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Complete List of Authors:	Virenfeldt, Jakob; Aarhus University Hospital, Department of Infectious Diseases Rudolf, Frauke; Bandim Health Project, Indepth Network Camara, Cidia; Bandim Health Project, Indepth Network FURTADO, Alcino; Bandim Health Project, Indepth Network Gomes, Victor; Bandim Health Project, Indepth Network Aaby, Peter; Bandim Health Project, Indepth Network Petersen, Eskild; Aarhus University Hospital, Department of Infectious Diseases Wejse, Christian; Bandim Health Project, Indepth Network
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Treatment delay affects clinical severity of tuberculosis;

A longitudinal cohort study

Virenfeldt J[1,2], Rudolf F[1,2], Camara C[1], Furtado A[1], Gomes V[1], Aaby P[1,3], Petersen E[2], Wejse C[1,4]

[1] Bandim Health Project, INDEPTH network, Apartado 861, Bissau, Guinea-Bissau

[2] Department of Infectious Diseases, Aarhus University Hospital, Denmark;

[3] Danish Epidemiology Science Center, Statens Serum Institut, Copenhagen, Denmark

[4] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Denmark

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ABSTRACT

Objectives

To describe the risk factors for treatment delay and the importance of delay for the severity of tuberculosis in a prospectively followed tuberculosis cohort at the Bandim Health Project in Guinea-Bissau.

Background

Treatment delay in patients with tuberculosis is associated with increased mortality and transmission of disease. However, it is not well described whether delay influences clinical severity at diagnosis. Reported risk factors for treatment delay vary in different geographical and cultural settings but have not been investigated in our setting.

Change in delay over time is rarely reported and our prospectively followed tuberculosis cohort makes such data available.

Participants

Patients were included at time of diagnosis at three local tuberculosis clinics and the national tuberculosis reference hospital. Inclusion criteria were age>15 years and diagnosis of tuberculosis by either sputum examination or by the WHO clinical criteria. Patients with extra-pulmonary tuberculosis were excluded.

Primary and secondary outcome measures

The primary outcome was treatment delay. Delay was assessed by patient questionnaires.

Secondary outcomes were TB morbidity assessed by Bandim TBscore and all cause mortality.

Results

A total of 1424 persons were diagnosed with tuberculosis in the study area between 2003 and 2010. We included 973 TB-patients in the study. The median treatment delay was 12.1 weeks. Risk factors for delay were low educational level, divorce, HIV-1+2 dual infection and negative sputum smear.

Tuberculosis treatment delay decreased with 10.3%[7.9%;12.6%] per year during the study period. Delay was significantly associated with clinical severity at presentation with 20.8% severe TB-cases in the low delay quartile compared to 33.9% if delay was over the median of 12.1 weeks.

Conclusion

Long treatment delay was associated with more severe clinical presentation. Treatment delay in tuberculosis cases is decreasing in Guinea Bissau. It is important for TB control programs to monitor treatment delay over time.

ARTICLE SUMMARY

Article focus

To investigate treatment delay in an urban tuberculosis cohort from West Africa including:

- Risk factors for delay
- Effects on clinical severity and mortality
- Changes in delay over the study period

Key messages

- Education, sputum smear, marital status and HIV-status were the most important risk factors for delay in our setting. Focus should be on uneducated and vulnerable groups when planning future health interventions concerning TB control.
- Delay in the initiation of TB-treatment is declining in this study though still a major issue in high burden areas. Treatment delay influences disease severity at diagnosis and health policy makers should maintain focus on awareness of TB symptoms and implement TB controls measures to detect the disease early.

Strengths and limitations of this study

- A continuously and well-described cohort of tuberculosis patients was used and a large number of TB-cases were included. Cases were followed with both clinical exams after treatment and mortality follow up.
- Division between patient delay and health care system delay was not possible since date of first contact to any health care provider could not be estimated validly. While this weakens the specificity of our findings it does not influence our results on severity of disease.
- There is a risk of recall bias since the data was collected by retrospective questionnaires. Cultural and educational factors may have influenced the estimated treatment delay.

INTRODUCTION

It is estimated that one third of the world's population is latently infected with tuberculosis (TB)[1]. In 2010 WHO estimated that there were 8.8 million cases of active TB with 1.45 million deaths annually[2]. Of these deaths, 98% occurred in developing countries[3]. Transmission of tuberculosis is difficult to control since one index case can infect a large number of secondary cases if left untreated[4-6]. Better management of TB should focus on reducing the time delay from symptom onset to initiating treatment[5 7]. Treatment delay is influenced by gender, age, sputum TB smear status, distance to nearest health care provider, educational level and TB and HIV status[8 9].

Previously reported treatment delays from different parts of the world vary considerably. A study from China found a total time delay of 3.6 weeks[7]; a study from Tanzania found a delay of 26 weeks[10]. The average delay in low income countries has recently been reported to be 9.7 weeks[8].

Factors affecting treatment delay are of key importance in TB management as delay may increase mortality[11,12]. However, there is limited data on the effect of delay on clinical severity at presentation. The aim of this study was to describe risk factors for treatment delay, to assess the possible effects of treatment delay on clinical severity at diagnosis and to monitor changes in delay over time in a prospective TB programme.

MATERIALS AND METHODS

Study design

Our study was a longitudinal prospective cohort including all patients diagnosed with TB within the Bandim Health Project study area. Inclusion criteria were age ≥ 15 years and diagnosed with TB by either sputum examination (smear microscopy; no culture was available) or by the World Health Organization clinical criteria[13].

Study location and population

The study was carried out in Bissau, the capital of Guinea-Bissau, at the Bandim Health Project (BHP). The BHP is a health and demographic surveillance site (HDSS), which has followed an urban population in Bissau since 1978. The study area consists of 6 suburban areas with a total population of 102,000, which is followed by regular demographic surveys. All individuals in the area are registered with ID-number, age, sex, ethnic group and socio-economic data. Censuses are performed at regular intervals and information on pregnancies, births, mortality and migration is collected on a daily basis. Since 1996 a surveillance system has detected all patients diagnosed with and treated for TB in the study area, and long-term follow-up has been performed. In 1997 an estimated TB incidence rate of the study area was one of the highest reported TB incidences in the world (470/100,000 person years)[14]; a recent study estimated a current incidence of 288/100,000 person years[15].

Health services and inclusion

Field assistants identified patients at the TB treatment facilities during daily visits. Patients were identified at treatment start and invited for consultation and enrolment. Patients were interviewed using a structured questionnaire including data on first signs and symptoms of TB and demographic

characteristics[16]. The inclusion period was from 1 November 2003 to 4 June 2010; data were analysed in June 2010 and the questionnaire was adapted for future studies.

Clinical exams were performed at the end of treatment and mortality follow up was performed after one and two years.

Definitions

Treatment delay was defined as the time from onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. Data on first contact to the health care system was incomplete, and a distinction between patient-related and health care system-related delay was not possible.

Many people from rural areas move temporarily to the capital city to work or to access the health services. These patients without a permanent address in the study area were classified as "non-residents".

The study was conducted in an urban environment and physical access to health care facilities was therefore not considered a major factor in delay.

Laboratory tests

An HIV-test was performed at inclusion using Enzygnost Anti-HIV 1+2 Plus (Behring Diagnostics Gmbh, Marburg, Germany) and confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) or Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-la Coquette, France). From January 2008, Determine HIV-1/2 (Alere Inc, Waltham, Massachusetts, USA) was used and positive results were confirmed with SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Korea). Tests for HIV were repeated at six months and for 13 cases with an inconclusive initial HIV-test the result of the next test after six months was used. Direct microscopy of sputum samples was performed using

Ziehl-Neelsen staining. Laboratory facilities for sputum cultures were destroyed during a civil war in Guinea-Bissau and were therefore not performed in the present study[17].

Bandim TBscore

Clinical severity was assessed by the Bandim TBscore. The TBscore is a newly developed tool assessing change in clinical status of patients with TB[18] expressed as a numeric index based on cough, haemoptysis, dyspnoea, chest pain and night sweating[18], and the following findings: Anaemic conjunctivae, tachycardia, positive lung auscultation, increased temperature, body mass index (BMI) and middle upper arm circumference (MUAC). The TBscore can be divided into three severity classes and a TBscore ≥8 correlates with mortality and lower TBscores with favourable outcomes, cure, and completed treatment as described elsewhere[18]. In consistency with previous findings "severe TB-cases" was defined as TBscore≥8.

Statistical analysis

Data were double entered and analyses were performed using Stata Statistical Software v.11 (Stata Corporation, College Station, TX, USA). To account for outliers in the reported treatment delay medians were chosen and logarithmic transformation was performed. The data was reanalyzed excluding these outliers but no significant change in estimates was observed. A multiple linear regression model was used to adjust for multiple risk factors in the treatment delay analysis. Cox-regression was used in the mortality analysis (Figure 3) and to estimate the development in initiation of TB treatment for the symptomatic TB-cases (Figure 2). Factors affecting the association between treatment delay and mortality with ≥10% were corrected for. No significant deviations from the proportional hazard assumption were found.

The Mann-Whitney test was applied for non-parametric testing of the group analysis on severity score. A two-tailed $p < 0.05$ was considered significant.

Ethics

The patients were informed in written Portuguese and verbally in the common language Creole and they gave consent by signature or fingerprint. The study was permitted by the Health Ministry of Guinea-Bissau and approved by the National Science and Ethics Committee in Guinea-Bissau as well as the Central Ethics Committee of Denmark.

RESULTS

Patient characteristics

A total of 1424 persons were diagnosed with TB in the study area. Of these, 58 were under the age of 15 years and 364 did not show up for enrolment. Thus, 1002 were enrolled in the cohort (Figure 1). We excluded 17 patients due to extra-pulmonary tuberculosis and 12 due to missing data at the time of symptom onset. Hence, 973 patients were included in the final analysis (62% males and 38% females). The mean age was 36.0 years (median 33; range 15-90) for men and 35.4 years (median 31; range 15-75) for women.

Information about non-included patients was generally limited to gender and age. Residential status was known for 153 of these patients. Non-included cases were older than included patients with a mean age of 38.0 years (median 35.0; range 15-98) compared with 35.7 years (median 32.0; range 15-90) ($p<0.01$). Non-included patients were more often not residents in the area; 34% (52/153) were from outside the study area, compared with 26% (252/973) in the included group ($p=0.03$), but there was no significant difference in gender ($p=0.87$).

The median treatment delay was 12.1 weeks; 10% of patients were still not in anti-tuberculosis treatment after 37 weeks (Figure 2).

Symptoms of TB

The first self-reported symptoms of TB are described in Table I. There were no data available on the type of first symptoms for 29 patients. The most frequent initial symptoms were cough, fever and chest pain. In total, 89.8% (874/973) reported cough at the time of treatment initiation and the median time with cough before diagnosis was 12.9 weeks (inter-quartile range (IQR) 8.6-21.4).

Risk factors for treatment delay

The risk factor analysis is shown in Table II. Female sex, age >45 years, marital status (divorced/widower), no education, negative smear, HIV-dual infection, traditional religion, non-residency and one ethnic group were significant risk factors for treatment delay in the univariate analyses. In the multiple regression analysis 22 cases were excluded due to missing information in one or more variables, and no education, negative smear, HIV-dual infection and being divorced or a widow remained significantly associated with treatment delay.

Of the 973 patients, 290 had never attended school and had a longer median time to treatment (15.1 weeks) than patients with a higher educational level (9.7 weeks; 10.0 weeks) ($p<0.001$). There was no significant difference in treatment delay between cases with an intermediate educational level (7-9 years) and a high educational level (>9 years) ($p=0.135$).

Patients with no expectoration or a negative TB-smear had a longer treatment delay than patients with a positive TB-smear (12,7 vs. 11.6 weeks).

Stratifying the risk factor analysis on TB-smear status did not change estimates significantly though ethnicity, religion and marital status were not found to be significant risk factors for sputum negative cases.

Bandim TBscore and mortality

Bandim TBscore was available for all 973 patients (Table III). We defined the lowest quartile as short treatment delay; followed by moderate, long and very long delay. The shortest treatment delay quartile (0-7.6 weeks) included a lower proportion of severe TB-cases at inclusion compared with the quartiles including patients with longer delays, though there was no difference in TBscore between the patients with a long and very long delay (Table III). The pooled percentage of severe TB-cases for these two groups was 33.9%.

In figure 3 we show the Kaplan Meier mortality curves for the four delay quartiles. A delay above 21 weeks (quartile 4) had a 59% significantly higher mortality than quartile 1 (delay below 7.6 weeks), Hazard Ratio (HR) 1.59 [95% CI 1.01-2.48], whereas quartiles 2 and 3 did not differ significantly from quartile 1. However when adjusting for HIV, age, education and civil status the HR was no longer significant.

We included 343 smear-negative TB-cases of whom 199 cases attended six months clinical follow up after completion of anti-tuberculosis treatment. During treatment thirty-six died and the remaining 108 declined further clinical follow-up or were lost to follow up. Mean TBscore for smear-negative TB-cases decreased from 6.46 to 1.31 ($p<0.00$) within 6 months of anti-tuberculosis treatment.

Changes in treatment delay

The median delay to treatment decreased during the study period. Within the first year the median delay was 14.6 weeks (IQR 9.3-26.1), but had dropped to 8.6 weeks (IQR: 5.7-16.7) in the last year. In a linear regression model the delay decreased with 10.3% [7.9%;12.6%] annually. The delay decreased with three weeks from 12.0 weeks (n=153) to 9.0 weeks (n=110) in 2007 and 2008 respectively. In 2007, free access to HIV-treatment was implemented and in 2006-2007 a TB incidence study was started[19]; this may be an important factor in the noticeable fall in delay between 2007 and 2008.

To account for changes in other factors affecting treatment delay we adjusted for changes in the significant risk factors for treatment delay from Table II. This did not change the estimates significantly and the adjusted decrease was 8.5%[6.0%;10.9%].

DISCUSSION

This study showed a long treatment delay in the diagnosis of TB in Guinea Bissau of 12.1 weeks equivalent to the delays found in comparable settings[20-24]. We found a clear association between treatment delay and clinical severity at inclusion, which has not been described before. This association is not surprising as patients in whom delay was long the disease had longer time to progress. Higher risk of hospitalization for patients with a longer treatment delay has been described previously[25], and educational level, marital status and TB-smear negativity affect delay in starting treatment for TB[9]; this was also seen in our study.

The clinical consequences of a short (but significant) difference in delay are difficult to measure. The smear-negative cases had only one week longer median treatment delay than smear-positive cases and though transmission of disease is time-dependent[4,5] the community implications are probably limited.

We found that smear-negative patients profited substantially from anti-tuberculosis treatment. Though the diagnosis was not confirmed by culture or smear, an empirical six months anti-tuberculosis treatment resulted in a significant decrease in clinical severity assessed by TBscore.

Non-residential status did not reach statistical significance in the multiple regression model, though it could be due to a limited sample size. Non-residents are a heterogeneous group in our setting; some arrive with TB symptoms attending the centralized health care facilities. However, many come to work or study in the capital and acquire the infection while being heavily exposed to TB living in an urban overpopulated environment. Previous studies have reported that residential status can be a risk factor for treatment delay[8 9] and we suggest that this parameter is included in future studies.

HIV-status influence treatment delay in some studies, but specific delay for HIV1+2 dual infection has not been described before. This could be a random finding but HIV1+2 dual infected cases are likely to be a vulnerable group with limited access to treatment.

Several factors may influence the observed change over time in delay to treatment. Socio-economic changes are likely to be the most important factor and the general economic situation in Bissau has improved within the last decade as the country has been rebuilt after the civil war in 1998[26]. Changes in risk factors for delay during the study period could only explain a minor part of the decrease in treatment delay. An increased awareness of signs and symptoms of TB after a large TB prevalence survey in the study area in 2006 may have contributed to the decrease in treatment delay[19]. We have seen a peak in incidence in 2007 following this survey, which is likely to have increased awareness of TB in the study area[27].

The study has several limitations; firstly we were not able to divide treatment delay into patient-related and health system-related delay due to limited information on the first contact to any health care provider.

Secondly, recall bias of treatment delay is a potential problem even though experienced local personnel and a comprehensive questionnaire was used [11,16]. Patients were included after a considerable time period of symptomatic TB and may have overestimated the delay. The most invalidating symptoms present at inclusion may also have been over-reported as a result of recall bias potentially influencing the reported symptoms analysis.

Thirdly, we did not have data on cause of death in the background population, and were not able to estimate the corresponding case detection rate (CDR) of pulmonary TB in the area. There may indeed be a small number of undiagnosed TB patients who die from tuberculosis as previously shown[27]. This is supported by our prevalence study finding undetected TB cases in the study area[19]. Treatment

delay may therefore have been underestimated since delay would be longer if undiagnosed cases with long delays had also been included.

Yet, our findings represent a population-based analysis over a long time period and are based on a large dataset. The results clearly show that delay to TB treatment is still a major issue in high burden areas. Our findings indicate that increased awareness of TB symptoms in the population and in health care systems is important to reduce the unacceptably long delay. Maintained focus is needed on awareness of TB and continuous implementation of TB control measures to detect the disease early and reduce unnecessary disability and loss of lives.

CONCLUSION

Delay of TB treatment in Bissau is significantly associated with disease severity at diagnosis. No education, being unmarried, HIV-dual infection and smear negative TB were significant risk factors for treatment delay. The treatment delay, though still unacceptably high, has decreased in Bissau during the past decade. TB control programmes may need to monitor treatment delay over time.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CW, FR and VG collected the clinical data. CC did the laboratory tests. JV and AF checked all variables and JV wrote the first draft of the manuscript. PA, EP and CW made the study logistically possible and edited the paper. All authors accepted the final version of the manuscript.

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FIGURE 1

Flowchart of participants

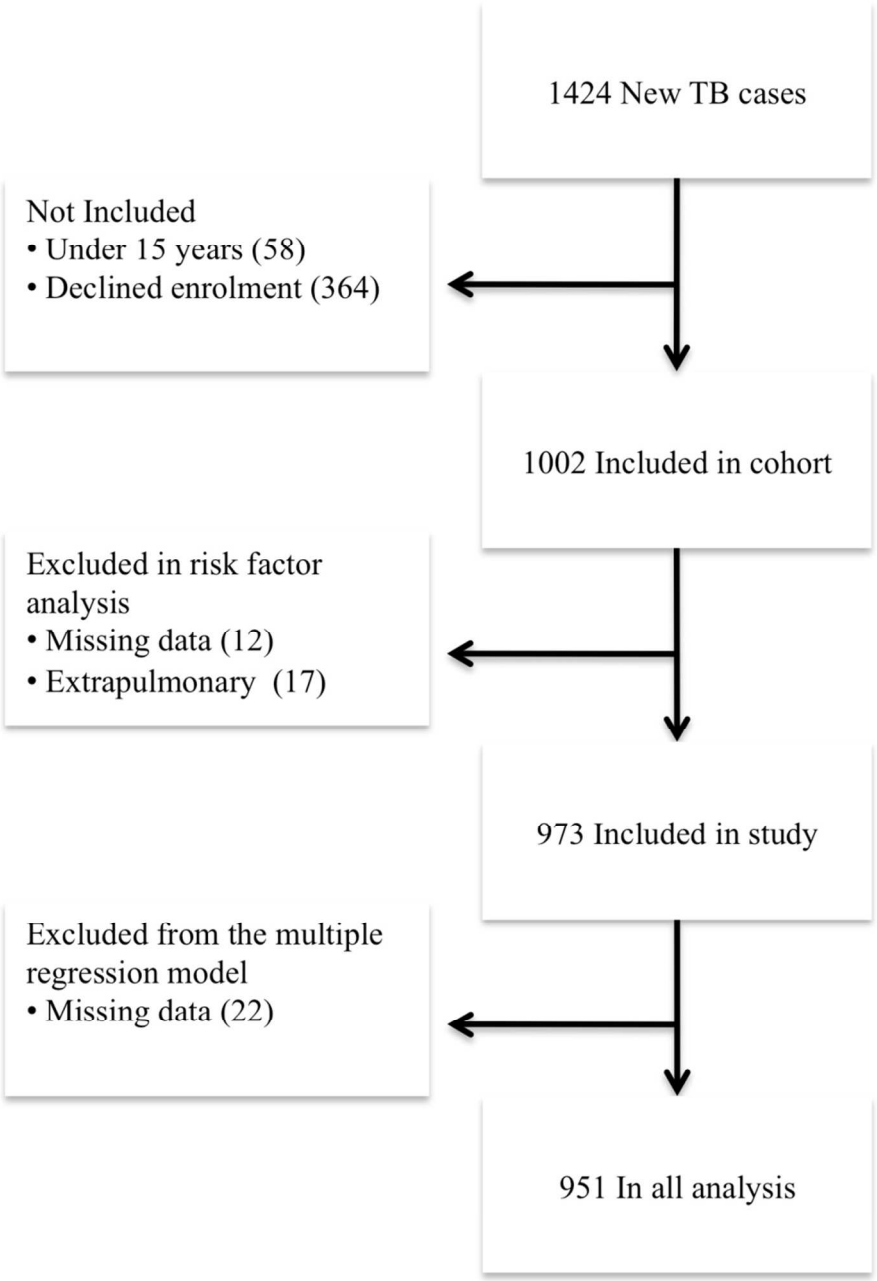


FIGURE 2

Proportion of TB cases in treatment

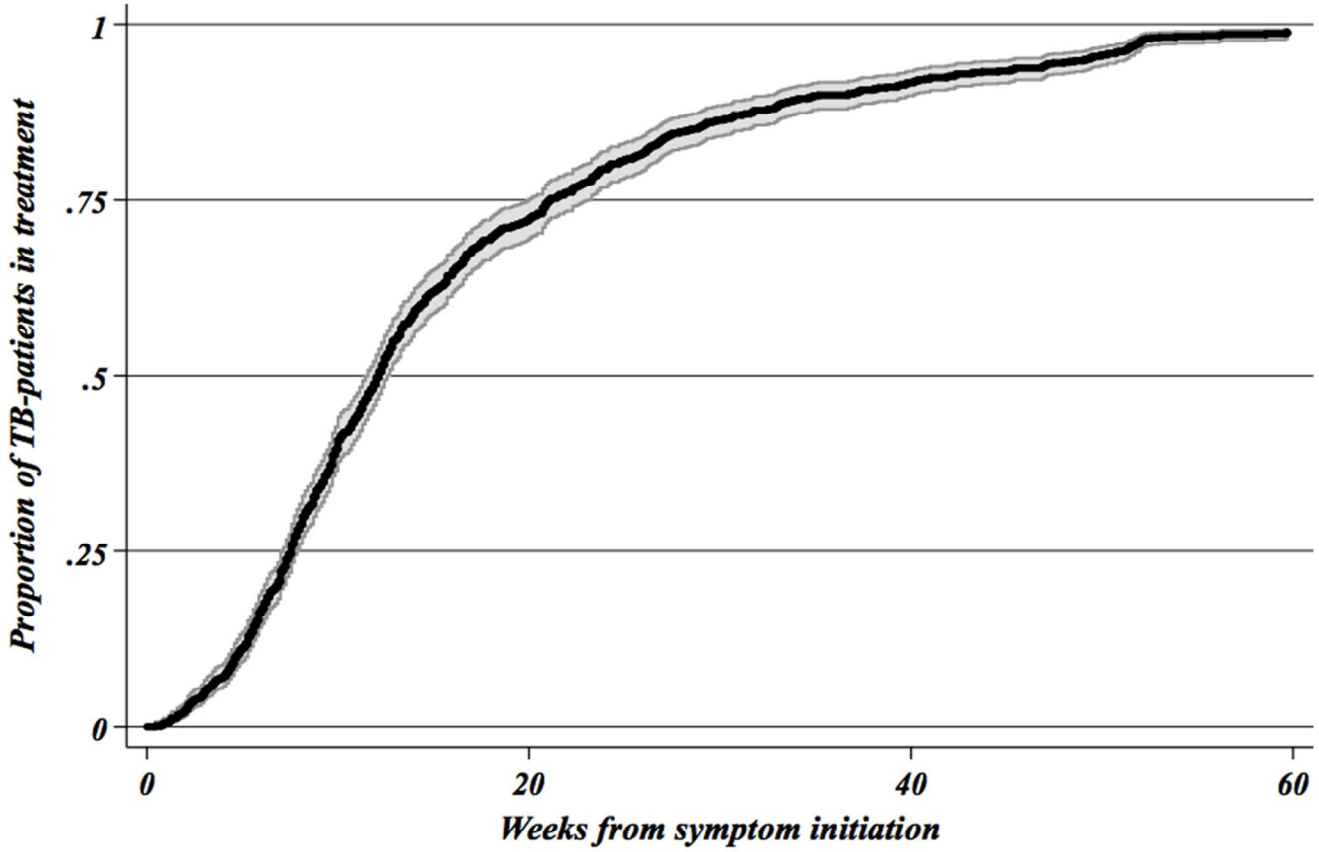


FIGURE 3

Mortality analysis

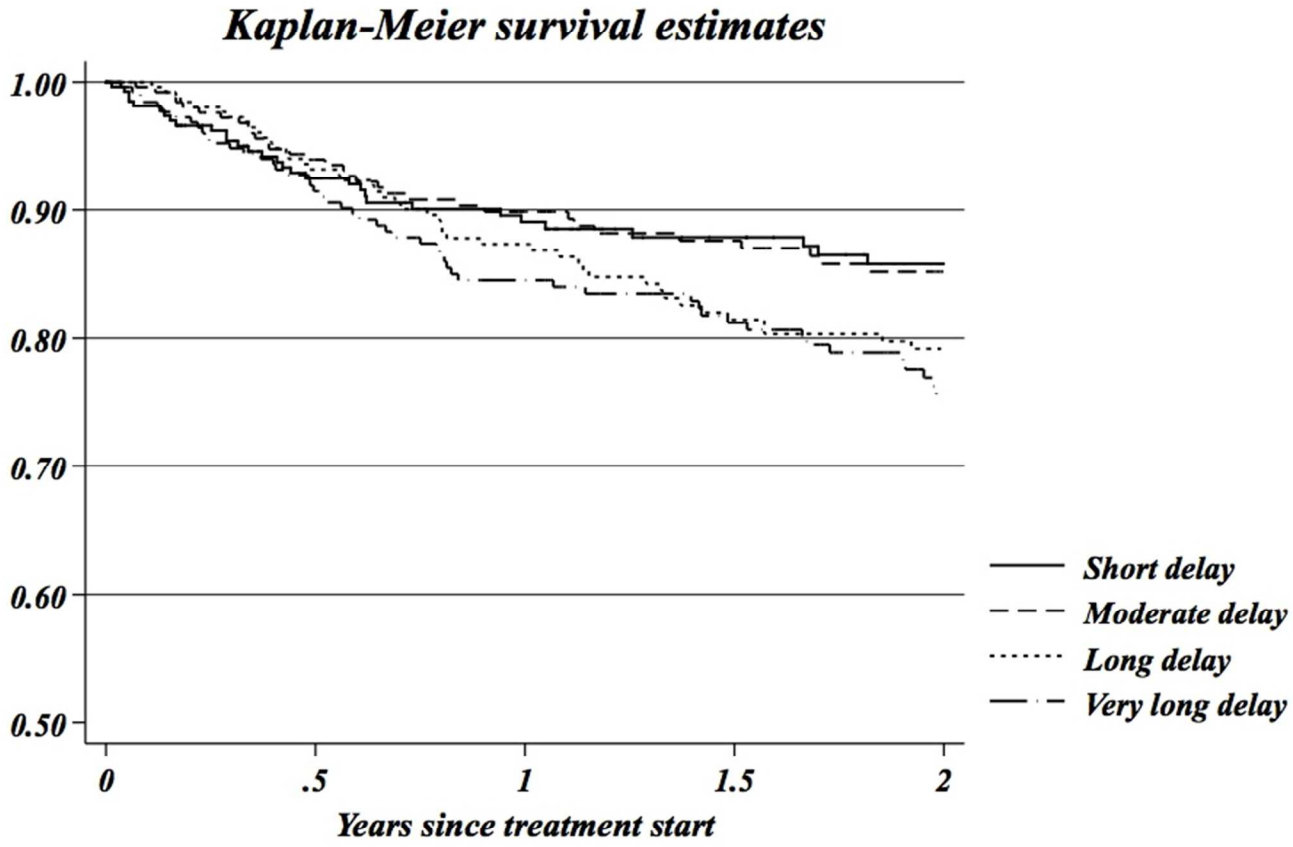


TABLE I

First self-reported symptoms of TB (n=930*)

	TB-smear positive (n=600)	TB-smear negative/no expectoration (n=330)**
Cough	88% (530/600)	87% (286/330)
Fever	80% (482/600)	85% (279/330)
Chest Pain	80% (477/600)	84% (276/330)
Weight loss	55% (330/600)	63% (208/330)
Night sweating	16% (95/600)	17% (55/330)
Breathlessness	14% (85/600)	16% (52/330)
Haemoptysis	6% (37/600)	8% (27/330)
Other symptoms	1% (6/600)	1% (2/330)

*43 cases did not provide information on the nature of first TB-symptoms.

** There were no significant differences between the two groups (p>0.05).

TABLE II

Risk factors for total treatment delay (n= 973*)

	N	Median (weeks)	RR	Adjusted RR
Gender				
- Male	602	11.4	1	1
- Female	371	12.7	1.15[1.03;1.28]**	1.03[0.91;1.16]**
Age (years)				
- 15-24	186	10.9	1	1
- 25-34	351	11.4	1.05[0.91;1.22]	1.01[0.86;1.17]
- 35-44	209	12.0	1.10[0.94;1.29]	0.93[0.78;1.12]
- 45+	227	14.9	1.31[1.12;1.54]	0.99[0.82;1.20]
Civil Status				
- Single	439	10.7	1	1
- Married	383	12.4	1.23[1.10;1.37]	1.07[0.93;1.22]
- Divorced/widow(er)	151	14.6	1.42[1.23;1.65]	1.21[1.01;1.46]
Education				
- 9+ years	199	9.7	1	1
- 7-9 years	208	10.0	1.13[0.96;1.31]	1.11[0.95;1.30]
- 1-6 years	264	12.6	1.36[1.17;1.57]	1.27[1.09;1.49]
- No education	290	15.1	1.55[1.34;1.79]	1.31[1.08;1.58]
- Data missing	12	17.2	1.50[0.94;2.38]	-
Residence				
- Resident	721	11.3	1	1
- Guest	252	15.1	1.32[1.18;1.48]	1.13[0.99;1.29]
HIV-status				
- HIV-neg	665	11.9	1	1
- HIV1	187	12.3	1.05[0.92;1.20]	1.04[0.91;1.19]
- HIV2	79	12.0	0.97[0.80;1.17]	0.89[0.73;1.08]
- HIV1+2	39	16.0	1.40[1.08;1.82]	1.34[1.02;1.75]
- Data missing	3	21.0	2.05[0.82;5.14]	-
Sputum smear status				
- Smear positive	630	11.6	1	1
- Smear neg/no expectoration	343	12.7	1.19[1.07;1.32]	1.16[1.05;1.30]
BMI				
- Normal (BMI>18.5)	435	11.1	1	
- Underweight(BMI<18.5)	524	12.6	1.10[0.99;1.22]	-
- Data missing	14	12.3	1.01[0.66;1.56]	-
Ethnic Group				
- Mancanha/Manjaco	251	11.9	1	1
- Balanta	143	13.4	1.25[1.06;1.48]	1.13[0.95;1.34]
- Fula	240	11.8	1.00[0.87;1.16]	1.10[0.80;1.51]
- Pepel	232	12.7	1.12[0.97;1.30]	1.10[0.95;1.27]
- Other	100	11.7	1.09[0.91;1.32]	1.10[0.91;1.34]
- Data missing	7	13.0	1.26[0.68;2.31]	-
Religion				
- Catholic	346	11.0	1	1
- Traditional Religion	265	14.7	1.32[1.16;1.50]	1.04[0.89;1.22]
- Protestant	84	11.1	0.97[0.80;1.17]	0.90[0.74;1.09]

-	Muslim	260	11.4	1.02[0.89;1.16]	0.87[0.64;1.17]
-	Other	17	12.4	1.17[0.79;1.73]	1.12[0.75;1.67]
-	Data missing	1	11.9	1.05[0.22;5.12]	-

*In the multiple regression model twenty-two patients were not included due to missing information on one or more risk factors (see figure 1).
**95% Confidence Intervals

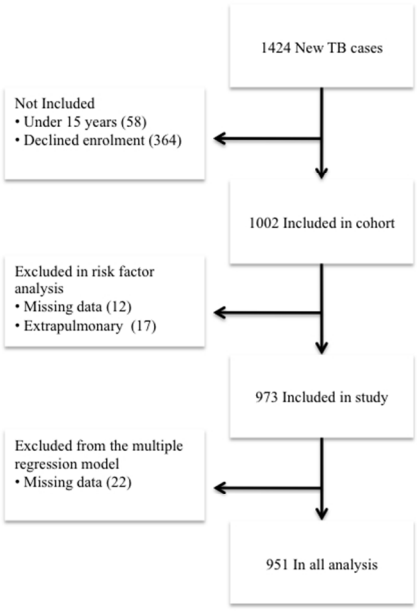
TABLE III

Treatment delay and clinical severity

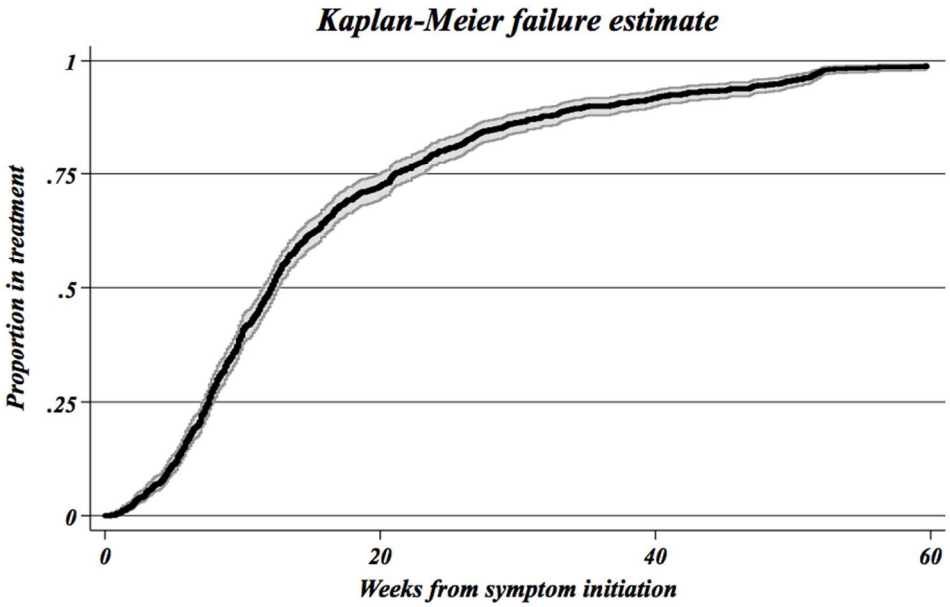
Centile	Delay (weeks)	Percentage of severe TB-cases*	p
0-25%	0-7.6	20.8% (50/241)	-
25-50%	7.6-12.1	26.1% (64/245)	-
50-75%	12.1-21.1	35.5% (87/245)	-
75%-	21.1-	32.4% (79/244)	0.001**

*Severe cases where defined as TBscore≥8

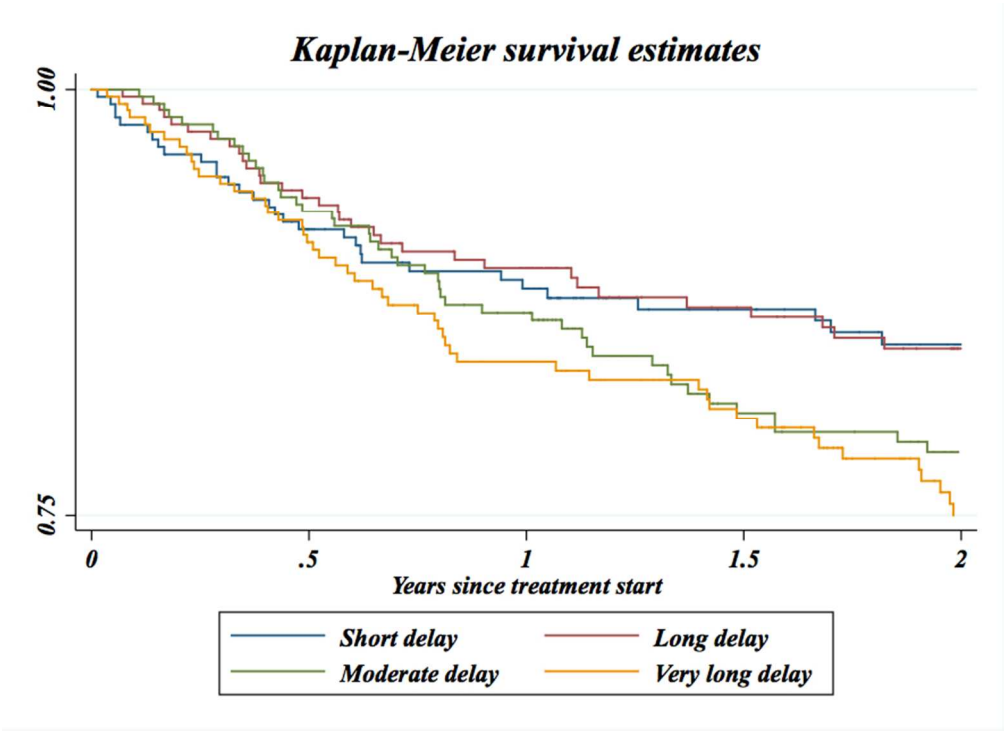
**Test for trend.



352x264mm (72 x 72 DPI)



339x226mm (72 x 72 DPI)



311x226mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	20
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9, 24
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Treatment delay affects clinical severity of tuberculosis; A longitudinal cohort study

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Treatment delay affects clinical severity of tuberculosis;

A longitudinal cohort study

Virenfeldt J[1,2], Rudolf F[1,2], Camara C[1], Furtado A[1], Gomes V[1], Aaby P[1,3], Petersen E[2], Wejse C[1,4]

[1] Bandim Health Project, INDEPTH network, Apartado 861, Bissau, Guinea-Bissau

[2] Department of Infectious Diseases, Aarhus University Hospital, Denmark;

[3] Danish Epidemiology Science Center, Statens Serum Institut, Copenhagen, Denmark

[4] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Denmark

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Keywords: HDSS, treatment delay, tuberculosis control

ABSTRACT

Objectives

To describe the risk factors for treatment delay and the importance of delay for the severity of tuberculosis in a prospectively followed tuberculosis cohort at the Bandim Health Project in Guinea-Bissau.

Background

Treatment delay in patients with tuberculosis is associated with increased mortality and transmission of disease. However, it is not well described whether delay influences clinical severity at diagnosis. Previously reported risk factors for treatment delay vary in different geographical and cultural settings. Such information has never been investigated in our setting.

Change in delay over time is rarely reported and our prospectively followed tuberculosis cohort gives an opportunity to present such data.

Participants

Patients were included at time of diagnosis at three local tuberculosis clinics and the national tuberculosis reference hospital. Inclusion criteria were age $\geq$ 15 years and diagnosis of tuberculosis by either sputum examination or by the WHO clinical criteria. Patients with extra-pulmonary tuberculosis were excluded.

Primary and secondary outcome measures

The primary outcome was treatment delay. Delay was assessed by patient questionnaires.

Secondary outcomes were Bandim TBscore as a measure of TB morbidity and all cause mortality.

Results

A total of 1424 persons were diagnosed with tuberculosis in the study area between 2003 and 2010. We included 973 TB-patients in the study. The median treatment delay was 12.1 weeks. Risk factors for delay were low educational level, HIV-1+2 dual infection and negative sputum smear. Tuberculosis

treatment delay decreased with 10.3% [7.9%;12.6%] per year during the study period. Delay was significantly associated with clinical severity at presentation with 20.8% severe TB-cases in the low delay quartile compared to 33.9% if delay was over the median of 12.1 weeks.

Conclusion

Long treatment delay was associated with more severe clinical presentation. Treatment delay in tuberculosis cases is decreasing in Guinea Bissau.

ARTICLE SUMMARY

Article focus

To investigate treatment delay in an urban tuberculosis cohort from West Africa including:

- Risk factors for delay
- Effects on clinical severity and mortality
- Changes in delay over the study period

Key messages

- Education, sputum smear and HIV-status were the most important risk factors for delay in our setting. Focus should be on uneducated and vulnerable groups when planning future health interventions concerning TB control.
- Delay in the initiation of TB-treatment is declining in this study though still a major issue in high burden areas. Treatment delay influences disease severity at diagnosis and health policy makers should maintain focus on awareness of TB symptoms and implement TB control measures to detect the disease early.

Strengths and limitations of this study

- A continuously and well-described cohort of tuberculosis patients was used and a large number of TB-cases were included. Cases were followed with both clinical exams after treatment and mortality follow up.
- Division between patient delay and health care system delay was not possible since date of first contact to any health care provider could not be estimated validly. While this weakens the specificity of our findings it does not influence our results on severity of disease.
- There is a risk of recall bias since the data was collected by retrospective questionnaires. Cultural and educational factors may have influenced the estimated treatment delay.

INTRODUCTION

It is estimated that one third of the world's population is latently infected with tuberculosis (TB)[1]. In 2013 WHO estimated that there were 8.6 million cases of active TB with 1.3 million deaths annually[2]. Of these deaths, over 95% occurred in developing countries[3]. Transmission of tuberculosis is difficult to control since one index case can infect a large number of secondary cases if left untreated[4-6]. Better management of TB should focus on reducing the time delay from symptom onset to initiating treatment[5 7]. Treatment delay is influenced by gender, age, sputum TB smear status, distance to nearest health care provider, educational level and HIV status[8 9].

Previously reported treatment delays from different parts of the world vary considerably. A study from China found a total time delay of 3.6 weeks[7]; a study from Tanzania found a delay of 26 weeks[10]. The average delay in low income countries has been reported to be 9.7 weeks[8].

Factors affecting treatment delay are of key importance in TB management as delay may increase mortality[11 12]. However, there is limited data on the effect of delay on clinical severity at presentation. The aim of this study was to describe risk factors for treatment delay, to assess the possible effects of treatment delay on clinical severity at diagnosis and to monitor changes in delay over time in a prospective TB programme.

MATERIALS AND METHODS

Study design

Our study was a longitudinal prospective cohort including all patients diagnosed with TB within the Bandim Health Project study area. Inclusion criteria were age ≥ 15 years and diagnosed with TB by either sputum examination (smear microscopy; no culture was available) or by the World Health Organizations clinical criteria[13].

Study location and population

The study was carried out in Bissau, the capital of Guinea-Bissau, at the Bandim Health Project (BHP). The BHP is a health and demographic surveillance site (HDSS), which has followed an urban population in Bissau since 1978. The study area consists of 6 suburban areas with a total population of 102,000, which is followed by regular demographic surveys. All individuals in the area are registered with ID-number, age, sex, ethnic group and socio-economic data. Censuses are performed at regular intervals and information on pregnancies, births, mortality and migration is collected on a daily basis. Since 1996 a surveillance system has detected all patients diagnosed with and treated for TB in the study area, and long-term follow-up has been performed. In 1997 an estimated TB incidence rate of the study area was one of the highest reported TB incidences in the world (470/100,000 person years)[14]; a recent study estimated a current incidence of 288/100,000 person years[15].

Health services and inclusion

Field assistants identified patients at the TB treatment facilities during daily visits. Patients were identified at treatment start and invited for consultation and enrolment. Not all patients showed up for enrolment, those not enrolled in the epidemiological study did not complete the questionnaire nor had the clinical examination, hence no delay or severity information was available for these patients.

Patients were interviewed using a structured questionnaire including data on first signs and symptoms of TB and demographic characteristics[16]. The inclusion period was from 1 November 2003 to 4 June 2010; data were analysed in June 2010 and the questionnaire was adapted for future studies.

During treatment patients were followed through regular clinical evaluations, the last being at the 6th and last month of treatment. Mortality follow-up was conducted through house visits 12 and 24 months after termination of treatment.

Definitions

Treatment delay was defined as the time from onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. At inclusion in the cohort, patients were asked when the first symptoms of the disease occurred and what the initial symptoms were. From the responses it was often difficult to make a clear assessment of the exact time of first contact to a health care facility, and a distinction between patient-related and health care system-related delay was not possible.

Many people from rural areas move temporarily to the capital city to work or to access the health services. These patients without a permanent address in the study area were classified as "non-residents".

The study was conducted in an urban environment and physical access to health care facilities was therefore not considered a major factor in delay.

Laboratory tests

An HIV-test was performed at inclusion using Enzygnost Anti-HIV 1+2 Plus (Behring Diagnostics Gmbh, Marburg, Germany) and confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) or Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-la Coquette, France). From January 2008, Determine HIV-1/2 (Alere Inc, Waltham, Massachusetts, USA) was used and positive results were confirmed with SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Korea). Tests

for HIV were repeated at six months and for 13 cases with an inconclusive initial HIV-test the result of the next test after six months was used. Direct microscopy of sputum samples was performed using Ziehl-Neelsen staining. Laboratory facilities for sputum cultures were destroyed during a civil war in Guinea-Bissau and were therefore not performed in the present study[17].

Bandim TBscore

Clinical severity was assessed by the Bandim TBscore. The TBscore is a newly developed tool assessing change in clinical status of patients with TB[18] expressed as a numeric index based on cough, haemoptysis, dyspnoea, chest pain and night sweating[18], and the following findings: Anaemic conjunctivae, tachycardia, positive lung auscultation, increased temperature, body mass index (BMI) and middle upper arm circumference (MUAC). The TBscore can be divided into three severity classes and a TBscore ≥8 correlates with mortality and lower TBscores with favourable outcomes, cure, and completed treatment as described elsewhere[18 19]. In these previous validations and also in other settings[20] "severe TB-cases" has been defined as TBscore≥8, which has a strong prognostic capacity for mortality.

Statistical analysis

Data were double entered and analyses were performed using Stata Statistical Software v.11 (Stata Corporation, College Station, TX, USA). To account for outliers in the reported treatment delay medians were presented in tables and figures and logarithmic transformation was performed. The data was reanalyzed excluding these outliers but no significant change in estimates was observed. A multiple linear regression model was used to adjust for multiple risk factors in the treatment delay analysis. Cox-regression was used in the mortality analysis (Figure 3) and in the depiction of time to TB treatment for the symptomatic TB-cases (Figure 2). Factors affecting the association between treatment delay and mortality with ≥10% were corrected for. No significant deviations from the

proportional hazard assumption were found. Factors affecting the association between treatment delay and severity with ≥10% were corrected for in the supplementary logistic regression model.

A two-tailed $p < 0.05$ was considered significant. Correction for calendar time was performed in the adjusted risk factor analysis (table 2).

Ethics

The patients were informed in written Portuguese and verbally in the common language Creole and they gave consent by signature or fingerprint. The study was permitted by the Health Ministry of Guinea-Bissau and approved by the National Science and Ethics Committee in Guinea-Bissau as well as the Central Ethics Committee of Denmark.

RESULTS

Patient characteristics

A total of 1424 persons were diagnosed with TB in the study area. Of these, 58 were under the age of 15 years and 364 were not enrolled. Thus, 1002 were enrolled in the cohort (Figure 1). We excluded 17 patients due to extra-pulmonary tuberculosis and 12 due to missing data at the time of symptom onset. Hence, 973 patients were included in the final analysis (62% males and 38% females). The mean age was 36.0 years (median 33; range 15-90) for men and 35.4 years (median 31; range 15-75) for women. Information about non-included patients was generally limited to gender and age. Residential status was known for 153 of these patients. Non-included cases were older than included patients with a mean age of 38.0 years (median 35.0; range 15-98) compared with 35.7 years (median 32.0; range 15-90) ($p<0.01$). Non-included patients were more often non-residents; 34% (52/153) were from outside the study area, compared with 26% (252/973) in the included group ($p=0.03$), but there was no significant difference in gender ($p=0.87$).

The median treatment delay was 12.1 weeks; 10% of patients were still not in anti-tuberculosis treatment after 37 weeks as described in Figure 2, which displays the proportion of the diagnosed and treated patients among the enrolled TB patients in this study.

Symptoms of TB

The first self-reported symptoms of TB are described in Table I, which comprises all symptoms, mentioned by patients as initial symptoms of their current illness, no attempt was made to differentiate which symptom was the first. There were no data available on the type of first symptoms for 43 patients. The most frequent initial symptoms were cough, fever and chest pain. In total, 89.8%

(874/973) reported cough at the time of treatment initiation and the median time with cough before diagnosis was 12.9 weeks (inter-quartile range (IQR) 8.6-21.4).

Risk factors for treatment delay

The risk factor analysis is shown in Table II. In the multiple regression analysis 22 cases were excluded due to missing information in one or more variables. No education, negative smear and HIV-dual infection remained significantly associated with treatment delay.

Of the 973 patients, 290 had never attended school and had a longer median time to treatment (15.1 weeks) than patients with schooling (p=0.02). There was no significant difference in treatment delay between cases with an intermediate educational level (7-9 years) and a high educational level (>9 years) (p=0.135).

Patients with no expectoration or a negative TB-smear had a longer treatment delay than patients with a positive TB-smear (12,7 vs. 11.6 weeks) (p=0.03).

Stratifying the risk factor analysis on TB-smear status did not change estimates significantly though ethnicity, religion and marital status were not found to be significant risk factors for sputum negative cases.

Bandim TBscore and mortality

Bandim TBscore was available for all 973 patients (Table III). We defined the lowest quartile as short treatment delay; followed by moderate, long and very long delay.

There was an increase in the proportion of severely ill patients as the delay increased and the association was significant (p=0.002) (Table III). There was no difference in TBscore between the patients with a long and very long delay. The pooled percentage of severe TB-cases for these two groups was 33.9%.

We also performed a logistic regression with severity (tbscore≥8) as the dependent variable and treatment delay (in 25% percentiles) as the explanatory variable. This resulted in an OR=2.08[1.39;3.13] for the long delay strata (7.6-12.1 weeks) and an OR=1.81[1.20;2.73] for very long delay strata (12.1-21.1 weeks). Correcting for possible confounders; HIV-status, residential status, education and sex the results were still significant - OR=1.57[1.21;2.82] and 1.85[1.21;2.82] respectively.

In figure 3 we show the Kaplan Meier mortality curves for the four delay quartiles. A delay above 21 weeks (quartile 4) had a higher mortality than quartile 1 (delay below 7.6 weeks), Hazard Ratio (HR) 1.59 [95% CI 1.01-2.48], whereas quartiles 2 and 3 did not differ significantly from quartile 1. However when adjusting for HIV, age, education and civil status the HR was no longer significant (Adjusted HR 1.10 [0.72;1.67]).

We included 343 smear-negative TB-cases of whom 199 cases attended six months clinical follow up after completion of anti-tuberculosis treatment. During treatment thirty-six died and the remaining 108 declined further clinical follow-up or were lost to follow up. Mean TBscore for smear-negative TB-cases decreased from 6.5 points to 1.3 points within 6 months of anti-tuberculosis treatment. The corresponding decrease in TBscore for smear positive patients was a decrease from a mean of 6.3 points to 0.9 points.

Changes in treatment delay

The median delay to treatment decreased during the study period. Within the first year the median delay was 14.6 weeks (IQR 9.3-26.1), but had dropped to 8.6 weeks (IQR: 5.7-16.7) in the last year. In a linear regression model the delay decreased with 10.3% [7.9%;12.6%] annually. The delay decreased with three weeks from 12.0 weeks (n=153) to 9.0 weeks (n=110) in 2007 and 2008 respectively. The trend in delay is displayed in Figure 4.

To account for changes in other factors affecting treatment delay we adjusted for changes in the significant risk factors for treatment delay from Table II. This did not change the estimates significantly and the adjusted decrease was 8.5%[6.0%;10.9%].

DISCUSSION

This study showed a long treatment delay in the diagnosis of TB in Guinea Bissau of 12.1 weeks equivalent to the delays found in comparable settings[21-25]. We found a clear association between treatment delay and clinical severity at inclusion, which has not been described before. This association is not surprising as patients in whom delay was long the disease had longer time to progress. We have shown in Table 3, that the proportion of severely ill is higher among those with a long delay, in the long delay groups it is a third of patients who are severely ill compared with a fifth in the short delay group. This translates directly into mortality risk, patients with a TBscore≥8 have a 60% higher mortality risk as previously shown[18] and a 6-fold higher risk of treatment failure[19]. Furthermore, a higher risk of hospitalization for patients with a longer treatment delay has also been described previously[26]. The clinical implications of shorter treatment delay is therefore likely to be less severe TB and subsequent lower mortality as well as less time to infect others.

There was a significant difference in mortality between patients with a short delay, compared with those with very long delay in the univariate analysis but when correcting for relevant factors this was no longer significant in the direct analysis.

The smear-negative cases had only one week longer median treatment delay than smear-positive cases and though transmission of disease is time-dependent[4 5] the community implications are probably limited.

We found that smear-negative patients profited substantially from anti-tuberculosis treatment. Though the diagnosis was not confirmed by culture or smear, an empirical six months anti-tuberculosis treatment resulted in a significant decrease in clinical severity assessed by TBscore.

Non-residential status did not reach statistical significance in the multiple regression model, though it could be due to a limited sample size. Non-residents are a heterogeneous group in our setting; some

arrive with TB symptoms attending the centralized health care facilities. However, many come to work or study in the capital and acquire the infection while being heavily exposed to TB living in an urban overpopulated environment. Previous studies have reported that residential status can be a risk factor for treatment delay[8 9] and we suggest that this parameter is included in future studies.

HIV-status influences treatment delay in some studies, but specific delay for HIV1+2 dual infection has not been described before. This could be a random finding but HIV1+2 dual infected cases are likely to be a vulnerable group with limited access to treatment. Furthermore HIV1+2 dual infected are more often sputum smear negative and present with fewer symptoms due to a weakened immune system[27].

Several factors may influence the observed change over time in delay to treatment. Socio-economic changes are likely to be the most important factor and the general economic situation in Bissau has improved within the last decade as the country has been rebuilt after the civil war in 1998[28]. Changes in risk factors for delay during the study period could only explain a minor part of the decrease in treatment delay. We hypothesize that an increased awareness of signs and symptoms of TB may have been a factor after a TB prevalence survey in the study area in 2006 which may have contributed to the decrease in treatment delay[29]. This survey among 2989 adults involved active case finding with the use of a questionnaire on TB symptoms and referral for sputum and x-ray according to an algorithm, and may have increased TB awareness in the involved 384 sampled houses. We have seen a peak in incidence in 2007 following this survey, which may have been a result of the survey and the increased awareness of TB in the study area[30].

The study has several limitations; firstly we were not able to divide treatment delay into patient-related and health system-related delay due to limited information on the first contact to any health care provider.

Secondly, recall bias is a potential problem even though experienced local personnel and a comprehensive questionnaire was used [11 16], and the self-reported duration of symptoms may not be an accurate assessment of treatment delay. Patients were included after a considerable time period of symptomatic TB and may have overestimated the delay. The most invalidating symptoms present at inclusion may also have been over-reported as a result of recall bias potentially influencing the reported symptoms analysis.

Thirdly, we did not have data on cause of death in the background population, and were not able to estimate the corresponding case detection rate (CDR) of pulmonary TB in the area. There may indeed be a small number of undiagnosed TB patients who die from tuberculosis as previously shown[30]. This is supported by our prevalence study finding undetected TB cases in the study area[29]. Treatment delay may therefore have been underestimated since delay would be longer if undiagnosed cases with long delays had also been included.

Fourthly, this study was not a prospective follow-up of TB suspects and therefore we have no information on a possible undetected TB cases or diagnosed yet untreated cases, and the study is therefore not able to assess how many patients with symptoms are under treatment. This has been done in a recent cohort-study on TB suspects[31], but this is not the scope of this study.

Yet, our findings represent a population-based analysis over a long time period and are based on a large dataset. The results clearly show that delay to TB treatment is still a major issue in high burden areas. Our findings indicate that increased awareness of TB symptoms in the population and in health care systems is important to reduce the unacceptable long delay. Maintained focus is needed on awareness of TB and continuous implementation of TB control measures to detect the disease early and reduce unnecessary disability and loss of lives.

CONCLUSION

Delay of TB treatment in Bissau is significantly associated with disease severity at diagnosis. No education, HIV-dual infection and smear negative TB were significant risk factors for treatment delay. The treatment delay, though still unacceptably high, has decreased in Bissau during the past decade. TB control programmes may need to monitor treatment delay over time.

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AUTHORS' CONTRIBUTIONS

CW, FR and VG collected the clinical data. CC did the laboratory tests. JV and AF checked all variables and JV wrote the first draft of the manuscript. PA, EP and CW made the study logistically possible and edited the paper. All authors accepted the final version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

Data Sharing Statement

Tecnical appendix, statistical code and dataset available from the corresponding author on request.

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FIGURE 1

Flowchart of participants

FIGURE 2

Proportion of TB-patient in treatment according to selfreported delay

FIGURE 3
Mortality analysis

FIGURE 4
Changes in treatment delay during the study period

TABLE I

First self-reported symptoms of TB (n=930*)

	TB-smear positive (n=600)	TB-smear negative/no expectoration (n=330)**
Cough	88% (530/600)	87% (286/330)
Fever	80% (482/600)	85% (279/330)
Chest Pain	80% (477/600)	84% (276/330)
Weight loss	55% (330/600)	63% (208/330)
Night sweating	16% (95/600)	17% (55/330)
Breathlessness	14% (85/600)	16% (52/330)
Haemoptysis	6% (37/600)	8% (27/330)
Other symptoms	1% (6/600)	1% (2/330)

*43 cases did not provide information on the nature of first TB-symptoms.

** There were no significant differences between the two groups (p>0.05).

TABLE II

Risk factors for total treatment delay (n= 973*)

	N	Median (weeks)	RR [95%CI]	Adjusted RR [95%CI]**
Gender				
- Male	602	11.4	1	1
- Female	371	12.7	1.15[1.03;1.28]	1.05[0.94;1.18]
Age (years)				
- 15-24	186	10.9	1	1
- 25-34	351	11.4	1.05[0.91;1.22]	0.99[0.85;1.15]
- 35-44	209	12.0	1.10[0.94;1.29]	0.95[0.79;1.14]
- 45+	227	14.9	1.31[1.12;1.54]	1.01[0.84;1.22]
Civil Status				
- Single	439	10.7	1	1
- Married	383	12.4	1.23[1.10;1.37]	1.02[0.89;1.17]
- Divorced/widow(er)	151	14.6	1.42[1.23;1.65]	1.15[0.86;1.37]
Education				
- 9+ years	199	9.7	1	1
- 7-9 years	208	10.0	1.13[0.96;1.31]	1.10[0.94;1.28]
- 1-6 years	264	12.6	1.36[1.17;1.57]	1.23[1.05;1.44]
- No education	290	15.1	1.55[1.34;1.79]	1.24[1.03;1.49]
- Data missing	12	17.2	1.50[0.94;2.38]	-
Residence				
- Resident	721	11.3	1	1
- Guest	252	15.1	1.32[1.18;1.48]	1.09[0.95;1.24]
HIV-status				
- HIV-neg	665	11.9	1	1
- HIV1	187	12.3	1.05[0.92;1.20]	1.03[0.90;1.17]
- HIV2	79	12.0	0.97[0.80;1.17]	0.86[0.71;1.04]
- HIV1+2	39	16.0	1.40[1.08;1.82]	1.31[1.01;1.70]
- Data missing	3	21.0	2.05[0.82;5.14]	-
Sputum smear status				
- Smear positive	630	11.6	1	1
- Smear neg/no expectoration	343	12.7	1.19[1.07;1.32]	1.13[1.02;1.25]
BMI				
- Normal (BMI>18.5)	435	11.1	1	
- Underweight(BMI<18.5)	524	12.6	1.10[0.99;1.22]	-
- Data missing	14	12.3	1.01[0.66;1.56]	-
Ethnic Group				
- Mancanha/Manjaco	251	11.9	1	1
- Balanta	143	13.4	1.25[1.06;1.48]	1.11[0.94;1.32]
- Fula	240	11.8	1.00[0.87;1.16]	1.12[0.82;1.52]
- Pepel	232	12.7	1.12[0.97;1.30]	1.13[0.98;1.31]
- Other	100	11.7	1.09[0.91;1.32]	1.10[0.90;1.33]
- Data missing	7	13.0	1.26[0.68;2.31]	-
Religion				
- Catholic	346	11.0	1	1
- Traditional Religion	265	14.7	1.32[1.16;1.50]	1.07[0.92;1.25]
- Protestant	84	11.1	0.97[0.80;1.17]	0.90[0.75;1.09]

-	Muslim	260	11.4	1.02[0.89;1.16]	0.88[0.65;1.18]
-	Other	17	12.4	1.17[0.79;1.73]	1.12[0.76;1.65]
-	Data missing	1	11.9	1.05[0.22;5.12]	-

*In the multiple regression model twenty-two patients were not included due to missing information on one or more risk factors (see figure 1).
**The adjusted analysis was corrected for calendar time.

TABLE III

Treatment delay and clinical severity

Centile	Delay (weeks)	TBscore	Percentage of severe TB-cases*	p
0-25%	0-7.6	5.7[5.4;5.9]	20.8% (50/241)	-
25-50%	7.6-12.1	6.3[5.9;6.7]	26.1% (64/245)	-
50-75%	12.1-21.1	6.8[6.4;7.2]	35.5% (87/245)	-
75%-	21.1-	6.7[6.3;7.1]	32.4% (79/244)	0.002**

*Severe cases were defined as TBscore≥8

**Fishers exact test

Treatment delay affects clinical severity of tuberculosis;

A longitudinal cohort study

Virenfeldt J[1,2], Rudolf F[1,2], Camara C[1], Furtado A[1], Gomes V[1], Aaby P[1,3], Petersen E[2], Wejse C[1,4]

[1] Bandim Health Project, INDEPTH network, Apartado 861, Bissau, Guinea-Bissau

[2] Department of Infectious Diseases, Aarhus University Hospital, Denmark;

[3] Danish Epidemiology Science Center, Statens Serum Institut, Copenhagen, Denmark

[4] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Denmark

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ABSTRACT

Objectives

To describe the risk factors for treatment delay and the importance of delay for the severity of tuberculosis in a prospectively followed tuberculosis cohort at the Bandim Health Project in Guinea-Bissau.

Background

Treatment delay in patients with tuberculosis is associated with increased mortality and transmission of disease. However, it is not well described whether delay influences clinical severity at diagnosis. Previously reported risk factors for treatment delay vary in different geographical and cultural settings. Such information has never been investigated in our setting.

Change in delay over time is rarely reported and our prospectively followed tuberculosis cohort gives an opportunity to present such data.

Participants

Patients were included at time of diagnosis at three local tuberculosis clinics and the national tuberculosis reference hospital. Inclusion criteria were age $\geq$ 15 years and diagnosis of tuberculosis by either sputum examination or by the WHO clinical criteria. Patients with extra-pulmonary tuberculosis were excluded.

Primary and secondary outcome measures

The primary outcome was treatment delay. Delay was assessed by patient questionnaires.

Secondary outcomes were Bandim TBscore as a measure of TB morbidity and all cause mortality.

Results

A total of 1424 persons were diagnosed with tuberculosis in the study area between 2003 and 2010. We included 973 TB-patients in the study. The median treatment delay was 12.1 weeks. Risk factors for delay were low educational level, HIV-1+2 dual infection and negative sputum smear. Tuberculosis

treatment delay decreased with 10.3% [7.9%;12.6%] per year during the study period. Delay was significantly associated with clinical severity at presentation with 20.8% severe TB-cases in the low delay quartile compared to 33.9% if delay was over the median of 12.1 weeks.

Conclusion

Long treatment delay was associated with more severe clinical presentation. Treatment delay in tuberculosis cases is decreasing in Guinea Bissau.

ARTICLE SUMMARY

Article focus

To investigate treatment delay in an urban tuberculosis cohort from West Africa including:

- Risk factors for delay
- Effects on clinical severity and mortality
- Changes in delay over the study period

Key messages

- Education, sputum smear and HIV-status were the most important risk factors for delay in our setting. Focus should be on uneducated and vulnerable groups when planning future health interventions concerning TB control.
- Delay in the initiation of TB-treatment is declining in this study though still a major issue in high burden areas. Treatment delay influences disease severity at diagnosis and health policy makers should maintain focus on awareness of TB symptoms and implement TB control measures to detect the disease early.

Strengths and limitations of this study

- A continuously and well-described cohort of tuberculosis patients was used and a large number of TB-cases were included. Cases were followed with both clinical exams after treatment and mortality follow up.
- Division between patient delay and health care system delay was not possible since date of first contact to any health care provider could not be estimated validly. While this weakens the specificity of our findings it does not influence our results on severity of disease.
- There is a risk of recall bias since the data was collected by retrospective questionnaires. Cultural and educational factors may have influenced the estimated treatment delay.

INTRODUCTION

It is estimated that one third of the world's population is latently infected with tuberculosis (TB)[1]. In 2013 WHO estimated that there were 8.6 million cases of active TB with 1.3 million deaths annually[2]. Of these deaths, over 95% occurred in developing countries[3]. Transmission of tuberculosis is difficult to control since one index case can infect a large number of secondary cases if left untreated[4-6]. Better management of TB should focus on reducing the time delay from symptom onset to initiating treatment[5 7]. Treatment delay is influenced by gender, age, sputum TB smear status, distance to nearest health care provider, educational level and HIV status[8 9].

Previously reported treatment delays from different parts of the world vary considerably. A study from China found a total time delay of 3.6 weeks[7]; a study from Tanzania found a delay of 26 weeks[10]. The average delay in low income countries has been reported to be 9.7 weeks[8].

Factors affecting treatment delay are of key importance in TB management as delay may increase mortality[11 12]. However, there is limited data on the effect of delay on clinical severity at presentation. The aim of this study was to describe risk factors for treatment delay, to assess the possible effects of treatment delay on clinical severity at diagnosis and to monitor changes in delay over time in a prospective TB programme.

MATERIALS AND METHODS

Study design

Our study was a longitudinal prospective cohort including all patients diagnosed with TB within the Bandim Health Project study area. Inclusion criteria were age ≥ 15 years and diagnosed with TB by either sputum examination (smear microscopy; no culture was available) or by the World Health Organizations clinical criteria[13].

Study location and population

The study was carried out in Bissau, the capital of Guinea-Bissau, at the Bandim Health Project (BHP). The BHP is a health and demographic surveillance site (HDSS), which has followed an urban population in Bissau since 1978. The study area consists of 6 suburban areas with a total population of 102,000, which is followed by regular demographic surveys. All individuals in the area are registered with ID-number, age, sex, ethnic group and socio-economic data. Censuses are performed at regular intervals and information on pregnancies, births, mortality and migration is collected on a daily basis. Since 1996 a surveillance system has detected all patients diagnosed with and treated for TB in the study area, and long-term follow-up has been performed. In 1997 an estimated TB incidence rate of the study area was one of the highest reported TB incidences in the world (470/100,000 person years)[14]; a recent study estimated a current incidence of 288/100,000 person years[15].

Health services and inclusion

Field assistants identified patients at the TB treatment facilities during daily visits. Patients were identified at treatment start and invited for consultation and enrolment. Not all patients showed up for enrolment, those not enrolled in the epidemiological study did not complete the questionnaire nor had the clinical examination, hence no delay or severity information was available for these patients.

Patients were interviewed using a structured questionnaire including data on first signs and symptoms of TB and demographic characteristics[16]. The inclusion period was from 1 November 2003 to 4 June 2010; data were analysed in June 2010 and the questionnaire was adapted for future studies.

During treatment patients were followed through regular clinical evaluations, the last being at the 6th and last month of treatment. Mortality follow-up was conducted through house visits 12 and 24 months after termination of treatment.

Definitions

Treatment delay was defined as the time from onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. At inclusion in the cohort, patients were asked when the first symptoms of the disease occurred and what the initial symptoms were. From the responses it was often difficult to make a clear assessment of the exact time of first contact to a health care facility, and a distinction between patient-related and health care system-related delay was not possible.

Many people from rural areas move temporarily to the capital city to work or to access the health services. These patients without a permanent address in the study area were classified as "non-residents".

The study was conducted in an urban environment and physical access to health care facilities was therefore not considered a major factor in delay.

Laboratory tests

An HIV-test was performed at inclusion using Enzygnost Anti-HIV 1+2 Plus (Behring Diagnostics Gmbh, Marburg, Germany) and confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) or Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-la Coquette, France). From January 2008, Determine HIV-1/2 (Alere Inc, Waltham, Massachusetts, USA) was used and positive results were confirmed with SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Korea). Tests

for HIV were repeated at six months and for 13 cases with an inconclusive initial HIV-test the result of the next test after six months was used. Direct microscopy of sputum samples was performed using Ziehl-Neelsen staining. Laboratory facilities for sputum cultures were destroyed during a civil war in Guinea-Bissau and were therefore not performed in the present study[17].

Bandim TBscore

Clinical severity was assessed by the Bandim TBscore. The TBscore is a newly developed tool assessing change in clinical status of patients with TB[18] expressed as a numeric index based on cough, haemoptysis, dyspnoea, chest pain and night sweating[18], and the following findings: Anaemic conjunctivae, tachycardia, positive lung auscultation, increased temperature, body mass index (BMI) and middle upper arm circumference (MUAC). The TBscore can be divided into three severity classes and a TBscore ≥8 correlates with mortality and lower TBscores with favourable outcomes, cure, and completed treatment as described elsewhere[18 19]. In these previous validations and also in other settings[20] "severe TB-cases" has been defined as TBscore≥8 which has a strong prognostic capacity for mortality.

Statistical analysis

Data were double entered and analyses were performed using Stata Statistical Software v.11 (Stata Corporation, College Station, TX, USA). To account for outliers in the reported treatment delay medians were presented in tables and figures and logarithmic transformation was performed. The data was reanalyzed excluding these outliers but no significant change in estimates was observed. A multiple linear regression model was used to adjust for multiple risk factors in the treatment delay analysis. Cox-regression was used in the mortality analysis (Figure 3) and in the depiction of time to TB treatment for the symptomatic TB-cases (Figure 2). Factors affecting the association between treatment delay and mortality with ≥10% were corrected for. No significant deviations from the

proportional hazard assumption were found. Factors affecting the association between treatment delay and severity with ≥10% were corrected for in the supplementary logistic regression model.

A two-tailed $p < 0.05$ was considered significant. Correction for calendar time was performed in the adjusted risk factor analysis (table 2).

Ethics

The patients were informed in written Portuguese and verbally in the common language Creole and they gave consent by signature or fingerprint. The study was permitted by the Health Ministry of Guinea-Bissau and approved by the National Science and Ethics Committee in Guinea-Bissau as well as the Central Ethics Committee of Denmark.

RESULTS

Patient characteristics

A total of 1424 persons were diagnosed with TB in the study area. Of these, 58 were under the age of 15 years and 364 were not enrolled. Thus, 1002 were enrolled in the cohort (Figure 1). We excluded 17 patients due to extra-pulmonary tuberculosis and 12 due to missing data at the time of symptom onset. Hence, 973 patients were included in the final analysis (62% males and 38% females). The mean age was 36.0 years (median 33; range 15-90) for men and 35.4 years (median 31; range 15-75) for women. Information about non-included patients was generally limited to gender and age. Residential status was known for 153 of these patients. Non-included cases were older than included patients with a mean age of 38.0 years (median 35.0; range 15-98) compared with 35.7 years (median 32.0; range 15-90) ($p<0.01$). Non-included patients were more often non-residents; 34% (52/153) were from outside the study area, compared with 26% (252/973) in the included group ($p=0.03$), but there was no significant difference in gender ($p=0.87$).

The median treatment delay was 12.1 weeks; 10% of patients were still not in anti-tuberculosis treatment after 37 weeks as described in Figure 2, which displays the proportion of the diagnosed and treated patients among the enrolled TB patients in this study.

Symptoms of TB

The first self-reported symptoms of TB are described in Table I, which comprises all symptoms mentioned by patients as initial symptoms of their current illness, no attempt was made to differentiate which symptom was the first. There were no data available on the type of first symptoms for 43 patients. The most frequent initial symptoms were cough, fever and chest pain. In total, 89.8%

(874/973) reported cough at the time of treatment initiation and the median time with cough before diagnosis was 12.9 weeks (inter-quartile range (IQR) 8.6-21.4).

Risk factors for treatment delay

The risk factor analysis is shown in Table II. In the multiple regression analysis 22 cases were excluded due to missing information in one or more variables. No education, negative smear and HIV-dual infection remained significantly associated with treatment delay.

Of the 973 patients, 290 had never attended school and had a longer median time to treatment (15.1 weeks) than patients with schooling (p=0.02). There was no significant difference in treatment delay between cases with an intermediate educational level (7-9 years) and a high educational level (>9 years) (p=0.135).

Patients with no expectoration or a negative TB-smear had a longer treatment delay than patients with a positive TB-smear (12,7 vs. 11.6 weeks) (p=0.03).

Stratifying the risk factor analysis on TB-smear status did not change estimates significantly though ethnicity, religion and marital status were not found to be significant risk factors for sputum negative cases.

Bandim TBscore and mortality

Bandim TBscore was available for all 973 patients (Table III). We defined the lowest quartile as short treatment delay; followed by moderate, long and very long delay.

There was an increase in the proportion of severely ill patients as the delay increased and the association was significant (p=0.002) (Table III). There was no difference in TBscore between the patients with a long and very long delay. The pooled percentage of severe TB-cases for these two groups was 33.9%.

We also performed a logistic regression with severity (tbscore≥8) as the dependent variable and treatment delay (in 25% percentiles) as the explanatory variable. This resulted in an OR=2.08[1.39;3.13] for the long delay strata (7.6-12.1 weeks) and an OR=1.81[1.20;2.73] for very long delay strata (12.1-21.1 weeks). Correcting for possible confounders; HIV-status, residential status, education and sex the results were still significant - OR=1.57[1.21;2.82] and 1.85[1.21;2.82] respectively.

In figure 3 we show the Kaplan Meier mortality curves for the four delay quartiles. A delay above 21 weeks (quartile 4) had a higher mortality than quartile 1 (delay below 7.6 weeks), Hazard Ratio (HR) 1.59 [95% CI 1.01-2.48], whereas quartiles 2 and 3 did not differ significantly from quartile 1. However when adjusting for HIV, age, education and civil status the HR was no longer significant (Adjusted HR 1.10 [0.72;1.67]).

We included 343 smear-negative TB-cases of whom 199 cases attended six months clinical follow up after completion of anti-tuberculosis treatment. During treatment thirty-six died and the remaining 108 declined further clinical follow-up or were lost to follow up. Mean TBscore for smear-negative TB-cases decreased from 6.5 points to 1.3 points within 6 months of anti-tuberculosis treatment. The corresponding decrease in TBscore for smear positive patients was a decrease from a mean of 6.3 points to 0.9 points.

Changes in treatment delay

The median delay to treatment decreased during the study period. Within the first year the median delay was 14.6 weeks (IQR 9.3-26.1), but had dropped to 8.6 weeks (IQR: 5.7-16.7) in the last year. In a linear regression model the delay decreased with 10.3% [7.9%;12.6%] annually. The delay decreased with three weeks from 12.0 weeks (n=153) to 9.0 weeks (n=110) in 2007 and 2008 respectively. The trend in delay is displayed in Figure 4.

To account for changes in other factors affecting treatment delay we adjusted for changes in the significant risk factors for treatment delay from Table II. This did not change the estimates significantly and the adjusted decrease was 8.5%[6.0%;10.9%].

DISCUSSION

This study showed a long treatment delay in the diagnosis of TB in Guinea Bissau of 12.1 weeks equivalent to the delays found in comparable settings[21-25]. We found a clear association between treatment delay and clinical severity at inclusion, which has not been described before. This association is not surprising as patients in whom delay was long the disease had longer time to progress. We have shown in Table 3, that the proportion of severely ill is higher among those with a long delay, in the long delay groups it is a third of patients who are severely ill compared with a fifth in the short delay group. This translates directly into mortality risk, patients with a TBscore≥8 have a 60% higher mortality risk as previously shown[18] and a 6-fold higher risk of treatment failure[19]. Furthermore, a higher risk of hospitalization for patients with a longer treatment delay has also been described previously[26]. The clinical implications of shorter treatment delay is therefore likely to be less severe TB and subsequent lower mortality as well as less time to infect others.

There was a significant difference in mortality between patients with a short delay, compared with those with very long delay in the univariate analysis but when correcting for relevant factors this was no longer significant in the direct analysis.

The smear-negative cases had only one week longer median treatment delay than smear-positive cases and though transmission of disease is time-dependent[4 5] the community implications are probably limited.

We found that smear-negative patients profited substantially from anti-tuberculosis treatment. Though the diagnosis was not confirmed by culture or smear, an empirical six months anti-tuberculosis treatment resulted in a significant decrease in clinical severity assessed by TBscore.

Non-residential status did not reach statistical significance in the multiple regression model, though it could be due to a limited sample size. Non-residents are a heterogeneous group in our setting; some

arrive with TB symptoms attending the centralized health care facilities. However, many come to work or study in the capital and acquire the infection while being heavily exposed to TB living in an urban overpopulated environment. Previous studies have reported that residential status can be a risk factor for treatment delay[8 9] and we suggest that this parameter is included in future studies.

HIV-status influences treatment delay in some studies, but specific delay for HIV1+2 dual infection has not been described before. This could be a random finding but HIV1+2 dual infected cases are likely to be a vulnerable group with limited access to treatment. Furthermore HIV1+2 dual infected are more often sputum smear negative and present with fewer symptoms due to a weakened immune system[27].

Several factors may influence the observed change over time in delay to treatment. Socio-economic changes are likely to be the most important factor and the general economic situation in Bissau has improved within the last decade as the country has been rebuilt after the civil war in 1998[28]. Changes in risk factors for delay during the study period could only explain a minor part of the decrease in treatment delay. We hypothesize that an increased awareness of signs and symptoms of TB may have been a factor after a TB prevalence survey in the study area in 2006 which may have contributed to the decrease in treatment delay[29]. This survey among 2989 adults involved active case finding with the use of a questionnaire on TB symptoms and referral for sputum and x-ray according to an algorithm, and may have increased TB awareness in the involved 384 sampled houses. We have seen a peak in incidence in 2007 following this survey, which may have been a result of the survey and the increased awareness of TB in the study area[30].

The study has several limitations; firstly we were not able to divide treatment delay into patient-related and health system-related delay due to limited information on the first contact to any health care provider.

Secondly, recall bias is a potential problem even though experienced local personnel and a comprehensive questionnaire was used [11 16], and the self-reported duration of symptoms may not be an accurate assessment of treatment delay. Patients were included after a considerable time period of symptomatic TB and may have overestimated the delay. The most invalidating symptoms present at inclusion may also have been over-reported as a result of recall bias potentially influencing the reported symptoms analysis.

Thirdly, we did not have data on cause of death in the background population, and were not able to estimate the corresponding case detection rate (CDR) of pulmonary TB in the area. There may indeed be a small number of undiagnosed TB patients who die from tuberculosis as previously shown[30]. This is supported by our prevalence study finding undetected TB cases in the study area[29]. Treatment delay may therefore have been underestimated since delay would be longer if undiagnosed cases with long delays had also been included.

Fourthly, this study was not a prospective follow-up of TB suspects and therefore we have no information on a possible undetected TB cases or diagnosed yet untreated cases, and the study is therefore not able to assess how many patients with symptoms are under treatment. This has been done in a recent cohort-study on TB suspects[31], but this is not the scope of this study.

Yet, our findings represent a population-based analysis over a long time period and are based on a large dataset. The results clearly show that delay to TB treatment is still a major issue in high burden areas. Our findings indicate that increased awareness of TB symptoms in the population and in health care systems is important to reduce the unacceptable long delay. Maintained focus is needed on awareness of TB and continuous implementation of TB control measures to detect the disease early and reduce unnecessary disability and loss of lives.

CONCLUSION

Delay of TB treatment in Bissau is significantly associated with disease severity at diagnosis. No education, HIV-dual infection and smear negative TB were significant risk factors for treatment delay. The treatment delay, though still unacceptably high, has decreased in Bissau during the past decade. TB control programmes may need to monitor treatment delay over time.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CW, FR and VG collected the clinical data. CC did the laboratory tests. JV and AF checked all variables and JV wrote the first draft of the manuscript. PA, EP and CW made the study logistically possible and edited the paper. All authors accepted the final version of the manuscript.

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FIGURE 1

Flowchart of participants

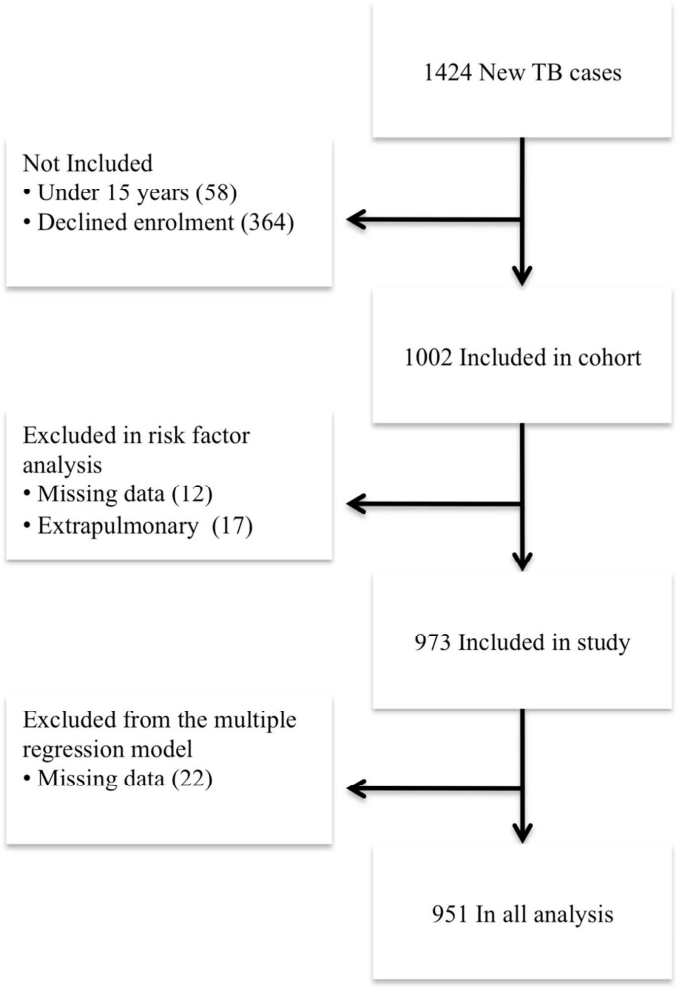

FIGURE 2

Proportion of TB-patient in treatment according to selfreported delay

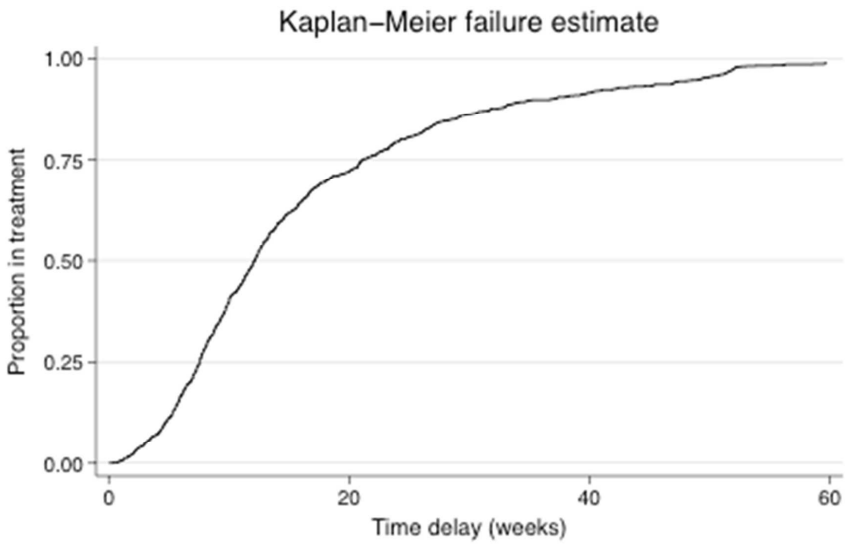

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FIGURE 3
Mortality analysis

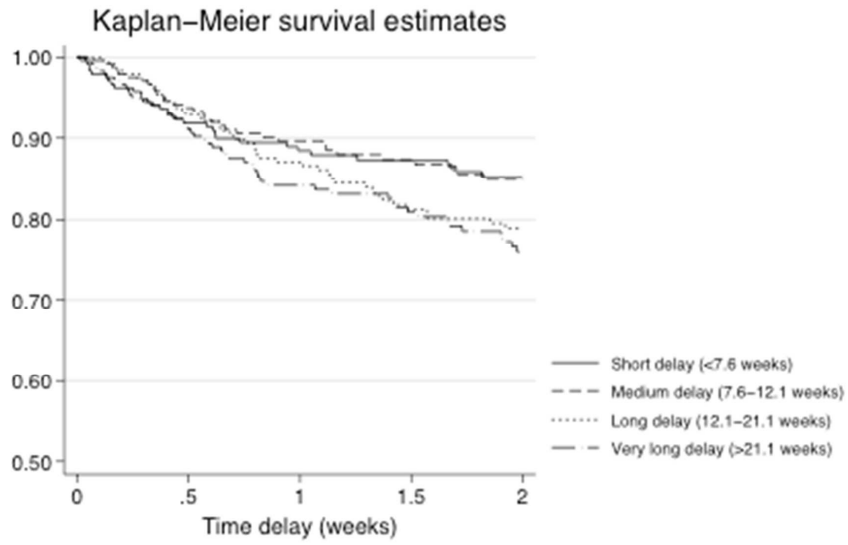

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FIGURE 4

Changes in treatment delay during the study period

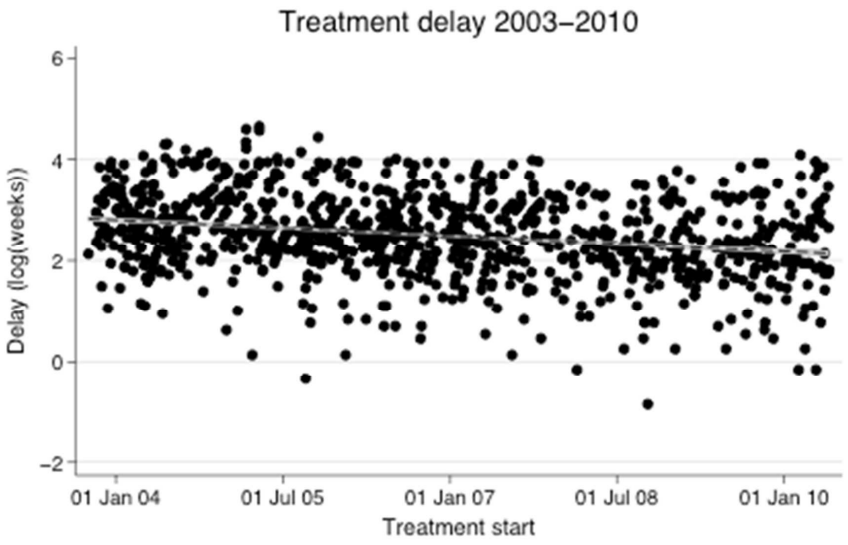

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TABLE I

First self-reported symptoms of TB (n=930*)

	TB-smear positive (n=600)	TB-smear negative/no expectoration (n=330)**
Cough	88% (530/600)	87% (286/330)
Fever	80% (482/600)	85% (279/330)
Chest Pain	80% (477/600)	84% (276/330)
Weight loss	55% (330/600)	63% (208/330)
Night sweating	16% (95/600)	17% (55/330)
Breathlessness	14% (85/600)	16% (52/330)
Haemoptysis	6% (37/600)	8% (27/330)
Other symptoms	1% (6/600)	1% (2/330)

*43 cases did not provide information on the nature of first TB-symptoms.

** There were no significant differences between the two groups (p>0.05).

TABLE II

Risk factors for total treatment delay (n= 973*)

	N	Median (weeks)	RR [95%CI]	Adjusted RR [95%CI]**
Gender				
- Male	602	11.4	1	1
- Female	371	12.7	1.15[1.03;1.28]	1.05[0.94;1.18]
Age (years)				
- 15-24	186	10.9	1	1
- 25-34	351	11.4	1.05[0.91;1.22]	0.99[0.85;1.15]
- 35-44	209	12.0	1.10[0.94;1.29]	0.95[0.79;1.14]
- 45+	227	14.9	1.31[1.12;1.54]	1.01[0.84;1.22]
Civil Status				
- Single	439	10.7	1	1
- Married	383	12.4	1.23[1.10;1.37]	1.02[0.89;1.17]
- Divorced/widow(er)	151	14.6	1.42[1.23;1.65]	1.15[0.86;1.37]
Education				
- 9+ years	199	9.7	1	1
- 7-9 years	208	10.0	1.13[0.96;1.31]	1.10[0.94;1.28]
- 1-6 years	264	12.6	1.36[1.17;1.57]	1.23[1.05;1.44]
- No education	290	15.1	1.55[1.34;1.79]	1.24[1.03;1.49]
- Data missing	12	17.2	1.50[0.94;2.38]	-
Residence				
- Resident	721	11.3	1	1
- Guest	252	15.1	1.32[1.18;1.48]	1.09[0.95;1.24]
HIV-status				
- HIV-neg	665	11.9	1	1
- HIV1	187	12.3	1.05[0.92;1.20]	1.03[0.90;1.17]
- HIV2	79	12.0	0.97[0.80;1.17]	0.86[0.71;1.04]
- HIV1+2	39	16.0	1.40[1.08;1.82]	1.31[1.01;1.70]
- Data missing	3	21.0	2.05[0.82;5.14]	-
Sputum smear status				
- Smear positive	630	11.6	1	1
- Smear neg/no expectoration	343	12.7	1.19[1.07;1.32]	1.13[1.02;1.25]
BMI				
- Normal (BMI>18.5)	435	11.1	1	
- Underweight(BMI<18.5)	524	12.6	1.10[0.99;1.22]	-
- Data missing	14	12.3	1.01[0.66;1.56]	-
Ethnic Group				
- Mancanha/Manjaco	251	11.9	1	1
- Balanta	143	13.4	1.25[1.06;1.48]	1.11[0.94;1.32]
- Fula	240	11.8	1.00[0.87;1.16]	1.12[0.82;1.52]
- Pepel	232	12.7	1.12[0.97;1.30]	1.13[0.98;1.31]
- Other	100	11.7	1.09[0.91;1.32]	1.10[0.90;1.33]
- Data missing	7	13.0	1.26[0.68;2.31]	-
Religion				
- Catholic	346	11.0	1	1
- Traditional Religion	265	14.7	1.32[1.16;1.50]	1.07[0.92;1.25]
- Protestant	84	11.1	0.97[0.80;1.17]	0.90[0.75;1.09]

-	Muslim	260	11.4	1.02[0.89;1.16]	0.88[0.65;1.18]
-	Other	17	12.4	1.17[0.79;1.73]	1.12[0.76;1.65]
-	Data missing	1	11.9	1.05[0.22;5.12]	-

*In the multiple regression model twenty-two patients were not included due to missing information on one or more risk factors (see figure 1).

**The adjusted analysis was corrected for calendar time.

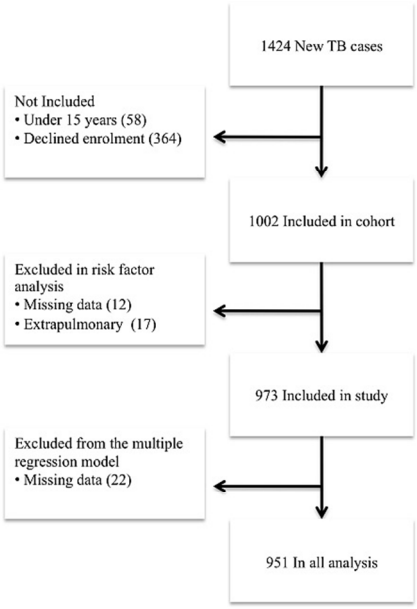
TABLE III

Treatment delay and clinical severity

Centile	Delay (weeks)	TBscore	Percentage of severe TB-cases*	p
0-25%	0-7.6	5.7[5.4;5.9]	20.8% (50/241)	-
25-50%	7.6-12.1	6.3[5.9;6.7]	26.1% (64/245)	-
50-75%	12.1-21.1	6.8[6.4;7.2]	35.5% (87/245)	-
75%-	21.1-	6.7[6.3;7.1]	32.4% (79/244)	0.002**

*Severe cases were defined as TBscore≥8

**Fishers exact test



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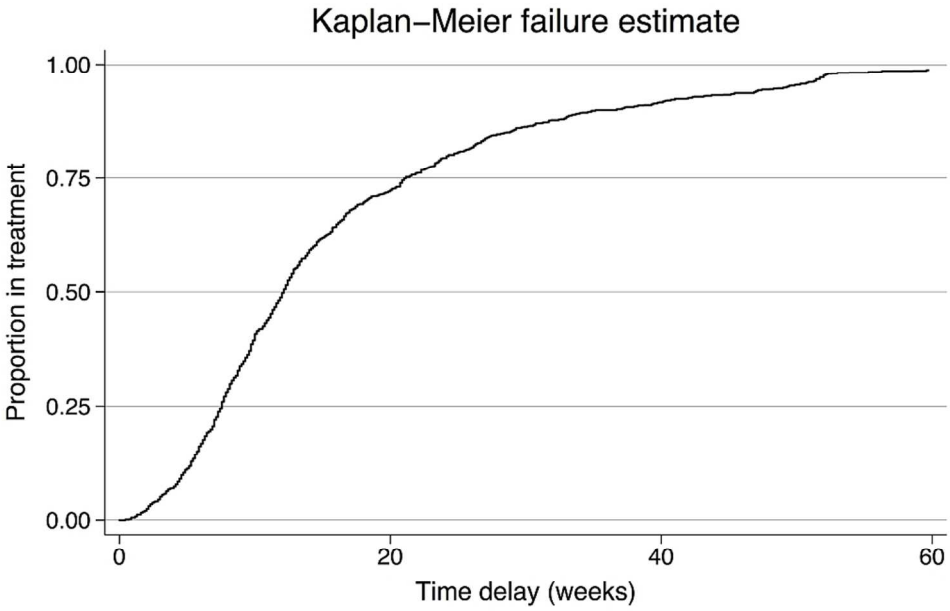

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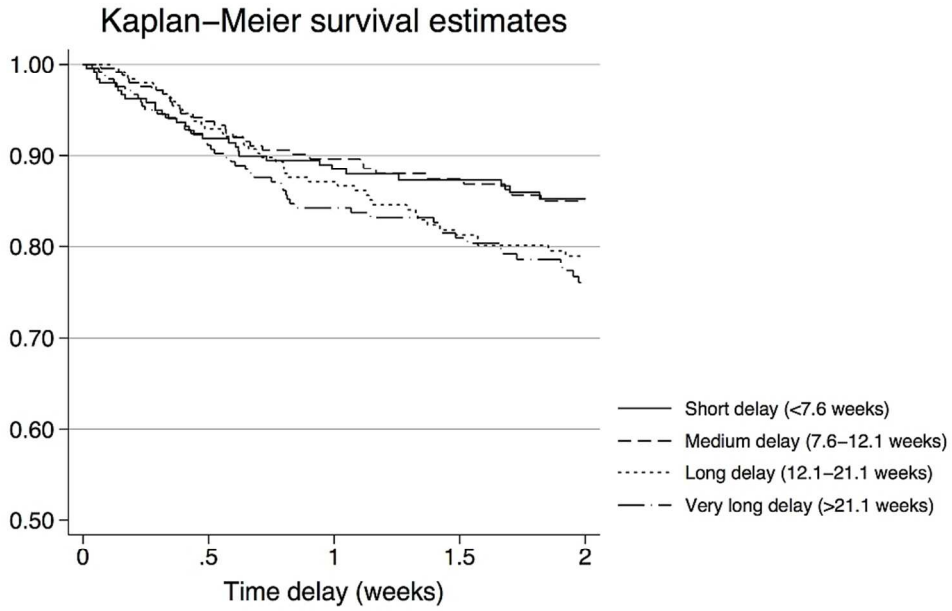

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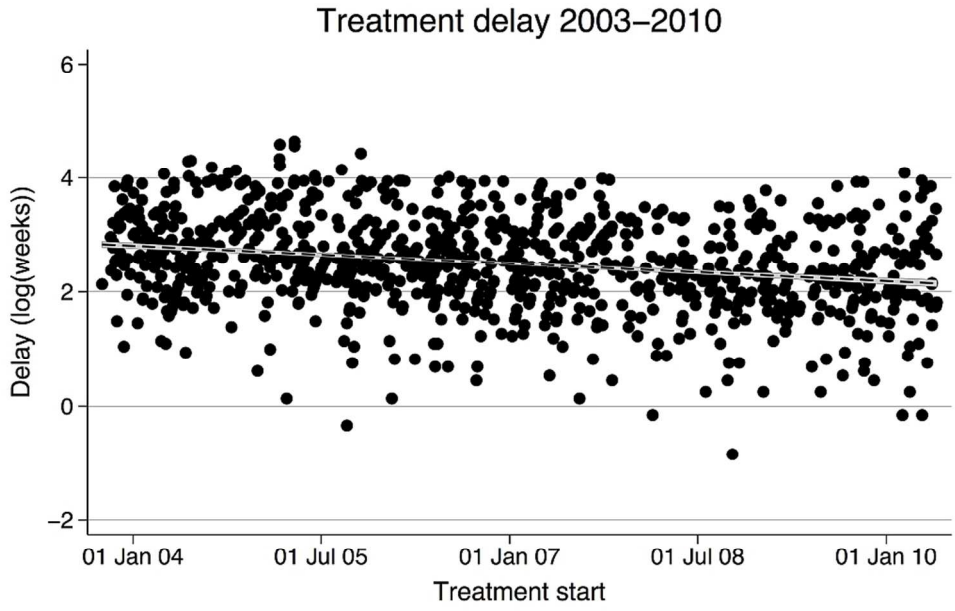

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	20
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9, 24
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Treatment delay affects clinical severity of tuberculosis; A longitudinal cohort study

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Global health, Epidemiology
Keywords:	Tuberculosis < INFECTIOUS DISEASES, Epidemiology < TROPICAL MEDICINE, Infection control < INFECTIOUS DISEASES

Treatment delay affects clinical severity of tuberculosis;

A longitudinal cohort study

Virenfeldt J[1,2], Rudolf F[1,2], Camara C[1], Furtado A[1], Gomes V[1], Aaby P[1,3], Petersen E[2], Wejse C[1,4]

[1] Bandim Health Project, INDEPTH network, Apartado 861, Bissau, Guinea-Bissau

[2] Department of Infectious Diseases, Aarhus University Hospital, Denmark;

[3] Danish Epidemiology Science Center, Statens Serum Institut, Copenhagen, Denmark

[4] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Denmark

Word Count: Abstract 294. Text 3107

Keywords: HDSS, treatment delay, tuberculosis control

ABSTRACT

Objectives

To describe the risk factors for treatment delay and the importance of delay for the severity of tuberculosis in a prospectively followed tuberculosis cohort at the Bandim Health Project in Guinea-Bissau.

Background

Treatment delay in patients with tuberculosis is associated with increased mortality and transmission of disease. However, it is not well described whether delay influences clinical severity at diagnosis. Previously reported risk factors for treatment delay vary in different geographical and cultural settings. Such information has never been investigated in our setting.

Change in delay over time is rarely reported and our prospectively followed tuberculosis cohort gives an opportunity to present such data.

Participants

Patients were included at time of diagnosis at three local tuberculosis clinics and the national tuberculosis reference hospital. Inclusion criteria were age $\geq$ 15 years and diagnosis of tuberculosis by either sputum examination or by the WHO clinical criteria. Patients with extra-pulmonary tuberculosis were excluded.

Primary and secondary outcome measures

The primary outcome was treatment delay. Delay was assessed by patient questionnaires.

Secondary outcomes were Bandim TBscore as a measure of TB morbidity and all cause mortality.

Results

A total of 1424 persons were diagnosed with tuberculosis in the study area between 2003 and 2010. We included 973 TB-patients in the study. The median treatment delay was 12.1 weeks. Risk factors for delay were low educational level, HIV-1+2 dual infection and negative sputum smear. Tuberculosis

treatment delay decreased with 10.3% [7.9%;12.6%] per year during the study period. Delay was significantly associated with clinical severity at presentation with 20.8% severe TB-cases in the low delay quartile compared to 33.9% if delay was over the median of 12.1 weeks.

Conclusion

Long treatment delay was associated with more severe clinical presentation. Treatment delay in tuberculosis cases is decreasing in Guinea Bissau.

ARTICLE SUMMARY

Article focus

To investigate treatment delay in an urban tuberculosis cohort from West Africa including:

- Risk factors for delay
- Effects on clinical severity and mortality
- Changes in delay over the study period

Key messages

- Education, sputum smear and HIV-status were the most important risk factors for delay in our setting. Focus should be on uneducated and vulnerable groups when planning future health interventions concerning TB control.
- Delay in the initiation of TB-treatment is declining in this study though still a major issue in high burden areas. Treatment delay influences disease severity at diagnosis and health policy makers should maintain focus on awareness of TB symptoms and implement TB control measures to detect the disease early.

Strengths and limitations of this study

- A continuously and well-described cohort of tuberculosis patients was used and a large number of TB-cases were included. Cases were followed with both clinical exams after treatment and mortality follow up.
- Division between patient delay and health care system delay was not possible since date of first contact to any health care provider could not be estimated validly. While this weakens the specificity of our findings it does not influence our results on severity of disease.
- There is a risk of recall bias since the data was collected by retrospective questionnaires. Cultural and educational factors may have influenced the estimated treatment delay.

INTRODUCTION

It is estimated that one third of the world's population is latently infected with tuberculosis (TB)[1]. In 2013 WHO estimated that there were 8.6 million cases of active TB with 1.3 million deaths annually[2]. Of these deaths, over 95% occurred in developing countries[3]. Transmission of tuberculosis is difficult to control since one index case can infect a large number of secondary cases if left untreated[4-6]. Better management of TB should focus on reducing the time delay from symptom onset to initiating treatment[5 7]. Treatment delay is influenced by gender, age, sputum TB smear status, distance to nearest health care provider, educational level and HIV status[8 9].

Previously reported treatment delays from different parts of the world vary considerably. A study from China found a total time delay of 3.6 weeks[7]; a study from Tanzania found a delay of 26 weeks[10]. The average delay in low income countries has been reported to be 9.7 weeks[8].

Factors affecting treatment delay are of key importance in TB management as delay may increase mortality[11 12]. However, there is limited data on the effect of delay on clinical severity at presentation. The aim of this study was to describe risk factors for treatment delay, to assess the possible effects of treatment delay on clinical severity at diagnosis and to monitor changes in delay over time in a prospective TB programme.

MATERIALS AND METHODS

Study design

Our study was a longitudinal prospective cohort including all patients diagnosed with TB within the Bandim Health Project study area. Inclusion criteria were age ≥ 15 years and diagnosed with TB by either sputum examination (smear microscopy; no culture was available) or by the World Health Organizations clinical criteria[13].

Study location and population

The study was carried out in Bissau, the capital of Guinea-Bissau, at the Bandim Health Project (BHP). The BHP is a health and demographic surveillance site (HDSS), which has followed an urban population in Bissau since 1978. The study area consists of 6 suburban areas with a total population of 102,000, which is followed by regular demographic surveys. All individuals in the area are registered with ID-number, age, sex, ethnic group and socio-economic data. Censuses are performed at regular intervals and information on pregnancies, births, mortality and migration is collected on a daily basis. Since 1996 a surveillance system has detected all patients diagnosed with and treated for TB in the study area, and long-term follow-up has been performed. In 1997 an estimated TB incidence rate of the study area was one of the highest reported TB incidences in the world (470/100,000 person years)[14]; a recent study estimated a current incidence of 288/100,000 person years[15].

Health services and inclusion

Field assistants identified patients at the TB treatment facilities during daily visits. Patients were identified at treatment start and invited for consultation and enrolment. Not all patients showed up for enrolment, those not enrolled in the epidemiological study did not complete the questionnaire nor had the clinical examination, hence no delay or severity information was available for these patients.

Patients were interviewed using a structured questionnaire including data on first signs and symptoms of TB and demographic characteristics[16]. The inclusion period was from 1 November 2003 to 4 June 2010; data were analysed in June 2010 and the questionnaire was adapted for future studies.

During treatment patients were followed through regular clinical evaluations, the last being at the 6th and last month of treatment. Mortality follow-up was conducted through house visits 12 and 24 months after termination of treatment.

Definitions

Treatment delay was defined as the time from onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. At inclusion in the cohort, patients were asked when the first symptoms of the disease occurred and what the initial symptoms were. From the responses it was often difficult to make a clear assessment of the exact time of first contact to a health care facility, and a distinction between patient-related and health care system-related delay was not possible.

Many people from rural areas move temporarily to the capital city to work or to access the health services. These patients without a permanent address in the study area were classified as "non-residents".

The study was conducted in an urban environment and physical access to health care facilities was therefore not considered a major factor in delay.

Laboratory tests

An HIV-test was performed at inclusion using Enzygnost Anti-HIV 1+2 Plus (Behring Diagnostics Gmbh, Marburg, Germany) and confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) or Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-la Coquette, France). From January 2008, Determine HIV-1/2 (Alere Inc, Waltham, Massachusetts, USA) was used and positive results were confirmed with SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Korea). Tests

for HIV were repeated at six months and for 13 cases with an inconclusive initial HIV-test the result of the next test after six months was used. Direct microscopy of sputum samples was performed using Ziehl-Neelsen staining. Laboratory facilities for sputum cultures were destroyed during a civil war in Guinea-Bissau and were therefore not performed in the present study[17].

Bandim TBscore

Clinical severity was assessed by the Bandim TBscore. The TBscore is a newly developed tool assessing change in clinical status of patients with TB[18] expressed as a numeric index based on cough, haemoptysis, dyspnoea, chest pain and night sweating[18], and the following findings: Anaemic conjunctivae, tachycardia, positive lung auscultation, increased temperature, body mass index (BMI) and middle upper arm circumference (MUAC). The TBscore can be divided into three severity classes and a TBscore ≥8 correlates with mortality and lower TBscores with favourable outcomes, cure, and completed treatment as described elsewhere[18 19]. In these previous validations and also in other settings[20] "severe TB-cases" has been defined as TBscore≥8, which has a strong prognostic capacity for mortality.

Statistical analysis

Data were double entered and analyses were performed using Stata Statistical Software v.11 (Stata Corporation, College Station, TX, USA). To account for outliers in the reported treatment delay medians were presented in tables and figures and logarithmic transformation was performed. The data was reanalyzed excluding these outliers but no significant change in estimates was observed. A multiple linear regression model was used to adjust for multiple risk factors in the treatment delay analysis. Cox-regression was used in the mortality analysis (Figure 3) and in the depiction of time to TB treatment for the symptomatic TB-cases (Figure 2). No significant deviations from the proportional hazard assumption were found. Factors affecting the association between treatment delay and mortality

with ≥10% were corrected for and the same cut-off was used in the supplementary logistic regression model on treatment delay and severity. A two-tailed $p < 0.05$ was considered significant. Correction for calendar time was performed in the adjusted risk factor analysis (table 2).

Ethics

The patients were informed in written Portuguese and verbally in the common language Creole and they gave consent by signature or fingerprint. The study was permitted by the Health Ministry of Guinea-Bissau and approved by the National Science and Ethics Committee in Guinea-Bissau as well as the Central Ethics Committee of Denmark.

RESULTS

Patient characteristics

A total of 1424 persons were diagnosed with TB in the study area. Of these, 58 were under the age of 15 years and 364 were not enrolled. Thus, 1002 were enrolled in the cohort (Figure 1). We excluded 17 patients due to extra-pulmonary tuberculosis and 12 due to missing data at the time of symptom onset. Hence, 973 patients were included in the final analysis (62% males and 38% females). The mean age was 36.0 years (median 33; range 15-90) for men and 35.4 years (median 31; range 15-75) for women. Information about non-included patients was generally limited to gender and age. Residential status was known for 153 of these patients. Non-included cases were older than included patients with a mean age of 38.0 years (median 35.0; range 15-98) compared with 35.7 years (median 32.0; range 15-90) ($p<0.01$). Non-included patients were more often non-residents; 34% (52/153) were from outside the study area, compared with 26% (252/973) in the included group ($p=0.03$), but there was no significant difference in gender ($p=0.87$).

The median treatment delay was 12.1 weeks; 10% of patients were still not in anti-tuberculosis treatment after 37 weeks as described in Figure 2, which displays the proportion of the diagnosed and treated patients among the enrolled TB patients in this study.

Symptoms of TB

The first self-reported symptoms of TB are described in Table I, which comprises all symptoms, mentioned by patients as initial symptoms of their current illness, no attempt was made to differentiate which symptom was the first. There were no data available on the type of first symptoms for 43 patients. The most frequent initial symptoms were cough, fever and chest pain. In total, 89.8%

(874/973) reported cough at the time of treatment initiation and the median time with cough before diagnosis was 12.9 weeks (inter-quartile range (IQR) 8.6-21.4).

Risk factors for treatment delay

The risk factor analysis is shown in Table II. In the multiple regression analysis 22 cases were excluded due to missing information in one or more variables. No education, negative smear and HIV-dual infection remained significantly associated with treatment delay.

Of the 973 patients, 290 had never attended school and had a longer median time to treatment (15.1 weeks) than patients with schooling (p=0.02). There was no significant difference in treatment delay between cases with an intermediate educational level (7-9 years) and a high educational level (>9 years) (p=0.135).

Patients with no expectoration or a negative TB-smear had a longer treatment delay than patients with a positive TB-smear (12,7 vs. 11.6 weeks) (p=0.03).

Stratifying the risk factor analysis on TB-smear status did not change estimates significantly though ethnicity, religion and marital status were not found to be significant risk factors for sputum negative cases.

Bandim TBscore and mortality

Bandim TBscore was available for all 973 patients (Table III). We defined the lowest quartile as short treatment delay; followed by moderate, long and very long delay.

There was an increase in the proportion of severely ill patients as the delay increased and the association was significant (p=0.002) (Table III). There was no difference in TBscore between the patients with a long and very long delay. The pooled percentage of severe TB-cases for these two groups was 33.9%.

We also performed a logistic regression with severity (tbscore≥8) as the dependent variable and treatment delay (in 25% percentiles) as the explanatory variable. This resulted in an OR=2.08[1.39;3.13] for the long delay strata (7.6-12.1 weeks) and an OR=1.81[1.20;2.73] for very long delay strata (12.1-21.1 weeks). Correcting for possible confounders; HIV-status, residential status, education, age and sex the results were still significant - 1.85[1.21;2.82] and OR=1.57[1.21;2.82] respectively.

In figure 3 we show the Kaplan Meier mortality curves for the four delay quartiles. A delay above 21 weeks (quartile 4) had a higher mortality than quartile 1 (delay below 7.6 weeks), Hazard Ratio (HR) 1.59 [95% CI 1.01-2.48], whereas quartiles 2 and 3 did not differ significantly from quartile 1. The majority of the excess mortality in people with a long treatment delay occurred after the first 6 months following presentation and when adjusting for HIV, age, education and civil status the HR was no longer significant (Adjusted HR 1.10 [0.72;1.67]).

We included 343 smear-negative TB-cases of whom 199 cases attended six months clinical follow up after completion of anti-tuberculosis treatment. During treatment thirty-six died and the remaining 108 declined further clinical follow-up or were lost to follow up. Mean TBscore for smear-negative TB-cases decreased from 6.5 points to 1.3 points within 6 months of anti-tuberculosis treatment. The corresponding decrease in TBscore for smear positive patients was a decrease from a mean of 6.3 points to 0.9 points.

Changes in treatment delay

The median delay to treatment decreased during the study period. Within the first year the median delay was 14.6 weeks (IQR 9.3-26.1), but had dropped to 8.6 weeks (IQR: 5.7-16.7) in the last year. In a linear regression model the delay decreased with 10.3% [7.9%;12.6%] annually. The delay decreased with three weeks from 12.0 weeks (n=153) to 9.0 weeks (n=110) in 2007 and 2008 respectively. The trend in delay is displayed in Figure 4.

To account for changes in other factors affecting treatment delay we adjusted for changes in the significant risk factors for treatment delay from Table II. This did not change the estimates significantly and the adjusted decrease was 8.5%[6.0%;10.9%].

DISCUSSION

This study showed a long treatment delay in the diagnosis of TB in Guinea Bissau of 12.1 weeks equivalent to the delays found in comparable settings[21-25]. We found a clear association between treatment delay and clinical severity at inclusion, which has not been described before. This association is not surprising as patients in whom delay was long the disease had longer time to progress. We have shown in Table 3, that the proportion of severely ill is higher among those with a long delay, in the long delay groups it is a third of patients who are severely ill compared with a fifth in the short delay group. This may translate into mortality risk, patients with a TBscore≥8 have a 60% higher mortality risk as previously shown[18] and a 6-fold higher risk of treatment failure[19], but in a multivariate model we were not able to show significance with this sample size. Furthermore, a higher risk of hospitalization for patients with a longer treatment delay has also been described previously[26]. The clinical implications of shorter treatment delay is therefore likely to be less severe TB and subsequent lower mortality as well as less time to infect others.

There was a significant difference in mortality between patients with a short delay, compared with those with very long delay in the univariate analysis but when correcting for relevant factors this was no longer significant in the direct analysis.

The smear-negative cases had only one week longer median treatment delay than smear-positive cases and though transmission of disease is time-dependent[4 5] the community implications are probably limited.

We found that smear-negative patients profited substantially from anti-tuberculosis treatment. Though the diagnosis was not confirmed by culture or smear, an empirical six months anti-tuberculosis treatment resulted in a significant decrease in clinical severity assessed by TBscore.

Non-residential status did not reach statistical significance in the multiple regression model, though it could be due to a limited sample size. Non-residents are a heterogeneous group in our setting; some arrive with TB symptoms attending the centralized health care facilities. However, many come to work or study in the capital and acquire the infection while being heavily exposed to TB living in an urban overpopulated environment. Previous studies have reported that residential status can be a risk factor for treatment delay[8 9] and we suggest that this parameter is included in future studies.

HIV-status influences treatment delay in some studies, but specific delay for HIV1+2 dual infection has not been described before. This could be a random finding but HIV1+2 dual infected cases are likely to be a vulnerable group with limited access to treatment. Furthermore HIV1+2 dual infected are more often sputum smear negative and present with fewer symptoms due to a weakened immune system[27].

Several factors may influence the observed change over time in delay to treatment. Socio-economic changes are likely to be the most important factor and the general economic situation in Bissau has improved within the last decade as the country has been rebuilt after the civil war in 1998[28]. Changes in risk factors for delay during the study period could only explain a minor part of the decrease in treatment delay. We hypothesize that an increased awareness of signs and symptoms of TB may have been a factor after a TB prevalence survey in the study area in 2006 which may have contributed to the decrease in treatment delay[29]. This survey among 2989 adults involved active case finding with the use of a questionnaire on TB symptoms and referral for sputum and x-ray according to an algorithm, and may have increased TB awareness in the involved 384 sampled houses. We have seen a peak in incidence in 2007 following this survey, which may have been a result of the survey and the increased awareness of TB in the study area[30].

The study has several limitations; firstly we were not able to divide treatment delay into patient-related and health system-related delay due to limited information on the first contact to any health care provider.

Secondly, recall bias is a potential problem even though experienced local personnel and a comprehensive questionnaire was used [11 16], and the self-reported duration of symptoms may not be an accurate assessment of treatment delay. Patients were included after a considerable time period of symptomatic TB and may have overestimated the delay. The most invalidating symptoms present at inclusion may also have been over-reported as a result of recall bias potentially influencing the reported symptoms analysis.

Thirdly, we did not have data on cause of death in the background population, and were not able to estimate the corresponding case detection rate (CDR) of pulmonary TB in the area. There may indeed be a small number of undiagnosed TB patients who die from tuberculosis as previously shown[30]. This is supported by our prevalence study finding undetected TB cases in the study area[29]. Treatment delay may therefore have been underestimated since delay would be longer if undiagnosed cases with long delays had also been included.

Fourthly, this study was not a prospective follow-up of TB suspects and therefore we have no information on a possible undetected TB cases or diagnosed yet untreated cases, and the study is therefore not able to assess how many patients with symptoms are under treatment. This has been done in a recent cohort-study on TB suspects[31], but this is not the scope of this study.

Yet, our findings represent a population-based analysis over a long time period and are based on a large dataset. The results clearly show that delay to TB treatment is still a major issue in high burden areas. Our findings indicate that increased awareness of TB symptoms in the population and in health care systems is important to reduce the unacceptable long delay. Maintained focus is needed on awareness of TB and continuous implementation of TB control measures to detect the disease early and reduce unnecessary disability and loss of lives.

CONCLUSION

Delay of TB treatment in Bissau is significantly associated with disease severity at diagnosis. No education, HIV-dual infection and smear negative TB were significant risk factors for treatment delay. The treatment delay, though still unacceptably high, has decreased in Bissau during the past decade. TB control programmes may need to monitor treatment delay over time.

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AUTHORS' CONTRIBUTIONS

CW, FR and VG collected the clinical data. CC did the laboratory tests. JV and AF checked all variables and JV wrote the first draft of the manuscript. PA, EP and CW made the study logistically possible and edited the paper. All authors accepted the final version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

Data Sharing Statement

Tecnical appendix, statistical code and dataset available from the corresponding author on request.

FIGURE LEGENDS:

Figure 1: Flow chart demonstrating included individuals

Figure 2: Treatment delay was defined as the time from self-reported onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. All patients were in treatment by the end of the analysis period because only those patients who ultimately began treatment were included in this analysis.

Figure 3: The graph describes the relation between treatment delay and mortality. Strata's were created from 25% percentiles of median treatment delay.

Figure 4: Median treatment delay during the study period divided into 6 months intervals starting from November 2003. The last interval is 8 months to include all included cases in the figure.

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TABLE I

First self-reported symptoms of TB (n=930*)

	TB-smear positive (n=600)	TB-smear negative/no expectoration (n=330)**
Cough	88% (530/600)	87% (286/330)
Fever	80% (482/600)	85% (279/330)
Chest Pain	80% (477/600)	84% (276/330)
Weight loss	55% (330/600)	63% (208/330)
Night sweating	16% (95/600)	17% (55/330)
Breathlessness	14% (85/600)	16% (52/330)
Haemoptysis	6% (37/600)	8% (27/330)
Other symptoms	1% (6/600)	1% (2/330)

*43 cases did not provide information on the nature of first TB-symptoms.

** There were no significant differences between the two groups ($p>0.05$).

TABLE II

Risk factors for total treatment delay (n= 973*)

	N	Median (weeks)	RR [95%CI]	Adjusted RR [95%CI]**
Gender				
- Male	602	11.4	1	1
- Female	371	12.7	1.15[1.03;1.28]	1.05[0.94;1.18]
Age (years)				
- 15-24	186	10.9	1	1
- 25-34	351	11.4	1.05[0.91;1.22]	0.99[0.85;1.15]
- 35-44	209	12.0	1.10[0.94;1.29]	0.95[0.79;1.14]
- 45+	227	14.9	1.31[1.12;1.54]	1.01[0.84;1.22]
Civil Status				
- Single	439	10.7	1	1
- Married	383	12.4	1.23[1.10;1.37]	1.02[0.89;1.17]
- Divorced/widow(er)	151	14.6	1.42[1.23;1.65]	1.15[0.86;1.37]
Education				
- 9+ years	199	9.7	1	1
- 7-9 years	208	10.0	1.13[0.96;1.31]	1.10[0.94;1.28]
- 1-6 years	264	12.6	1.36[1.17;1.57]	1.23[1.05;1.44]
- No education	290	15.1	1.55[1.34;1.79]	1.24[1.03;1.49]
- Data missing	12	17.2	1.50[0.94;2.38]	-
Residence				
- Resident	721	11.3	1	1
- Guest	252	15.1	1.32[1.18;1.48]	1.09[0.95;1.24]
HIV-status				
- HIV-neg	665	11.9	1	1
- HIV1	187	12.3	1.05[0.92;1.20]	1.03[0.90;1.17]
- HIV2	79	12.0	0.97[0.80;1.17]	0.86[0.71;1.04]
- HIV1+2	39	16.0	1.40[1.08;1.82]	1.31[1.01;1.70]
- Data missing	3	21.0	2.05[0.82;5.14]	-
Sputum smear status				
- Smear positive	630	11.6	1	1
- Smear neg/no expectoration	343	12.7	1.19[1.07;1.32]	1.13[1.02;1.25]
BMI				
- Normal (BMI>18.5)	435	11.1	1	
- Underweight(BMI<18.5)	524	12.6	1.10[0.99;1.22]	-
- Data missing	14	12.3	1.01[0.66;1.56]	-
Ethnic Group				
- Mancanha/Manjaco	251	11.9	1	1
- Balanta	143	13.4	1.25[1.06;1.48]	1.11[0.94;1.32]
- Fula	240	11.8	1.00[0.87;1.16]	1.12[0.82;1.52]
- Pepel	232	12.7	1.12[0.97;1.30]	1.13[0.98;1.31]
- Other	100	11.7	1.09[0.91;1.32]	1.10[0.90;1.33]
- Data missing	7	13.0	1.26[0.68;2.31]	-
Religion				
- Catholic	346	11.0	1	1
- Traditional Religion	265	14.7	1.32[1.16;1.50]	1.07[0.92;1.25]
- Protestant	84	11.1	0.97[0.80;1.17]	0.90[0.75;1.09]

-	Muslim	260	11.4	1.02[0.89;1.16]	0.88[0.65;1.18]
-	Other	17	12.4	1.17[0.79;1.73]	1.12[0.76;1.65]
-	Data missing	1	11.9	1.05[0.22;5.12]	-

*In the multiple regression model twenty-two patients were not included due to missing information on one or more risk factors (see figure 1).
**The adjusted analysis was corrected for calendar time.

TABLE III

Treatment delay and clinical severity

Centile	Delay (weeks)	TBscore	Percentage of severe TB-cases*	p**
0-25%	0-7.6	5.7[5.4;5.9]	20.8% (50/241)	-
25-50%	7.6-12.1	6.3[5.9;6.7]	26.1% (64/245)	0.199
50-75%	12.1-21.1	6.8[6.4;7.2]	35.5% (87/245)	0.001
75%-	21.1-	6.7[6.3;7.1]	32.4% (79/244)	0.005

*Severe cases were defined as TBscore≥8

**Fishers exact test comparing each stratum with baseline.

Treatment delay affects clinical severity of tuberculosis;

A longitudinal cohort study

Virenfeldt J[1,2], Rudolf F[1,2], Camara C[1], Furtado A[1], Gomes V[1], Aaby P[1,3], Petersen E[2], Wejse C[1,4]

[1] Bandim Health Project, INDEPTH network, Apartado 861, Bissau, Guinea-Bissau

[2] Department of Infectious Diseases, Aarhus University Hospital, Denmark;

[3] Danish Epidemiology Science Center, Statens Serum Institut, Copenhagen, Denmark

[4] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Denmark

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ABSTRACT

Objectives

To describe the risk factors for treatment delay and the importance of delay for the severity of tuberculosis in a prospectively followed tuberculosis cohort at the Bandim Health Project in Guinea-Bissau.

Background

Treatment delay in patients with tuberculosis is associated with increased mortality and transmission of disease. However, it is not well described whether delay influences clinical severity at diagnosis. Previously reported risk factors for treatment delay vary in different geographical and cultural settings. Such information has never been investigated in our setting.

Change in delay over time is rarely reported and our prospectively followed tuberculosis cohort gives an opportunity to present such data.

Participants

Patients were included at time of diagnosis at three local tuberculosis clinics and the national tuberculosis reference hospital. Inclusion criteria were age ≥ 15 years and diagnosis of tuberculosis by either sputum examination or by the WHO clinical criteria. Patients with extra-pulmonary tuberculosis were excluded.

Primary and secondary outcome measures

The primary outcome was treatment delay. Delay was assessed by patient questionnaires.

Secondary outcomes were Bandim TBscore as a measure of TB morbidity and all cause mortality.

Results

A total of 1424 persons were diagnosed with tuberculosis in the study area between 2003 and 2010. We included 973 TB-patients in the study. The median treatment delay was 12.1 weeks. Risk factors for delay were low educational level, HIV-1+2 dual infection and negative sputum smear. Tuberculosis

treatment delay decreased with 10.3% [7.9%;12.6%] per year during the study period. Delay was significantly associated with clinical severity at presentation with 20.8% severe TB-cases in the low delay quartile compared to 33.9% if delay was over the median of 12.1 weeks.

Conclusion

Long treatment delay was associated with more severe clinical presentation. Treatment delay in tuberculosis cases is decreasing in Guinea Bissau.

ARTICLE SUMMARY

Article focus

To investigate treatment delay in an urban tuberculosis cohort from West Africa including:

- Risk factors for delay
- Effects on clinical severity and mortality
- Changes in delay over the study period

Key messages

- Education, sputum smear and HIV-status were the most important risk factors for delay in our setting. Focus should be on uneducated and vulnerable groups when planning future health interventions concerning TB control.
- Delay in the initiation of TB-treatment is declining in this study though still a major issue in high burden areas. Treatment delay influences disease severity at diagnosis and health policy makers should maintain focus on awareness of TB symptoms and implement TB control measures to detect the disease early.

Strengths and limitations of this study

- A continuously and well-described cohort of tuberculosis patients was used and a large number of TB-cases were included. Cases were followed with both clinical exams after treatment and mortality follow up.
- Division between patient delay and health care system delay was not possible since date of first contact to any health care provider could not be estimated validly. While this weakens the specificity of our findings it does not influence our results on severity of disease.
- There is a risk of recall bias since the data was collected by retrospective questionnaires. Cultural and educational factors may have influenced the estimated treatment delay.

INTRODUCTION

It is estimated that one third of the world's population is latently infected with tuberculosis (TB)[1]. In 2013 WHO estimated that there were 8.6 million cases of active TB with 1.3 million deaths annually[2]. Of these deaths, over 95% occurred in developing countries[3]. Transmission of tuberculosis is difficult to control since one index case can infect a large number of secondary cases if left untreated[4-6]. Better management of TB should focus on reducing the time delay from symptom onset to initiating treatment[5 7]. Treatment delay is influenced by gender, age, sputum TB smear status, distance to nearest health care provider, educational level and HIV status[8 9].

Previously reported treatment delays from different parts of the world vary considerably. A study from China found a total time delay of 3.6 weeks[7]; a study from Tanzania found a delay of 26 weeks[10]. The average delay in low income countries has been reported to be 9.7 weeks[8].

Factors affecting treatment delay are of key importance in TB management as delay may increase mortality[11 12]. However, there is limited data on the effect of delay on clinical severity at presentation. The aim of this study was to describe risk factors for treatment delay, to assess the possible effects of treatment delay on clinical severity at diagnosis and to monitor changes in delay over time in a prospective TB programme.

MATERIALS AND METHODS

Study design

Our study was a longitudinal prospective cohort including all patients diagnosed with TB within the Bandim Health Project study area. Inclusion criteria were age ≥ 15 years and diagnosed with TB by either sputum examination (smear microscopy; no culture was available) or by the World Health Organizations clinical criteria[13].

Study location and population

The study was carried out in Bissau, the capital of Guinea-Bissau, at the Bandim Health Project (BHP). The BHP is a health and demographic surveillance site (HDSS), which has followed an urban population in Bissau since 1978. The study area consists of 6 suburban areas with a total population of 102,000, which is followed by regular demographic surveys. All individuals in the area are registered with ID-number, age, sex, ethnic group and socio-economic data. Censuses are performed at regular intervals and information on pregnancies, births, mortality and migration is collected on a daily basis. Since 1996 a surveillance system has detected all patients diagnosed with and treated for TB in the study area, and long-term follow-up has been performed. In 1997 an estimated TB incidence rate of the study area was one of the highest reported TB incidences in the world (470/100,000 person years)[14]; a recent study estimated a current incidence of 288/100,000 person years[15].

Health services and inclusion

Field assistants identified patients at the TB treatment facilities during daily visits. Patients were identified at treatment start and invited for consultation and enrolment. Not all patients showed up for enrolment, those not enrolled in the epidemiological study did not complete the questionnaire nor had the clinical examination, hence no delay or severity information was available for these patients.

Patients were interviewed using a structured questionnaire including data on first signs and symptoms of TB and demographic characteristics[16]. The inclusion period was from 1 November 2003 to 4 June 2010; data were analysed in June 2010 and the questionnaire was adapted for future studies.

During treatment patients were followed through regular clinical evaluations, the last being at the 6th and last month of treatment. Mortality follow-up was conducted through house visits 12 and 24 months after termination of treatment.

Definitions

Treatment delay was defined as the time from onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. At inclusion in the cohort, patients were asked when the first symptoms of the disease occurred and what the initial symptoms were. From the responses it was often difficult to make a clear assessment of the exact time of first contact to a health care facility, and a distinction between patient-related and health care system-related delay was not possible.

Many people from rural areas move temporarily to the capital city to work or to access the health services. These patients without a permanent address in the study area were classified as "non-residents".

The study was conducted in an urban environment and physical access to health care facilities was therefore not considered a major factor in delay.

Laboratory tests

An HIV-test was performed at inclusion using Enzygnost Anti-HIV 1+2 Plus (Behring Diagnostics Gmbh, Marburg, Germany) and confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) or Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-la Coquette, France). From January 2008, Determine HIV-1/2 (Alere Inc, Waltham, Massachusetts, USA) was used and positive results were confirmed with SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Korea). Tests

for HIV were repeated at six months and for 13 cases with an inconclusive initial HIV-test the result of the next test after six months was used. Direct microscopy of sputum samples was performed using Ziehl-Neelsen staining. Laboratory facilities for sputum cultures were destroyed during a civil war in Guinea-Bissau and were therefore not performed in the present study[17].

Bandim TBscore

Clinical severity was assessed by the Bandim TBscore. The TBscore is a newly developed tool assessing change in clinical status of patients with TB[18] expressed as a numeric index based on cough, haemoptysis, dyspnoea, chest pain and night sweating[18], and the following findings: Anaemic conjunctivae, tachycardia, positive lung auscultation, increased temperature, body mass index (BMI) and middle upper arm circumference (MUAC). The TBscore can be divided into three severity classes and a TBscore ≥8 correlates with mortality and lower TBscores with favourable outcomes, cure, and completed treatment as described elsewhere[18 19]. In these previous validations and also in other settings[20] "severe TB-cases" has been defined as TBscore≥8, which has a strong prognostic capacity for mortality.

Statistical analysis

Data were double entered and analyses were performed using Stata Statistical Software v.11 (Stata Corporation, College Station, TX, USA). To account for outliers in the reported treatment delay medians were presented in tables and figures and logarithmic transformation was performed. The data was reanalyzed excluding these outliers but no significant change in estimates was observed. A multiple linear regression model was used to adjust for multiple risk factors in the treatment delay analysis. Cox-regression was used in the mortality analysis (Figure 3) and in the depiction of time to TB treatment for the symptomatic TB-cases (Figure 2). No significant deviations from the proportional hazard assumption were found. Factors affecting the association between treatment delay and mortality

with ≥10% were corrected for and the same cut-off was used in the supplementary logistic regression model on treatment delay and severity. A two-tailed $p < 0.05$ was considered significant. Correction for calendar time was performed in the adjusted risk factor analysis (table 2).

Ethics

The patients were informed in written Portuguese and verbally in the common language Creole and they gave consent by signature or fingerprint. The study was permitted by the Health Ministry of Guinea-Bissau and approved by the National Science and Ethics Committee in Guinea-Bissau as well as the Central Ethics Committee of Denmark.

RESULTS

Patient characteristics

A total of 1424 persons were diagnosed with TB in the study area. Of these, 58 were under the age of 15 years and 364 were not enrolled. Thus, 1002 were enrolled in the cohort (Figure 1). We excluded 17 patients due to extra-pulmonary tuberculosis and 12 due to missing data at the time of symptom onset. Hence, 973 patients were included in the final analysis (62% males and 38% females). The mean age was 36.0 years (median 33; range 15-90) for men and 35.4 years (median 31; range 15-75) for women. Information about non-included patients was generally limited to gender and age. Residential status was known for 153 of these patients. Non-included cases were older than included patients with a mean age of 38.0 years (median 35.0; range 15-98) compared with 35.7 years (median 32.0; range 15-90) ($p<0.01$). Non-included patients were more often non-residents; 34% (52/153) were from outside the study area, compared with 26% (252/973) in the included group ($p=0.03$), but there was no significant difference in gender ($p=0.87$).

The median treatment delay was 12.1 weeks; 10% of patients were still not in anti-tuberculosis treatment after 37 weeks as described in Figure 2, which displays the proportion of the diagnosed and treated patients among the enrolled TB patients in this study.

Symptoms of TB

The first self-reported symptoms of TB are described in Table I, which comprises all symptoms, mentioned by patients as initial symptoms of their current illness, no attempt was made to differentiate which symptom was the first. There were no data available on the type of first symptoms for 43 patients. The most frequent initial symptoms were cough, fever and chest pain. In total, 89.8%

(874/973) reported cough at the time of treatment initiation and the median time with cough before diagnosis was 12.9 weeks (inter-quartile range (IQR) 8.6-21.4).

Risk factors for treatment delay

The risk factor analysis is shown in Table II. In the multiple regression analysis 22 cases were excluded due to missing information in one or more variables. No education, negative smear and HIV-dual infection remained significantly associated with treatment delay.

Of the 973 patients, 290 had never attended school and had a longer median time to treatment (15.1 weeks) than patients with schooling (p=0.02). There was no significant difference in treatment delay between cases with an intermediate educational level (7-9 years) and a high educational level (>9 years) (p=0.135).

Patients with no expectoration or a negative TB-smear had a longer treatment delay than patients with a positive TB-smear (12,7 vs. 11.6 weeks) (p=0.03).

Stratifying the risk factor analysis on TB-smear status did not change estimates significantly though ethnicity, religion and marital status were not found to be significant risk factors for sputum negative cases.

Bandim TBscore and mortality

Bandim TBscore was available for all 973 patients (Table III). We defined the lowest quartile as short treatment delay; followed by moderate, long and very long delay.

There was an increase in the proportion of severely ill patients as the delay increased and the association was significant (p=0.002) (Table III). There was no difference in TBscore between the patients with a long and very long delay. The pooled percentage of severe TB-cases for these two groups was 33.9%.

We also performed a logistic regression with severity (tbscore≥8) as the dependent variable and treatment delay (in 25% percentiles) as the explanatory variable. This resulted in an OR=2.08[1.39;3.13] for the long delay strata (7.6-12.1 weeks) and an OR=1.81[1.20;2.73] for very long delay strata (12.1-21.1 weeks). Correcting for possible confounders; HIV-status, residential status, education, age and sex the results were still significant - 1.85[1.21;2.82] and OR=1.57[1.21;2.82] respectively.

In figure 3 we show the Kaplan Meier mortality curves for the four delay quartiles. A delay above 21 weeks (quartile 4) had a higher mortality than quartile 1 (delay below 7.6 weeks), Hazard Ratio (HR) 1.59 [95% CI 1.01-2.48], whereas quartiles 2 and 3 did not differ significantly from quartile 1. The majority of the excess mortality in people with a long treatment delay occurred after the first 6 months following presentation and when adjusting for HIV, age, education and civil status the HR was no longer significant (Adjusted HR 1.10 [0.72;1.67]).

We included 343 smear-negative TB-cases of whom 199 cases attended six months clinical follow up after completion of anti-tuberculosis treatment. During treatment thirty-six died and the remaining 108 declined further clinical follow-up or were lost to follow up. Mean TBscore for smear-negative TB-cases decreased from 6.5 points to 1.3 points within 6 months of anti-tuberculosis treatment. The corresponding decrease in TBscore for smear positive patients was a decrease from a mean of 6.3 points to 0.9 points.

Changes in treatment delay

The median delay to treatment decreased during the study period. Within the first year the median delay was 14.6 weeks (IQR 9.3-26.1), but had dropped to 8.6 weeks (IQR: 5.7-16.7) in the last year. In a linear regression model the delay decreased with 10.3% [7.9%;12.6%] annually. The delay decreased with three weeks from 12.0 weeks (n=153) to 9.0 weeks (n=110) in 2007 and 2008 respectively. The trend in delay is displayed in Figure 4.

To account for changes in other factors affecting treatment delay we adjusted for changes in the significant risk factors for treatment delay from Table II. This did not change the estimates significantly and the adjusted decrease was 8.5%[6.0%;10.9%].

DISCUSSION

This study showed a long treatment delay in the diagnosis of TB in Guinea Bissau of 12.1 weeks equivalent to the delays found in comparable settings[21-25]. We found a clear association between treatment delay and clinical severity at inclusion, which has not been described before. This association is not surprising as patients in whom delay was long the disease had longer time to progress. We have shown in Table 3, that the proportion of severely ill is higher among those with a long delay, in the long delay groups it is a third of patients who are severely ill compared with a fifth in the short delay group. This may translate into mortality risk, patients with a TBscore≥8 have a 60% higher mortality risk as previously shown[18] and a 6-fold higher risk of treatment failure[19], but in a multivariate model we were not able to show significance with this sample size. Furthermore, a higher risk of hospitalization for patients with a longer treatment delay has also been described previously[26]. The clinical implications of shorter treatment delay is therefore likely to be less severe TB and subsequent lower mortality as well as less time to infect others.

There was a significant difference in mortality between patients with a short delay, compared with those with very long delay in the univariate analysis but when correcting for relevant factors this was no longer significant in the direct analysis.

The smear-negative cases had only one week longer median treatment delay than smear-positive cases and though transmission of disease is time-dependent[4 5] the community implications are probably limited.

We found that smear-negative patients profited substantially from anti-tuberculosis treatment. Though the diagnosis was not confirmed by culture or smear, an empirical six months anti-tuberculosis treatment resulted in a significant decrease in clinical severity assessed by TBscore.

Non-residential status did not reach statistical significance in the multiple regression model, though it could be due to a limited sample size. Non-residents are a heterogeneous group in our setting; some arrive with TB symptoms attending the centralized health care facilities. However, many come to work or study in the capital and acquire the infection while being heavily exposed to TB living in an urban overpopulated environment. Previous studies have reported that residential status can be a risk factor for treatment delay[8 9] and we suggest that this parameter is included in future studies.

HIV-status influences treatment delay in some studies, but specific delay for HIV1+2 dual infection has not been described before. This could be a random finding but HIV1+2 dual infected cases are likely to be a vulnerable group with limited access to treatment. Furthermore HIV1+2 dual infected are more often sputum smear negative and present with fewer symptoms due to a weakened immune system[27].

Several factors may influence the observed change over time in delay to treatment. Socio-economic changes are likely to be the most important factor and the general economic situation in Bissau has improved within the last decade as the country has been rebuilt after the civil war in 1998[28]. Changes in risk factors for delay during the study period could only explain a minor part of the decrease in treatment delay. We hypothesize that an increased awareness of signs and symptoms of TB may have been a factor after a TB prevalence survey in the study area in 2006 which may have contributed to the decrease in treatment delay[29]. This survey among 2989 adults involved active case finding with the use of a questionnaire on TB symptoms and referral for sputum and x-ray according to an algorithm, and may have increased TB awareness in the involved 384 sampled houses. We have seen a peak in incidence in 2007 following this survey, which may have been a result of the survey and the increased awareness of TB in the study area[30].

The study has several limitations; firstly we were not able to divide treatment delay into patient-related and health system-related delay due to limited information on the first contact to any health care provider.

Secondly, recall bias is a potential problem even though experienced local personnel and a comprehensive questionnaire was used [11 16], and the self-reported duration of symptoms may not be an accurate assessment of treatment delay. Patients were included after a considerable time period of symptomatic TB and may have overestimated the delay. The most invalidating symptoms present at inclusion may also have been over-reported as a result of recall bias potentially influencing the reported symptoms analysis.

Thirdly, we did not have data on cause of death in the background population, and were not able to estimate the corresponding case detection rate (CDR) of pulmonary TB in the area. There may indeed be a small number of undiagnosed TB patients who die from tuberculosis as previously shown[30]. This is supported by our prevalence study finding undetected TB cases in the study area[29]. Treatment delay may therefore have been underestimated since delay would be longer if undiagnosed cases with long delays had also been included.

Fourthly, this study was not a prospective follow-up of TB suspects and therefore we have no information on a possible undetected TB cases or diagnosed yet untreated cases, and the study is therefore not able to assess how many patients with symptoms are under treatment. This has been done in a recent cohort-study on TB suspects[31], but this is not the scope of this study.

Yet, our findings represent a population-based analysis over a long time period and are based on a large dataset. The results clearly show that delay to TB treatment is still a major issue in high burden areas. Our findings indicate that increased awareness of TB symptoms in the population and in health care systems is important to reduce the unacceptable long delay. Maintained focus is needed on awareness of TB and continuous implementation of TB control measures to detect the disease early and reduce unnecessary disability and loss of lives.

CONCLUSION

Delay of TB treatment in Bissau is significantly associated with disease severity at diagnosis. No education, HIV-dual infection and smear negative TB were significant risk factors for treatment delay. The treatment delay, though still unacceptably high, has decreased in Bissau during the past decade. TB control programmes may need to monitor treatment delay over time.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CW, FR and VG collected the clinical data. CC did the laboratory tests. JV and AF checked all variables and JV wrote the first draft of the manuscript. PA, EP and CW made the study logistically possible and edited the paper. All authors accepted the final version of the manuscript.

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FIGURE 1

Flowchart of participants

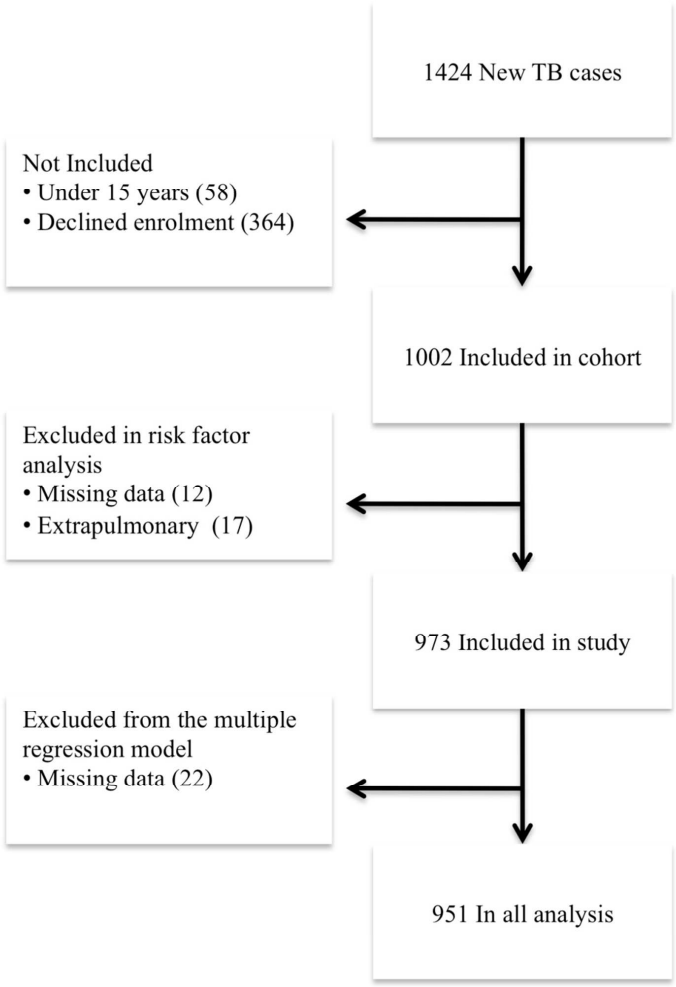

FIGURE 2

Proportion of TB-patient in treatment, according to treatment delay

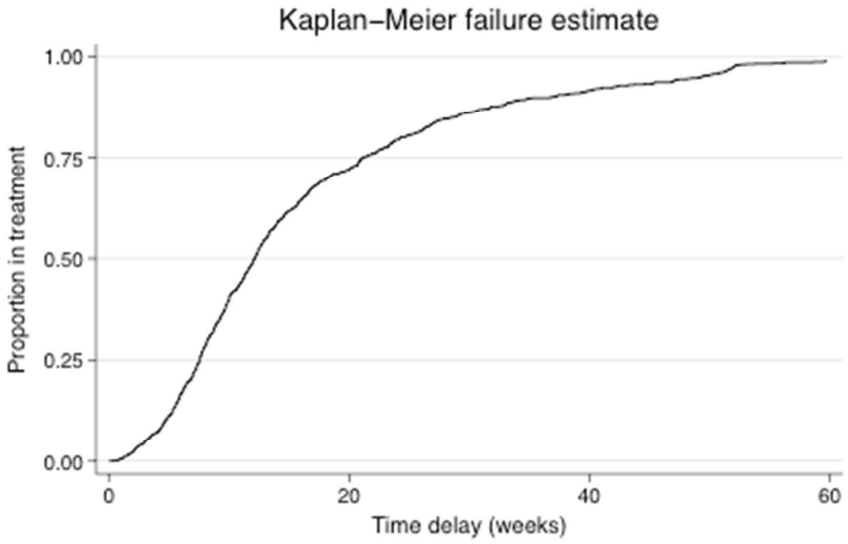

FIGURE 3
Mortality analysis

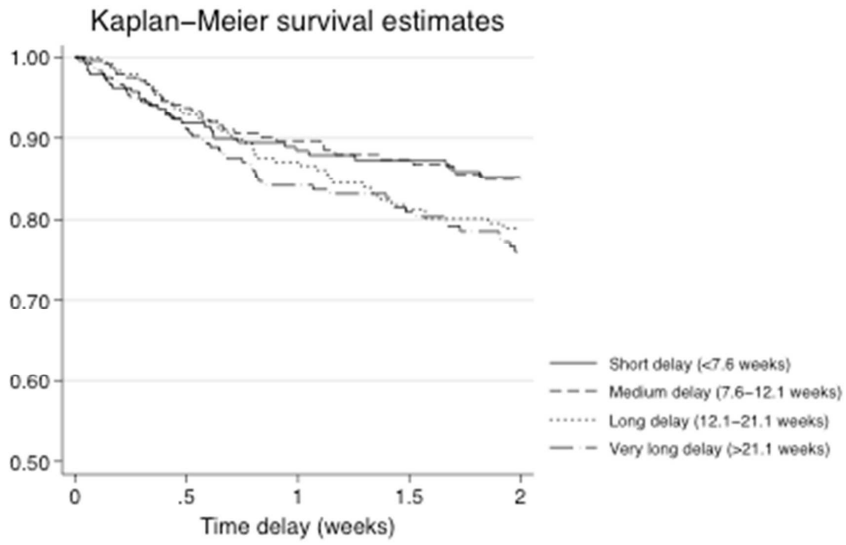

FIGURE 4

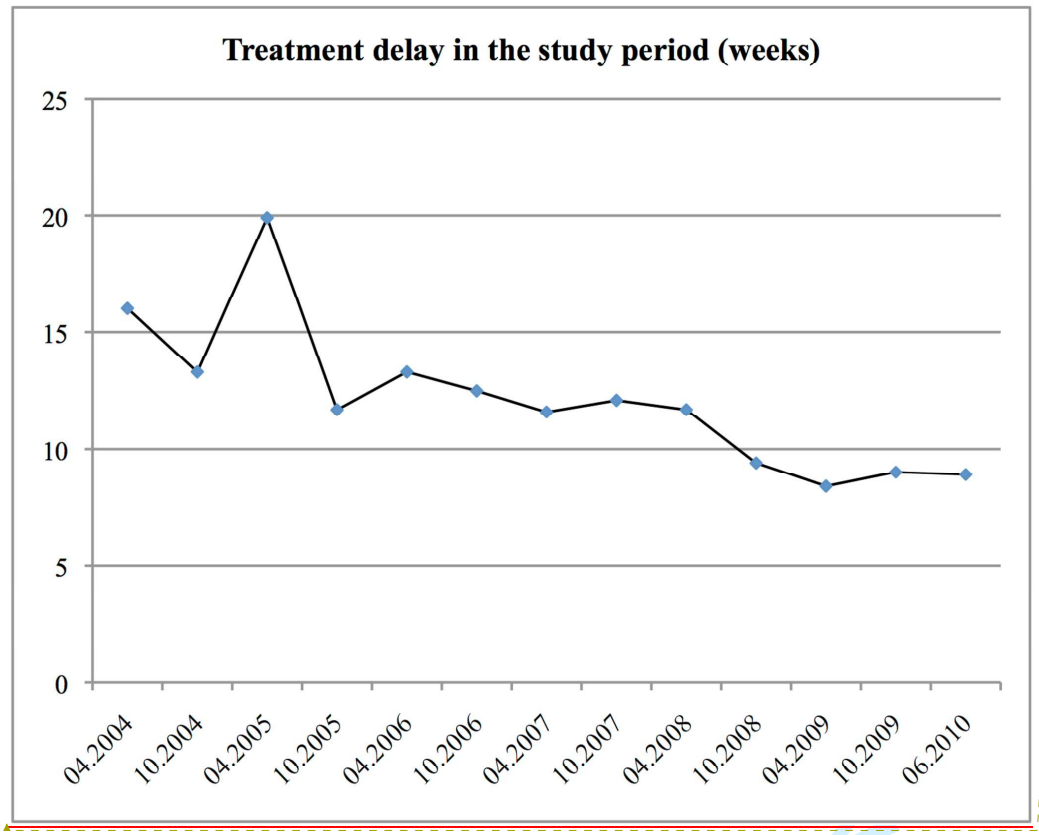

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TABLE I

First self-reported symptoms of TB (n=930*)

	TB-smear positive (n=600)	TB-smear negative/no expectoration (n=330)**
Cough	88% (530/600)	87% (286/330)
Fever	80% (482/600)	85% (279/330)
Chest Pain	80% (477/600)	84% (276/330)
Weight loss	55% (330/600)	63% (208/330)
Night sweating	16% (95/600)	17% (55/330)
Breathlessness	14% (85/600)	16% (52/330)
Haemoptysis	6% (37/600)	8% (27/330)
Other symptoms	1% (6/600)	1% (2/330)

*43 cases did not provide information on the nature of first TB-symptoms.

** There were no significant differences between the two groups (p>0.05).

TABLE II

Risk factors for total treatment delay (n= 973*)

	N	Median (weeks)	RR [95%CI]	Adjusted RR [95%CI]**
Gender				
- Male	602	11.4	1	1
- Female	371	12.7	1.15[1.03;1.28]	1.05[0.94;1.18]
Age (years)				
- 15-24	186	10.9	1	1
- 25-34	351	11.4	1.05[0.91;1.22]	0.99[0.85;1.15]
- 35-44	209	12.0	1.10[0.94;1.29]	0.95[0.79;1.14]
- 45+	227	14.9	1.31[1.12;1.54]	1.01[0.84;1.22]
Civil Status				
- Single	439	10.7	1	1
- Married	383	12.4	1.23[1.10;1.37]	1.02[0.89;1.17]
- Divorced/widow(er)	151	14.6	1.42[1.23;1.65]	1.15[0.86;1.37]
Education				
- 9+ years	199	9.7	1	1
- 7-9 years	208	10.0	1.13[0.96;1.31]	1.10[0.94;1.28]
- 1-6 years	264	12.6	1.36[1.17;1.57]	1.23[1.05;1.44]
- No education	290	15.1	1.55[1.34;1.79]	1.24[1.03;1.49]
- Data missing	12	17.2	1.50[0.94;2.38]	-
Residence				
- Resident	721	11.3	1	1
- Guest	252	15.1	1.32[1.18;1.48]	1.09[0.95;1.24]
HIV-status				
- HIV-neg	665	11.9	1	1
- HIV1	187	12.3	1.05[0.92;1.20]	1.03[0.90;1.17]
- HIV2	79	12.0	0.97[0.80;1.17]	0.86[0.71;1.04]
- HIV1+2	39	16.0	1.40[1.08;1.82]	1.31[1.01;1.70]
- Data missing	3	21.0	2.05[0.82;5.14]	-
Sputum smear status				
- Smear positive	630	11.6	1	1
- Smear neg/no expectoration	343	12.7	1.19[1.07;1.32]	1.13[1.02;1.25]
BMI				
- Normal (BMI>18.5)	435	11.1	1	
- Underweight(BMI<18.5)	524	12.6	1.10[0.99;1.22]	-
- Data missing	14	12.3	1.01[0.66;1.56]	-
Ethnic Group				
- Mancanha/Manjaco	251	11.9	1	1
- Balanta	143	13.4	1.25[1.06;1.48]	1.11[0.94;1.32]
- Fula	240	11.8	1.00[0.87;1.16]	1.12[0.82;1.52]
- Pepel	232	12.7	1.12[0.97;1.30]	1.13[0.98;1.31]
- Other	100	11.7	1.09[0.91;1.32]	1.10[0.90;1.33]
- Data missing	7	13.0	1.26[0.68;2.31]	-
Religion				
- Catholic	346	11.0	1	1
- Traditional Religion	265	14.7	1.32[1.16;1.50]	1.07[0.92;1.25]
- Protestant	84	11.1	0.97[0.80;1.17]	0.90[0.75;1.09]

-	Muslim	260	11.4	1.02[0.89;1.16]	0.88[0.65;1.18]
-	Other	17	12.4	1.17[0.79;1.73]	1.12[0.76;1.65]
-	Data missing	1	11.9	1.05[0.22;5.12]	-

*In the multiple regression model twenty-two patients were not included due to missing information on one or more risk factors (see figure 1).
**The adjusted analysis was corrected for calendar time.

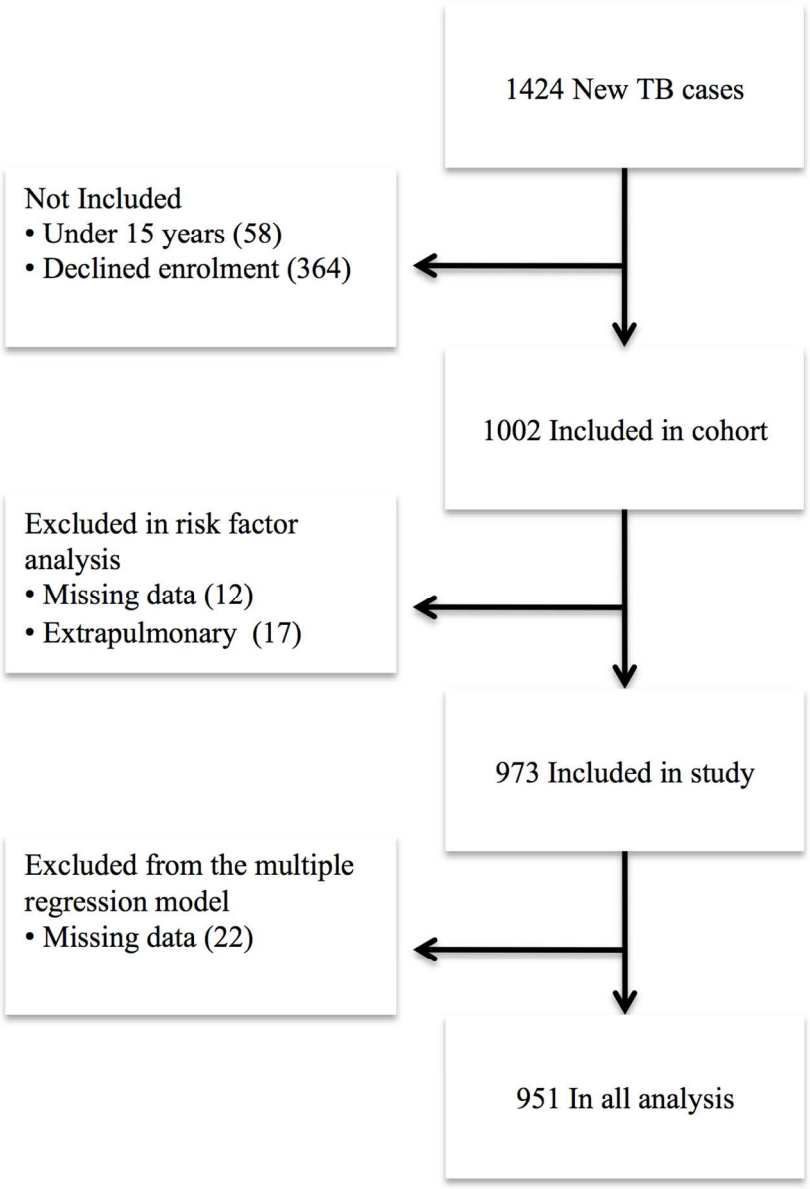
TABLE III

Treatment delay and clinical severity

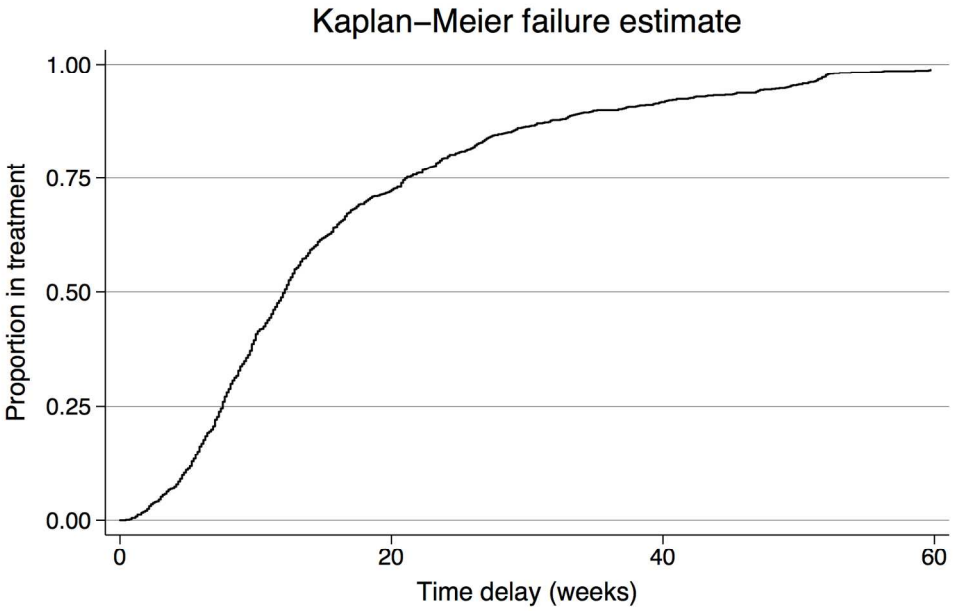
Centile	Delay (weeks)	TBscore	Percentage of severe TB-cases*	p**
0-25%	0-7.6	5.7[5.4;5.9]	20.8% (50/241)	-
25-50%	7.6-12.1	6.3[5.9;6.7]	26.1% (64/245)	0.199
50-75%	12.1-21.1	6.8[6.4;7.2]	35.5% (87/245)	0.001
75%-	21.1-	6.7[6.3;7.1]	32.4% (79/244)	0.005

*Severe cases were defined as TBscore≥8

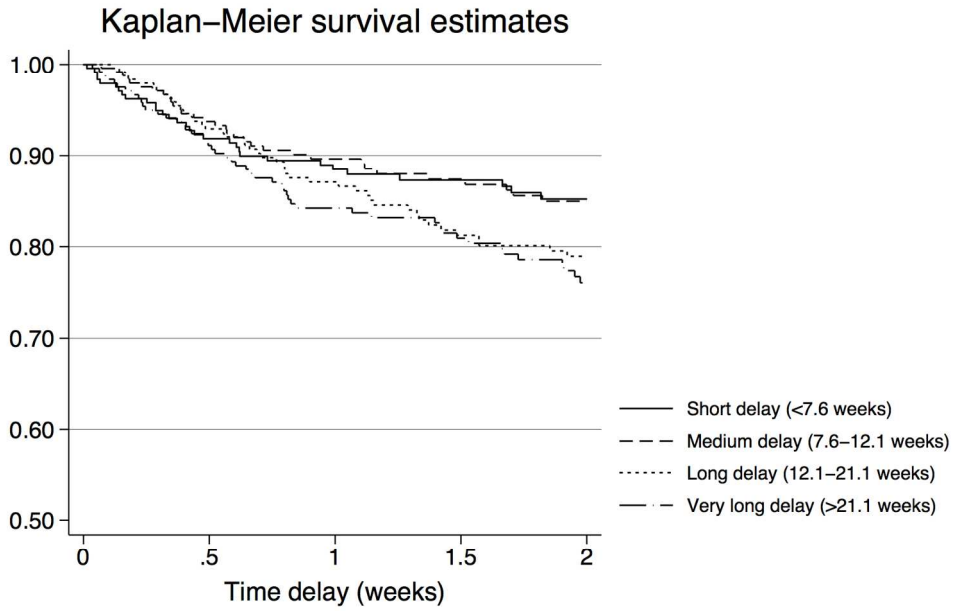
**Fishers exact test comparing each stratum with baseline.



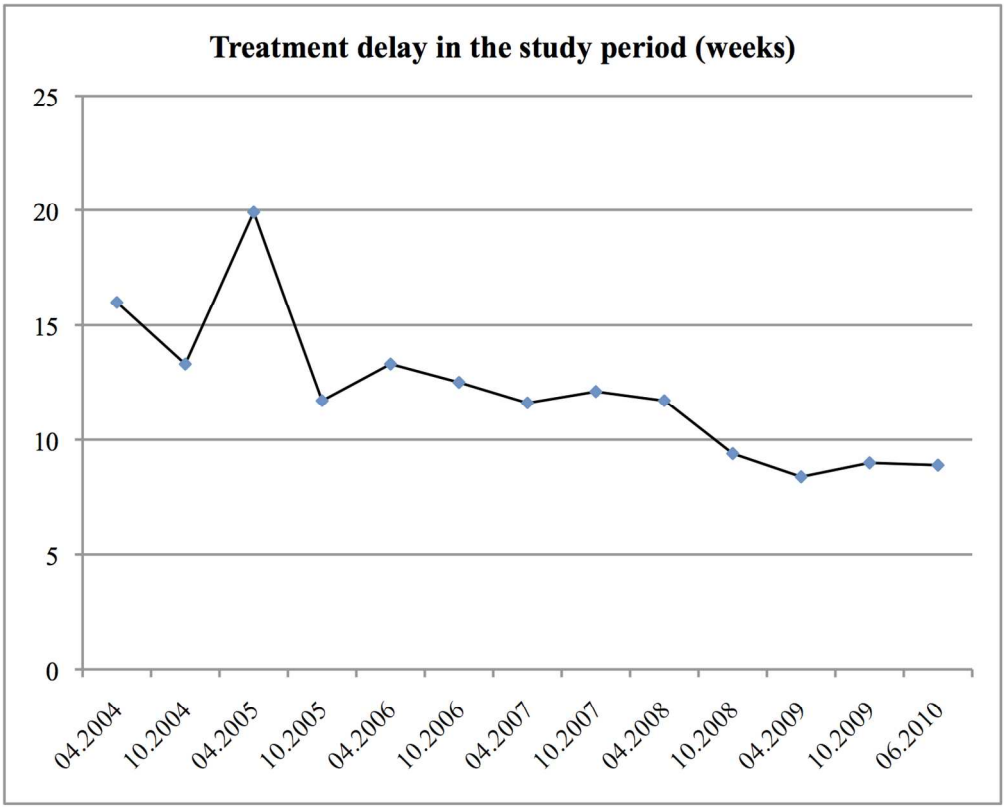
Flow chart demonstrating included individuals
107x155mm (300 x 300 DPI)



Treatment delay was defined as the time from self-reported onset of TB symptoms to the initiation of specific anti-tuberculosis treatment. All patients were in treatment by the end of the analysis period because only those patients who ultimately began treatment were included in this analysis.
152x101mm (300 x 300 DPI)



The graph describes the relation between treatment delay and mortality. Strata's were created from 25% percentiles of median treatment delay.
152x101mm (300 x 300 DPI)



Median treatment delay during the study period divided into 6 months intervals starting from November 2003. The last interval is 8 months to include all included cases in the figure.
135x108mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	20
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9, 24
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.