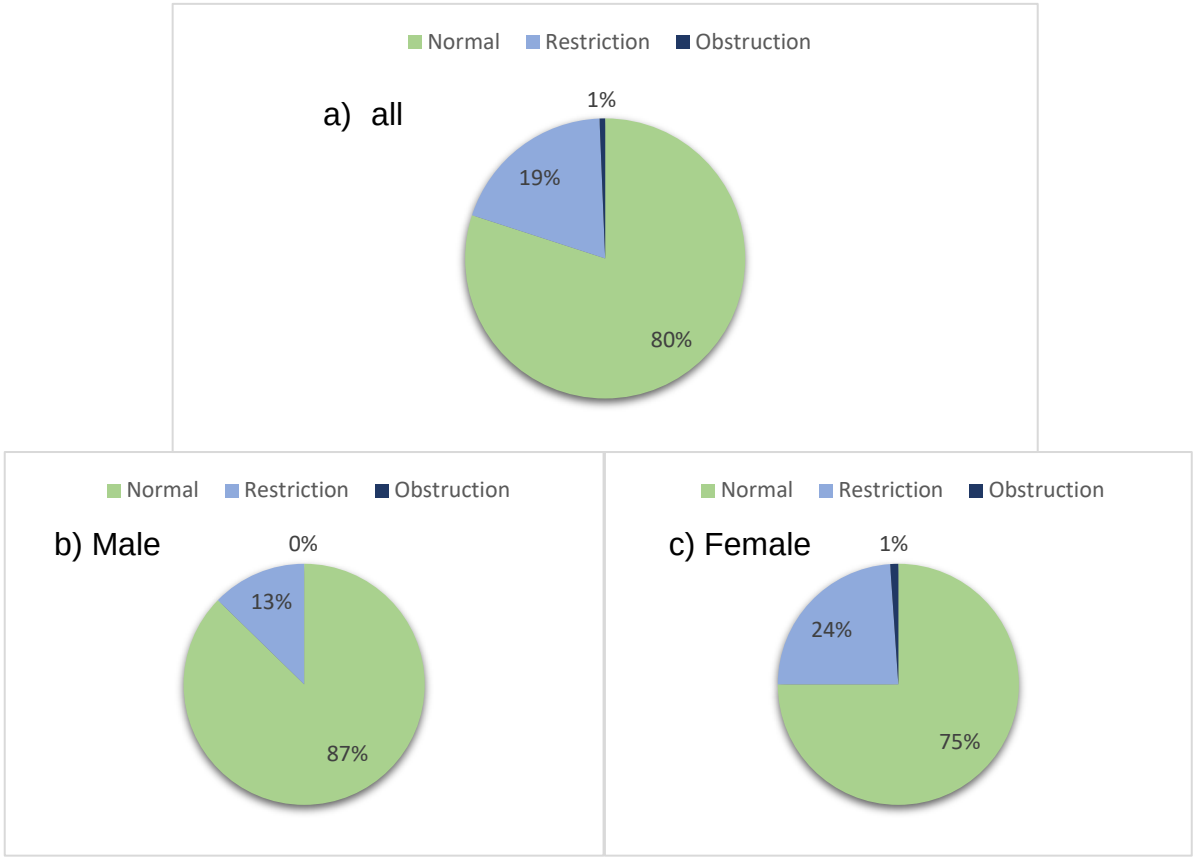


Figure 5-5 Proportions of types of Lung impairment a) all study participants b) Male participants c) Female participants

Type of lung impairment in a) all b) Male and c) Female

All but one case (mild obstruction in a female participant) are of mild and moderate impairment. The severity of impairment is describe in Table 5-9 and Table 5-10.

Table 5-9 Severity of restriction

	Male, n=8	Female, n=22	All, n=30
Mild	6	16	22 (73.33%)
Moderate	2	6	8 (26.67%)

Severity in case of lung impairment according to z scores obtained by comparing to the health volunteer predictions for the South African healthy population.

Table 5-10 Severity of obstruction

	Male, n=0	Female, n=1	All, n=1
Mild	0	1	1 (100.00%)

Severity in case of lung impairment according to z scores obtained by comparing to the health volunteer predictions for the South African healthy population.

d. Functional capacity

The healthy volunteers participants travelled a distance of 441 meters (IQR 405-480.50). The Spo2, fatigue and dyspnoea scores were with normal ranges in this group (Table 5-11). No interruptions or adverse events were registered during the walks.

Table 5-11Minute Walk Test parameters after walk (N=155)

Parameters	**Median peripheral capillary oxygen saturation - Spo2 (IQR)**	**Median fatigue (IQR)***	**Median Dyspnoea (IQR)***	**Median 6-Minute walk distance in meters (IQR)**
	99 (99-99)	1 (0-2)	0 (0-0.5)	441 (405, 480.5)

IQR = Interquartile Range, *fatigue and dyspnoea were self-reported according to the Modified Borg Dyspnoea and Fatigue Scale (Scale 0-10) by patients after the walk (See Appendix 10 – MaTuTU Standard Operating Procedure - 6 Minute Walk Test)

III. Output III

a. Comparison of TB patient versus healthy participants

The main characteristics of the TB cohort versus healthy participants are summarized in Table 5-12. There was a significant lower proportion of men (40.65% versus 67.7%), smokers (12.3% versus 37.1%) and HIV-positives (26.5%, self-reported, versus 62.9%) among the healthy controls, p<0.001. The age median and proportion of participants younger than 40 years old was similar in both groups (33.9 years versus 29.5 years, p = 0.13 and 67.74% versus 74.19%, p = 0.35).

Table 5-12 Baseline characteristics of TB cohort and healthy volunteers

Characteristic	TB cohort (N=62)	Healthy volunteers (N=155)	p-value**
Sex			
Male, % (n/N)	67.74 (42/62)	40.65 (63/155)	<0.001
Age			
median (IQR)	29.5 (25, 40)	33.9 (26, 42)	0.13***
< 40 years old, % (n/N)	74.19 (46/62)	67.74 (105/155)	0.35
HIV-status			
Positive, % (n/N)	62.90 (39/62)	26.45 (41/155) *	<0.001
History of TB			
Yes, % (n/N)	6.45 (4/62)	0.00 (0/155)	0.001
Ever smoked			
Yes, % (n/N)	37.10 (23/62)	12.26 (19/155)	<0.001

*missing/unknown self-reported HIV-status in 47 (47 of 155, 30.32%) subjects of healthy volunteers, **p-value for difference in proportions, *** rank sum test

We generated density distribution of adjusted z-scores of FVC and FEV1 for the TB patients at week 52 (red), healthy participants (green) and the South African reference standard (black). The TB cohort demonstrated a much more pronounced shift of both curves (FVC and FEV1) to lower z-scores when compared to the healthy group and there is also a large left-side tail below a z-score of -1.64, which represents a higher proportion of subjects with abnormal lung function.

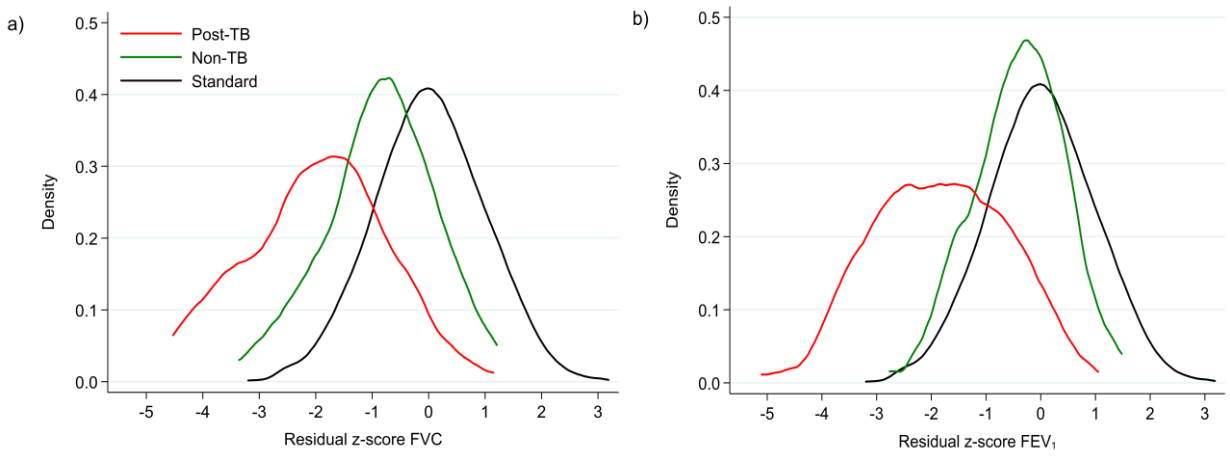


Figure 5-6 Residual Z-scores density plots for standard population (black - south Africa reference population), healthy control (green) and week 52 Post-TB (red). a)-FVC and b)-FEV1

Source: Adapted from table 3, Khosa *et al*. "Development of chronic lung impairment in Mozambican TB patients and associated risks"

Even after improvement in FVC and FEV z scores from week 8 to week 52 after treatment for TB, the z scores are still statistically significantly lower than the controls ($p<0.0001$ for FVC Z scores and $p <0.0001$ for FEV1 Z scores). As expected from the predominant type of lung impairment the FVC have a greater affection than FEV1 when controls are compared to week 52 (Figure 5-7 and Figure 5-8).

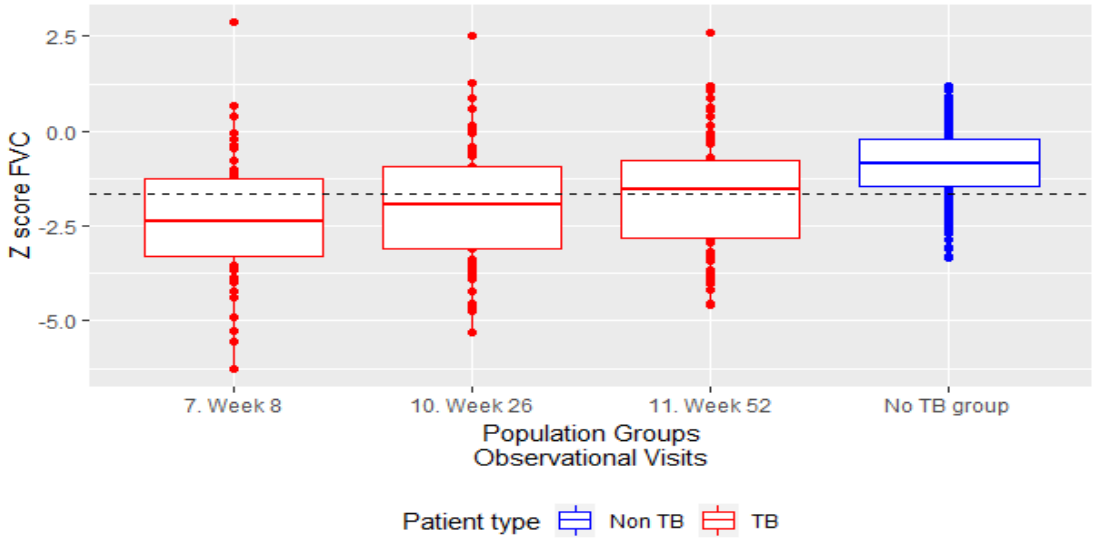


Figure 5-7 FVC Z Scores Boxplot

Comparison of FVC Z scores using the global test (Kruskal Wallis) followed by the post hoc analysis using Dunn's test for multiple comparison. There is a statistical evidence for a difference between the mean FVC at week 52 and healthy volunteers, $p< 0.0001$.

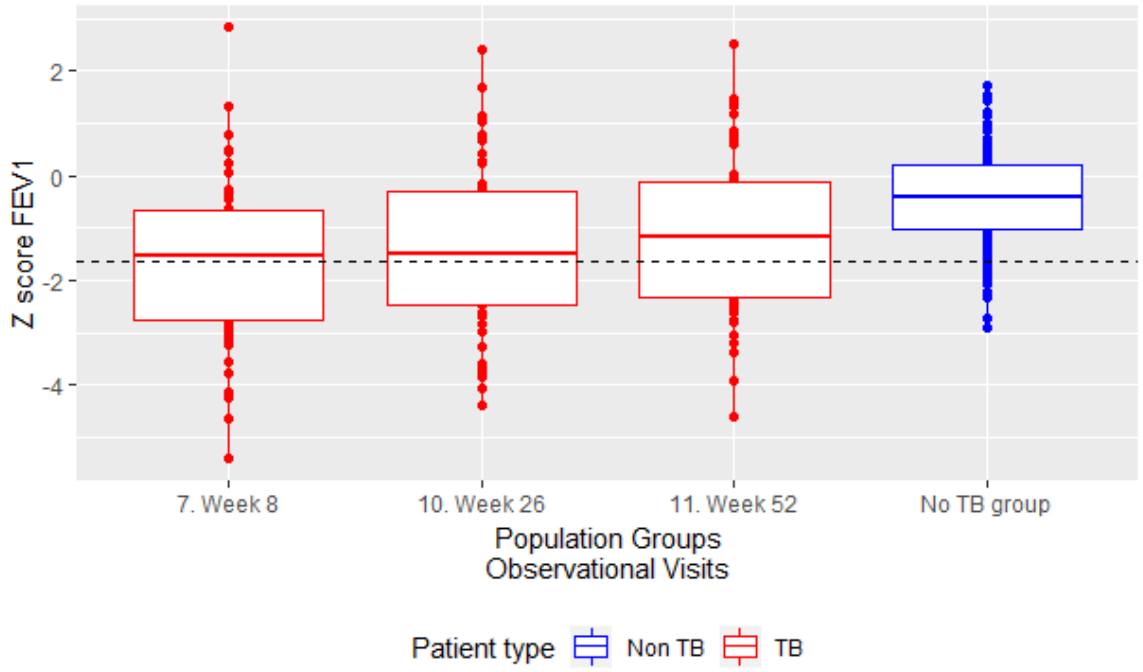


Figure 5-8 FEV Z Scores Boxplot

Comparison of FEV1 Z scores using the global test (Kruskal Wallis) followed by the post hoc analysis using Dunn's test for multiple comparison. There is a statistical evidence for a difference between the mean FEV1 Z scores at at week 52 and healthy volunteers, p< 0.0001.

The Comparison of median 6MWT distance using the global test (Kruskal Wallis) there is no statistical difference be-tween different study visits and healthy participants: week 8 (n=58) and healthy participants, p. adjusted = 0.053, week 26 (n=60) and non TB participants, p. adjusted = 0.232, and week 52 (n=60) and healthy participants, p. adjusted = 0.690.

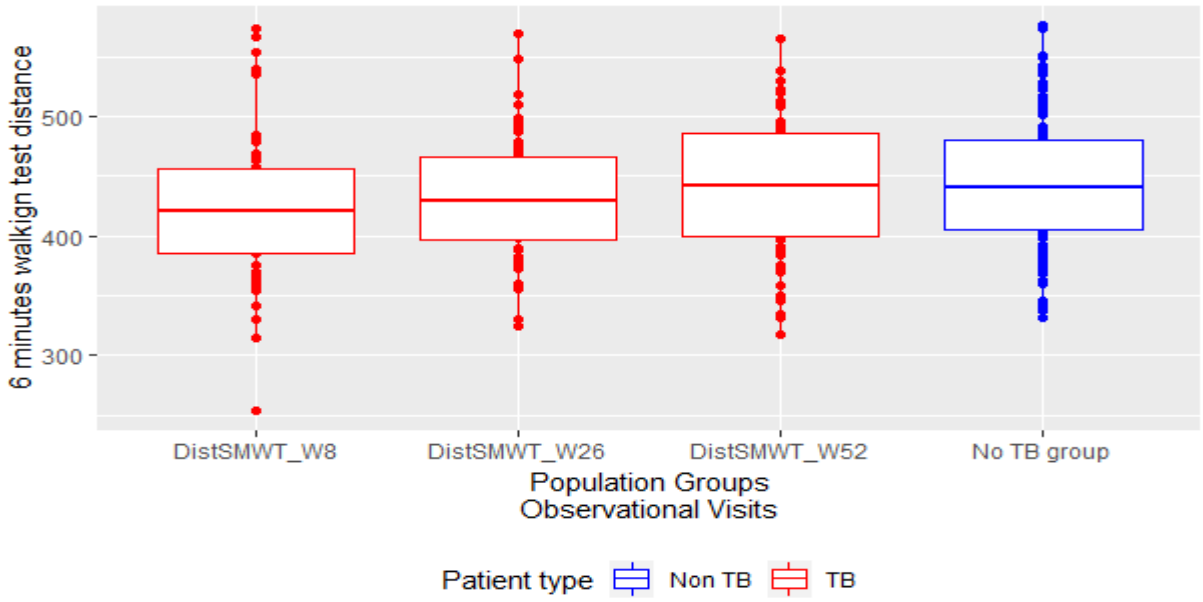


Figure 5-9 Box plot of 6-minute distance in TB patients (all study visits) and healthy participants

b. Comparison of references equations for the Mozambican population

As discussed in chapter iii the GLI worldwide equations do not include African populations and we did not find studies that establish equations for Mozambican population. From this healthy controls Ivanova and Khosa *et al* (unpublished) established reference equations applicable for this specific region of Mozambique.

The Table 5-13 Equation established in South African population (Workers cohort) are shown together with those from the healthy Mozambican cohort.

Table 5-13 Comparison of equations based on South African and Mozambican volunteers

Outcome (Sex specific)	South African population	Mozambique population
FVC Males	-3.08-0.024*Age+4.8*Height	-2.268-0.020*Age + 4.000* Height
FVC Females	-3.04-0.023*Age+4.5*Height	-2.750-0.020*Age + 4.000* Height
FEV1 Males	-0.54-0.027*Age+2.9*Height	-1.845-0.025*Age + 3.467* Height
FEV1 Females	-1.87-0.028*Age+3.4*Height	-2.223-0.025*Age + 3.467* Height

Source: Adapted Ivanova & Khosa *et al*. "Lung function testing and predictive equations in adult population in Maputo, Mozambique"

The equations derived from the healthy Mozambican participants have different coefficients when compared to the south African equations, this will lead to different diagnosis and classification of impairment between the equations. In regards to FVC equations Mozambique (MZ) equations have a smaller coefficient for height and bigger coefficient for age when compared to South Africa (SA) equations. For FEV1 MZ equations have both coefficients for height and age higher.

We have used the MZ equations above to reassess impairment, type of impairment in the TB cohort and compared the outcome when using the south African reference equations **(different methodology was used for the determination of LI)**. It is clear that there is some misclassification with the South African reference equations leading to more Lung imparity and severity. Using the non-TB participants equations 13/33 cases of restriction (SA equations) are classified as normal (MZ equations). In terms of severity 13/22 mild impairment cases (SA equations) change to no impairment (MZ equations) and 7/22 mild impairment (SA equations) change to moderate/severe cases.

The agreement on the mild cases is only in two cases.

From this analysis the proportion of lung impairment decreases from 61.29% to 43.55%, decreasing the overall proportion of mild cases and increasing the overall and relative proportion of moderate/severe cases (Additional 6 cases). Therefore, we can speculate that TB is responsible for around 40% of cases of lung impairment in this community.

Table 5-14 Lung impairment type misclassification when compared to the Mozambican non-TB cohort reference equations to the South African reference equations

		Predictions (South African MLR)			
	Impairment Outcome	Normal	Obstruction	Restriction	Mixed
Predictions (Mozambique individual (FEV1/FVC) MLR)	Normal	22	0	13	0
	Obstruction	0	1	0	1
	Restriction	0	0	20	0
	Mixed	0	0	0	4

Table 5-15 Lung impairment severity misclassification when compared to the Mozambican non-TB cohort reference equations to the South African reference equations

		South African healthy volunteer standards		
	Impairment severity	No impairment	Mild impairment	Moderate/severe impairment
Mozambique healthy volunteer standards	No impairment	22	13	0
	Mild impairment	1	2	1
	Moderate/severe impairment	0	7	15

6. DISCUSSION

IV. Output I

This is the first report describing lung impairment in TB patients from Mozambique. Our results showed that 12 months after TB treatment initiation more than half (61%) of TB patients presented lung impairment and even a quarter of them had moderate or severe impairment. This finding is in line with that of similar studies from African countries: two studies conducted in Benin and Botswana the prevalence of lung impairment were about 45% and 56%, respectively [68, 69], in a study from Tanzania the prevalence of LI was 74% [7]. Also study data from Asia [17] and America [2, 18] indicate, that on average half of previous TB patients show abnormalities in spirometry. Smaller differences in proportions among these studies (about 10%) can –most likely- be explained by differences in data analysis, e.g. selection of best spirometry result, selection of reference values (see below) and threshold for abnormality.

However, in many studies, the majority –or at least a relevant proportion- of post TB patients presented with pulmonary obstruction, while in our study almost all patients had pulmonary restriction, represented by a low FVC but normal FEV1/FVC ratio. The different pattern may result from the presence of different background risk factors, e.g., environmental or indoor air pollution, smoking behaviour in communities, lung infections in childhood, etc., in the different setting the study were performed, including genetic factors determining the immune response pathway [33]. Further age per se is a risk factor for chronic lung diseases and also COPD. This might also explain why we saw almost no obstructive ventilation impairment in our relatively young patients with a median age of about 30 years.

Due to a prospective study design, we were able to measure FEV1 and FVC during and after TB treatment. Like in the few other prospective studies [2, 17], an increase of both parameters could be observed over time in most subjects, although this improvement was only small and borderline significant. Importantly, the majority of subjects and all women with lung impairment at week 8 still had lung impairment at week 52. Further, only very few patients with normal values for FEV1 and FVC and week 8 (three and four, respectively), showed lung impairment later in the follow up, at week 52. This finding indicates that LI is usually developing early during the course of active TB disease, e.g. before treatment start or early during treatment and that a large part of the disease is not significantly reversible under TB treatment. Further research studies are needed in which lung function is measured before treatment start or early during treatment (e.g., until week 4) in order to further define the best timepoint for potential therapeutic interventions which are

aiming for preservation of lung function, such as host directed therapies or immune-modulation. These types of treatment are currently not available, however, several trials are currently ongoing (not published data) in order to assess such treatments in addition to anti-microbial treatment of TB.

In our study, female sex was associated with lung impairment. This finding is surprising as in many other studies on microbiological TB outcome or lung function as outcome men showed an elevated risk for poor outcome categories [7]. Often, this association was confounded by additional risk behaviour such as alcohol drinking and smoking. As female participants did not smoke and were less likely to drink critical amounts of alcohol, other factors, such as indoor air pollution, access to health care or immunological/hormonal factors, might be contributing to the higher risk for LI in women compared to men.

Of note, in our study, smoking and alcohol consumption per se were no risk factors for LI. This observation might be based on a healthy smoker/drinker effect, when either subjects with a good physical condition or those who have access or can afford the consumption of these stimulants do not develop lung impairment due to the presence of other, protective, factors. This thinking is further supported by the finding that the consumption of higher amounts of tobacco (>10PY) and alcohol (critical alcohol consumption) showed a positive association with LI, indicating that at a certain threshold the "healthy smoker effect" is not compensating the actual damage of tobacco and or alcohol to the lung. It is commonly known and was shown by several studies that alcohol consumption and smoking can lead to poor TB outcomes. Also with regards to lung outcome, smoking was shown to be a risk factor in several studies worldwide [7, 18], although this could not be confirmed in few other studies from Africa and Asia [17, 21, 68]. Alcohol, was so far not assessed as risk factor for lung outcome. However, it is known that alcohol can impair the immune response of a subject and thus, could lead via this pathway to increased lung damage in TB patients [70]. However, especially with regards to smoking and alcohol drinking as risk factors for post TB LI, it cannot be excluded that in a certain proportion of study participants, a certain level of lung impairment was existing before active TB disease was developed (see below). A positive HIV-status was not a risk factor for lung impairment after TB. This is in line with few other studies from Africa [68] but contrary to what Manji *et al* found [7]. However, those HIV-positives with CD4 higher than 200 cels/ul at baseline visit had an higher risk for post TB restriction compared to those with lower CD4 count. This effect was already observed in another study by Ravimohan *et al* [69]. Interestingly, anaemia was a risk factor in this study. Iron metabolism is closely related

to pro inflammatory status [71], and several inflammatory diseases like TB are related to a shift of iron into the bone narrow macrophages, which could also be the reason for anaemia [72] in our study participants, who developed LI after TB.

In the absence of a local spirometry reference standard, we used South African prediction equations as basis for the analysis of spirometry data. The spirometry results of the healthy volunteers (see discussion below) indicate that a major part of the observed LI in TB patients was very likely associated with TB. Although the healthy volunteers were on average 5 years older than the TB patients, only about 20% of them had abnormal lung parameters and mostly of mild grade only.

One limitation of our study was the sample size, which prevented a more in dept analysis of some other risk factors like time to TB diagnosis or ART initiation in HIV-positives, TB severity on chest x-ray or microbiological markers such as bacterial load. Also, the risk factors shown in this study could not be controlled for bias such as confounding or effect modification. Further, the small samples size and also the limitation of study subjects to one neighbourhood only does not allow the generalization of our findings to the general population of TB patients in Mozambique.

V. Output II

In our study, the healthy volunteers were in majority females, had a higher BMI, lower HIV rates and less history of smoking when compared to TB participants. There are no significant differences related to age and proportion of patients below 40 years old. Individuals with history of TB were non eligible for the study.

One in every five healthy participant had LI, not surprisingly restriction was the most prevalent type of LI and more prominent in females. Participants are susceptible to other lung injuries factors like childhood respiratory infection, indoor air pollution and air pollution. Three in each four participants referred the use of charcoal for cooking, females might be more exposed to the fumes leading to more severe LI.

The South African prediction equation are derived from a healthy worker; therefore, a healthy worker effect might be responsible for higher rates of LI in our healthy volunteers as working individual usually have better lung function [65, 73]. Further, reference values are usually derived from non-smokers. Although only 12.3% of our participants were smokers, this might be another reason for impaired lung function in our healthy volunteers. Finally, as restriction was the prevailing lung function impairment found in the healthy volunteers, it is also thinkable, that the South African standard cannot be applied to Mozambican population due to physiologic differences that lead to a smaller total lung capacity in Mozambicans compared to South Africans.

Finally, we have used equations derived from the healthy volunteers to access the potential misclassification derived from the use of South African equations, i.e. in case the South African standard cannot be applied to Mozambicans. Having in account that the control group differ mainly from the TB participant by the inexistence of history of TB, the difference in prevalence of LI might be attributed to TB.

Using the MZ equations generated from the healthy volunteers there is not a significant decrease in the proportion or type of lung impairment.

The main type of lung impairment, restriction, does not change when healthy volunteers lung function is assessed, leading the conclusion that other lung insults lead to this specific lung remodelation.

Our study had some limitations: 1/ HIV status was self-reported leading to missing data although this was not a relevant risk factor in our TB cohort, 2/ Exposure to biomass fuel and other lung insults was not collected in a quantifiable manner and 3/Smoker were not excluded and absence of lung disease not ascertained via imagiology (e.g Xray).

7. CONCLUSION

1. More than half of those cured from TB on their first episode still present with lung impairment 52 weeks after TB diagnosis. The majority had restrictive lung impairment and in 25% the grade of impairment was severe or moderate. The pattern of lung impairment in different study populations most likely depends on several risk factors, including indoor air pollution or other aggressors such as childhood respiratory infections or dusty environments. It might also be that the genetic background also plays a relevant role in this context. In our setting, female gender, the severity of HIV related immune-depression and low haemoglobin are important risk factors for lung impairment.

2. South African prediction equations might not be completely applicable in Mozambican population. In addition, other factors besides TB related may drive the occurrence of restrictive pattern of lung impairment, and being more prevalent in female participants.

3. There is a huge effort to control and end TB, however, Post TB Chronic lung disease (PTBLD) and also the resulting burden on quality of life is overlooked. Although the early diagnosis and treatment might lead to fewer sequel[6], our data also support the thinking that additional risk factors can lead to LI which could be controlled or treated. New guidelines for diagnosis and treatment of PTBLD are urgently needed in order to reduce TB related morbidity. For now, for many TB patients the end of anti-microbiological treatment is just the beginning of a long journey of suffering and even early death in some cases[2, 18].

4. In high TB burden settings, chronic pulmonary services are scarce and former TB patients presents with symptoms similar to their previous episodes. The health professional may misinterpret as another episode of TB treatment with consequent treatment. Some of those cases are indeed cases of PTBLD. Therefore, our work shows how closely pulmonary infectious diseases and non-communicable diseases (NCDs) of the lung can be interrelated. More efforts in the development of pulmonary health services are needed in Mozambique in order to improve long-term lung outcome in TB patients.

8. RECOMMENDATIONS

There is a need of more studies on lung outcome in PTB patients and a necessity of integrated pulmonary function testing and rehabilitation during and after TB treatment. Those studies should focus on:

- Increase the knowledge on prevalence, type and severity of PTBLD;
- Understand the underlying pathophysiological pathways and risk factors in order to develop new interventions;
- Trials on preventive therapies (host directed therapies) and supportive/ symptomatic therapies including rehabilitation.

Additional studies also are needed to establish reference equation applicable to the Mozambican population.

Spirometry and inhalers should be available at TB treatment center to support diagnosis and treatment of PTBLD. Respiratory and rehabilitation should be available for TB patients. Further studies are needed to understand the lung remodulation post TB, the risk factors and measures to improve the lung function during TB treatment.

Finally, new drug and new regimens should include lung function evaluation in order to identify drug-regimens with better lung outcomes.

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10. APPENDIXES

II. Appendix 2 – List of publications

1. Sabiiti W, Azam K, Kuchaka D, Mtafya B, Bowness RE, Oravcova K, Farmer E, Honeyborne I, Evangelopoulos D, McHugh TD, Xiao H, **Khosa C**, Rachow A, Heinrich N, Kampira E, Davies G, Bhatt N, Ntinginya N, Viegas S, Jani I, Kamdolozi M, Mdolo a, Khonga M, Boeree MJ, Philips PP, Sloan DJ, Hoelscher M, Kibiki G, Gillespie SH. Improving diagnosis and monitoring of treatment response in pulmonary tuberculosis using the molecular bacterial load assay (MBLA). *bioRxiv* 2019: 555995.

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III. Appendix 3 - Statement on Pre-release and Contribution

At least 5 articles are being written from the data collected from this project:

1. **Improving diagnosis and monitoring of treatment response in pulmonary tuberculosis using the molecular bacterial load assay (MBLA).**
   Submission: No (available at bioRxiv see list of publications) | **Journal:** To be determined
   Aim: Evaluation of the utility of a marker of M. tuberculosis viable count, the Molecular Bacterial Load assay (MBLA) for diagnosis and treatment monitoring of tuberculosis in a high burden setting
   Role of the PhD candidate: Data acquisition, Samples processing, interpretation of data, revision of manuscript, final approval of the version to be published.

2. **Development of chronic lung impairment in Mozambican TB patients and associated risk**
   Submission: Yes | **Journal:** European Respiratory Journal (ERS) | Under review
   Aim: Description of LI and risk factor in a cohort of TB patients
   Role of the PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data, drafting and revision of manuscript, final approval of the version to be published and accountability for aspect of the study.

3. **Lung function testing and predictive equations in adult population in Maputo, Mozambique**
   Submission: No (Under internal review) | **Journal:** To be determined
   Aim: A cross-sectional study on healthy individual and establishment of reference equations.
   Role of PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data, drafting of manuscript.

4. **Prevalence of Aspergillosis and risk factors in a cohort of TB patients in Mozambique**
   Submission: No (Data analyses) | **Journal:** To be determined
   Aim: A longitudinal assessment of the prevalence of Aspergillosis during and after TB treatment
   Role of PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data.

5. **Long-term Pulmonary Outcomes in Patients with Tuberculosis - Systematic Review and Meta- Analysis**
   Submission: No (Under writing) | **Journal:** To be determined
   Aim: Describe and analyse available spirometry, St. George Respiratory Questionnaire (SGRQ) and the Six-Minute-Walking-Test (6MWT) in Patients
   Role of PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data.

   Note: Teams from INS and LMU actively supported all steps described above. Supervisors provided close support.

IV. Appendix 4 – Acknowledgement

This project would not be a reality without the unconditional support, inputs and encouragement from several parties, therefore I would like to thank, first and foremost God.

Supervisors:
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Dr Rachow and Dr Bhatt for the friendship and support, guided me in all the steps to become a better researcher, physician and person. For always being there, thank you so much.

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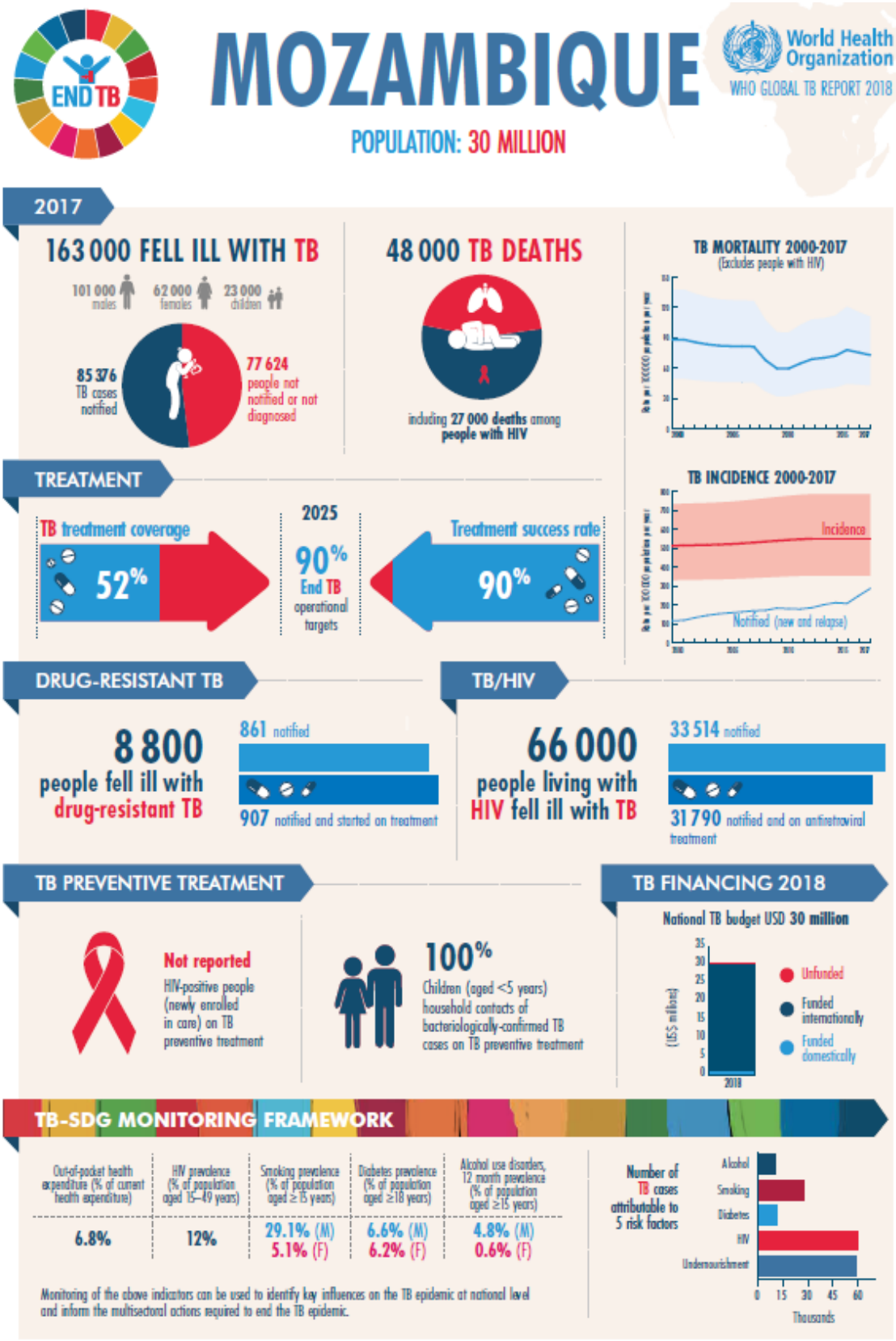
Further, I would like to thank the members of the TB Sequel Consortium for the fruitful discussions during the monography writing process.

Study participants:
Without any doubt the participants collaboration was the key factor for the success of this project. To the participants my deepest appreciation for the support and for study participation.

Family and friends:
Last but not least, my wife Vanessa, my daughter Andressa and baby Elon who provided me all the social support during this journey. My Father, mother and brothers I have also been supportive during all time.

VI. Appendix 6 – TB burden in Mozambique (source: World TB Report 2018[1])



VII. Appendix 7 – Supplementary table 1: Distribution of risk factors in participants from TB cohort without and with LI (and with mild or moderate/severe LI among those with LI)

Risk factor, total N= 62	Total	No LI, % (n) (N=24)	Any LI, % (n) (N=38)	Mild LI, (n) (N=22)	Moderate/ severe LI, (n) (N=16)
Sex					
Male	42	47.62 (20)	52.38 (22)	14	8
Female	20	20.00 (4)	80.00 (16)	8	8
Age					
< 40 years old	46	36.96 (17)	63.04 (29)	17	12
≥40 years old	16	43.75 (7)	56.25 (9)	5	4
BMI*					
< 18.5	25	32 (8)	68 (17)	10	7
≥ 18.5	36	41.57 (15)	58.33 (21)	12	9
HIV-status					
Positive	39	38.46 (15)	61.54 (24)	14	10
CD4- cells < 200/µl	18	55.56 (10)	44.44 (8)	3	5
CD4- cells ≥ 200/µl	21	23.81 (5)	76.19 (16)	11	5
Negative	23	39.13 (9)	60.87 (14)	8	6
Culture Conversion at week 8					
Yes	35	42.86 (15)	57.14 (20)	12	8

No	27	33.33 (9)	66.67 (18)	10	8
Culture conversion at week 26					
Yes	58	39.66 (23)	60.34 (35)	19	16
No	4	25 (1)	75.00 (3)	3	-
Ever smoked					
Yes	22	59.09 (13)	40.91 (9)	4	5
No	40	27.50 (11)	72.50 (29)	18	11
**Pack Years*, ** **					
<10	13	76.92 (10)	23.08 (3)	1	2
≥10	7	28.57 (2)	71.43 (5)	2	3
Critical alcohol consumption*,**					
Yes	32	43.75 (14)	56.25 (18)	8	10
No	30	33.33 (10)	66.67 (20)	14	6
C-reactive protein*					
<100 mg/dl	41	36.59 (15)	63.41 (26)	15	11
≥100 mg/dl	17	41.18 (7)	58.82 (10)	5	5
Hemoglobin					
Median male, g/dl (IQR)	42	11.95 (11.12, 12.82)	11.15 (9.75, 12.55)	11.45 (10.10,12.60)	10.5 (9.38,12.18)
Median female, g/dl (IQR)	20	11.1 (10.62, 12.00)	9.8 (9.08,10.78)	9.7 (8.10, 10.78)	9.8 (9.48, 10.70)

Overall affected lung at baseline, Ralph *et al*.**					
Median, % (IQR)	62	17.5 (15,20)	15 (13, 25)	15 (10,25)	20 (15, 21.25)

Legend to supplementary table 2:

*missing observations: CRP for 4 participants, BMI for 1 participant, Pack Years (PY) for 2 participants, amount of alcohol consumption for 2 participants.

**definitions:

- Anaemia, definition according to WHO, non-anaemia: 12mg/dl or higher (women) or 13 mg/dl or higher (men), mild: 11.0-11.9 g/dl (women) and 11.0-12.9 g/dl (men), moderate: 8.0g/dl-10.9g/dl (both sexes), severe: <8.0g/dl (both sexes)[66].
- X-ray scoring system for affected lung, according to Ralph *et al* [74].
- Pack Years: numbers of years a person smoked 1 pack (20 cigarettes) per day, ≥10 pack years means that the respective study participant smoked an equivalent of one pack of cigarettes every day for 10 years (e.g. 1 pack of cigarettes for 10 years, or 2 packs of cigarettes for 5 years, or ½ packs of cigarettes for 20 years) at baseline study visit.
- Critical alcohol consumption: in men (women) more than 60g (30g) alcohol per occasion of alcohol drinking and/or more than 150g (80g) alcohol per week, according to the International Alliance for Responsible Drinking for general population[67]

Source: Adapted from supplementary table 2, Khosa *et al*. "Development of chronic lung impairment in Mozambican TB patients and associated risk"

VIII. Appendix 8 – MaTuTU Standard Operating Procedure - Spirometry and Reversibility Test

MaTuTU Standard Operating Procedure

Spirometry and Reversibility Test

Version: **V0.1 (2015-09-18)** Valid from: **August 20, 2015**

Translation of: --- Dated ---

Author:
Celso Khosa/Andrea Rachow _____ Date _____ Author signature

Translator:
_____ Date _____ Translator signature

Change history:

Version – Date	Content changes	Format Changes
V0.1 (2013-07-20)	First draft ready for circulation for review.	

Appendixes:

Contents

1. Introduction

I. Objective

The objective of this SOP is to provide general guidance to study personnel on indications and procedures to perform a Spirometry and Reversibility Test, specifically it reviews details that influences results, step-by-step protocol, proper patient preparation and procedures, performing the test.

II. Background

Spirometry is a form of pulmonary function test. Patients blow into a tube as strongly as possible, expiring until they have no breath left. The Reversibility test consists in the use of a bronchodilator to evaluate whether a measured obstruction is fixed or reversible. It is important that all clinical staff use the same procedure to this test as specified on this SOP, to ensure continuity, comparability and consistency in results.

III. References

- SOP **CISPOC- POP-1001**
- EasyGuide Operator's Manual
- SA Guidelines for office spirometry 2012
- Spirometry testing (Reversibility testing), Sandwell and West Birmingham Hospitals NHS Trust, www.swbh.nhs.uk
- Study protocol;
- Study Manual of Procedures;
- Videos:

https://www.youtube.com/playlist?list=PLEFvavfsN26YG8MJqJ7VCu4_8UmTXoBsc
https://www.youtube.com/watch?v=6kbgZWS5wH0

2. Technical aspects

I. Location

The Spirometry and Reversibility test should be performed in a specialized examination room. Ensure a quiet environment, always maintain room well ventilated and adequate infection control measures (Use respirator and gloves).

II. Required equipment

1. Spirometer
2. Individual spirette
3. Noise clip
4. Inhaler*****
5. Metered Salbutamol*****
6. Absorbent paper

*** Reversibility test only**

III. Contraindications

1. Hemoptysis
2. Pneumothorax
3. Recent MI (UA)
4. Aortic Aneurysm
5. Cerebral Aneurysm
6. Recent eye surgery
7. Recent thoracic & abdominal surgery
8. Contraindication for ß2 agonists use (hyper sensibility)*****

*** Reversibility test only**

IV. Patient Preparation

1. Explain the procedure to the participant and answer any questions he/she may have before performing the Spirometry (for the first spirometry let the patient watch the video, explain and demonstrate the maneuver for the patient).
2. Emphasize the essential elements of the test:
   - filling lungs completely
   - sealing lips around the spirette so that there are no leaks, taking care not to block its opening with teeth or tongue or bite down excessively lasting out as hard and fast as possible
   - continue blowing out until the lungs are completely empty
3. Obtain verbal consent to perform the Spirometry.
4. Ask patient to stop taking the following medication for the following time periods before your test unless you need to use them because patients symptoms are worsening:

Medication Type	Drug Name	Brand Name
For 4-8 hours before		
Inhaler or nebuliser	Salbutamol	Ventolin, Salamol, Asamol clickhaler, Salbumin, Airomir, Combivent
Inhaler	Terbutaline	Bricanyl
For 6 hours before		
Inhaler or nebuliser	Ipratropium bromide	Atrovent
For 24 hours before		
Inhaler	Salmeterol	Seretide, serevent
Inhaler	Fometerol	Symbicort, Oxis, Fostair, Foradil, Atimos Modulite
Tablet	Theophylline	Slo-phylline, Uniphylline Continus, Nuelin SA
Tablet	Aminophylline	Phyllocontin continus
Tablet or syrup	Salbutamol	Ventolin, ventmax
For 24-36 hours		
Inhaler	Tiotropium	Spiriva

5. Ask the patient to refrain from smoking 24 hours before the test. Avoid eating a large meal for 2 hours before the test and avoid vigorous exercise for 30 minutes before the test
6. Ask the patient to avoid alcohol on the day before the test also ask wear comfortable and loose clothes, remove dentures, and relax.

V. Spirometry procedures

The appointment will take up to 1 ½ hours; this includes the time for the medication to work if necessary (after the first test) and the time to do the 2nd spirometry test.

Measure patient's weight and body temperature and record all data needed in the spirometer to generate results, check patient file.

1. Ask patient to seat on a fixed chair. Switch on the EasyOne Worldspirometer.

- Choose "Perform Test" in the main menu and then NEW. Confirm withNTER. The instrument will now allow you to enter the patient data.
- Enter the corresponding patient data line by line. Use the keys and confirm with ENTER each time.
- After entering the patient data, you then move on to the "Test selection" menu. Choose the FVC Expiratory test and confirm with ENTER.
- Insert a spirette into the instrument. Ensure that the arrow on the spirette is lined up with the arrow on the instrument.
- Once again briefly prepare the patient for the test. When the patient is ready, press ENTER. You will now hear the sensor buzzing.
- The instrument now prompts you to avoid air flow in the spirette since it is setting the baseline. It is advisable to block off the spirette on one end in order to ensure that the baseline is set precisely even if the room is draughty. An audible signal will sound when the baseline has been set. You will see prompt "Blast out" on the screen.
- Hand the instrument to the patient. Ask the patient to breathe in deeply, insert the spiretteTM correctly into his or her mouth. Now ask the patient to exhale as firmly and as quickly as possible, and continue exhaling until all air has been exhaled.

- At the end of the maneuver, you will see a message on the display indicating whether the maneuver was acceptable. At least three acceptable maneuvers must have been performed before you see message "Session complete".

2. After each maneuver recognize a poor effort by observing the patient and/or interpreting the Quality Messages displayed by the spirometer. After a poor effort you must explain what went wrong.

3. The patient will be asked to repeat this a few times (at least 3 times, maximum 8 times) trying to achieve the best possible quality section (quality A). Develop enthusiastic coaching techniques to use during the maneuver to maximize your chances of getting quality results with a minimum number of efforts.

VI. Reversibility test

If FEV1/FVC < 0.7 is Important to determine if a fixed or reversible air narrowing is present. If FEV1 increases by more than 12% or 200ml after application of a bronchodilatator (e.g. Salbutamol) there is a high likelihood for asthma, instead of or in addition to COPD.

1. The patient should be clinically stable and there should be no contra-indications for the use of Salbutamol or other bronchodilating drugs.

2. Confirm if Measured FEV1/FVC before bronchodilator was below 0.7.

3. Use metered dose inhaler through a spacer.

4. After a gentle expiration to Forced Residual Capacity, a dose of 100 µg of salbutamol (or equivalent) is inhaled in one breath to Total Lung Capacity. The breath is then held for 10 s before exhaling. Four separate doses (total dose 400 µg) are delivered at 30 s intervals.

5. A waiting period of 15 - 20 minutes follows.

6. Perform 2nd spirometry test (see instruction above).

7. Calculating bronchodilator reversibility:

$$\%\ FEV_1\ \text{Reversibility} = \frac{\text{Post-bronchodilator } FEV_1 - \text{Pre bronchodilator } FEV_1}{\text{Pre-bronchodilator } FEV_1} \times 100$$

IX. Appendix 9 – MaTuTU Standard Operating Procedure - 6 Minute Walk Test

MaTuTU Standard Operating Procedure

6 Minute Walk Test

Version: **V0.1 (2013-12-06)** Valid from: **August 7, 2014**

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Date _____ Author signature _____

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Version - Date	Content changes	Format Changes
V0.1 (2013-07-20)	First draft ready for circulation for review.	

Appendixes: MaTuTU 6MWT
MaTuTU Borg scale

Contents

1. Introduction

I. Objective

The objective of this SOP is to provide practical guideline for 6 Minute Walk Test (6MWT), specifically it reviews details factor that influences results, step-by-step protocol, safety measures, proper patient preparation and procedures and clinical interpretation of results.

II. Background

Several modalities are available for the objective of functional exercise capacity. Some provide a very complete assessment of all systems involved, whereas others provide lower information and are simpler to perform. 6MWT is a practical simple test that requires a 30 m hallway but no advanced equipment or advanced training for technician.
This test measures the distance the patient can quickly walk in a flat, hard surface in a period of 6 minutes, it evaluates global integrated response of all systems integrated during the exercise including pulmonary and cardiovascular systems, systemic circulation, blood, neuromuscular units and muscle metabolism.

The self-paced 6MWT access the sub maximal level of functional capacity, most patients does not achieve maximal exercise capacity during 6MWT instead they choose their own intensity of exercise and are allowed to stop and rest during the test. However most activities of daily living are performed at submaximal levels of exertion, the 6MWT may better reflect the functional exercise level for daily physical activities.

III. References

- ATS statement: Guidelines for the Six Minute Walk Test;
- Study protocol;
- Study Manual of Procedures;

2. Contraindications

- Unstable angina or Myocardial infarction during previous month;
- Resting heart rate > 120 bpm;
- Systolic Blood pressure > 180 mmHg or Diastolic >100 mmHg;
- Abnormal ECG;

3. Safety Issues

- Testing should performed in a location where rapid and appropriate response to an emergency is possible; the appropriate position of the crash cart should be determined by the supervising clinician of the facility;
- Oxygen must be available; a telephone must be in place to enable a call for help;
- A certified individual should be available if necessary;
- Physicians a not necessary to be present during all the test;
- If patient is in on oxygen therapy, oxygen should be given in standard rate or has direct by Physician or protocol;

Reasons for immediately stopping 6 MWT include the following:
Chest pain, Intolerable Dyspnoea, Leg cramps, Staggering, Diaphoresis, Pale or ashen appearance.

Technicians must be trained to recognize any of these situations and respond accordingly. If test is stopped due to any of these conditions patients should sit or lie supine.
The following should be obtained: Blood pressure, Heart rate, Oxygen saturation and physical examination. Oxygen should be administered as appropriate.

4. Technical aspects

Location

The 6 MWT should be performed in a flat, straight, hard surface, seldom travelled corridor.
The walking course must be 30 m and marked each 3 m, the turnaround point should be marked with a cone and starting line marked with brightly tape. If test is done outside take particularly attention to weather conditions.

Required equipment

1. Countdown timer (or stopwatch)
2. Mechanical lap counter
3. Two small cones to mark the turnaround points
4. A chair that can be easily moved along the walking course
5. Worksheets on a clipboard
6. A source of oxygen
7. Sphygmomanometer
8. Telephone

Patient Preparation

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn.
3. Patients should use their usual walking aids during the test (cane, walker, etc.).
4. The patient's usual medical regimen should be continued.

5. A light meal is acceptable before early morning or early afternoon tests.
6. Patients should not have exercised vigorously within 2 hours of beginning the test.

Measurements

1. Repeat testing should be performed about the same time of day to minimize intraday variability;

2. "Warm-up" period before the test should not be performed.

3. The patient should sit at rest in a chair, located near the starting position, for at least 10 minutes before the test starts. During this time, check for contraindications, **measure pulse, respiratory rate** and **blood pressure**, and make sure that clothing and shoes are appropriate. Complete the first portion of the work-sheet (see the APPENDIX).

4. Measure and record **baseline heart rate** and **oxygen saturation (SpO2)**). Make sure the readings are stable before recording. Note pulse regularity and whether the oximeter signal quality is acceptable.

5. Have the **patient stand** and rate their baseline dyspnoea and overall fatigue using the Borg scale.

6. Set the **lap counter to zero** or **tick marks on the worksheet** and the **timer to 6 minutes**. Assemble all necessary equipment (lap counter, timer, clipboard, Borg Scale, worksheet) and move to the starting point.

7. Instruct the patient as follows:

"The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself.
You will probably get out of breath or become exhausted.
You are permitted to slow down, to stop, and to rest as necessary.
You may lean against the wall while resting, but resume walking as soon as you are able. You will be walking back and forth around the cones.
You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."
Demonstrate by walking one lap yourself. Walk and pivot around a cone briskly.
"Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Start now, or whenever you are ready."

8. Position the patient at the starting line. Stand near the starting line during the test, do not walk with the patient. As long as the patient starts walking start the timer.

9. Do not talk to anyone during the walk. Use an even tone of voice when using the standard phrases of encouragement. Watch the patient. Do not get distracted and lose count of the laps. Each time the participant returns to the starting line, click the lap counter once (or mark the lap on the worksheet). Let the participant see you do it. Exaggerate the click using body language, like using a stopwatch at a race.

After the first minute, tell the patient the following (in even tones):

"You are doing well. You have 5 minutes to go."

When the timer shows 4 minutes remaining, tell the patient
the following: "Keep up the good work. You have 4 minutes to go."

When the timer shows 3 minutes remaining, tell the patient the following: “You are doing well. You are halfway done.”

When the timer shows 2 minutes remaining, tell the patient the following: “Keep up the good work. You have only 2 minutes left.”
When the timer shows only 1 minute remaining, tell the patients: “You are doing well. You have only 1 minute to go.”Do not use other words of encouragement (or body language to speed up).

If the patient stops walking during the test and needs a rest, say this: “You can lean against the wall if you would like; then continue walking whenever you feel able.” Do not stop the timer. If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely.

When the timer is 15 seconds from completion, say this: “In a moment I’m going to tell you to stop. When I do, just stop right where you are and I will come to you.” When the timer rings (or buzzes), say this: “Stop!” Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a bean bag or a piece of tape on the floor.

10. Post-test: Record the post walk Borg dyspnoea and fatigue levels and ask this: “What, if anything, kept you from walking farther?”

11. If using a pulse oximeter, measure SpO2 and pulse rate from the oximeter and then remove the sensor.

12. Record the number of laps from the counter or tick marks on the worksheet.

13. Record the additional distance covered (the number of meters in the final partial lap) using the markers on the floor as distance guides. Calculate the total distance walked, rounding to the nearest meter, and record it on the worksheet.

14. Congratulate the patient on good effort and offer a drink of water.

APPENDIX

1. Modified Borg Dyspnoea and Fatigue Scale