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Research Article

Tuberculous Pericarditis is Multibacillary and Bacterial Burden Drives High Mortality

Jotam G. Pasipanodya [e], Mwenya Mubanga [a], Mpiko Ntsekhe [a], Shaheen Pandie [a], Beki T. Magazi [d], Freedom Gumedze [b], Landon Myer [c], Tawanda Gumbo [a,e,*], Bongani M. Mayosi [a]

[a] The Cardiac Clinic, Department of Medicine, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa
[b] Department of Statistical Sciences, University of Cape Town, Cape Town, South Africa
[c] School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa
[d] National Health Laboratory Services (Tshwane Academic Division), Department of Medical Microbiology, University of Pretoria, South Africa
[e] Center for Infectious Diseases Research & Experimental Therapeutics, Baylor Research Institute, Baylor University Medical Center, Dallas, TX, USA

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ABSTRACT

Background: Tuberculous pericarditis is considered to be a paucibacillary process; the large pericardial fluid accumulation is attributed to an inflammatory response to tuberculoproteins. Mortality rates are high. We investigated the role of clinical and microbial factors predictive of tuberculous pericarditis mortality using the artificial intelligence algorithm termed classification and regression tree (CART) analysis.

Methods: Patients were prospectively enrolled and followed in the *I*nvestigation of the *M*anagement of *P*ericarditis (IMPI) registry. Clinical and laboratory data of 70 patients with confirmed tuberculous pericarditis, including time-to-positive (TTP) cultures from pericardial fluid, were extracted and analyzed for mortality outcomes using CART. TTP was translated to $\log_{10}$ colony forming units (CFUs) per mL, and compared to that obtained from sputum in some of our patients.

Findings: Seventy patients with proven tuberculous pericarditis were enrolled. The median patient age was 35 (range: 20–71) years. The median, follow up was for 11.97 (range: 0·03–74.73) months. The median TTP for pericardial fluid cultures was 22 (range: 4–58) days or 3.91(range: 0·5–8·96) $\log_{10}$CFU/mL, which overlapped with the range of 3.24–7.42 $\log_{10}$CFU/mL encountered in sputum, a multi-bacillary disease. The overall mortality rate was 1.43 per 100 person-months. CART identified follow-up duration of 5·23 months on directly observed therapy, a CD4+ count of ≤199.5/mL, and TTP ≤ 14 days (bacillary load ≥ 5.53 $\log_{10}$ CFU/mL) as predictive of mortality. TTP interacted with follow-up duration in a non-linear fashion.

Interpretation: Patients with culture confirmed tuberculous pericarditis have a high bacillary burden, and this bacterial burden drives mortality. Thus proven tuberculosis pericarditis is not a paucibacillary disease. Moreover, the severe immunosuppression suggests limited inflammation. There is a need for the design of a highly bactericidal regimen for this condition.

1. Introduction

Tuberculous (TB) pericarditis accounts for 50–70% of pericardial disease in Africa (Mayosi et al., 2005, 2006, 2008). Mortality rates range between 17 and 60% (Mayosi et al., 2008; Pusch et al., 2014; Shaw et al., 2010). The *I*nvestigation of the *M*anagement of *P*ericarditis (IMPI) registry, a prospective observational study, revealed a case fatality rate of 26% within 6-months of diagnosis (Mayosi et al., 2008). Several host-factors were independent predictors of this early mortality, including the presence of HIV infection, increasing age, and concurrent pulmonary TB (Mayosi et al., 2008). However, these patients were followed up for only 6 months, and a definitive TB pericarditis diagnosis was confirmed in only 7% of patients. Thus, factors predictive of long-term outcomes in patients with proven TB pericarditis still need to be identified. Here, we identified factors predictive of long-term outcome using classification and regression tree (CART) analyses. We used CART because we did not want to use a model that pre-specified the important potential predictors. Instead we wanted a distribution- and assumption-free method to identify the predictors in the context of all potential clinical and laboratory factors. We also wanted to rank the predictors, in order to allow clinical decision making as to which factors to modify first to have the largest impact on reducing TB pericarditis mortality.

* Corresponding author at: Center for Infectious Diseases Research and Experimental Therapeutics, Baylor Research Institute, Baylor University Medical Center, 3434 Live Oak Street, Dallas, TX 75204, USA.
E-mail address: tawanda.gumbo@baylorhealth.edu (T. Gumbo).

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TB pericarditis is considered to be a paucibacillary process, and the large pericardial fluid accumulation is attributed to an inflammatory response caused by a few tuberculoproteins (Cherian, 2004; Fowler, 1991). For that reason, the same regimen and doses used for pulmonary TB, and for the same duration, are administered to patients with extra-pulmonary TB including TB pericarditis (Mayosi et al., 2005, 2006, 2008, 2002; Pusch et al., 2014; Shaw et al., 2010; Cherian, 2004; Fowler, 1991). However, the baseline bacillary burden or temporal changes in bacillary load with therapy are yet to be rigorously quantified. These microbial factors are known to be important determinants of outcome in patients on the same type of standard therapy for pulmonary TB (Bowness et al., 2015; Diacon et al., 2010; Chigutsa et al., 2013, 2015). Here, we used the IMPI registry to investigate microbial, clinical, echocardiography and hemodynamic factors as possible predictors of long term death. Uniquely, we had an access to quantitative microbiology information based on liquid culture, which is known to better capture larger populations of *Mycobacterium tuberculosis* (*Mtb*) than solid agar techniques.

2. Methods

2.1. Study Setting and Study Design

The goal of the IMPI registry is to improve the outcomes of patients with TB pericarditis in resource-limited settings by improving access to clinical decision-making information (Mayosi et al., 2006). IMPI prospectively recruits patients with presumptive TB pericarditis from clinics across several African countries, collects and records observational data and then systematically analyzes these data to answer specific questions. We used the registry to investigate the role of several microbial and clinical factors as predictors of early and late mortality in patients with microbiologically proven TB pericarditis. We specifically focused on patients treated at Groote Schuur Hospital, a tertiary and teaching facility in Cape Town, South Africa, in order to control for uniformity of care, especially microbiology laboratory use and access. We examined study patients who had been treated at Groote Schuur Hospital between January 2006 and December 2010, and followed-up until January 1st 2012.

All patients received standard first-line anti-TB therapy recommended by the South African National Tuberculosis Program. Patients with prior TB history or retreatment were put on a longer regimen of the same first-line anti-TB drugs supplemented with streptomycin in the intensive phase. HIV-infected patients were treated with anti-retroviral drugs in line with local practice guidelines at the time (National Department of Health, 2004). (http://southafrica.usembassy.gov/media/2004-doh-art-guidelines.pdf) Progression of HIV and response to antiretroviral therapy was monitored using CD4 + T cell counts.

2.2. Definition of Terms

Pericardial constriction was defined using clinical and echocardiographic criteria described in the IMPI trial (Mayosi et al., 2014). The vital status of the patient (i.e. whether the patient was alive or dead by the censure date) was based on death certificate copies from the medical records and use of the eKapa and Clinicom databases, the Department of Home Affairs death records database and via verbal autopsy from family members. January 1st 2012 was set as the censure date.

2.3. Inclusion and Exclusion Criteria

The inclusion criteria for the IMPI registry is pericardial effusion confirmed via echocardiography in a patient with suspected TB (Mayosi et al., 2006). Patients with a presumptive diagnosis of tuberculous pericarditis and met eligibility for the IMPI registry also met study inclusion criteria. However, we excluded patients who did not have proven or definite tuberculous pericarditis for this sub-study of the IMPI registry. Additionally, only those patients who had pericardiocentesis within one week of diagnosis were included in this study. We included only patients who had been treated for 3 days or less with anti-TB antibiotics.

2.4. Modeling Bacillary Burden in Pericardial Fluid and Sputum

The standard laboratory measure of *Mtb* burden is colony-forming units per milliliter (CFU/mL); in sputum a smear grade is also used in clinical practice (Bowness et al., 2015; Diacon et al., 2010; Chigutsa et al., 2013, 2015; Epstein et al., 1998; Pfyffer et al., 1997; Giampaglia et al., 2007; Kolibab et al., 2014; Davies, 2010). Unfortunately, CFU/mL determinations are labor-intensive, time-consuming, require a biosafety level 3 facility, and at least 3 weeks of incubation. On the other hand, the mycobacterium growth indicator tube (MGIT) [Becton Dickinson Microbiology Systems, NJ, USA] liquid media system has become the standard in *Mtb* cultures for clinical specimens in South Africa (Diacon et al., 2010; Chigutsa et al., 2013, 2015). The MGIT reports a culture's time-to-positivity (TTP); the higher the bacillary load, the shorter the TTP (Epstein et al., 1998; Pfyffer et al., 1997; Giampaglia et al., 2007; Kolibab et al., 2014; Davies, 2010). Since TTP is highly correlated with CFU/mL, we utilized the TTP of pericardial cultures from 70 samples from the IMPI patients and a comparable randomly selected sputum samples from 18 patients with confirmed pulmonary TB from the South African TB reference laboratory in the Gauteng province, South Africa, to calculate the $\log_{10}$ CFU/mL. In the MGIT, a specimen without growth in over 60 days of incubation was considered negative for *Mtb* infection. Ziehl–Neelsen staining was used routinely to confirm positive MGIT results at Groote Schuur Hospital.

We used two approaches to translate TTP (in days) to CFU/mL, based on methods published in the literature (Bowness et al., 2015; Chigutsa et al., 2013, 2015; Davies, 2010). The first method is more conservative because it does not account for therapy received, has an r^2 of 0.998, and employs the following formula:

$$\log_{10}\mathrm{CFU/mL} = 2.6818e^{-0.046*\mathrm{TTP}}. \quad (1)$$

The second method is derived from the Gompertz model and was recently used by Bowness et al. (2015). This method accounts for bacterial decline during the first week of therapy. The formula is as follows:

$$\log_{10}\mathrm{CFU/mL} = \frac{\mathrm{TTP} - \left(562.318e^{-0.789e^{-0.195t}}\right)}{-64.111e^{1.002e^{-0.248t}}}, \quad (2)$$

where t denotes the days on therapy. The final pericardial fluid CFU/mL was adjusted for dilution made during sample preparation for each patient. Data extracted from the patients' notes did not indicate the number of days the patients was on therapy prior to pericardiocentesis. We assumed that most patients have the initial pericardiocentesis after receiving at least three days' worth of antibiotics (empirical anti-TB therapy is recommended for suspected pericardial TB by the national program). Given that we had excluded patients who received more than 3 days of therapy, we used the $t = 3$ for baseline $\log_{10}$CFU using this method.

2.5. Classification and Regression Tree Analysis

CART analysis is a machine-learning method that is distribution-free and can be used to assist in decision-making in the clinic (Breiman et al., 1984; Steinberg and Colla, 1995). CART analyses reveal trees that rank the important selected predictors and are akin to decision-making trees. Patients' demographic and clinical information including age, gender, HIV-infection, CD4 + T cell counts, receipt of anti-retroviral therapy, and history of active TB were examined using CART. Additional variables such as measures of hemodynamic stability (initial systolic, diastolic blood pressure and mean arterial pressure), heart rate, presence of pericardial constriction, receipt of pericardiectomy, and TTP derived

bacterial burden, were also included in the analysis. Several of these independent variables (such as measures of HIV-infection related immunosuppression) are highly correlated, and interact with both host and pathogen factors in a nonlinear fashion. This would make it difficult to separate them out using standard multivariate logistic or linear regression models (Breiman et al., 1984; Steinberg and Colla, 1995; Shadish et al., 2001; Breiman, 2001). Our approach to using CART for variable selection, and threshold identification of diseases outcomes in patients has been well described in the past (Pasipanodya et al., 2013; Gumbo et al., 2014; Jain et al., 2013; Pasipanodya and Gumbo, 2011). The main outcome we examined was mortality on follow-up. Priors were set to equal (i.e., all categories had equal probability assignment), with no penalty sets for misclassification. GINI methods were used for building trees together with V-fold cross-validation of each tree (Steinberg and Colla, 1995; Breiman, 2001). CART analyses were performed using the Salford Predictive Miner System software (San Diego, CA).

2.6. Standard Statistical Analysis

After CART use, standard frequentist inference analyses were performed to estimate associations and parameter estimates of time-to-death for comparison with existing literature. Cox proportional hazard models that used variables and thresholds identified by the CART analysis were utilized. Proportional hazard assumptions were examined by examining plots as residuals (Shadish et al., 2001). All analyses were two-sided with alpha at 0.05 performed with STATA version 12, College Station, Texas.

3. Results

The laboratory and clinical characteristics of patients at enrolment are shown in Table 1. These were the same in patients with TB confined to pericardium compared to those with TB pericarditis and evidence of TB in another body organ; the only difference was that antiretrovirals were used more commonly in the latter group (4/6 patients) compared to the former (5/38). Cardiac tamponade, either at presentation or in the course of TB therapy, was observed in 3/70 (4%) patients. The CD4+ T cell counts for 53/70 (80%) of patients shown Fig. 1 reflect overall immunosuppression in study patients. The median follow-up duration was 11.97 (range: 0·03–74.73) months. Of the 70 patients, 16 (23%) died during the 75-month follow-up period. The overall mortality rate was 1.43 per 100 person-month follow-up.

To put the pericardial *Mtb* burden into context, 18 sputum samples from 18 randomly chosen patients with pulmonary TB, based on a South African reference laboratory, were compared to 70 pericardial fluid samples from IMPI patients. The pulmonary TB patients had a median age of 40 (range 22–44) years compared to 35 (range 30–71) years of those with pericardial TB ($p = 0.600$). Fig. 2A shows the bacillary burden in terms of TTP, while Fig. 2B shows the bacillary burden as $\log_{10}$CFU/mL based on the more conservative formula. The median TTP for pericardial samples was 22 (range 4 to 58) days and that for sputum was 12 days ($p < 0.001$). Fig. 2B shows that even though overall bacillary burden was significantly higher in sputum samples (mean difference 2.22 ± 0.34 $\log_{10}$CFU, $p < 0.001$), the mean baseline bacillary load for pericardial fluid samples (3.86 ± 0.21 $\log_{10}$CFU), was substantial and inconsistent with a paucibacillary process. The baseline CFU/mL in pericardial fluid is higher (median = 8.55 $\log_{10}$CFU/mL) using a different but more stringent modeling approach (Fig. 3).

CART models identified the most important predictors of mortality as duration of therapy or follow-up (importance score = 100%), CD4+ T cell counts (score = 47%), TTP (score = 38%), and age (score = 21%). The identified thresholds (which partitioned patients to homogenous groups with similar mortality risk) shown in Table 2, were a follow-up or therapy duration of 5.23 months, a CD4+ T cell count of 199·5, and a TTP ≤14 days. CART identified an interaction between TTP and follow-up duration; patients with TTP ≤14 days (i.e. high bacillary loads) had significantly higher mortality rates earlier than 5.3 months. The meaning of an interaction in machine learning is that of a predictor modifying another predictor's effect on the outcome (i.e., mortality in this case). A TTP of ≤14 days translates to ≥5.65 $\log_{10}$CFU/mL. Notably, measures of hemodynamic instability, heart rate, use of oral steroids, adenosine deaminase level or gender were not ranked. HIV-infection status or use of anti-retrovirus medications was also not ranked. The best CART model receiver-operating

Table 1
Comparison of clinical and laboratory characteristics of 58 patients with exclusive tuberculous pericarditis and 12 patients who also had tuberculosis elsewhere.

Variable		Total (n = 70)	Tuberculosis elsewhere (n = 12)	Exclusive TB pericarditis (n = 58)	p-Value
Sex	Female	22 (31)	5 (42)	17 (29)	0.401
	Male	48 (69)	7 (58)	41 (71)	
Age (years)	Median (IQR)	35 (30–48)	31.5 (29·5–39·5)	35·5 (29–50)	0.663
Previous TB[a]	No	53 (87)	10 (91)	43 (86)	0.662
	Yes	8 (13)	1 (9)	7 (12)	
HIV-test result	Negative/Not Done	23 (33)	4 (33)	19 (33)	1.000
	Positive	47 (67)	8 (67)	39 (67)	
Pericardial calcification[a]	No	16 (70)	3 (100)	13 (65)	0.526
	Yes	7 (30)	0	7 (35)	
Pericardial Constriction	None	62 (89)	12 (100)	50 (86)	0·340
	Yes	8 (11)	0	8 (14)	
Pericardiectomy	No	67 (96)	12 (100)	55 (95)	0.590
	Yes	3 (4)	0	3 (5)	
Comorbid conditions	None	56 (80)	9 (75)	47 (87)	0.372
	Some	14 (20)	3 (25)	11 (13)	
Baseline NYHA[a]	II	9 (15)	1 (9)	8 (16)	0.674
	III	30 (50)	5 (45)	25 (51)	
	IV	21 (35)	5 (46)	16 (33)	
Oral steroids[a]	None	17 (44)	3 (38)	14 (45)	1.000
	Some	22 (56)	5 (62)	17 (55)	
Antiretroviral therapy	None	35 (80)	2 (33)	33 (87)	*0.011*
	Some	9 (20)	4 (67)	5 (13)	
Adenine deaminase level (IU/L)	Median (IQR)	74.7 (53–107.6)	67 (52–85)	76 (51–110)	0.909
Globulin (g/L)	Median (IQR)	53.5 (47–58)	53 (43–54)	56 (51–60)	0.416
CD4+ counts (per mL)	Median (IQR)	116 (61·5–266)	111 (22–254)	107 (15–254)	0.204

[a] Total does not add to 70 because data for some patient categories were missing; IQR — Interquartile range: NYHA — New York Heart Association functional classification; Italics — statistically significant difference.

characteristic was 0·95 on the learning set and 0·68 on the test sample. This means that the probability of obtaining similar results with an independent dataset is at least 68%.

CART output was examined in standard logistic regression, with results shown in Table 2. Among patients with *Mtb* > 5.53 $\log_{10}$CFU/mL mortality incidence was 6/197·7 person-months and the mortality incidence rate was 0·03 compared to 10/920 person-months and mortality rate of 0·01 (Fig. 4). The incidence rate difference was −0.02 (95% −0.04 to 0.01), p = 0.031. Multivariable analysis based on time-to-death as an outcome on follow-up in a Cox proportional hazards model revealed a hazards-ratio of 3.57 (95% confidence interval: 1.27–10·00; $p = 0.016$) in patients >29 years and 2.91 (95% confidence interval: 1.03–8·21; $p = 0.044$) in patients with pericardial Mtb >5.53 $\log_{10}$CFU/mL.

4. Discussion

There were three main findings in this study. First, mortality rates in proven TB pericarditis were high. The fact that the overwhelming majority of deaths in this cohort occurred during directly observed therapy is troubling, and the timing of 5.3 months suggests that this is more a failure of therapy rather than death from chronic complications such as constriction. Since all patients were on directly observed therapy, failure in this case may have had little to do with poor adherence. There are two possible explanations for this failure of therapy, both pharmacokinetic/pharmacodynamic (PK/PD) in nature (Shenje et al., 2015; Pasipanodya and Gumbo, 2011). First, it is most likely that there are inadequate antibiotic concentrations in TB pericardial fluid, especially of the primary sterilizing effect drugs rifampin and pyrazinamide, due to poor penetration, as we have shown elsewhere (Shenje et al., 2015). There is now sufficient hollow fiber and prospective clinical studies evidence to show that both the bactericidal activities and the sterilizing effect of antibiotics in TB is driven by high peak concentration and the area under the concentration-time curve of each drug, and that evolution-mandated pharmacokinetic variability drives many patients on recommended doses to not achieve optimal peak and area under the concentration–time curve concentrations (Shenje et al., 2015; Pasipanodya and Gumbo, 2011; Gumbo et al., 2015; Pasipanodya et al., 2013; Srivastava et al., 2011). Secondly, CART identified a CD4 + T cell count ≤199.5 as a major predictor. This happens to be the exact CD4 + T cell count cut-off point that defines severe

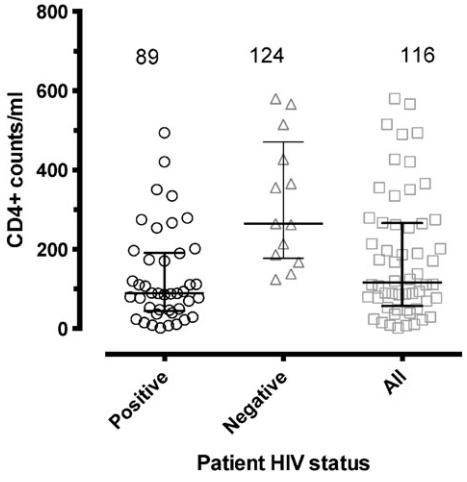

Fig. 1. CD4 + T cell count distribution in patients with proven tuberculous pericarditis. The figure shows the CD4 + T cell count distribution in patients with and without confirmed HIV and those with HIV test result missing demonstrating remarkably low CD4 + T cell counts in all study patients. CD4 + T cell counts data were missing in 17/70 (20%) of patients including 4/5 (80%) of patients with missing HIV test result. Baseline CD4 + T cell counts were significantly higher in HIV negative patients than positive, p < 0·001. The median values are shown in figure.

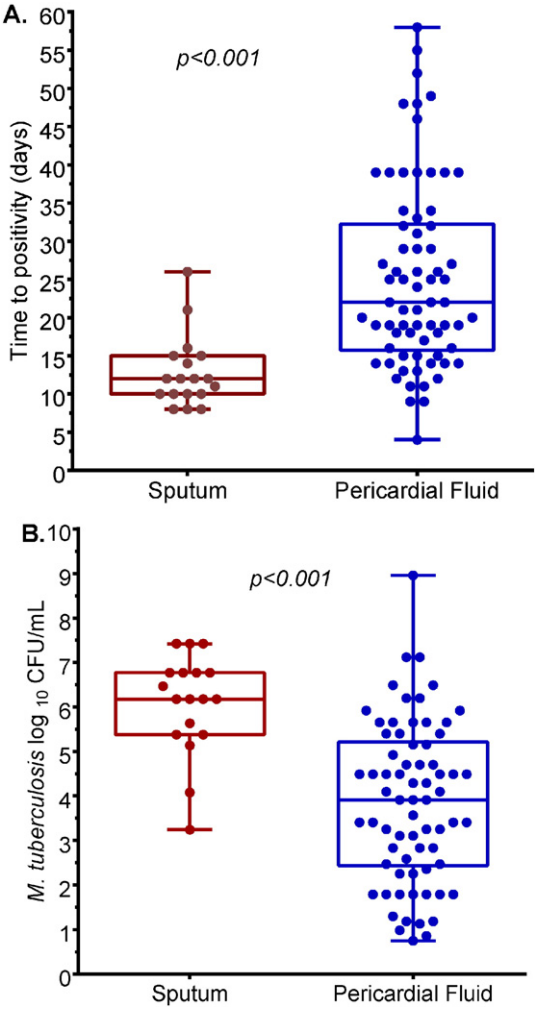

Fig. 2. *Mycobacterium tuberculosis* burden in pericardial fluid compared to sputum The baseline bacillary load in 18 sputa produced by 18 different patients and in 70 pericardial fluid samples also from 70 separate patients is shown. The box and whisker plots in both panel Fig. 2A and B show all data points, while the lines denote the median, interquartile as well as the minimum and maximum values. The p-values are for both the Mann–Whitney test to compare median values and the Kolmogorov–Smirnov test to examine and compare the variability and shape of the distributions between the two samples. Panel A shows the bacillary burden in time-to-positive (TTP) cultures in days. Panel B shows the bacillary burden in Log_{10} colony forming Units per mL (CFU/mL).

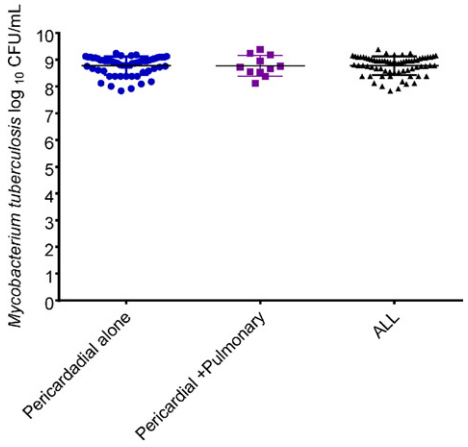

Fig. 3. *Mycobacterium tuberculosis* burden using the less conservative method. This less conservative method by Bowness et al., which takes into account days of therapy, leads to higher bacterial burdens that average about 8.55 $\log_{10}$CFU/mL in pericardial fluid.

Table 2
Mortality proportions for CART identified predictors of mortality.

Variable[a]		Died n = 16 (%)	Alive n = 54 (%)	p-Value[b]	Unadjusted OR (95% CI)
CD4 + T cell counts	≤199.5/mL	11 (30)	26 (70)	0.266	Ref
	>199.50	2 (11)	17 (89)		0.28 (0.05, 1.41)
	Missing	3 (21)	11 (79)		0.64 (0.15, 2.77)
Age	≤29	7 (41)	10 (59)	*0.039*	Ref
	>29	9 (17)	44 (83)		0.29 (0.09, 0.97)
Follow-up	≤157 days	11 (58)	8 (42)	*<0.001*	Ref
	>157 days	5 (10)	46 (90)		0.08 (0.02, 0.29)
Bacillary burden					
Mtb $\log_{10}$ CFU/mL	≤5.53	10 (18)	46 (82)	*0.046*	3.45 (0.98, 12.17)
	>5.53	6 (43)	8 (57)		Ref
Time to positivity	≤14 days	6 (43)	8 (57)		Ref
	>14 days	10 (18)	46 (82)	*0.046*	0.29 (0.08, 1.02)

[a] Row (%) shown.
[b] Chi-square tests; computed odds ratio (OR) univariate logistic regression; CFU-Colony forming unit; Italics — statistically significant difference.

immunosuppression. The immune system is an efficient antimicrobial agent (that is why it exists), and these patients would have been deprived of that advantage.

The second main finding is that proven TB pericarditis may not be a paucibacillary disease as previously proposed; (Theron et al., 2014) indeed patients who died had pericardial bacterial burden of >5.53 $\log_{10}$CFU/mL. This result is with the more conservative method to correlate TTP to CFU/mL of pericardial fluid. Use of the less conservative method revealed bacterial burdens in Fig. 3, much higher than even those in sputum. In our study, patients had pericardiocentesis within a week of starting anti-TB therapy. Thus, the initial bacterial burden may actually be falsely lower in some patients because of effects of therapy. However, the caveat is that these were patients chosen based on the basis of a positive *Mtb* culture in pericardial fluid, which could skew the bacterial burden.

The third important finding was that the bacillary load was a significant predictor for both early and late mortality outcomes. This aspect outranked well-known hemodynamic predictors of mortality in TB pericarditis such as hypotension. Moreover, this even outranked type of pericardial syndromes such as constrictive pericarditis versus effusive pericarditis versus effusive–constrictive disease. Thus, in prioritizing decisions to decrease the high mortality, a priority should be to find rapidly bactericidal drugs, or even exploration of the notion of decreasing the large bacterial burden via drainage (i.e., draining the cold abscesses). It is a well-known principle of antimicrobial PK/PD science, and indeed of standard microbiology, that the higher the bacterial burden, the greater the chances of treatment failure and development of acquired drug resistance (Gumbo et al., 2015; Jumbe et al., 2003; Gumbo, 2011). Indeed, for some patients, the initial bacillary burden was above the inverse of the mutation frequency of 10^{-7} of the only effective antibiotic that penetrates well into pericardial fluid: isoniazid (Shenje et al., 2015). This means that acquired resistance due to effective monotherapy could be a problem at these high pericardial fluid bacterial burdens. Moreover, time to total sterilization of bacteria in higher bacterial burden disease will be longer than with smaller bacterial burdens, given that dose–response curves of antibiotics reveal a ceiling killing rate. Thus, time to cure is longer with high burden disease, especially given the limited number of drugs in the standard regimen that penetrate into pericardium(Shenje et al., 2015).

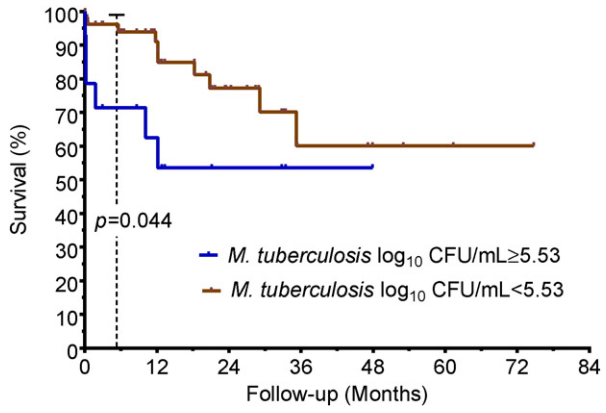

Fig. 4. Survival in patients with CART identified predictors of high mortality Patients with bacterial burden of >553 $\log_{10}$CFU experienced significantly higher overall mortality and higher hazards over the entire follow-up. The hazard rate was 2.86 (95% CI 1.01, 7.69), p = 0·044. The vertical line indicates the 5.23 months follow-up time point identified by classification and regression tree (CART) analysis.

There are limitations to these data. First, we only examined proven TB pericarditis and excluded suspected pericarditis. This criterion might have biased against suspected TB pericarditis patients with such low bacillary burden that cultures fail to grow: a phenomenon also encountered with culture negative pulmonary TB. Nevertheless, our comparison was with culture positive pulmonary TB, a disease universally considered multi-bacillary. The second limitation is that CART identifies predictors, but does not assign a causal pathway. Our findings thus constitute a hypothesis, which should be tested in a large prospective dataset. Second longer follow-up and data may be required to delineate effects of TB/HIV related cardiomyopathy as well as the effect of sustained antiretroviral therapy. Data of antiretroviral therapy were available in 44/70 (63%) of patients and only 8/47 (17%) of known HIV positive patients were on therapy. The low % of patients on antiretroviral drugs is because the study recruitment was during a period when the timing of initiation of antiretroviral therapy in patients with tuberculosis was still being studied, and also occurred during the early rollout period for antiretroviral drugs.

Author Contributions

J.G. Pasipanodya and T.G. were responsible for data analyses, calculation of CFU/mL of *M. tuberculosis*, CART models, multivariable regression, interpretation, and, writing manuscript. M. Mubanga, M. Ntsekhe, S. Pandie, F. Gumedze, and L. Myer, were involved in study design, collection of pericardial tuberculosis data, data analysis and interpretation, and writing of manuscript. B.T. Magazi was responsible for collection of sputum tuberculosis data and writing manuscript. B. Mayosi was responsible for study design, collection of data, data analysis and interpretation, and, writing of manuscript.

Conflicts of Interests

T.Gumbo has been a consultant for Astellas Pharma USA and Founded Jacaranda Biomed Inc. None of the other authors have any conflicts of interests to disclose.

Role of Funding Source

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References

Bowness, R., Boeree, M.J., Aarnoutse, R., et al., 2015. The relationship between *Mycobacterium tuberculosis* MGIT time to positivity and CFU in sputum samples demonstrates changing bacterial phenotypes potentially reflecting the impact of chemotherapy on critical sub-populations. J. Antimicrob. Chemother. 70, 448–455.

Breiman, L., 2001. Statistical modeling: the two cultures. Stat. Sci. 16, 199–231.

Breiman, L., Friedman, J., Olshen, R., et al., 1984. Classification and regression trees. Chapman & Hall/CRC, Washington DC.

Cherian, G., 2004. Diagnosis of tuberculous aetiology in pericardial effusions. Postgrad. Med. J. 80, 262–266.

Chigutsa, E., Pasipanodya, J.G., Visser, M.E., et al., 2015. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. Antimicrob. Agents Chemother. 59, 38–45.

Chigutsa, E., Patel, K., Denti, P., et al., 2013. A time-to-event pharmacodynamic model describing treatment response in patients with pulmonary tuberculosis using days to positivity in automated liquid mycobacterial culture. Antimicrob. Agents Chemother. 57, 789–795.

Davies, G.R., 2010. Early clinical development of anti-tuberculosis drugs: science, statistics and sterilizing activity. Tuberculosis (Edinb.) 90, 171–176.

Diacon, A.H., Maritz, J.S., Venter, A., et al., 2010. Time to detection of the growth of *Mycobacterium tuberculosis* in MGIT 960 for determining the early bactericidal activity of antituberculosis agents. Eur. J. Clin. Microbiol. Infect. Dis. 29, 1561–1565.

Epstein, M.D., Schluger, N.W., Davidow, A.L., et al., 1998. Time to detection of *Mycobacterium tuberculosis* in sputum culture correlates with outcome in patients receiving treatment for pulmonary tuberculosis. Chest 113, 379–386.

Fowler, N.O., 1991. Tuberculous pericarditis. JAMA 266, 99–103.

Giampaglia, C.M., Martins, M.C., Vieira, G.B., et al., 2007. Multicentre evaluation of an automated bactec 960 system for susceptibility testing of *Mycobacterium tuberculosis*. Int. J. Tuberc. Lung Dis. 11, 986–991.

Gumbo, T., 2011. General principles of chemotherapy of infectious diseases. In: Brunton, L.L., Chabner, B., Knollmann, B. (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics. McGraw Hill Medical, New York, NY.

Gumbo, T., Chigutsa, E., Pasipanodya, J., et al., 2014. The pyrazinamide susceptibility breakpoint above which combination therapy fails. J. Antimicrob. Chemother. 69, 2420–2425.

Gumbo, T., Pasipanodya, J.G., Romero, K., Hanna, D., Nuermberger, E., 2015. Forecasting accuracy of the hollow fiber system model of tuberculosis for clinical therapeutic outcomes. Clin. Infect. Dis. 61 (suppl 1), S25–S31.

Jain, M.K., Pasipanodya, J.G., Alder, L., et al., 2013. Pegylated interferon fractal pharmacokinetics: individualized dosing for hepatitis C virus infection. Antimicrob. Agents Chemother. 57, 1115–1120.

Jumbe, N., Louie, A., Leary, R., et al., 2003. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. J. Clin. Invest. 112, 275–285.

Kolibab, K., Yang, A., Parra, M., et al., 2014. Time to detection of *Mycobacterium tuberculosis* using the MGIT 320 system correlates with colony counting in preclinical testing of new vaccines. Clin. Vaccine Immunol. 21, 453–455.

Mayosi, B.M., Burgess, L.J., Doubell, A.F., 2005. Tuberculous pericarditis. Circulation 112, 3608–3616.

Mayosi, B.M., Ntsekhe, M., Bosch, J., et al., 2014. Prednisolone and *Mycobacterium indicus pranii* in tuberculous pericarditis. N. Engl. J. Med. 371, 1121–1130.

Mayosi, B.M., Ntsekhe, M., Volmink, J.A., et al., 2002. Interventions for treating tuberculous pericarditis. Cochrane Database Syst. Rev., CD000526

Mayosi, B.M., Wiysonge, C.S., Ntsekhe, M., et al., 2006. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. BMC Infect. Dis. 6, 2.z.

Mayosi, B.M., Wiysonge, C.S., Ntsekhe, M., et al., 2008. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. S. Afr. Med. J. 98, 36–40.

National Department of Health, 2004. Republic of South Africa. National Antiretroviral Treatment Guidelines. Jacana Publishers (ISBN: 1–919931–69–4). (http://southafrica.usembassy.gov/media/2004-doh-art-guidelines.pdf (last accessed 9th August 2015)).

Pasipanodya, J., Gumbo, T., 2011. An oracle: antituberculosis pharmacokinetics-pharmacodynamics, clinical correlation, and clinical trial simulations to predict the future. Antimicrob. Agents Chemother. 55, 24–34.

Pasipanodya, J.G., McIlleron, H., Burger, A., et al., 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J. Infect. Dis. 208, 1464–1473.

Pfyffer, G.E., Welscher, H.M., Kissling, P., et al., 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J. Clin. Microbiol. 35, 364–368.

Pusch, T., Pasipanodya, J.G., Hall, R.G., et al., 2014. Therapy duration and long-term outcomes in extra-pulmonary tuberculosis. BMC Infect. Dis. 14, 115.

Shadish, R.S., Cook, T.D., Campbell, D.T., 2001. Experimental and quasi-experimental designs for generalized causal inference. Belmont, Cengage Learning.

Shaw, J.E., Pasipanodya, J.G., Gumbo, T., 2010. Meningeal tuberculosis: high long-term mortality despite standard therapy. Medicine (Baltimore) 89, 189–195.

Shenje, J., Adimora-Nweke, I.F., Ross, I.L., et al., 2015. Penetration of antibiotics in tuberculous pericarditis. EBiomedicine (Accepted).

Srivastava, S., Pasipanodya, J.G., Meek, C., Leff, R., Gumbo, T., 2011. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J. Infect. Dis. 204, 1951–1959.

Steinberg, D., Colla, P., 1995. CART: Tree-Structured Non-parametric Data Analysis. Salford Systems, San Diego.

Theron, G., Peter, J., Calligaro, G., et al., 2014. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for tb diagnosis using specimens from different body compartments. Sci. Rep. 4, 5658.