

The relationship between chest X-ray findings, bacterial load and treatment-related outcomes in persons with extensively drug resistant TB

BY

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A. Protocol

A1. Study Title

The relationship between chest X-ray findings, bacterial load and treatment-related outcomes in persons with extensively drug resistant tuberculosis.

A2. Introduction, background and motivation

Multi-drug resistant tuberculosis (MDR-TB) was first recognized in the 1990s with an increase in caseload of eighty-two percent between 2000 and 2007 (1). Extensively drug-resistant tuberculosis (XDR-TB), a more difficult and more expensive form of TB to treat with poorer outcomes (1), emerged in South Africa in 2006. The prevalence of XDR-TB is likely to be underestimated in South Africa as a result of incomplete detection and notification. In peri-urban areas like Khayelitsha where there are high rates of HIV, TB and poverty, the prevalence of MDR-TB is estimated at 51/100 000 (2). A significant proportion of these cases are indeed undetected pre-XDR-TB (MDR and resistance to either a fluoroquinolone or a 2nd line injectable drug) and XDR (MDR and resistance to both fluoroquinolones and any one of the 2nd line injectable drugs) cases with inadequate access to drug sensitivity testing (2). Treatment outcomes of XDR-TB have been variable with countries like Peru showing a 60% overall cure (or completed treatment) rate, and studies in KwaZulu Natal in South Africa showing much poorer outcomes (3, 4).

The reasons for the poor outcomes in XDR-TB remain unclear. We are continuing to investigate the role of strain-type and several other factors including nutritional status, degree of drug resistance, HIV status and drug regimens in determining outcomes.

There is a paucity of literature describing the chest X-ray (CXR) findings in patients with XDR-TB, and whether disease extent is related to treatment outcomes and the evolution of resistance remains unclear. It has been shown that patients with radiological extensive drug-sensitive TB have higher initial sputum mycobacterial loads and take a longer time to sputum conversion than those without (5, 6). The extent of disease on the CXR at baseline has been used as a tool to inform and predict the need for infection control measures, treatment duration, and outcomes (7, 8).

The time-to-positivity (TTP) of mycobacterium tuberculosis in a liquid medium culture has become a validated indicator of bacterial sputum load (9, 10) and indeed a surrogate bio-marker of treatment response to anti-tuberculosis drugs. The relationships between mycobacterial sputum load, radiological disease and treatment outcomes have been studied in drug-sensitive TB (5, 8), but little is known about XDR-TB.

A3. Hypothesis

More extensive disease, and cavitary vs. non-cavitary disease, are associated with higher bacterial load and unfavorable outcomes in patients with XDR-TB i.e. poorer rates of culture conversion and higher mortality.

A4. Study Aim

- (i) To explore the relationship between the radiological features and treatment outcomes (culture conversion, mortality, and treatment failure) in patients with XDR-TB.
- (ii) To explore the relationship between drug susceptibility profiles and radiological features on CXR.

(iii) To study the relationship between measures of bacterial load (TTP and smear grade) and radiological features.

A5. Outcome Measures

(i) Cavitation and disease extent (cavitation vs. no cavitation; unilateral vs. bilateral; and total scores)

(ii) Time to culture conversion and mortality, number of previous drug-resistant TB episodes, and the number of drugs the isolate is resistant to.

(iii) Bacterial load (smear grade and TTP on MGIT culture)

A6. Study Design

This is a cross-sectional study with a follow-up component.

Characteristics of the study population

Patients admitted to BCH for XDR-TB treatment from October 2008 to October 2012.

Inclusion criteria: All patients with a first-time diagnosis of XDR-TB who completed at least six months of treatment. Missing data will not be included but will be noted.

Research procedures and data collection methods

The data collection will occur at Brooklyn Chest TB hospital. Chest x-rays will be analyzed for cavitation and extent of disease using a scoring system (see Appendix 2).

Patient folders will be examined for the relevant information on treatment-related

outcomes (these have already been performed as part of the ongoing clinical registry).

Smear status and TTP values at baseline will be recorded. The relationship between

disease extent and cavitation, and the evolution of resistance from pre-XDR to XDR-TB will be measured.

A7. Data Analysis

The frequency of the main radiological outcomes (e.g. number of zones, disease extent etc.) will be tabulated for each of the treatment-related outcome categories (e.g. mortality vs. no mortality). Other factors known to be associated with outcomes will also be tabulated (history of previous MDR-TB, total number of drugs the organism is resistant to, weight under 50kg, HIV status, CD4 count, and HAART usage). A multivariable analysis will be calculated for each outcome analysis (mortality and culture conversion). Data is to be extracted from folders and CXRs onto a hard copy CRF and then an Excel spreadsheet.

A8. Time Frame and Budget

Submission of research proposal: May 2012

Collection of data: June 2012 to April 2014

Data analysis: April 2014

Writing up and completion: May 2014

The current clinical registry is being funded through a NRF SARChI grant held by Prof. Dheda.

A9. Ethical Considerations and Reporting of Results

The study already has ethical approval as part of the ongoing prospective clinical registry. Results of the study will be submitted in fulfillment of a Masters of Medicine degree in Family Medicine at the University of Cape Town. The results of the study will be made available to all involved in the diagnosis and management of drug-resistant TB in Cape Town, and the National and Provincial Departments of Health. The findings of the study are expected to assist clinicians in providing better care for these and other patients. Once this study is completed, there is an intention to publish this study as a paper. The PI declares no conflict of interests

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A11. Appendix 2 (See Section D2.2 Appendices)

B. Literature review

B1. Introduction

The purpose of this literature review is to critically assess the current level of knowledge of chest radiography in drug-resistant TB and in particular extensively drug-resistant TB. To identify knowledge gaps in XDR-TB chest radiography and its relationship to sputum bacillary load and treatment-related outcomes (conversion; treatment failure and death).

A Medline Pubmed search was conducted with filters including “core journal”; “last 10 years”; “English”. Key search terms used included: “tuberculosis”; “chest radiography”; “cavitation”; “predictors”; “extensively drug-resistant “and “outcomes”

B2. CXR Findings in Tuberculosis

Chest radiography has been used in the diagnosis of TB for about a century. Today it remains an important tool alongside clinical and microbiological indicators in the management of TB. The chest X-ray (CXR) findings in drug-sensitive tuberculosis (DS-TB) are well documented. These may be categorised broadly into parenchymal disease (consolidation, cavitation, nodular and military patterns); pleural disease (thickening, calcification, effusion); and lymph node disease. Upper zone involvement with cavitation is the most common pattern seen in post primary disease in HIV uninfected patients. CXR findings in HIV infected patients are commonly bilateral, diffuse and involve the middle or lower zones (1). The CXR findings in drug resistant TB (with and without HIV co-infection) are not as clear.

Drug-resistant TB

There have been few studies comparing the CXR findings in DS-TB with those of drug-resistant tuberculosis (DR-TB). Some studies have reported more extensive disease in DR-TB (2, 3). In an HIV uninfected cohort of 68 patients with DR TB (2), significantly more cavities but fewer large nodules were found in patients with primary multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB compared with 141 patients with primary DS-TB. There was no difference found between the CXRs in MDR and XDR-TB. In a retrospective review of 78 chest CT scans comparing primary MDR-TB with primary DS-TB Yeom JA (3) et al found bilateral involvement; consolidation and multiple cavities on CT scan more frequently in primary MDR-TB than DS-TB.

Fishman JE (4) however, recorded similar radiographic findings for DS- and MDR-TB groups but more cavitation and consolidation in the acquired MDR group compared with the primary MDR group. Lessnau et al found no significant difference in chest radiography at presentation between DS and MDR-TB in 72 HIV infected patients residing in New York (5). In all these retrospective studies the time from infection to diagnosis (ie: the duration of active untreated disease) is a significant confounding variable. More rapid PCR-based diagnostics have reduced the time to diagnosis for MDR-TB to that of DS-TB and future studies may be able to answer this question more accurately.

Table 1. X-ray findings in patients with drug-resistant (DR) TB in comparison with DS-TB

	Setting	Sample size	HIV prevalence	Outcome data	Cavities	Consolidation	Large nodules
Cha J et al (2)	South Korea	n=68	None	No	DR>DS-TB		DR<DS-TB
Fishman et al (4)	Florida, USA	n=60	HIV and non-HIV	No	DR=DS-TB	DR=DS-TB	DR=DS-TB
Lessnau et al (5)	New York, USA	n=36	All HIV	No	DR=DS-TB	DR=DS-TB	DR=DS-TB
Brust et al (12)	South Africa	n=56 (DR-TB only)	High	Yes	39%	66%	Not described

HIV co-infection

CXR of patients with HIV tend to have more frequent lower lobe involvement, effusions and lymphadenopathy. There have been several studies which examine the differences in CXR findings in HIV infected and uninfected patients in the setting of DS-TB (6-9) but none in the context of DR-TB. In DS-TB it has been found that there is less consolidation and cavitation in HIV infected compared to uninfected patients and that patients with low CD4 counts are unlikely to have cavitation and may have normal chest x-rays (1, 10, 11).

In contrast to this: of a cohort of 56 patients with MDR-TB (88% HIV positive) in Tugela Ferry Kwa-Zulu Natal South Africa, most were found to have severe pathology (

a total of 39% with cavities and 62% with consolidation in 2 or more zones) on baseline CXR despite high rates of advanced immunodeficiency (12). The number of previous TB episodes, the time to diagnosis and time to treatment were not included here. Factors affecting host immunity such as nutrition and bacterial factors such as strain type are likely to play a role. These factors make it difficult to draw any conclusions between two different patient settings.

B3. Scoring Systems for Chest Radiography in Tuberculosis

Chest radiography is regularly used by clinicians to assist with diagnosis and monitoring of treatment in tuberculosis. Its usefulness, however, has been limited by poor specificity and high inter- and intra-observer variability. A simple standardised scoring system could assist in conjunction with other clinical parameters in case detection in an out-patient setting. It may be of value in predicting and monitoring response to treatment. In the setting of XDR-TB, where drug sensitivities are often not fully known and drugs generally less effective, a CXR scoring system may assist in tailoring individual regimens that are more likely to be successful.

In a systematic review of scoring systems for the diagnosis of TB (13) it was found that all the included scoring systems combined radiological and clinical data rather than using radiological data alone. This limits the versatility of the system for use in other settings such as XDR-TB prognostic studies. The majority of scoring systems were designed as screening tools to assess the likelihood of a TB diagnosis to inform infection control measures in a hospital setting. Most studies revealed a high sensitivity and a low specificity and have been in well resourced, low burden countries. Most scoring systems have not been validated in populations with a high prevalence of HIV co-infection.

Anna P Ralph et al (14) developed and validated a simple numerical scoring system for use in smear positive adults with DS TB to assess severity of disease and predict outcome. The outcome measure used was the 2 month smear status. The baseline CXR score was found to significantly predict the 2 month smear status and smear grade and the score was found to decrease over time with response to treatment.

The CXR reading and recording system (CRRS) is a purely radiographic tool that was developed for use in epidemiological surveys for TB (15). It was developed and validated in a high TB burden setting in Cape Town South Africa. The overall intra and inter-reader agreement was found to be satisfactory and was confirmed among culture proven HIV co-infected cases (16). CRRS was designed as an epidemiological prevalence tool rather than a tool for diagnosing TB or monitoring treatment response in culture proven TB. Pinto et al (13) developed a numerical scoring system based on CRRS and suitable for use in the out-patient setting. It was found to have a high negative predictive value (a good rule out test) for smear negative and smear positive patients clinically suspected of having tuberculosis. Furthermore this system has advantages over other scoring systems in three areas:

- Clinical data is not included in the score which allows it to be used by trained, non-clinical staff. This could make it more accessible as an adjunct to clinical tools and allows more versatility for use in a wider variety of settings including out-patient, hospital and research.
- It has a weighted scoring system accounting the most prevalent X-ray findings in TB.
- It appears to perform well in HIV infected patients (although this needs further testing in the field).

Reliability of scoring systems

None of the studies in a recent systematic review reported on the reliability of the scoring systems (17). In all the studies which have reported on reliability the intra- and inter-reader variability the range was from “fair” to “substantial” on variables such as consolidation or cavitation with most achieving a “moderate” score. Much lower levels of agreement were reached on variables such as “nodules” and “lymphadenopathy”.

In many of the studies the CXR readers have been radiologists or experienced clinicians. The CRRS explores the idea that trained readers need not be clinicians. Notably in the CRRS study (15) an open training session of 50 CXRs in which some agreement was achieved preceded the study group analysis. Relatively higher levels of agreement were achieved with this system.

B4. The CXR for Predicting Response to Treatment (smear or culture conversion) and Outcomes (cure, death and treatment failure)

There are a number of studies which examine the relationship between X-ray findings at baseline and smear or culture conversion in DS-TB (14, 18, 19). It has been found that cavity and extent of disease on baseline CXR are associated with longer times to smear and culture conversion.(20) Most studies are in HIV uninfected patients or study populations with a low prevalence of HIV. This relationship is not as clear for HIV infected patients.

There are few studies examining the baseline CXR of drug-resistant TB in relation to outcomes and fewer still in DR-TB populations with high HIV co-infection rates. In a study of 167 patients in Latvia with MDR-TB (21) bilateral cavitation, a history of previous MDR-TB and a high baseline bacterial load were found to be independent predictors of a longer conversion time. The prevalence of HIV in this study was not commented on, however.

Brust et al (12) reported no relationship between cavitation or consolidation and conversion time (after adjusting for smear status) in their study of 56 patients with MDR-TB (88% HIV co-infected). Here the only independent predictor of culture conversion was baseline smear status. The authors concluded, however, that smear status may have mediated the effect of cavitation on culture conversion.

In a study in South Korea of 176 XDR-TB patients (22) (HIV uninfected) the previous use of second line drugs, linezolid, surgical resection and baseline cavitary disease were found to be independent predictors of a poor outcome (death, failure and default) on multivariate analysis.

Table 2. X-ray analysis and scoring approaches in relation to TB treatment outcomes

	Resistance pattern	Outcome indicators	HIV prevalence	Radiological features (associated outcome)	Numerical score
Holtz et al (21)	MDR	Culture conversion	Unknown	Bilateral cavitation (delayed conversion)	No
Brust et al (12)	MDR	Culture conversion	88%	Cavitation. Consolidation (score not associated with delayed conversion) 6 zones Bilateral lymphadeopathy	Yes
Jeon D.S. et al (22)	XDR	WHO outcomes	0%	Bilateral cavitation (poor outcome) Bilateral disease	No
Hesseling et al (20)	DS-TB	2 month smear and culture	0%	Extensive disease (Y/N) Cavity and no. of cavities (culture conversion at 2 months)	No
Ozsahin et al (18)	DS-TB	2 month smear and culture	Unknown	Cavitation. Consolidation (2 month smear negative status) 6 zones	No
Ralph et al (14)	DS-TB	2 month smear	13%	Disease extent Cavitation (Y/N) (Score predicts negative 2 month smear status)	Yes
Visser et al (19)	DS-TB	Culture conversion	10%	Cavities (delayed culture conversion) 6 zones	No

Culture conversion in relation to outcomes

Culture conversion and time to culture conversion is recognised as an early indicator for treatment outcome in tuberculosis. In a cohort of 167 patients with MDR-TB the median baseline sputum culture conversion time was 48 days among those who had a favourable outcome and 169 days among those with a poor outcome ($P < 0.001$) (21). Delayed culture conversion has also been associated with an increased risk of relapse in some studies (20). In a recent prospective cohort of 107 patients with XDR-TB (23), time to conversion was found not to be associated with a favourable outcome. However, net culture conversion (in a series of conversions and reversions) was found to be independently predict survival. Time to culture conversion may be a less useful indicator of outcome in settings of high levels of drug-resistance and poor treatment response rates.

There are limitations to conversion as an early surrogate indicator of outcome. In a systematic review Horne et al (24) found low sensitivity and only moderate specificity for smear and culture conversion as predictors of relapse or failure. The studies included here were predominantly of patients with DS-TB and the indicator used was the smear and culture status at 2 months.

B5. Chest Radiography and Sputum Bacterial Load

The time-to-positivity (TTP) of mycobacterium tuberculosis in a liquid medium is a more sensitive indicator of bacterial load than smear status (25). It has become a

validated indicator of bacterial sputum load (26) and has been found to be a viable alternative to CFU (colony forming units) on solid media (27). Patients with more extensive disease and cavitation on CXR are known to have higher bacillary burdens and shorter TTPs (14, 28). In general, HIV infected patients have paucibacillary disease with longer TTPs and less disease and cavitation on CXR (10)

Bacterial load in relation to outcomes

In a study of 263 HIV uninfected, previously untreated, smear positive patients (20) culture conversion at 2 months was found to be associated with baseline extent of disease; cavities; smear grade and a baseline TTP>3 days. In multivariate analyses baseline TTP≤3 days and an extensive disease pattern on CXR were found to predict a delayed 2 month culture conversion, while a baseline TTP≤3 days also independently predicted relapse and recurrence of disease.

In a small retrospective study, Epstein et al (25) looked at 26 consecutive patients with active TB and found serial TTPs to correlate well with treatment response and to be a valuable and more sensitive prognostic indicator than serial microscopy. In a study of 167 patients in Latvia with MDR-TB (21) bilateral cavitation, a history of previous MDR-TB and a high baseline bacterial load (as measured by CFU count) were found to be an independent predictors of a longer conversion time.

B6. Needs for Further Research

Chest radiography remains a useful tool in the management of tuberculosis. There is, however, a need for further comparative descriptive studies in multidrug-resistant TB

with and without HIV co-infection. There is a knowledge gap in chest radiography in XDR-TB and XDR-TB HIV co-infection. The role of the CXR in predicting bacterial load (smear and TTP) and response to treatment in XDR-TB has not been described. The relevance of baseline and sequential TTP in predicting and monitoring response to treatment in MDR and XDR-TB needs to be clarified.

There is a lack of standardisation of a simple, valid and reliable scoring system for CXRs in TB in high prevalent settings. A purely radiographic, numerical scoring system is relevant for future clinical research. The CRRS based numerical scoring system shows promise in this area and needs further validation studies in different settings. It may also prove to be very valuable in both the detection of cases and the management of DR-TB.

Future prognostic studies in XDR-TB should quantify and consider the extent of disease on baseline and sequential chest radiography.

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C. Article. Publication-ready format

Mini-Citation

Julian te Riele

Thesis Title: The relationship between baseline chest X-ray findings, treatment-related outcomes, and sputum bacterial load in persons with extensively drug-resistant tuberculosis (XDR-TB).

For the degree of MMED Family Medicine

Julian te Riele has an MBBCh degree from the University of the Witwatersrand. His thesis emerged as a result of his interest in drug-resistant tuberculosis.

His thesis reports a relationship between radiological parameters, bacterial load, and clinical outcome in patients with extensively drug-resistant TB. The roles of quantitative chest radiography in the management of XDR-TB and in future prognostic studies are highlighted.

Supervisor: (Prof K. Dheda) (Department of Medicine)

Co-supervisor: (Dr Grant Theron)

C.1 Title page

Relationship between chest radiography, bacterial load, and outcomes in XDR-TB

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Take-home message: Radiological features may inform clinical decision-making and prognosis in XDR-TB

C.2 Abstract

Background: The relationship between chest radiography, sputum bacillary load, and treatment outcome in patients with extensively drug-resistant tuberculosis (XDR-TB) has, hitherto, not been evaluated in TB and HIV endemic settings.

Methods: Chest X-rays (CXRs) at diagnosis from 97 XDR-TB patients from South Africa were scored by 2 observers and findings correlated with sputum load at diagnosis (smear grade and culture time-to-positivity) and clinical outcomes (culture conversion and all-cause mortality).

Results: 75/97(77%) had bilateral disease and 69/97(71%) had parenchymal involvement of $\geq 50\%$ in extent. There was an association between the total radiographic disease score and risk of death [HR1.16 (1.05-1.28) $p=0.003$]. The total disease and cavitation score were inversely associated with culture conversion ($p<0.0001$ and $p = 0.0047$, respectively) and time-to-conversion [HR0.85 (0.74-0.97) $p=0.02$]. The intraclass correlation coefficient for two CXR readers was 71.4% for the total score. Cavity and total scores were significantly lower in HIV-infected persons and did not correlate with outcome or bacterial load.

Conclusions: Radiological disease extent and cavitatory scores correlated with outcomes and the latter correlated with bacterial load. Thus, quantitative radiographic scores may inform clinical decision-making and prognosis, and should be taken into account when determining independent prognostic biomarkers in future studies of XDR-TB.

C.3 Introduction

Tuberculosis remains a global health problem. Drug-resistant strains of TB have poorer outcomes, are extremely costly to treat [1] and threaten the viability of already overburdened national TB programs. According to the WHO there has been a 42% increase in patients eligible for multi-drug resistant (MDR-TB) treatment since 2011 [2]. In South Africa, more than 8000 cases of MDR-TB were identified in 2012 [2]. There is a growing epidemic of XDR-TB - defined as MDR-TB with resistance to a fluoroquinolone and either capreomycin, amikacin, or kanamycin [3]. It is estimated that 9.6% of all MDR-TB patients have XDR-TB [2]. Outcomes for patients with XDR-TB are extremely poor [4]. In a prospective cohort of 107 patients with XDR-TB Pietersen et al [5] reported a mortality of 78% and a favourable outcome of 11% at 60 months from diagnosis. Thus, targeting more aggressive regimens at those with the worst prognosis is important.

Chest X-rays (CXRs) have been used in the diagnosis of tuberculosis for about a century [6]. It is now used in screening, diagnosing and monitoring response to treatment. Despite being limited by poor specificity and high inter-observer variability it remains a useful adjunct to clinical and microbiological tools. Several CXR scoring systems have been developed to quantify disease and some have shown the potential to limit observer variability and increase the clinical value of the chest radiograph in TB [7, 8].

The extent of disease and cavities on baseline chest radiography are known to be associated with an increased sputum bacterial load [8-10], delayed smear and culture conversion [8, 11-13] and an increased risk of treatment failure [13, 14] in drug sensitive TB. Some authors have found cavities on the baseline chest radiograph in MDR-TB to be an independent predictor of bacterial load, longer conversion times [15] and poor outcomes [16] though relatively little has been reported in MDR-TB-HIV co-infected patients [17-18].

There have been very few studies describing the chest radiograph in XDR-TB [19] or relating chest radiograph findings to outcomes [16] and none from TB endemic countries where the diagnosis is often delayed, and previous TB and other respiratory insults including HIV co-infection, exposure to mining dusts and biomass fuel exposure are common. Thus, radiographic findings and their prognostic value may differ considerably in such settings. Surprisingly, given the burden of XDR-TB in countries like South Africa there are no published studies evaluating the relationship between baseline chest radiography and bacterial load and outcomes.

To address this issue we evaluated radiographic scores in a prospectively recruited XDR-TB cohort in Cape Town, South Africa.

C.4 Methods

Setting

In this prospective cohort study baseline chest radiographs of patients admitted to Brooklyn Chest TB Hospital (BCH) with XDR-TB between October 2008 and June 2012 were evaluated. Brooklyn Chest Hospital is the primary TB referral hospital in the Western Cape and is situated in Cape Town. Most patients were managed as in-patients for the duration of the intensive (or injectable) phase of treatment and most remain in-patients for longer than 6 months due to low conversion rates and poor treatment response [5, 20].

XDR-TB treatment regimen and outcomes

Patients received a standard regimen containing Capreomycin and PAS with a combination of some Group 5 drugs added at the discretion of the treating doctor [21]. Linezolid was not accessible. TB medication administered was directly observed by the ward staff. Side effects and co-morbidities were actively managed by doctors experienced in managing TB. Patients were seen regularly by adherence counsellors, social workers and occupational therapists while in hospital. Audiology screening for drug induced ototoxicity and chest radiography was done every two months. Treatment outcomes were assigned at censor date (31st October 2013) and regarded as favourable (cure or treatment completion) or unfavourable (default; treatment failure and death). Definitions were based on Laserson's definitions of 2005 [22]. (Appendix 1)

Ethics Statement

Ethics approval was obtained as a sub-study from the human research ethics committees at the University of Cape Town.

Study Population

All included patients had isolates that were resistant to Rifampacin and Isoniazid. They were also resistant to Amikacin and Ofloxacin on phenotypic testing. A sub-group of 26 of the 97 patients included in the study had extended drug sensitivity testing (DST) with a range of 4 to 13 drugs to which the isolate was resistant.

Although the cohort was prospectively enrolled at Brooklyn Chest Hospital and basic radiographic information was captured at diagnosis (e.g. unilateral vs bilateral disease and presence or absence of cavitation) the detailed radiographic evaluation was only conducted towards the end of the parent study, which recruited 222 patients with XDR-TB. However, at the time of detailed radiographic evaluation 109 patients had already been transferred out of the facility to peripheral clinics and other hospitals. Thus, only 113 patient CXRs were available for detailed analysis. However, a sensitivity analysis did not show any significant differences between the study group of 97 patients and the total group of 222 patients in respect of age, gender, HIV status, and CD4 count.

Chest radiographs dated closest to the date of diagnosis (sputum DST showing XDR-TB) were chosen and those dated more than 3 months from the date of diagnosis were excluded (n=13). A further 3 CXRs were unreadable according to Chest Radiograph Recording and Reporting (CRRS) criteria (23). Thus, 97 CXRs were included in the analysis.

Chest radiograph analysis

For the sake of external validity and generalisability the 97 chest radiographs were analysed independently by two non-specialist medical doctors. Prior to the study, both readers were trained by two specialist pulmonologists experienced in TB diagnosis using over 20 chest radiographs of patients with drug-resistant TB. The readers also attended a two day CRRS radiographic reading [23] course prior to the analysis. Criteria for cavities and disease extent were agreed before independently analysing the study radiographs. To delineate zonal borders two horizontal lines were measured at the lower border of the 2nd and 5th anterior costochondral junctions thus dividing the lungs into 6 zones. Each of the 6 zones and was given a numerical score for the extent of disease (Disease Score) and for the number and size of the cavities (Cavity Score). Any parenchymal or pleural abnormalities were regarded as diseased and the area involved was estimated as more than or less than 50% of the total area. Cavities were declared where there was a clearly visible ring opacity of greater than 50% of the total circumference. Cavities less than 1 cm were not counted. Only the zone containing the majority of each cavity was selected. A “Total Score” was derived by the sum of the total disease and total cavitation scores for all 6 zones (Appendix 2).

Statistical analysis

Selected factors (history of previous multi-drug-resistant, total number of drugs the organism is resistant to, weight under 50kg, HIV status and CD4 count) known to be associated with outcomes (mortality and culture conversion) from previously published research were tested for association in this cohort using Wilcoxon-rank sum (for continuous variables) and Fisher’s exact test (for discrete variables). Anti-retroviral

therapy was not considered as all but 2 HIV-infected patients were on ARV treatment at the time of XDR-TB diagnosis. Univariate and multivariable logistic regression was applied, with variables carried forward to the multivariable case if $p < 0.1$ in the univariate analysis. Culture conversion was assessed via a non-parametric competing risk model with death as the competing risk.

C.5 Results

Patient demographic and clinical outcomes

Of the 97 patients 63 (65%) were male. The median (IQR) body weight was 50 (46-59) kg. 53/97 (55%) had a previous history of drug-resistant TB. 44/97 (45%) were HIV infected with a median (IQR) CD4 of 132 (74.5- 301.5) cells/ml³ (Table 1.). The overall mortality rate was 64/97 (66%) for the whole cohort and 31/44 (71%) HIV infected and 33/53 (62%) uninfected groups $p=0.53$ (Table 2). Treatment outcomes were poor: 86/97 (89%) had an unfavourable outcome (Table 1). Of these, 7/86 (8%) defaulted, 40/86 (47%) failed treatment and 39/86 (45%) died by censor date. Only 4 (4%) patients were cured.

Chest radiograph findings

Patients had extensive disease on chest radiograph at diagnosis. 75/97 (77%) had bilateral disease and 55/97 (57%) had cavitation (Table 2.). Patients with a history of previous drug-resistant-TB were more likely to have bilateral disease ($p=0.026$) but there was no significant difference in disease ($p=0.68$) or cavity scores ($p=0.42$). In the sub-group of 26 patients with extended DST we did not find an association between the number of drugs the isolate was resistant to and the baseline X-ray scores using Kendall's tau test ($p=0.78$ ($\text{tau}=-0.043$) cavity and $p=0.87$ ($\text{tau}=0.024$) disease).

Disease extent and cavitation scores were lower for the HIV infected group: Median total score 7 (4-11) infected and 9 (6-12) uninfected ($p=0.02$) and median (IQR) cavity scores 0(0-0.9) infected and 1(0-2) uninfected ($p<0.0001$) (Table 2). 2 of the chest radiographs that were recorded as normal were both of HIV infected patients with CD4 counts less than $200 \times 10^6/l$.

Chest radiographs related to conversion and mortality

Patients who achieved culture conversion had lower total scores (disease plus cavitation scores): Median (IQR) total score 5 (4-7) conversion and 10 (6.38-12) no conversion ($p<0.0001$). The total score on chest radiograph was found to be inversely associated with conversion and time to conversion on multivariate analysis in the presence of death as a competing risk factor (HR=0.85 (0.74-0.97) $p=0.02$) (Table 4.) In the HIV subgroup the cavity and total scores did not independently predict the likelihood of culture conversion. The total score was also found to independently predict the risk of death (HR=1.16 (1.05-1.28) $p=0.003$) (Table 5).

Chest radiographs related to bacterial load

Smear status was not significantly associated with disease ($p=0.23$) or cavity scores ($p=0.76$). Disease and cavity scores were however associated with time-to-positivity (TTP) (both $p < 0.0001$). There was a correlation found between disease score and TTP ($p=0.071$), and between cavity score and TTP ($p=0.038$), however chest radiograph scores were not found to predict TTP (Fig.2).

The Pearson intraclass coefficient (ICC) for reliability between two readers was 0.34 (0.18 to 0.48) for cavity score - “moderate” correlation and 0.714 (0.62 to 0.79) for disease score - “strong” correlation.

C.6 Discussion

Summary of main findings

In this study total score (disease plus cavitation scores) strongly predicted the risk of death (HR=1.16 (1.05-1.28) p=0.003) (Table 5). Cavitation and the extent of disease on baseline chest radiography were found to be directly related to bacterial load at diagnosis. Both cavity and total scores were significantly lower in the HIV-infected group and did not independently predict the likelihood of culture conversion or death in this sub-group. We have demonstrated the value of a valid and reliable scoring system in XDR-TB for both clinical and research purposes. Our study supports the current practice of using the chest radiograph as a tool for clinical management including selection of more aggressive regimens and to prognosticate in patients with XDR-TB. Interestingly, hardly any published data on XDR-TB takes into account radiographic scores when computing independent prognostic variables and biomarkers. These are important as there are few drugs to treat XDR-TB and the precise time of cure (culture negativity) is often difficult to establish. Thus, erroneous information may be obtained if radiographic scores are omitted from multivariable analyses performed in cohorts of XDR-TB.

Treatment outcomes for this group of patients have been poor with high rates of death and treatment failure [5]. 89% of our patients had died or failed treatment at the censor date. In view of the extremely poor mortality outcomes (thus impossible to sensibly compare groups) conversion and time to conversion were used as surrogate markers of treatment response. Conversion has been recognised as an early though imperfect indicator of treatment response [5, 15]. Indeed, we found initial culture conversion to independently predict the risk of death for both HIV infected and uninfected patients (Table 5.).

Cavitatory disease on chest radiograph was first shown to be an independent predictor of time to culture conversion in 167 patients with MDR-TB [15] Our findings are similar with total disease score (disease plus cavitation scores) predicting culture conversion and time to culture conversion on multivariate analysis with death as a competing risk factor (Table 4). We have also found a previous history of drug-resistant TB [15] to independently predict the likelihood of culture conversion. In a study by Jeon et al [16] cavitation was shown to be an independent risk factor for poor outcomes in HIV un-infected patients with XDR-TB. Supporting this we found total score (disease plus cavity scores) to strongly predict the risk of death (Table 5.) in HIV uninfected but not HIV-infected persons (in the latter cavitation did not predict bacterial load). These observations in HIV infected persons is likely related to the low bacterial load in such patients, modulation of HIV on radiographic findings, and the substantial interaction between other factors such as CD4 count and mortality.

The TTP of *Mycobacterium tuberculosis* in a liquid medium is a more sensitive indicator of bacterial load than smear status [24]. Patients with more extensive disease and cavitation on chest radiograph are known to have higher bacillary burdens and shorter TTPs [8, 9]. In this study we have shown an inverse relationship between disease and cavity scores and TTP (Fig. 2). Patients who experienced culture conversion had significantly longer TTP's at diagnosis [median (IQR) 18 (13-22) in converters versus 14 (10-21) non-converters $p=0.03$; Table. 1]. However, TTP did not independently predict culture conversion in the multivariate analysis. TTP is likely to be a less powerful indicator of treatment response in XDR-TB than in the drug sensitive [13] or MDR-TB group [15]. Reasons for this may include poor treatment response rates (thus higher bacterial load) and greater pre-existing lung damage in XDR TB (delay in the diagnosis).

HIV-infected patients with drug sensitive TB are known to have less immunopathology and cavitation on the chest radiograph [25-27]. In this study of we have found lower chest radiograph scores in the HIV infected sub-group (Table 2). Despite advanced immune deficiency patients had advanced disease on chest radiograph at the time of diagnosis. This may reflect disease chronicity. In Tugela Ferry, KwaZulu Natal, Brust et al also found advanced radiological disease at diagnosis in MDR-TB (88% HIV-infected) [17]. Factors that may account for this finding include more previous TB in patients with drug resistant-TB and delayed diagnosis. We found no association between baseline chest radiography and conversion or mortality in the HIV sub-group. This suggests that chest radiograph analysis in the context of HIV infected XDR-TB patients may have limited prognostic value. HIV co-infection may cause higher death rates related to disseminated TB and non-TB related causes, which may affect the

impact of the chest radiograph. These causes may be amplified by delayed diagnosis in paucibacillary TB.

We highlight the importance of quantitative radiological analysis as a factor in predicting treatment response and prognosis in XDR-TB. In a recent systematic review it was found that most scoring systems combine clinical and radiological data to inform respiratory isolation in a hospital setting [28]. There is a need for a standardized radiological scoring system that is suitable for use in HIV infected and uninfected patients. We used a simple zonal scoring system that generates a numerical score and found it to be reliable for 2 readers. Since starting this study Pinto et al [7] developed a numerical scoring system based on CRRS [23] which was found to have a high negative predictive value (a good rule out test) for smear negative; smear positive and HIV-infected patients clinically suspected of having tuberculosis. This has promise as a more comprehensive scoring system for use in clinical out-patient settings and in future research.

The limitations identified in this study include the limited sample size as many patients had already been transferred out. Thus, the majority of patients who had been transferred out to clinics did not have accessible radiographs. It is possible therefore that the study group included a greater proportion of patients with more advanced disease. A sensitivity analysis did not however reveal any significant differences between the study group and the main cohort in respect of age; gender; HIV status or CD4 count. Furthermore, the sample size is still substantial for an XDR-TB-related study.

A small number of patients (n=26) had extended drug sensitivity available and the lack of association between the number of drugs to which the isolates were resistant and the baseline X-ray scores should be interpreted with caution.

Conversion and time to conversion has limitations in predicting response to treatment and treatment-related outcomes [29]. We considered “initial” conversion in our study whereas “net conversion” (in a series of culture conversions and reversions) may be a more powerful indicator of treatment response [5].

In conclusion we have shown that quantitative baseline chest radiography analysis in XDR-TB is independently associated with treatment response (culture conversion) and survival. We have also demonstrated a relationship between cavity and disease extent on chest radiograph and bacterial load (TTP). Thus, quantitative chest radiography remains relevant in the clinical management of HIV-uninfected patients with XDR-TB as it portends prognosis and informs the selection of more robust regimens when considering the cost-benefit and toxicity-harm analysis of an individualised regimen. Our findings also underscore the precept that radiological scores should be used to accurately compute the relative importance of prognostic biomarkers for XDR-TB. This has, hitherto, not been undertaken in published studies.

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Word counts:

Title: 78 characters/spaces

Abstract: 198 words

Article: 3217 words

Tables: 5

Figures: 2

References: 29

Figure 1. Flow diagram

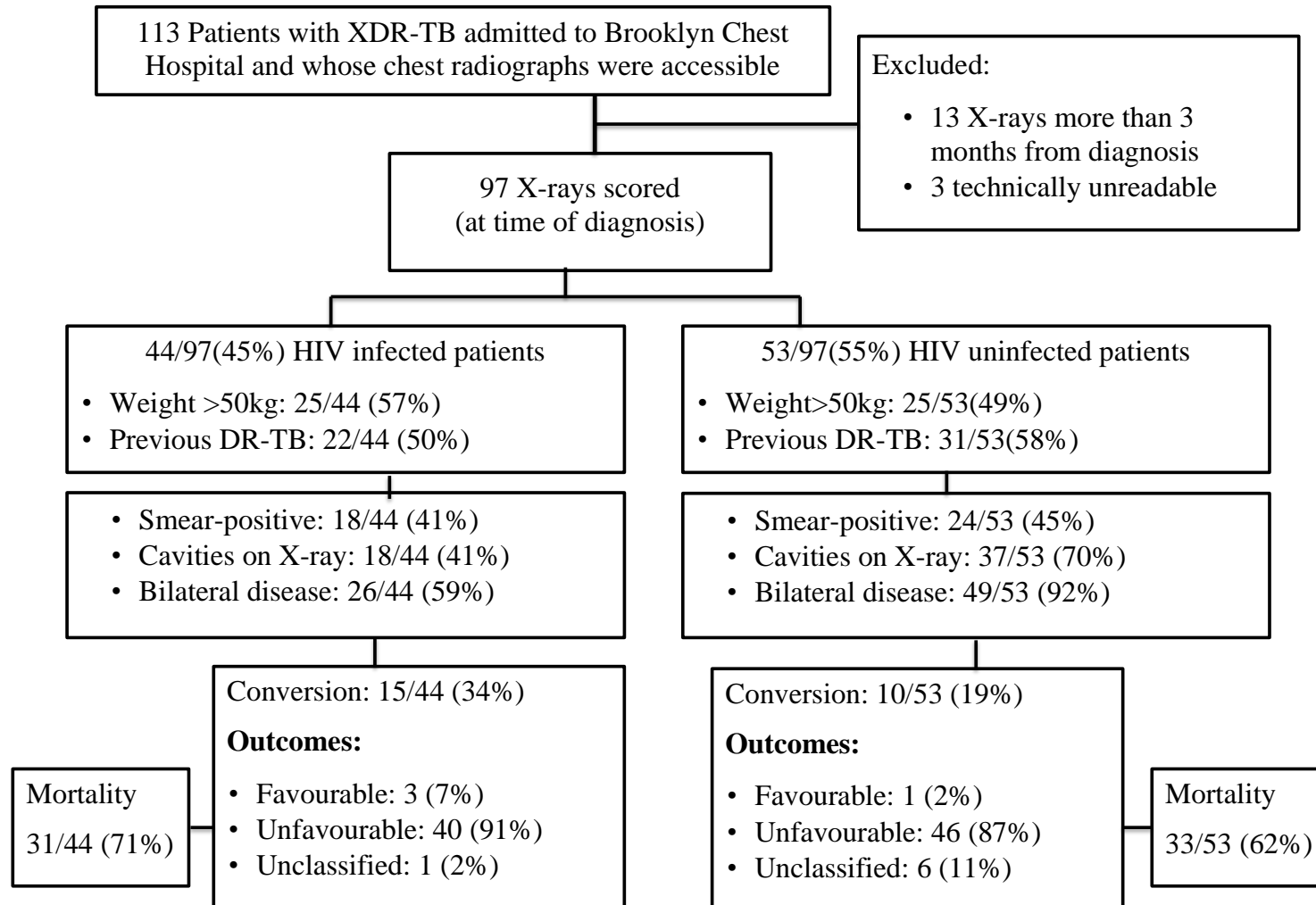


Figure 2. Scatter plot of chest radiograph scores (cavity, disease, total) and time-to-positivity (days) in patients with XDR-TB.

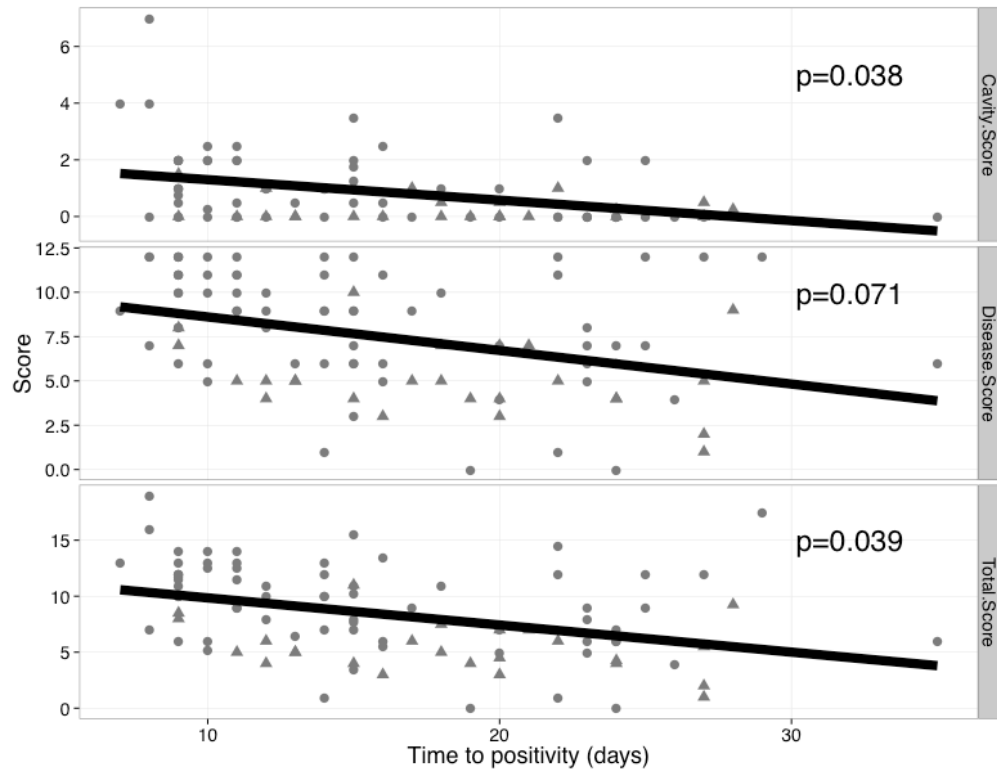


Table 1. Clinical and radiographic characteristics of the cohort according to outcomes

CLINICAL	Total	Alive	Deceased	P value	Conversion	No Conversion	P value
	n=97	n=33/97 (34%)	n=64/97 (66%)		n=25/97 (26%)	n=72/97 (74%)	
Age years (median, IQR)	34 (27-42)	34 (27-37)	35 (27-45)	0.25	36 (29-41)	34 (25-43)	0.51
Gender male	63/97 (65%)	23/33 (70%)	40/64 (62%)	0.63	17/25 (68%)	46/72 (64%)	0.9
Weight kg (median, IQR)	50.3 (46-59)	53.2 (48-60)	50 (44-58)	0.05	55.25 (52-63)	49.7 (45-57)	0.01
Diabetes	9/97 (9%)	4/33 (12%)	5/64 (8%)	0.75	3/25 (12%)	6/72 (8%)	0.89
HIV-infected positive	44/97(45%)	13/33(39%)	31/64 (48%)	0.53	15/25 (60%)	29/72 (40%)	0.14
CD4* 10 ⁶ /l (median, IQR)	123 (70-266)	122 (65-264)	123 (72-266)	0.91	119 (58-207)	124 (92-306)	0.34
Previous DR-TB	53/97 (55%)	15/33 (45%)	38/64 (59%)	0.28	9/25 (36%)	44/72 (61%)	0.05
Number of drugs resistant(median, IQR)	11 (8-12)	11 (9-12)	11 (8-12)	1	11.5 (11-12)	10 (8-12)	0.34
Smear-positive	42/97 (43%)	10/33 (30%)	32/64 (50%)	0.03	5/25(20%)	37/72 (51%)	0.02
Culture conversion	25/97 (26%)	20/33 (61%)	5/64 (8%)	< 0.0001			
Time-to-positivity (TTP) days (median, IQR)	15 (11-22)	16 (13-22)	14 (10-21)	0.08	18 (13-22)	14 (10-21)	0.03
Final treatment outcome (FTO) unfavourable	77/97(79%)	22/33(67%)	55/64(85%)	0.03			
Alive					20/25 (80%)	13/72 (18%)	< 0.0001
Died					5/25(20%)	59/72 (82%)	< 0.0001
X-RAY							
Bilateral disease	75/97 (77%)	22/33 (67%)	53/64 (83%)	0.06	14/25 (56%)	61/72 (85%)	< 0.0001
Cavity Score(median, IQR)	0.5 (0-1)	0.5 (0-1)	0.5 (0-1.12)	0.76	0 (0-0.5)	1 (0-2)	< 0.0001
Disease Score(median, IQR)	7 (5-10)	5 (5-7)	9 (6-11)	< 0.0001	5 (4-7)	9 (6-11)	< 0.0001
Total Score*(median, IQR)	8 (5.5-11.75)	6 (5-8.5)	10 (6-12)	< 0.0001	5 (4-7)	10 (6.38-12)	< 0.0001

Data are number(%)unless otherwise indicated. *Total score = disease score plus cavitation score

Table 2. Clinical and radiological characteristics of the cohort according to HIV status

CLINICAL	Total n=97	HIV infected n=44/97(45%)	HIV non-infected n=53(55%)	P value
Age years (median, IQR)	34 (27- 42)	36 (32- 41)	33 (24-45)	0.17
Gender male	63/97 (65%)	24/44 (55%)	39/53 (74%)	0.08
Weight kg (median, IQR)	50 (46-59)	54 (46-63)	50 (46-55)	0.22
Diabetes	9/97(9%)	6/44 (14%)	3/53(6%)	0.32
CD4* 106/l (median, IQR)				
Previous DR-TB	53/97 (55%)	22/44 (50%)	31/53 (59%)	0.53
Number of drugs resistant(median, IQR)	11(8-12)	11.5 (11-12)	9.5 (8-12)	0.15
Smear-positive	42/97 (43%)	18/44 (41%)	24/53 (45%)	0.78
Culture conversion	25/97 (26%)	15/44 (34%)	10/53 (19%)	0.14
Time-to-positivity (TTP) days (median, IQR)	15 (11-22)	15 (11-23)	15 (11-20)	0.54
Final treatment outcome (FTO) unfavourable	86/97 (89%)	40/44 (91%)	46/53 (87%)	0.12
Alive	33/97(34%)	13/44 (30%)	20/53 (38%)	0.53
Died	64/97 (66%)	31/44 (71%)	33/53(62%)	0.53
X-RAY				
Bilateral disease	75/97 (77%)	26/44 (59%)	49/53 (93%)	< 0.0001
Cavity Score (median, IQR)	0.5 (0-1)	0 (0-0.9)	1 (0-2)	< 0.0001
Disease Score(median, IQR)	7 (5-10)	6 (4-10)	8 (6-10)	0.08
Total Score*(median, IQR)	8 (6- 12)	7 (4-11)	9 (6-12)	0.02

Data are median (IQR) or n (%), unless otherwise stated. *Total score = disease score plus cavitation score

Table 3. Univariate Cox proportional hazards regression model of clinical and radiographic factors associated with risk of death for all patients given treatment for XDR-TB and in HIV-infected patients only.

	FULL COHORT		HIV COHORT	
	HR**(95%CI)	pvalue	HR**(95%CI)	pvalue
Age (years)	1.01 (0.99-1.04)	0.24	0.98 (0.93-1.02)	0.3
Gender male	0.93 (0.56- 1.55)	0.79	1.45 (0.70-3.01)	0.31
Weight (kg)	0.97 (0.95- 0.997)	0.03	0.99 (0.95- 1.02)	0.34
Diabetes	0.67 (0.27- 1.68)	0.39	0.29 (0.07- 1.24)	0.1
HIV-infected positive	1.38 (0.84- 2.27)	0.2		
CD4 (*106/L)	0.999 (0.997-1.00)	0.63	0.999 (0.997- 1.00)	0.63
Previous DR-TB	1.35 (0.82- 2.23)	0.24	3.13 (1.43- 6.83)	0.004
Previous MDR-TB	1.05 (0.63- 1.73)	0.9	2.43 (1.14- 5.20)	0.02
Previous Pre XDR-TB	1.15 (0.66- 2.00)	0.63	1.87 (0.86- 4.05)	0.11
Number of drugs resistant	1.00 (0.81- 1.25)	0.99	0.96 (0.61- 1.52)	0.86
Smear positive	1.96 (1.11- 3.46)	0.02	2.28 (0.96- 5.43)	0.062
Smear no data	1.71 (0.84- 3.47)	0.14	1.77 (0.66- 4.74)	0.259
Culture conversion	0.11 (0.05- 0.28)	< 0.0001	0.10 (0.03- 0.32)	< 0.0001
TTP(days)	0.96 (0.92- 1.00)	0.07	0.97 (0.92- 1.03)	0.35
FTO Unfavourable	1.3 (0.40-4.21)	0.66	1.15 (0.15- 8.60)	0.89
FTO Unclassified	0.49 (0.12- 1.98)	0.32	0.79 (0.09- 6.84)	0.83
Diagnosis to treatment (days)	0.998 (0.997- 1.00)	0.05	0.999 (0.996- 1.00)	0.24
Diagnosis to x-ray(days)	0.996 (0.988- 1.00)	0.27	0.99 (0.98- 1.00)	0.09
X-RAY				
Unilateral disease	0.52 (0.26- 1.05)	0.07	0.5 (0.22- 1.14)	0.1
Clear x-ray	6.61 (1.52-28.79)	0.01	4.32 (0.93- 19.99)	0.06
Cavity score	1.26 (0.99- 1.6)	0.06	1.48 (0.94- 2.35)	0.09
Disease score	1.24 (1.13- 1.36)	< 0.0001	1.21 (1.07- 1.37)	0.002
Total score*	1.18 (1.10- 1.28)	< 0.0001	1.18 (1.07- 1.31)	0.001

*Total score = disease score plus cavitation score

Table 4. Multivariate Cox proportional hazards model of clinical and radiographic factors associated with culture conversion in the presence of the competing risk of death.

FULL COHORT		
	HR**(95%CI)	P value
Age (years)	0.99(0.95-1.04)	0.79
Weight (kg)	1.03(0.99-1.07)	0.15
Diabetes	0.65(0.14-2.98)	0.58
HIV-infected	1.33(0.50-3.50)	0.57
Previous DR-TB	0.36(0.15-0.91)	0.03
X-RAY		
Total Score*	0.85(0.74-0.97)	0.02

*Total score = disease score plus cavitation score

Table 5. Multivariate Cox proportional hazards model of clinical and radiographic factors associated with risk of death for all patients given treatment for extensively drug-resistant tuberculosis, and in HIV-infected patients only.

	FULL COHORT		HIV COHORT	
	HR**(95%CI)	P value	HR**(95%CI)	P value
Weight (kg)	0.98(0.95-1.01)	0.13	–	–
Diabetes	–	–	0.23(0.05-1.02)	0.05
Previous MDR-TB	–	–	1.89(0.80-4.45)	0.15
		<		
Culture conversion	0.11(0.03-0.37)	0.0001	0.21(0.05-0.93)	0.04
TTP(days)	0.99(0.95-1.04)	0.79	–	–
Diagnosis to treatment(days)	1.00(1.00-1.00)	0.67	0.99(0.98-1.01)	0.31
X-RAY				
Unilateral disease	2.05(0.82-5.14)	0.13	1.26(0.34-4.67)	0.73
Disease score	–	–	1.19(0.95-1.48)	0.13
Total score*	1.16(1.05-1.28)	0.003	–	–

*Total score = disease score plus cavitation score

D. Appendices

D1. Ethical approvals



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • Facsimile [021] 406 6411
Email: Sumayah.arietdien@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

30 May 2014

HREC/REF: 351/2014

Prof K Dheda
Lung Infection & Immunity Unit
H46.41
OMB

Dear Prof Dheda

Project Title: THE RELATIONSHIP BETWEEN CHEST RADIOGRAPHIC FINDINGS, BACTERIAL LOAD AND TREATMENT-RELATED OUTCOMES IN PERSONS WITH EXTENSIVELY DRUG RESISTANT TUBERCULOSIS-MMed-J te Riele

Thank you for submitting your sub-study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned sub-study.

Approval is granted for one year until the 30 May 2015.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:- Dr Julian te Riele is also involved in this sub-study.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

signature removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Hrec/ref:351/2014

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Hrec/ref:351/2014



UNIVERSITY OF CAPE TOWN

**Health Sciences Faculty
Research Ethics Committee**

Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925

Telephone (021) 406 6492 • Facsimile (021) 406 6411
e-mail: Non.Tywala@uct.ac.za

16 April 2008

REC REF: 038/2008

Prof K Dheda
Lung Infection & Immunity Unit
Division of Pulmonology
Medicine Department

Dear Prof Dheda

PROJECT TITLE: XDR AND MDR TB: A STUDY OF TREATMENT-RELATED OUTCOMES, COST ANALYSIS, EVALUATION OF RAPID DIAGNOSTICS AND IMMUNE PROFILING.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above mentioned study.

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: 1RB00001938

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC.REF in all your correspondence.

Yours Sincerely

signature removed

PROF M DLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

lemjedi

D2. Technical appendices

D2.1 Laserson's definitions (38)

Cure

An MDR-TB patient who has completed treatment according to country protocol and has been consistently culture-negative (with at least five results) for the final 12 months of treatment. If only one positive culture† is reported during that time, and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures, taken at least 30 days apart.

Treatment completed

An MDR-TB patient who has completed treatment according to country protocol but does not meet the definition for cure or treatment failure due to lack of bacteriologic results (i.e., fewer than five cultures were performed in the final 12 months of therapy).

Death

An MDR-TB patient who dies for any reason during the course of MDR-TB treatment.

Treatment default

An MDR-TB patient whose MDR-TB treatment was interrupted for 2 or more consecutive months for any reason.

*Treatment failure**

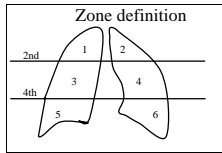
Treatment will be considered to have failed if two or more of the five cultures recorded in the final 12 months are positive, or if any one of the final three cultures is positive. Treatment will also be considered to have failed if a clinical decision has been made to terminate treatment early due to poor response or adverse events.

Transfer out

An MDR-TB patient who has been transferred to another reporting and recording unit and for whom the treatment outcome is unknown.

D2.2 Appendix 2

CXR scoring sheet for drug-Resistant tuberculosis



- Bilateral
- Effusion
- Glands
- Unilateral

Legend	Symbol	Score
Disease (a)		
No disease		
<50% of area affected	<	1
≥ 50% of area affected	>	2
Cavitation (b)		
No cavitation		
Single cavity, <2cm diameter		
Single cavity, 2- 4cm diameter	1a	0.25
Single cavity, >4cm diameter	1b	0.50
Multiple cavities, largest <2cm diameter	1c	1.00
Multiple cavities, largest 2-4cm diameter	2a	0.50
Multiple cavities, largest >4cm diameter	2b	1.00
	2c	2.00

DATE:						
Zones affected	1	2	3	4	5	6
Disease						
Score (a)						
Cavitation						
Score(b)						
Total score(a) + (b)						
↓						
Composite score all zones		.				

DATE:						
Zones affected	1	2	3	4	5	6
Disease						
Score (a)						
Cavitation						
Score(b)						
Total score(a) + (b)						
↓						
Composite score all zones		.				

DATE:						
Zones affected	1	2	3	4	5	6
Disease						
Score (a)						
Cavitation						
Score(b)						
Total score(a) + (b)						
↓						
Composite score all zones		.				

D3. European Respiratory Society research journals manuscript submission guidelines

Before submitting a manuscript to the ERS research journals (the *European Respiratory Journal* and *ERJ Open Research*), please read these guidelines carefully. Adherence to the guidelines will help to ensure smooth and prompt peer review. The other ERS journals (*European Respiratory Review* and *Breathe*) have their own specific manuscript preparation guidelines.

All submissions to the ERS research journals are handled via the [ScholarOne Manuscripts](#) platform, which provides detailed instructions about the submission process. If you experience problems or require any further assistance, please contact the submission helpline direct on +44 114 2672864 or contact [Gill Archer](#), ScholarOne Manuscripts coordinator.

- [Overlapping publications and publication ethics](#)
- [Manuscript preparation](#)
- [Original articles](#)
- [Review and series articles](#)
- [Letters and correspondence](#)
- [Online supplementary material and video summaries](#)
- [Guidelines for reporting research findings](#)
- [Data availability and publication](#)
- [Registering clinical trials](#)
- [Permission to re-publish materials](#)
- [Authorship](#)
- [Conflict of interest](#)
- [Proofs](#)
- [Correction policy](#)
- [Appendix: sources of statistical information](#)

Overlapping publications and publication ethics top

Authors submitting a paper to the ERS research journals do so on the understanding that neither the work nor any part of its essential substance, tables or figures have been or will be published or submitted to another scientific journal or are being considered for publication elsewhere. This must be stated in the cover letter. This restriction does not apply to conference abstracts or material published under legal requirements for clinical trials reporting, but includes work published in another language.

It is the authors' responsibility to ensure that submitted manuscripts are not duplicate publications; they must declare any simultaneous submissions of similar or related manuscripts at the point of submission and must include electronic copies of these manuscripts as a supplement to their submission. If there are any concerns following submission, the editors reserve the right to take appropriate action.

The ERS uses iThenticate plagiarism detection software (www.ithenticate.com). Submitted articles are screened and compared to previously published sources. Manuscripts revealing a high proportion of similarity to single or multiple published sources will be examined carefully, and the Chief Editors reserve the right to approach authors for an explanation (as per the Committee on Publication Ethics recommendations of procedures to follow in the event of suspected plagiarism in a submitted manuscript).

As a member of the Committee on Publication Ethics, the ERS follows the [COPE](#) codes of conduct and best practice guidelines.

Manuscript preparation top

Presentation of manuscripts should be consistent with the [Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals](#), as recommended by the International Committee of Medical Journal Editors ([ICMJE](#)).

Brief requirements for journal articles are summarised in the following table. The requirements are outlined in more detail below.

Article type	Word limit	Figures and tables*	References	Online supplement	Abstract
Original research	3000	8	40	Accepted	Yes, 200 wor
Editorials	1500	2	30	Not accepted	No
Review and series articles	5000	5	200	Accepted	Yes, 200 wor
Research letters	1200	1	15	Not accepted	No
Correspondence	800	1	10	Not accepted	No
Task force reports, guidelines and consensus statements	8000	15	250	Accepted	Yes, 200 wor

*The number of figures and tables in the above summary refers to the combined number; for example, letters can have either one figure or one table, not one of each.

For further general guidance on how to write papers, please refer to: Sterk PJ, Rabe KF. [The joy of writing a paper](#). *Breathe* 2008; 4: 224-232, and guidelines for authors on how to write scientific articles to be published in English at <http://www.ease.org.uk/pdfguidelines/AuthorGuidelinesHighRes.pdf>. Authors are reminded that they should not imply that any opinions or views expressed in their article are those of the journal.

General

- Write the manuscript in UK English.
- The manuscript file you submit must be saved in rich text format (.rtf) or as a Microsoft Word document (.doc or .docx).
- Describe abbreviations and unusual terms at the first time of use.
- Symbols as defined by the ad hoc working group of the Commission of the European Communities (see [Eur Respir J 1993; 6: Suppl. 16](#)) are recommended.
- Système International (SI) units are recommended.
- Equations should be created as normal text.

Title page

- Provide a concise and informative title, limited to 90 characters (including spaces).
- Include a list of all contributing authors and all of their affiliations, with a clear indication of who is associated with each institution.
- Supply the full correspondence details for the corresponding author, including e-mail address. Only one corresponding author per manuscript should be provided.
- Provide a 120-character (including spaces) summary of the "take home" message of your paper, which can be used to publicise your study *via* social media.

Tables

- Insert tables into the main text document using the Table function in your word processing package. Do not supply tables in a separate file.
- Number tables consecutively with Arabic numerals.
- Limit data to a sensible number of significant figures.
- Avoid large tables if possible. Large tables are difficult to display on small screens or A4 printouts.
- Provide a clear footnote for each table, making sure all abbreviations and symbols used are defined.
- For reference numbering schemes, citations made in tables should continue in numerical order from the point in the main body text where the table is first cited.

Figures

File format

- Supply line-art figures in JPEG, TIFF, Adobe Illustrator (.ai), PDF, SVG or EPS format. Graphs or bar charts may be supplied in Excel or similar spreadsheet format.
- Supply halftone and photographic images in PSD, JPEG or TIFF format. Minimum resolution should be 300 dpi at the final printable size (90 mm wide or greater).
- Don't embed images in the main manuscript file. Supply them separately.
- If your figures were originally created in another format that contains extra information (*e.g.* embedded data in an Excel graph), consider supplying them as supplementary material (Original Articles only).

Size and quantity

- Figures constitute a key element of manuscripts submitted to the ERS research journals. However, figures should be limited (both in size and number) to those required to show the essential features described in the manuscript.
- Avoid large figures comprising many individual parts: as a maximum, each individual figure must fit to a single PDF page of the journal, with sufficient space for its accompanying caption.
- If you have a large number of figures, consider publishing some of them as an online supplement.
- Images should be submitted in as close a size as possible to the final print size. There are three options: 90, 140 or 190 mm.

Colour figures

- There is a charge for presenting figures in colour in the printed edition of the *European Respiratory Journal*; figures can be presented in colour online free of charge in both the *European Respiratory Journal* and *ERJ Open Research*.
- There is no charge for presenting figures in colour in *ERJ Open Research*.
- Please remember that people are likely to print your manuscript on a black-and-white printer. Your colour figures need to be comprehensible when printed in this format.

Figure presentation

- All submitted figures must be clearly named and numbered.
- Whether for images, drawings or graphs, use no more than four panels for a single figure. These should be labelled as a), b), c) and d).
- In photographic and halftone images, show only the areas of interest with enough surrounding area for orientation purposes.

- Radiographic images should be of high quality and combined into one array, such as posteroanterior and lateral views. Each panel should be sized identically.
- When several photographic or halftone images of a given type are being shown, please reproduce them all at the same magnification.
- Photomicrographs must have internal linear scale markers (scale bars), since the size and magnification may be altered when the figure is printed or displayed on screen.
- Images should correspond in appearance to the tonal relations of the original radiograph (*i.e.* showing the bones white on a dark background), with the patient's right to the observer's left. CT scans and magnetic resonance images should employ the internationally accepted 'view from below'.
- Label your images such that all important details are clearly marked, but avoid obscuring large areas of the images with excessive labelling.
- Use a sans serif font (such as Arial or Helvetica) for labelling, and ensure that the font is legible, of reasonable size and uniform throughout all the figures in your manuscript.
- Ensure that bar charts and graphs have a white background, with no shading or gridlines.
- Use greyscale shading on bar charts and graphs (different weights can be used, e.g. from 0% (white) to 100% (black) for purposes of differentiation), in preference to hatching and patterning.
- Do not use three-dimensional effects in the presentation of bar charts.
- For reference numbering schemes, citations made in figures should continue in numerical order from the point in the main body text where the figure is cited.

Guidelines for handling image data

- If an image has been enhanced electronically, please explain the alterations that have been made and submit the original image along with the enhanced one. Keep an electronic set of original images, since our reviewers might ask you to modify their content and the display modus.
- The Council of Science Editors has established four basic guidelines for handling image data, which authors submitting to the ERS research journals are urged to comply with. 1) No specific feature within an image may be enhanced, obscured, removed or introduced. 2) Adjustments of brightness, contrast or colour balance are acceptable if they are applied to the whole image and as long as they do not obscure, eliminate or misrepresent any information present in the original. 3) The grouping of images from different parts of the same gel, or from different gels, fields or exposures must be made explicit by the arrangement of the figure (*e.g.* by using dividing lines) and in the text of the figure legend. 4) If the original data cannot be produced by an author when asked to provide it, the acceptance of the manuscript may be revoked.

Captions

- Provide a clear caption for each figure.
- Captions should be brief and not repetitive of information given in the text.
- All abbreviations should be expanded.
- Where appropriate, captions should include the imaging technique used, the body part imaged and any noteworthy details.
- Mention any use of internal scale bars.

Acknowledgements

- Acknowledgements should be grouped into a single paragraph placed after the Discussion section.
- Only acknowledge people who have made substantial contributions to the study, and provide the affiliation of those you name.
- Provide the names and affiliation details of members of collaborating bodies.
- Financial support for the study should be acknowledged in a separate support statement; financial support provided to individuals must be disclosed on the conflict of interest declaration.

References

- Number references consecutively in the order in which they first appear in the text, using full-sized Arabic numerals in square brackets to cite references.
- All authors must be included for each cited item.
- References should contain at all the information shown in the following examples:
 1. Bannerjee D, Khair OA, Honeybourne D. Impact of sputum bacteria on airway inflammation and health status in clinical stable COPD. *Eur Respir J* 2004; 23: 685-692.
 2. Bourbon J, Henrion-Caude A, Gaultier C. Molecular basis of lung development. In: Gibson GJ, Geddes DM, Costable U, Sterk PJ, Corrin B, eds. *Respiratory Medicine*. 3rd Edn. Edinburgh/Philadelphia, Elsevier Science, 2002; pp. 64-81.
- Documents published online, and individual web pages, should be listed in the reference list, not in the text, and only used when an original citation is unavailable; citations should contain at all the information shown in this example (include the author of the webpage, its title, the URL on which the cited material can be found, and the dates on which the webpage was last accessed by you, and on which it was last updated):
 3. WHO. Severe Acute Respiratory Syndrome (SARS). www.who.int/csr/sars/en/index.html. Date last updated: June 1 2004. Date last accessed: June 1 2004.

- References to websites as a whole or sections of websites (rather than particular pages or documents on a website) should be included directly in the text:
...data was sourced from the WHO Global Health Observatory
(<http://www.who.int/gho/en/>)...
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Original articles should not exceed 3000 words (you do not need to include the abstract, references, tables and figure captions in this word count). If your manuscript exceeds this limit, please state the final word count and explicit reasons for exceeding the limit in your covering letter.

The total number of figures and/or tables should be limited to no more than eight. Large figures with more than four parts should be avoided: these can be presented as online supplementary material. More information regarding figures can be found above.

Abstract

Please provide an abstract of 200 words or fewer, which is easily understood without reference to the text (see *Ann Intern Med* 1987; 106: 598-604).

The abstract should have four separate paragraphs, which correspond to: the question addressed by the study; materials/patients and methods; results; and the answer to the question. One or two sentences of background information can be included in the opening paragraph if necessary. The question and answer should be the same as those in the text.

Include only the most important numbers and results, and avoid using abbreviations.

Introduction

In the introduction, state the question you asked (or hypothesis to be tested) and your considerations leading to the formulation of the question. Give only pertinent references.

Material and methods

Study subjects or animals

- Clearly describe how the subjects or experimental animals were identified, including the control subjects when used. For animals, see [Laboratory Animals 1985; 19: 106-108](#).
- Clearly state the eligibility criteria for cases and controls in observational studies, or for subjects in clinical trials.
- All work involving studies on human subjects must have received approval from local ethics committees and the regulatory authority (when appropriate: for example, for drug trials). Written informed consent must also have been obtained from all subjects and this must be clearly indicated in the paper. See also guidance on the reporting of clinical trials, below.
- Animal experimentation must have been performed according to the Helsinki convention for the use and care of animals.
- Provide details of the species and/or strain and number of animals involved in the study.
- The editors will reject work that does not conform to acceptable ethical criteria.

Study design

- Clearly state the main study objective(s).
- Consider sample size and whether you have enough subjects to reliably address the research question.
- Manuscripts reporting clinical trials should include details of the sample size calculation (*i.e.* the expected effect size, power, level of statistical significance and one- or two-sided test).
- For systematic reviews, make sure that the keywords used to search electronic medical databases cover different terminology (for example, tumour or cancer) and spelling (for example, randomised or randomized).

Methods

- Provide an overview of the main tests or experiments.
- Describe the methods and apparatus in sufficient detail to allow other workers to evaluate or reproduce the tests/experiments.
- For methods that have been published before, provide a reference only, or a reference and brief description.
- Identify drugs and chemicals, including generic name, dosage and route of administration.
- Provide manufacturers' names and addresses (city and country) for equipment, drugs, chemicals and software as necessary, but not in a separate section.

Analysis

- Clearly state and define the main outcome measure(s).
- Briefly state the statistical methods used during the analysis if they are standard. Describe any new methods and justify their use.
- In the case of single- or multicentre trials with blinded intervention, the code must have been broken at the end of the study in the presence of the responsible investigator of each centre. The code and the

data will then be available to each participating centre. The first author should make provisions so that if needed, the data are available to the editors for independent statistical analysis.

- Seek advice from a statistician on the appropriate methods of analysis and whether results have been interpreted correctly.

Results

- Keep the results section brief.
- Describe the baseline characteristics or condition of patients or animals.
- Focus on the important results, *i.e.* those that help to address the research question.
- Present most data in figures or tables, not in the text. Use the text to emphasise or summarise the most important observations.

Discussion

- At the beginning of the discussion, summarise the main results, and show how they have addressed the research question.
- Make sure that the conclusions are consistent with the results and are pertinent to the research question.
- Describe the limitations of the study and/or analysis, and discuss their possible implications for the conclusions.
- Emphasise the new and important aspects of the study.
- Try to explain contradictory or unexpected results, or discrepancies with previous findings.
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