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Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

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ABSTRACT

Background and objective: In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

Setting: This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

Participants: All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

Main outcome measures (end points): The all cause mortality rate ratio (MRR) between exposed children and unexposed community control children.

Results: A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The mortality in IPT children compared with community controls in the 2005-2008 period differed significantly from the mortality of exposed but untreated children in the 1996-1998 cohort (test of interaction, p=0.01).

Conclusion: The mortality among children who received IPT was significantly lower than the previously observed in the 1996-1998 period. The excess mortality was

completely removed in the cohort of children receiving IPT in the 2005-2008 period. Children exposed who received IPT had a lower mortality than children exposed who did not receive IPT.

Article summary

This article focuses on:

- impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home and
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent

at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study.

Finally, due to the current WHO recommendation it was decided not to conduct a randomised study, hence there are a number of theoretical biases.

Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the tuberculosis epidemic[1;2]. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe tuberculosis-related morbidity and mortality[2]. Of the estimated 8.3 million new tuberculosis cases diagnosed in 2000, almost 900,000 were children[3], and the proportion of children in the high-burden countries is estimated to be higher[4;5]. Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home[6] and reliable medical information on causes of death is therefore lacking[7]. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries[7;8]. Necropsy studies conducted in Africa have shown that tuberculosis rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas[9].

An intervention known to contribute to the reduction of morbidity and mortality due to tuberculosis is isoniazid preventive therapy (IPT). Isoniazid (INH) was recommended for tuberculosis chemoprophylaxis during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages[10]. After all this time, INH continues to be the drug of choice[11], and WHO recommends that all TB contacts under the age of 5 should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane

review concluded that there was not enough evidence for general recommendation of IPT for HIV infected children[12]. The use of INH in low income countries is, however, limited by several circumstances: difficulties of ruling out active tuberculosis in children, mainly those infected with HIV, before initiation of prophylaxis[13], liver toxicity[14-16], and poor adherence[17]. These circumstances may limit the widespread use of IPT in the resource-constrained settings, where provision of TB care often falls short of internationally recommended standards[13]. Isoniazid chemoprophylaxis has been shown to be effective in recent skin test converters and recent contacts of identified cases of active tuberculosis[17;18]. In one study of tuberculosis preventive therapy with isoniazid in HIV-positive subjects, a 20% reduction of mortality was found in those with positive tuberculin skin test (TST)[18]. The effect of such prophylaxis in children, however, is not well established.

The present study aimed to assess the impact of IPT on mortality in children less than 5 years of age exposed to intrathoracic TB at home in an urban area of Bissau. We compared the mortality of children on IPT with the mortality of community control children who had not been exposed to TB at home. In a previous study in the same community, we found that exposure to TB at home was associated with a 66% excess mortality among children under five years of age[19]. Hence, the purpose of the present study was to assess whether this differential excess mortality could be removed.

Materials and methods

Setting

The study was conducted as a prospective cohort study from September 2005 to October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years[20].

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.
The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

Houses and household contacts

- Houses in the study area are one-storey, rectangular constructions, usually with six to eight rooms and are inhabited by 2 to 4 households (families). The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the

same space in the house, eating from the same pot and recognizing one person as the head of the household.

Recruitment of participants and patients

Identification of adult TB index cases

Since 1996 a TB surveillance system, implemented in collaboration with the national TB hospital ("Hospital Raoul Follereau"), has identified adult (≥15 years) intrathoracic TB cases using passive and active case finding[20]. The procedures for identification and diagnosis of the cases within this TB surveillance system have previously been described[20].

Enrolment in the IPT cohort

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for active TB in a clinical examination for signs and symptoms using the Keith Edwards score[21]. If the investigation suggested active TB the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and chest x-ray. Broad spectrum antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically

after 2 weeks of broad spectrum antibiotics, and without other explanatory disease were given a full TB treatment regimen according to the national protocol. Children who developed signs and symptoms suggestive of active TB while on IPT were evaluated and treated in a similar way.

Children with active TB, those who did not give consent, who were absent from first, second or third visit or at enrolment consultation were excluded from the IPT cohort.

There were several steps in the enrolment procedure as depicted in Figure 1. Once an adult TB case from the study area was identified a project assistant went to the patient's house to update the census for the families living in the house, and socio-economic and demographic information was noted on the questionnaire. Following the census-update, a field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house was visited by the nurse who read the TST and referred potential TB cases for further clinical examination. Children less than 5 years of age without active TB were eligible for enrolment in the IPT cohort and were invited to attend the enrolment visit at the local health centre. Eligible children who did not show up at inclusion were traced again. If not found they were considered absent, but still followed up using basic census information. Due to limited time frame, logistic reasons and limited funding they were not included later.

For children enrolled in the IPT programme, INH tablets were administered at 5 mg/kg/day together with Pyridoxine (Vitamin B6) tablets. The Vitamin B6 dosage was 25 mg for children receiving <100 mg of INH and 50 mg for children receiving >100 mg of INH[22]. The medicine was provided at the house every two weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of INH treatment. The follow-up

visits at 1 and 7 months were performed by the research clinician at the local health centre and at 4 and 9 months by a field assistant at the child´s home. Evaluation at follow-up visits included questions about side effects and a physical assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study routines are described in detail elsewhere[23]. The initially intended IPT enrolment period was September 2005 to October 2007 with 9 months of follow-up to June 2008. However, children continued to be enrolled on IPT until the end of the study period in June 2008.

Pre-IPT cohort

As previously described; children less than 5 years of age living in the same house as an adult index TB case at the time of initiation of treatment during the period May 1996 to July 1998 were retrieved from the BHP register[19]. To assess the impact of TB exposure at home in the absence of IPT, their mortality was compared with the mortality of children living in the study area who had not been exposed to TB at home, during the same period.

Effect-size calculation

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases.

An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

Ethical approval

The parents or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before enrolment. The study protocol was approved by the Guinea-Bissau National Research Coordination and Ethics Committee.

Statistical analysis

Data regarding adult TB cases were obtained from the general TB identification system in the study area while demographic information was taken from the basic surveillance system of the BHP. Statistical analyses were conducted in STATA version 10.

Similar to the analysis of the impact of TB exposure in the absence of IPT, the average delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months. Hence, children registered in the same house as an adult index TB case 0-3 months before treatment were considered exposed. The effect of exposure on mortality was evaluated by rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in Guinea-Bissau is very high. To be exposed a child had to be born and registered at the time of exposure. Consequently only few children were exposed before 3 months of age. Therefore we have chosen to commence the analyses at 3 months of age.

IPT treatment was initiated in September 2005. To allow exposure time before IPT, follow-up started in July 2005. IPT enrolment for the present study ended in October 2007 with treatment ending in June 2008. Thus, the study period for the present study is July 2005 to June 2008. Exposed children counted as unexposed controls until the time of exposure. Exposed children never receiving IPT counted as exposed without IPT from the start of exposure to the end of follow-up. Children subsequently enrolled on IPT counted as exposed without IPT until the start of IPT and then as exposed with IPT to the end of follow-up. However, enrolment into the IPT program continued after the enrolment period of the present study. Children enrolled on IPT after October 2007 were also counted as exposed on IPT even though they did not finish the treatment within the study period. Censoring the children enrolled on IPT after October 2007 at the time of IPT had little impact on the results. Some children who were present when the TB team visited the TB case house (figure 1) were enrolled on IPT even though they were not born or registered in the house before the TB case initiated treatment. According to the

epidemiological definitions these children were not exposed and they have counted as unexposed with IPT. A separate analysis was conducted excluding these subjects.

An adjusted analysis was conducted including possible confounders related to child mortality: gender, ethnicity, district, socio-economic status, schooling of the mother, child crowding (<5 years) and crowding among older individuals (>5 years). A score for socio-economic status was calculated adding house indicators (yes=1; no/missing=0): corrugated iron roof, electricity, television and in-door toilet. A separate "Missing" category was constructed when information was missing on all four variables. Crowding was defined as the number of individuals in the house of the TB case on January 1, 2007, the mid-point of the examined period. Crowding was included in the analysis as a linear predictor.

It was further examined whether differential mortality not related to TB exposure may have existed in the exposed houses. Mortality was compared between children living in the house of the adult TB case three years before TB exposure began and children living in the remaining houses. As for the main study[19], the comparison was made over a 3-year period from July 2002 to June 2005. The period was chosen not to overlap the study period.

Results:

Index TB cases and included children

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from "case houses with no inclusion" and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Follow-up ended before 3 months of age for 14 of these children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

TB among exposed children

One child was on TB treatment at the time of inclusion. Active TB was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical

and chest X-ray findings. One of these children tested HIV-positive and was enrolled in an antiretroviral (ARV) programme.

TB exposure and mortality

Two children died during IPT. For a 6-month old boy, hospital records stated the cause of death as severe malaria and anaemia. The mother of a 2-year old girl, who died two months after the initiation of the IPT program, reported that the child had had diarrhoea, cough and fever prior to death. Antibiotics and other medications had been prescribed. The research clinician had requested a chest X-ray, but the result was never received. No further IPT children died during the follow-up period.

In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT contributed with 1023 PYO and the controls contributed with 30713 PYO.

Though not statistically significant, the exposed children receiving IPT had a lower mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-1.2) (Table 1). This estimate changed little when controlled for background factors (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and some exposed children did not receive IPT (Figure 1). In this group of TB-exposed children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7) (Table 1).

There were 68 children on IPT who were not formally exposed to TB because the child was born or registered after exposure occurred in the house. Excluding these from the IPT group we observed one death from 642 person years of observation giving a MRR of 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

Comparison of mortality among TB-exposed children in the absence and presence of IPT

We previously found that the excess mortality after exposure to TB only started 6 months after exposure[19]. We made a similar analysis for the period 2005-2008, table 3 shows the comparison of exposed children without IPT and unexposed children, stratified by time since exposure and age at exposure.

Restricted to the period after 6 months, the mortality rate relative to community controls in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

It was furthermore examined whether the effect in the exposed house was caused by lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among children in the houses which later had TB cases was the same as the mortality in the control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

The purpose of the present study was to assess whether the excess mortality associated with TB exposure at home could be removed by implementing an IPT programme. In a

previous study in the same community, exposure to TB was associated with an MRR of 1.66 (1.2-2.3) among children under five years of age[19]. In the present study both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction, p=0.01 and p=0.004).

It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

Discussion

In the present study we have shown a considerable impact of the IPT programme on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed children. This excess mortality was completely removed in the cohort of children receiving IPT from 2005-2008. It should be emphasized that comparing data from different time-periods is not straight-forward, as the conditions have changed in many ways that cannot be completely deduced, and the results can be biased. In our situation, the child mortality has dropped markedly between

these two time-periods. However, the excess mortality from the 1996-1998 cohort has changed to a trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child mortality, and our data suggest that this is partly due to the introduction of IPT.

Unexpected observations

Mortality in the study area declined dramatically between the two study periods and mortality declined more than expected among both exposed children receiving IPT and exposed children not receiving IPT. Based on the experience from the 1996-1998 period, TB exposure at home in the absence of IPT should have been associated with a 19/1000 person-years excess mortality.

Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa. However, despite the recent HIV epidemic, mortality rates have decreased drastically over the last decade following the same pattern observed in the other sub-Saharan African countries[24-26]. The reasons for the mortality decline are not fully understood but systematic annual vitamin A campaigns and the marked decrease in malaria incidence are likely to have contributed.

The strong reduction in mortality among IPT-treated children could possibly be linked to increased attention to these children including easier access to other forms of treatment and more attention from the parents. However, this would be unlikely to explain why mortality also declined more than expected among the children who did not receive IPT. This may suggest that all TB-associated mortality is not directly due to clinical TB disease, but may be due to interactions with other infections. If the incidence or severity

of these other infections goes down, as has happened in Bissau, the mortality associated with TB exposure would also decline.

Interpretation and consistency with previous studies

Children exposed to TB at home had excess mortality from around 6 months after exposure in the present study as well as in the previous study from 1996-1998. In both studies, we showed that the TB houses 3 years earlier had exactly the same mortality as community controls. It therefore seems unlikely that general social conditions in houses with TB cases explain the higher mortality of TB-exposed children. Furthermore, children enrolled on IPT, compared with community controls, had significantly lower mortality ratio than the TB exposed children not receiving IPT, compared with community controls, in both study periods. Hence, our results suggest that use of isoniazid plays an important role in decreasing mortality in children exposed to tuberculosis.

Studies conducted in South Africa and Zambia have reported isoniazid to be highly effective in reducing the mortality and incidence of tuberculosis in HIV-infected children and adults living in an area with a high prevalence of tuberculosis[14;27;28]. Our findings support those studies, but also observations made by Dr. Lincoln in the early 1950s showing that isoniazid chemotherapy reduced the case fatality from primary tuberculosis among children[29].

Implications and conclusions

In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was completely removed in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance. When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. More, in the 2005-2008 cohort, there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT.

Our study clearly shows that children less than 5 years of age exposed to TB at home have a high mortality that can be prevented with IPT. All our data indicate that IPT should be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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Author's contributions

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

Conflict of interest: There are no competing or conflicting interests.

Table 1: The effect of exposure on mortality according to age

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR[#1] IPT/Unexposed	MRR[#2] IPT/Unexposed	MRR[#1] No IPT/Unexposed	MRR[#2] No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

* Include unexposed children on IPT

#1 Mortality Rate Ratio from a model with age as underlying time

#2 Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.

	Age at exposure (Months)			
Months since exposure	0-11	12-35	36-59	Total
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	**2005-2008**
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

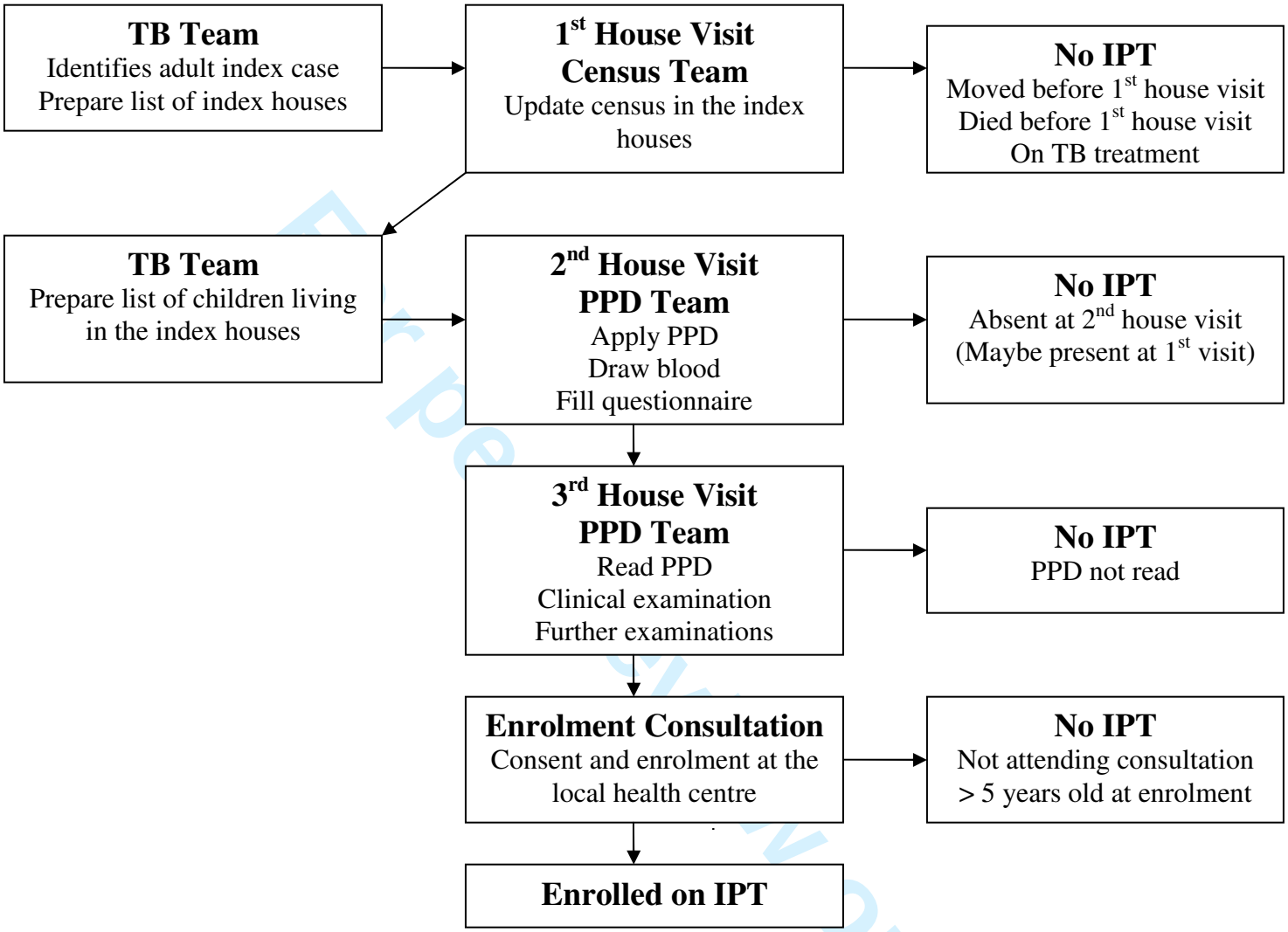

Figure 2: Flow chart of inclusion

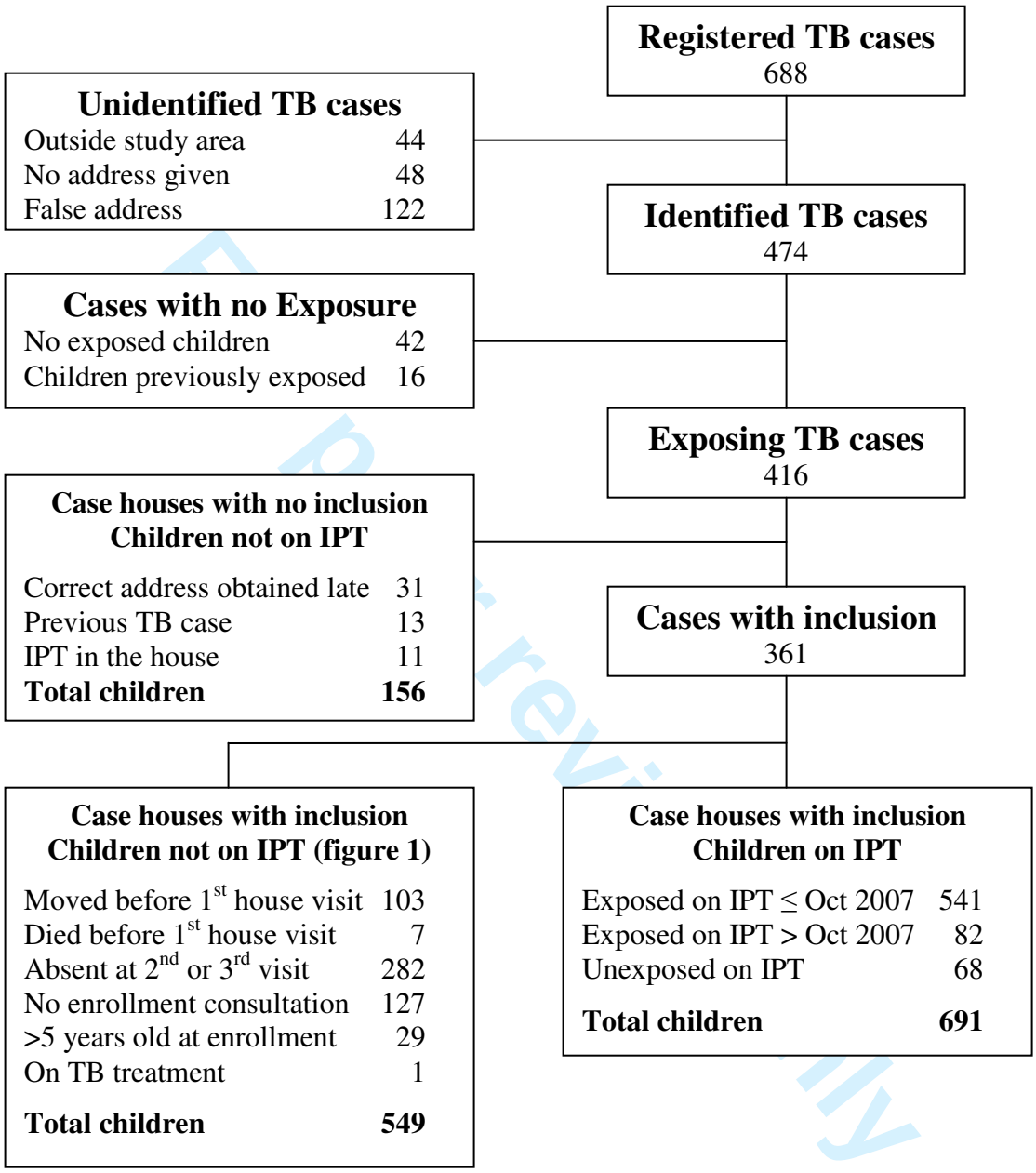

Figure 3: Person years of observation (PYO)

Children not on IPT		Children on IPT	
Exposed		**Exposed**	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months of age at death/censoring		**Unexposed**	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

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ABSTRACT

Background and objective: In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

Setting: This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

Participants: All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

Main outcome measures (end points): The all-cause mortality rate ratio (MRR) between exposed children on IPT and unexposed community control children.

Results: A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction, p=0.01).

Conclusion: In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control

children, the mortality among TB-exposed children on IPT in 2005-2008 was significantly lower than the mortality among TB-exposed children not on IPT in 1996-1998.

Article summary

This article focuses on:

- impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home and
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent

at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study. Due to the current World Health Organisation (WHO) recommendation it was decided not to conduct a randomised study, hence, there are a number of theoretical biases. Furthermore, the previous study was conducted 10 years earlier than the present study and many things changed in the meantime. Another limitation was that the children were not HIV tested, it might bias the results if there were more HIV infected children in the IPT group compared to the no IPT group. However, we would then expect higher mortality in the no IPT group compared to the community controls.

Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic[1;2]. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality[2]. Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were children[3], and the proportion of children in the high-burden countries is estimated to be higher[4;5].

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home[6] and reliable medical information on causes of death is therefore lacking[7]. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries[7;8]. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas[9].

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages[10]. After all this time, isoniazid continues to be the drug of choice[11], and WHO recommends that all TB contacts under the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded

that there was not enough evidence for general recommendation of IPT for HIV infected children[12]. The use of isoniazid in low income countries is, however, limited by several circumstances: difficulties of ruling out TB disease in children, mainly those infected with HIV, before initiation of IPT[13], liver toxicity[14-16], and poor adherence[17]. These circumstances may limit the widespread use of IPT in the resource-constrained settings, where provision of TB care often falls short of internationally recommended standards[13]. IPT has been shown to be effective in recent tuberculin skin test (TST) converters and recent contacts of identified cases of TBdisease[17;18]. In one study of IPT in HIV-positive subjects, a 20% reduction of mortality was found in those with positive TST[18]. The effect of such preventive therapy in children, however, is not well established.

The present study examined the impact of IPT on mortality in children less than 5 years of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous study in the same community, we found that exposure to TB at home was associated with 66% excess mortality compared to community control children not exposed to TB at home[19]. The aim of the present study was to compare mortality between exposed children on IPT and community control children, and to compare this relative mortality to the previously observed excess mortality.

Materials and methods

Setting

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years[20].

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.
The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

Houses and household contacts

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

Recruitment of participants and patients

Identification of adult TB index cases

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital ("Hospital Raoul Follereau"), has identified adult (≥15 years) intrathoracic TB cases using passive and active case finding[20]. As previously described in more detail[20], a TB case was defined as an adult with symptoms of TB disease with sputum smear positive or negative for AFB, presenting abnormalities in CX-ray with no improvement under treatment with broad spectrum antibiotics for two weeks.

Enrolment in the IPT cohort

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score[21]. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and chest x-ray. Broad spectrum antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically after 2 weeks of broad spectrum antibiotics, and without other

explanatory disease were given a full TB treatment regimen according to the national protocol. Antiretroviral treatment was not available at the time of the study and HIV testing was therefore not generally performed. It was only performed when the investigation suggested TB.

Children who developed signs and symptoms suggestive of TB disease while on IPT were evaluated and treated in a similar way.

Children with TB disease, those who did not give consent, who were absent from first, second or third visit or at enrolment consultation were excluded from the IPT cohort.

There were several steps in the enrolment procedure as depicted in Figure 1. Once an adult TB case from the study area was identified a project assistant went to the patient's house to update the census for the families living in the house, and socio-economic and demographic information was noted on the questionnaire. Following the census-update, a field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house was visited by the nurse who read the TST and referred potential TB cases for further clinical examination. Children without TB disease were eligible for enrolment in the IPT cohort regardless of the TST result and were invited to attend the enrolment visit at the local health centre. Eligible children who did not show up at inclusion were traced again. If not found they were considered absent, but still followed up using basic census information. Due to limited time frame, logistic reasons and limited funding they were not included later.

For children enrolled in the IPT programme, isoniazid tablets were administered at 5 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children

receiving >100 mg of isoniazid [22]. The medicine was provided at the house every two weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT. The follow-up visits at 1 and 7 months were performed by the research clinician at the local health centre and at 4 and 9 months by a field assistant at the child´s home. Evaluation at follow-up visits included questions about side effects and a physical assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study routines are described in detail elsewhere[23]. The initially intended IPT enrolment period was September 2005 to October 2007 with 9 months of follow-up to June 2008. However, children continued to be enrolled on IPT until the end of the study period in June 2008.

Pre-IPT cohort

As previously described; children less than 5 years of age living in the same house as an adult index TB case at the time of initiation of treatment during the period May 1996 to July 1998 were retrieved from the BHP register[19]. To assess the impact of TB exposure at home in the absence of IPT, their mortality was compared with the mortality of children living in the study area who had not been exposed to TB at home, during the same period.

Groups in the study

In the Pre-IPT cohort we had two groups: TB-exposed children and community control children. In the IPT cohort we intended also to have two groups: TB-exposed children on IPT and community control children. As we failed to include all exposed children in the IPT programme, a third group arose: TB-exposed children not on IPT.

Effect-size calculation

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

Ethical approval

The legal guardians (parents) or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before

enrolment. The study protocol was approved by the Guinea-Bissau National Research Coordination and Ethics Committee.

Statistical analysis

Data regarding adult TB cases were obtained from the general TB identification system in the study area while demographic information was taken from the basic surveillance system of the BHP. Statistical analyses were conducted in STATA version 10.

Similar to the analysis of the impact of TB exposure in the absence of IPT, the average delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months. Hence, children registered in the same house as an adult index TB case 0-3 months before treatment were considered exposed. The effect of exposure on mortality was evaluated by rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in Guinea-Bissau is very high. To be exposed a child had to be born and registered at the time of exposure. Consequently only few children were exposed before 3 months of age. Therefore we have chosen to commence the analyses at 3 months of age.

IPT treatment was initiated in September 2005. To allow exposure time before IPT, follow-up started in July 2005. IPT enrolment for the present study ended in October 2007 with treatment ending in June 2008. Thus, the study period for the present study is July 2005 to June 2008. Exposed children counted as unexposed controls until the time of exposure. Exposed children never receiving IPT counted as exposed without IPT from the start of exposure to the end of follow-up. Children subsequently enrolled on IPT

counted as exposed without IPT from the start of exposure until the start of IPT and then as exposed with IPT to the end of follow-up. However, enrolment into the IPT program continued after the enrolment period of the present study. Children enrolled on IPT after October 2007 were also counted as exposed on IPT even though they did not finish the treatment within the study period. Censoring the children enrolled on IPT after October 2007 at the time of IPT had little impact on the results. Some children who were present when the TB team visited the TB case house (figure 1) were enrolled on IPT even though they were not born or registered in the house before the TB case initiated treatment. According to the epidemiological definitions these children were not exposed and they have counted as unexposed with IPT. A separate analysis was conducted excluding these subjects.

An adjusted analysis was conducted including possible confounders related to child mortality: gender, ethnicity, district, socio-economic status, schooling of the mother, child crowding (<5 years) and crowding among older individuals (>5 years). A score for socio-economic status was calculated adding house indicators (yes=1; no/missing=0): corrugated iron roof, electricity, television and in-door toilet. A separate "Missing" category was constructed when information was missing on all four variables. Crowding was defined as the number of individuals in the house of the TB case on January 1, 2007, the mid-point of the examined period. Crowding was included in the analysis as a linear predictor.

It was further examined whether differential mortality not related to TB exposure may have existed in the exposed houses. Mortality was compared between children living in

the house of the adult TB case three years before TB exposure began and children living in the remaining houses. As for the main study[19], the comparison was made over a 3-year period from July 2002 to June 2005. The period was chosen not to overlap the study period.

Results:

Index TB cases and included children

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from "case houses with no inclusion" and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

TB among exposed children

One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical and chest X-ray findings. One of these children tested HIV-positive and was enrolled in an antiretroviral (ARV) programme.

TB exposure and mortality

Two children died during IPT. For a 6-month old boy, hospital records stated the cause of death as severe malaria and anaemia. The mother of a 2-year old girl, who died two months after the initiation of the IPT program, reported that the child had had diarrhoea, cough and fever prior to death. Antibiotics and other medications had been prescribed. The research clinician had requested a chest X-ray, but the result was never received. No further IPT children died during the follow-up period.

In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT contributed with 1023 PYO and the controls contributed with 30713 PYO.

Though not statistically significant, the exposed children receiving IPT had a lower mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-1.2) (Table 1). This estimate changed little when controlled for background factors (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and some exposed children did not receive IPT (Figure 1). In this group of TB-exposed children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7) (Table 1).

There were 68 children on IPT who were not formally exposed to TB because the child was born or registered after exposure occurred in the house. Excluding these from the IPT group we observed one death from 642 person years of observation giving a MRR of 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

Comparison of mortality among TB-exposed children in the absence and presence of IPT

We previously found that the excess mortality after exposure to TB only started 6 months after exposure[19]. We made a similar analysis for the period 2005-2008; table 3 shows the comparison of exposed children without IPT and unexposed community control children, stratified by time since exposure and age at exposure.

Restricted to the period after 6 months, the mortality rate relative to community controls in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

It was furthermore examined whether the effect in the exposed house was caused by lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among children in the houses which later had TB cases was the same as the mortality in the control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

In a previous study in the same community, exposure to TB at home was associated with an MRR of 1.66 (1.2-2.3) compared to community control children[19]. The present study assessed whether the excess mortality could be reduced by implementing an IPT programme. Both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction, p=0.01 and p=0.004).

It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

Discussion

In the present study we have shown an impact of the IPT programme on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed community control children. The mortality rate ratio was inverted in 2005-2008 with markedly (though not significantly) lower mortality among exposed children on IPT compared to the community control children.. It should be emphasized

that comparing data from different time-periods is not straight-forward, as the conditions have changed in many ways that cannot be completely deduced, and the results can be biased. In our situation, the child mortality has dropped markedly between these two time-periods. However, the excess mortality from 1996-1998 has changed to a marked trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child mortality, and our data suggest that this is partly due to the introduction of IPT.

Unexpected observations

Mortality in the study area declined dramatically between the two study periods and mortality declined more than expected among both exposed children receiving IPT and exposed children not receiving IPT. Based on the experience from the 1996-1998 period, TB exposure at home in the absence of IPT should have been associated with a 19/1000 person-years excess mortality.

Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa. However, despite the recent HIV epidemic, mortality rates have decreased drastically over the last decade following the same pattern observed in the other sub-Saharan African countries[24-26]. The reasons for the mortality decline are not fully understood but systematic annual vitamin A campaigns and the marked decrease in malaria incidence are likely to have contributed.

The strong trend toward less mortality among IPT-treated children could possibly be linked to increased attention to these children including easier access to other forms of treatment and more attention from the parents. However, this would be unlikely to explain why mortality also declined more than expected among the children who did not

receive IPT. This may suggest that all TB-associated mortality is not directly due to clinical TB disease, but may be due to interactions with other infections. If the incidence or severity of these other infections goes down, as has happened in Bissau, the mortality associated with TB exposure would also decline.

Interpretation and consistency with previous studies

Children exposed to TB at home had excess mortality from around 6 months after exposure in the present study as well as in the previous study from 1996-1998. In both studies, we showed that the TB houses 3 years earlier had exactly the same mortality as community controls. It therefore seems unlikely that general social conditions in houses with TB cases explain the higher mortality of TB-exposed children. Furthermore, children enrolled on IPT in the IPT cohort (compared with community controls) had significantly lower mortality than the TB exposed children not receiving IPT in the pre-IPT cohort (compared with community controls). Hence, our results suggest that use of isoniazid plays an important role in decreasing mortality in children exposed to TB. Studies conducted in South Africa and Zambia have reported isoniazid to be highly effective in reducing the mortality and incidence of TB in HIV-infected children and adults living in an area with a high prevalence of TB[14;27;28]. Our findings support those studies, but also observations made by Dr. Lincoln in the early 1950s showing that isoniazid chemotherapy reduced the case fatality from primary TB among children[29].

Implications and conclusions

In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was reduced in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance. When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. Furthermore, in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT.

. All our data indicate that IPT should be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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Author's contributions

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

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Table 1: The effect of exposure on mortality according to age

Age Months	On IPT* D/PYO	Exposed No IPT	Unexposed D/PYO	MRR[#1] IPT/Unexposed	MRR[#2] IPT/Unexposed	MRR[#1] No	MRR[#2] No

		D/PYO				IPT/Unexposed	IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

[*] Include unexposed children on IPT

[#1] Mortality Rate Ratio from a model with age as underlying time

[#2] Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.

	Age at exposure (Months)			
Months since exposure	0-11	12-35	36-59	Total
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	**2005-2008**
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

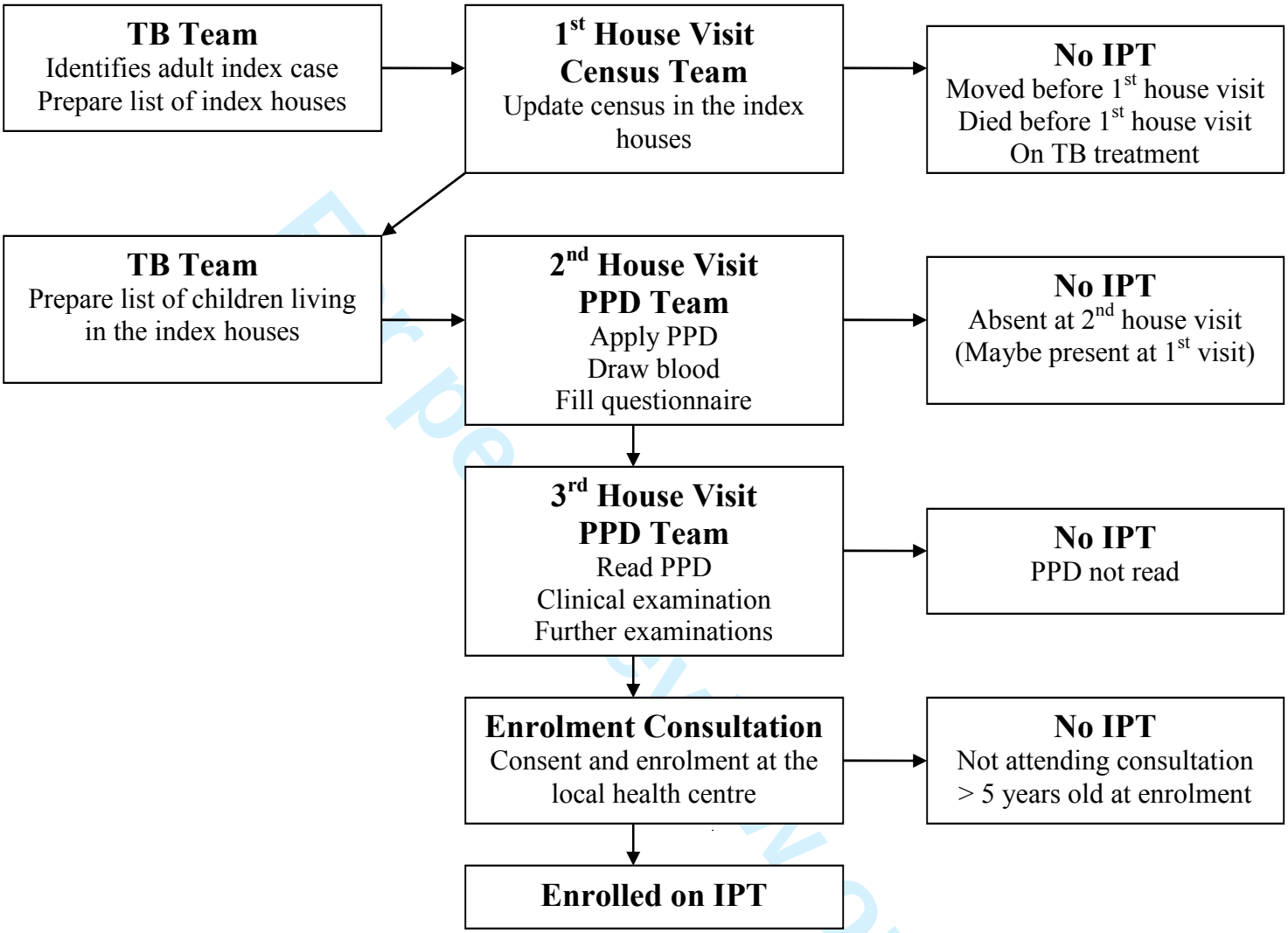

Figure 2: Flow chart of inclusion

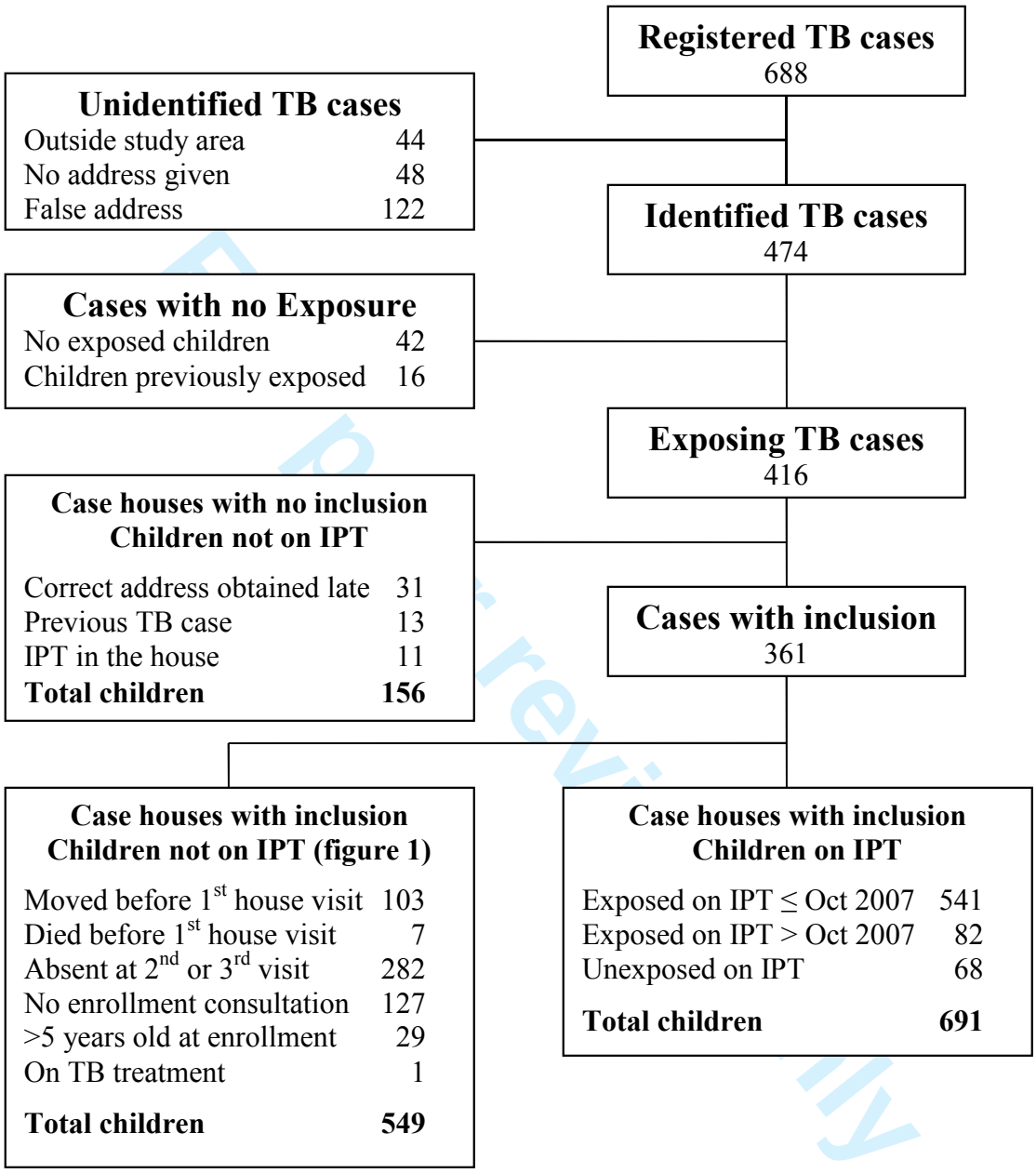

Figure 3: Person years of observation (PYO)

Children not on IPT		Children on IPT	
Exposed		**Exposed**	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		**Unexposed**	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

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ABSTRACT

Background and objective: In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

Setting: This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

Participants: All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

Main outcome measures (end points): The all-cause mortality rate ratio (MRR) between exposed children on IPT and unexposed community control children.

Results: A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction, p=0.01).

Conclusion: In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control

children, the mortality among TB-exposed children on IPT in 2005-2008 was significantly lower than the mortality among TB-exposed children not on IPT in 1996-1998.

Article summary

This article focuses on:

- impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home and
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent

at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study. Due to the current World Health Organisation (WHO) recommendation it was decided not to conduct a randomised study, hence, there are a number of theoretical biases. Furthermore, the previous study was conducted 10 years earlier than the present study and many things changed in the meantime. Another limitation was that the children were not HIV tested, it might bias the results if there were more HIV infected children in the IPT group compared to the no IPT group. However, we would then expect higher mortality in the no IPT group compared to the community controls.

Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic[1;2]. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality[2]. Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were children[3], and the proportion of children in the high-burden countries is estimated to be higher[4;5].

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home[6] and reliable medical information on causes of death is therefore lacking[7]. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries[7;8]. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas[9].

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages[10]. After all this time, isoniazid continues to be the drug of choice[11], and WHO recommends that all TB contacts under the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded

that there was not enough evidence for general recommendation of IPT for HIV infected children[12]. The use of isoniazid in low income countries is, however, limited by several circumstances: difficulties of ruling out TB disease in children, mainly those infected with HIV, before initiation of IPT[13], liver toxicity[14-16], and poor adherence[17]. These circumstances may limit the widespread use of IPT in the resource-constrained settings, where provision of TB care often falls short of internationally recommended standards[13]. IPT has been shown to be effective in recent tuberculin skin test (TST) converters and recent contacts of identified cases of TBdisease[17;18]. In one study of IPT in HIV-positive subjects, a 20% reduction of mortality was found in those with positive TST[18]. The effect of such preventive therapy in children, however, is not well established.

The present study examined the impact of IPT on mortality in children less than 5 years of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous study in the same community, we found that exposure to TB at home was associated with 66% excess mortality compared to community control children not exposed to TB at home[19]. The aim of the present study was to compare mortality between exposed children on IPT and community control children, and to compare this relative mortality to the previously observed excess mortality.

Materials and methods

Setting

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years[20].

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.
The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

Houses and household contacts

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

Recruitment of participants and patients

Identification of adult TB index cases

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital ("Hospital Raoul Follereau"), has identified adult (≥15 years) intrathoracic TB cases using passive and active case finding[20]. As previously described in more detail[20], a TB case was defined as an adult with symptoms of TB disease with sputum smear positive or negative for AFB, presenting abnormalities in CX-ray with no improvement under treatment with broad spectrum antibiotics for two weeks. ~~The procedures for identification and diagnosis of the cases within this TB surveillance system have previously been described[20].~~

Enrolment in the IPT cohort

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score[21]. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and chest x-ray. Broad spectrum

antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically after 2 weeks of broad spectrum antibiotics, and without other explanatory disease were given a full TB treatment regimen according to the national protocol. Antiretroviral treatment was not available at the time of the study and HIV testing was therefore not generally performed. It was only performed when the investigation suggested TB.

Children who developed signs and symptoms suggestive of TB disease while on IPT were evaluated and treated in a similar way.

Children with TB disease, those who did not give consent, who were absent from first, second or third visit or at enrolment consultation were excluded from the IPT cohort.

There were several steps in the enrolment procedure as depicted in Figure 1. Once an adult TB case from the study area was identified a project assistant went to the patient's house to update the census for the families living in the house, and socio-economic and demographic information was noted on the questionnaire. Following the census-update, a field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house was visited by the nurse who read the TST and referred potential TB cases for further clinical examination. Children without TB disease were eligible for enrolment in the IPT cohort regardless of the TST result and were invited to attend the enrolment visit at the local health centre. Eligible children who did not show up at inclusion were traced again. If not found they were considered absent, but still followed up using basic census information. Due to limited time frame, logistic reasons and limited funding they were not included later.

For children enrolled in the IPT programme, isoniazid tablets were administered at 5 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children receiving >100 mg of isoniazid [22]. The medicine was provided at the house every two weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT. The follow-up visits at 1 and 7 months were performed by the research clinician at the local health centre and at 4 and 9 months by a field assistant at the child´s home. Evaluation at follow-up visits included questions about side effects and a physical assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study routines are described in detail elsewhere[23]. The initially intended IPT enrolment period was September 2005 to October 2007 with 9 months of follow-up to June 2008. However, children continued to be enrolled on IPT until the end of the study period in June 2008.

Pre-IPT cohort

As previously described; children less than 5 years of age living in the same house as an adult index TB case at the time of initiation of treatment during the period May 1996 to July 1998 were retrieved from the BHP register[19]. To assess the impact of TB exposure at home in the absence of IPT, their mortality was compared with the mortality of children living in the study area who had not been exposed to TB at home, during the same period.

Groups in the study

In the Pre-IPT cohort we had two groups: TB-exposed children and community control children. In the IPT cohort we intended also to have two groups: TB-exposed children on IPT and community control children. As we failed to include all exposed children in the IPT programme, a third group arose: TB-exposed children not on IPT.

Effect-size calculation

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

Ethical approval

The legal guardians (parents) or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before enrolment. The study protocol was approved by the Guinea-Bissau National Research Coordination and Ethics Committee.

Statistical analysis

Data regarding adult TB cases were obtained from the general TB identification system in the study area while demographic information was taken from the basic surveillance system of the BHP. Statistical analyses were conducted in STATA version 10.

Similar to the analysis of the impact of TB exposure in the absence of IPT, the average delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months. Hence, children registered in the same house as an adult index TB case 0-3 months before treatment were considered exposed. The effect of exposure on mortality was evaluated by rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in Guinea-Bissau is very high. To be exposed a child had to be born and registered at the time of exposure. Consequently only few children were exposed before 3 months of age. Therefore we have chosen to commence the analyses at 3 months of age.

IPT treatment was initiated in September 2005. To allow exposure time before IPT, follow-up started in July 2005. IPT enrolment for the present study ended in October 2007 with treatment ending in June 2008. Thus, the study period for the present study is

July 2005 to June 2008. Exposed children counted as unexposed controls until the time of exposure. Exposed children never receiving IPT counted as exposed without IPT from the start of exposure to the end of follow-up. Children subsequently enrolled on IPT counted as exposed without IPT from the start of exposure until the start of IPT and then as exposed with IPT to the end of follow-up. However, enrolment into the IPT program continued after the enrolment period of the present study. Children enrolled on IPT after October 2007 were also counted as exposed on IPT even though they did not finish the treatment within the study period. Censoring the children enrolled on IPT after October 2007 at the time of IPT had little impact on the results. Some children who were present when the TB team visited the TB case house (figure 1) were enrolled on IPT even though they were not born or registered in the house before the TB case initiated treatment. According to the epidemiological definitions these children were not exposed and they have counted as unexposed with IPT. A separate analysis was conducted excluding these subjects.

An adjusted analysis was conducted including possible confounders related to child mortality: gender, ethnicity, district, socio-economic status, schooling of the mother, child crowding (<5 years) and crowding among older individuals (>5 years). A score for socio-economic status was calculated adding house indicators (yes=1; no/missing=0): corrugated iron roof, electricity, television and in-door toilet. A separate "Missing" category was constructed when information was missing on all four variables. Crowding was defined as the number of individuals in the house of the TB case on January 1, 2007, the mid-point of the examined period. Crowding was included in the analysis as a linear predictor.

It was further examined whether differential mortality not related to TB exposure may have existed in the exposed houses. Mortality was compared between children living in the house of the adult TB case three years before TB exposure began and children living in the remaining houses. As for the main study[19], the comparison was made over a 3-year period from July 2002 to June 2005. The period was chosen not to overlap the study period.

Results:

Index TB cases and included children

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from "case houses with no inclusion" and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

TB among exposed children

One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical and chest X-ray findings. One of these children tested HIV-positive and was enrolled in an antiretroviral (ARV) programme.

TB exposure and mortality

Two children died during IPT. For a 6-month old boy, hospital records stated the cause of death as severe malaria and anaemia. The mother of a 2-year old girl, who died two months after the initiation of the IPT program, reported that the child had had diarrhoea, cough and fever prior to death. Antibiotics and other medications had been prescribed. The research clinician had requested a chest X-ray, but the result was never received. No further IPT children died during the follow-up period.

In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT contributed with 1023 PYO and the controls contributed with 30713 PYO.

Though not statistically significant, the exposed children receiving IPT had a lower mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-1.2) (Table 1). This estimate changed little when controlled for background factors (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and some exposed children did not receive IPT (Figure 1). In this group of TB-exposed children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7) (Table 1).

There were 68 children on IPT who were not formally exposed to TB because the child was born or registered after exposure occurred in the house. Excluding these from the IPT group we observed one death from 642 person years of observation giving a MRR of 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

Comparison of mortality among TB-exposed children in the absence and presence of IPT

We previously found that the excess mortality after exposure to TB only started 6 months after exposure[19]. We made a similar analysis for the period 2005-2008; table 3 shows the comparison of exposed children without IPT and unexposed community control children, stratified by time since exposure and age at exposure.

Restricted to the period after 6 months, the mortality rate relative to community controls in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

It was furthermore examined whether the effect in the exposed house was caused by lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among children in the houses which later had TB cases was the same as the mortality in the control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

~~The purpose of the present study was to assess whether the excess mortality associated with TB exposure at home could be reduced by implementing an IPT programme.~~ In a previous study in the same community, exposure to TB at home was associated with an MRR of 1.66 (1.2-2.3) compared to community control children[19]. The present study assessed whether the excess mortality could be reduced by implementing an IPT programme. ~~In the present study b~~Both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction, p=0.01 and p=0.004).

It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

Discussion

In the present study we have shown an ~~considerable~~ impact of the IPT programme on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed community control children. The mortality rate ratio

was inverted in 2005-2008 with markedly (though not significantly) lower mortality among exposed children on IPT compared to the community control children. ~~This excess mortality was completely removed in the cohort of children receiving IPT from 2005-2008~~. It should be emphasized that comparing data from different time-periods is not straight-forward, as the conditions have changed in many ways that cannot be completely deduced, and the results can be biased. In our situation, the child mortality has dropped markedly between these two time-periods. However, the excess mortality from 1996-1998 has changed to a marked trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child mortality, and our data suggest that this is partly due to the introduction of IPT.

Unexpected observations

Mortality in the study area declined dramatically between the two study periods and mortality declined more than expected among both exposed children receiving IPT and exposed children not receiving IPT. Based on the experience from the 1996-1998 period, TB exposure at home in the absence of IPT should have been associated with a 19/1000 person-years excess mortality.

Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa. However, despite the recent HIV epidemic, mortality rates have decreased drastically over the last decade following the same pattern observed in the other sub-Saharan African countries[24-26]. The reasons for the mortality decline are not fully understood but systematic annual vitamin A campaigns and the marked decrease in malaria incidence are likely to have contributed.

The strong trend toward less mortality among IPT-treated children could possibly be linked to increased attention to these children including easier access to other forms of treatment and more attention from the parents. However, this would be unlikely to explain why mortality also declined more than expected among the children who did not receive IPT. This may suggest that all TB-associated mortality is not directly due to clinical TB disease, but may be due to interactions with other infections. If the incidence or severity of these other infections goes down, as has happened in Bissau, the mortality associated with TB exposure would also decline.

Interpretation and consistency with previous studies

Children exposed to TB at home had excess mortality from around 6 months after exposure in the present study as well as in the previous study from 1996-1998. In both studies, we showed that the TB houses 3 years earlier had exactly the same mortality as community controls. It therefore seems unlikely that general social conditions in houses with TB cases explain the higher mortality of TB-exposed children. Furthermore, children enrolled on IPT in the IPT cohort (compared with community controls) had significantly lower mortality than the TB exposed children not receiving IPT in the pre-IPT cohort (compared with community controls). Hence, our results suggest that use of isoniazid plays an important role in decreasing mortality in children exposed to TB. Studies conducted in South Africa and Zambia have reported isoniazid to be highly effective in reducing the mortality and incidence of TB in HIV-infected children and adults living in an area with a high prevalence of TB[14;27;28]. Our findings support those

studies, but also observations made by Dr. Lincoln in the early 1950s showing that isoniazid chemotherapy reduced the case fatality from primary TB among children[29].

Implications and conclusions

In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was reduced in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance. When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. Furthermore, in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT.

. All our data indicate that IPT should be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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Author's contributions

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

Conflict of interest: There are no competing or conflicting interests.

Table 1: The effect of exposure on mortality according to age

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR[#1] IPT/Unexposed	MRR[#2] IPT/Unexposed	MRR[#1] No IPT/Unexposed	MRR[#2] No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

[*] Include unexposed children on IPT

[#1] Mortality Rate Ratio from a model with age as underlying time

[#2] Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.

	Age at exposure (Months)			
Months since exposure	0-11	12-35	36-59	Total
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	**2005-2008**
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

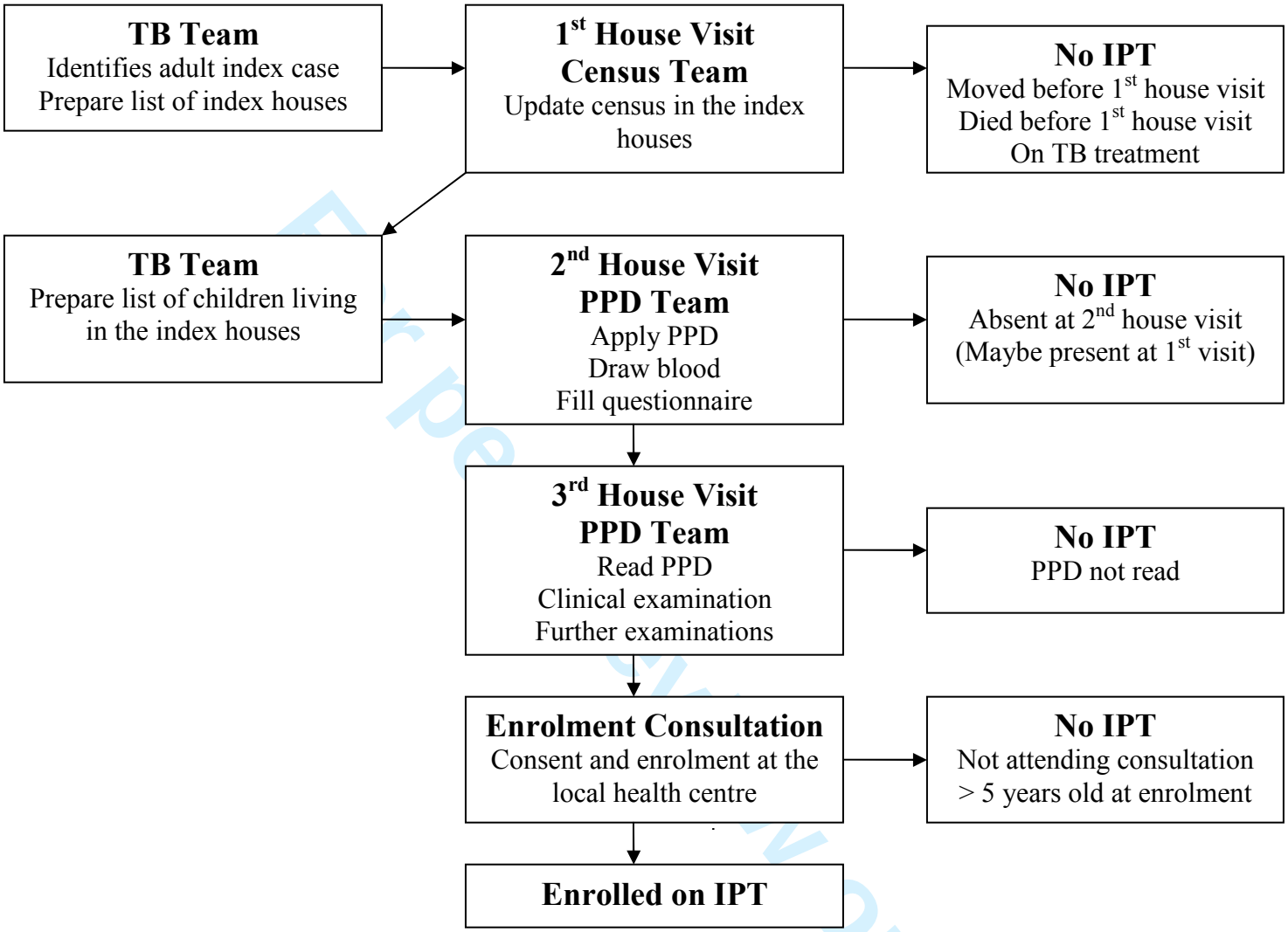

Figure 2: Flow chart of inclusion

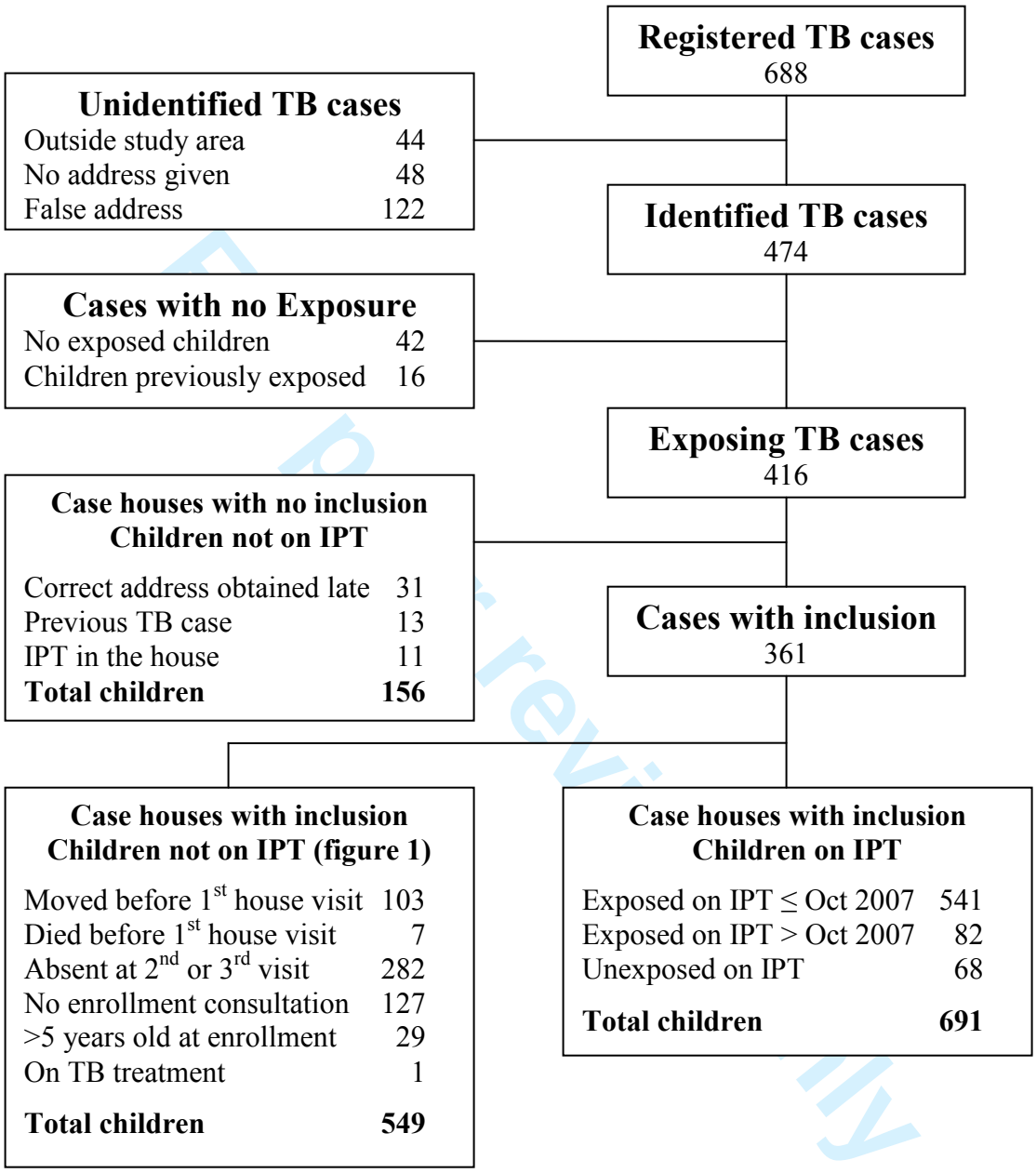

Figure 3: Person years of observation (PYO)

Children not on IPT		Children on IPT	
Exposed		**Exposed**	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		**Unexposed**	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

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ABSTRACT

Background and objective: In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

Setting: This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

Participants: All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT program.

Main outcome measures (end points): The all-cause mortality rate ratio (MRR) between exposed children on IPT, exposed without IPT and unexposed community control children.

Results: A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT program. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction, p=0.01).

Conclusion: In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control children, the mortality among TB-exposed children on IPT in 2005-2008 was significantly lower than the mortality among TB-exposed children not on IPT in 1996-1998.

Article summary

This article focuses on:

- Impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home: comparing 1) exposed children who received IPT to unexposed children and 2) exposed children who did not received IPT to unexposed children
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study. Due to the current World Health Organisation (WHO) recommendation it was decided not to conduct a randomised study, hence, there are a number of theoretical biases. Furthermore, the previous study was conducted 10 years earlier than the present study and many things changed in the meantime. Another limitation was that the children were not HIV tested, it might bias the results if there were more HIV-infected children in the IPT group compared to the no IPT group. However, we would then expect higher mortality in the no IPT group compared with the community controls in the present IPT cohort.

Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic[1;2]. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality[2]. In 2011 TB incidence among children was estimate at 490,000, equivalent to about 6% of the total number 8.7 million incident cases [3], and the proportion of children in the high-burden countries is estimated to be higher[4;5]. Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home[6] and reliable medical information on causes of death is therefore lacking[7]. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries[7;8]. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas[9].

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages[10]. After all this time, isoniazid continues to be the drug of choice[11], and WHO recommends that all TB contacts under the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded

that there was not enough evidence for general recommendation of IPT for HIV-infected children[12]. The use of isoniazid in low income countries is limited by difficulties of ruling out TB disease before initiation[13], liver toxicity[14-16], and poor adherence[17]. IPT has been shown to be effective in recent tuberculin skin test (TST) converters and recent contacts of identified cases of TB disease[17;18]. In one study of IPT in HIV-infected subjects, a 20% reduction of mortality was found in those with positive TST[18]. The effect of such preventive therapy in children, however, is not well established.

The present study examined the impact of IPT on mortality in children less than 5 years of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous study in the same community, we found that exposure to TB at home was associated with 66% excess mortality compared to community control children not exposed to TB at home[19]. The aim of the present study was to compare mortality between exposed children on IPT and community control children, and to compare this relative mortality to the previously observed excess mortality.

Materials and methods

Setting

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau, where the prevalence of HIV1 and HIV2 among adults was 4.6% and 4.4%, respectively[20]. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. Information about children older than 3 years of age and adults is obtained from general censuses carried out approximately every 3rd year. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years[21].

Due to difficulties in obtaining specific causes of deaths in our setting the all-cause mortality was used as the main outcome measurement of the effect of IPT.
The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

Houses and household contacts

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families

or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

Recruitment of participants and patients

Identification of adult TB index cases

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital ("Hospital Raoul Follereau"), has identified adult (≥15 years) intrathoracic TB cases using passive and active case finding[21]. As previously described in more detail[21], an intrathoracic TB case was defined as an adult with symptoms of TB with sputum smear microscopy positive or negative for acid-fast bacilli, presenting abnormalities in the chest X-ray (CXR) with no improvement on treatment with broad spectrum antibiotics for two weeks.

Enrolment in the IPT cohort

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score[22]. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment

of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and CXR. Broad spectrum antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically after 2 weeks of broad spectrum antibiotics, and without other explanatory disease were given a full TB treatment regimen according to the national protocol. Antiretroviral treatment was not available at the time of the study and HIV testing was therefore not generally performed. It was only performed when the investigation suggested TB. Children who developed signs and symptoms suggestive of TB disease while on IPT were evaluated and treated in a similar way.

Children with TB disease, those who did not give consent, who were absent from first, second or third visit or at enrolment consultation were excluded from the IPT cohort.

There were several steps in the enrolment procedure as depicted in Figure 1. Once an adult TB case from the study area was identified a project assistant went to the patient's house to update the census for the families living in the house, and socio-economic and demographic information was noted on the questionnaire. Following the census-update, a field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house was visited by the nurse who read the TST and referred potential TB cases for further clinical examination. Children without TB disease were eligible for enrolment in the IPT cohort regardless of the TST result and were invited to attend the enrolment visit at the local health centre. Eligible children who did not show up at inclusion were traced again. If not found they were considered absent, but still followed up using basic census information. Due to limited time frame, logistic reasons and limited funding they were not included later.

For children enrolled in the IPT program, isoniazid tablets were administered at 5 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children receiving >100 mg of isoniazid [23]. The medicine was provided at the house every two weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT. The follow-up visits at 1 and 7 months were performed by the research clinician at the local health centre and at 4 and 9 months by a field assistant at the child´s home. Evaluation at follow-up visits included questions about side effects and a physical assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study routines are described in detail elsewhere[24]. The initially intended IPT enrolment period was September 2005 to October 2007 with 9 months of follow-up to June 2008. However, children continued to be enrolled on IPT until the end of the study period in June 2008.

Pre-IPT cohort

As previously described; children less than 5 years of age living in the same house as an adult index TB case at the time of initiation of treatment during the period May 1996 to July 1998 were retrieved from the BHP register[19]. To assess the impact of TB exposure at home in the absence of IPT, their mortality was compared with the mortality of children living in the study area who had not been exposed to TB at home, during the same period.

Groups in the study

In the Pre-IPT cohort we had two groups: TB-exposed children and community control children. In the IPT cohort we intended also to have two groups: TB-exposed children on IPT and community control children. As we failed to include all exposed children in the IPT program, a third group arose: TB-exposed children not on IPT.

Effect-size calculation

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

Ethical approval

The legal guardians (parents) or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before enrolment. The study protocol was approved by the Guinea-Bissau National Research Coordination and Ethics Committee.

Statistical analysis

Data regarding adult TB cases were obtained from the general TB identification system in the study area while demographic information was taken from the basic surveillance system of the BHP. Statistical analyses were conducted in STATA version 10.

Similar to the analysis of the impact of TB exposure in the absence of IPT, the average delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months. Hence, children registered in the same house as an adult index TB case 0-3 months before treatment were considered exposed. The effect of exposure on mortality was evaluated by rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in Guinea-Bissau is very high. To be exposed a child had to be born and registered at the time of exposure. Consequently only few children were exposed before 3 months of age. Therefore we have chosen to commence the analyses at 3 months of age.

IPT treatment was initiated in September 2005. To allow exposure time before IPT, follow-up started in July 2005. IPT enrolment for the present study ended in October 2007 with treatment ending in June 2008. Thus, the study period for the present study is

July 2005 to June 2008. Exposed children counted as unexposed controls until the time of exposure. Exposed children never receiving IPT counted as exposed without IPT from the start of exposure to the end of follow-up. Children subsequently enrolled on IPT counted as exposed without IPT from the start of exposure until the start of IPT and then as exposed with IPT to the end of follow-up. However, enrolment into the IPT program continued after the enrolment period of the present study. Children enrolled on IPT after October 2007 were also counted as exposed on IPT even though they did not finish the treatment within the study period. Censoring the children enrolled on IPT after October 2007 at the time of IPT had little impact on the results. Some children who were present when the TB team visited the TB case house (figure 1) were enrolled on IPT even though they were not born or registered in the house before the TB case initiated treatment. According to the epidemiological definitions these children were not exposed and they have counted as unexposed with IPT. A separate analysis was conducted excluding these subjects.

An adjusted analysis was conducted including possible confounders related to child mortality: gender, ethnicity, district, socio-economic status, schooling of the mother, child crowding (<5 years) and crowding among older individuals (>5 years). A score for socio-economic status was calculated adding house indicators (yes=1; no/missing=0): corrugated iron roof, electricity, television and in-door toilet. A separate "Missing" category was constructed when information was missing on all four variables. Crowding was defined as the number of individuals in the house of the TB case on January 1, 2007, the mid-point of the examined period. Crowding was included in the analysis as a linear predictor.

It was further examined whether differential mortality not related to TB exposure may have existed in the exposed houses. Mortality was compared between children living in the house of the adult TB case three years before TB exposure began and children living in the remaining houses. As for the main study[19], the comparison was made over a 3-year period from July 2002 to June 2005. The period was chosen not to overlap the study period.

Results:

Index TB cases and included children

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from "case houses with no inclusion" and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

TB among exposed children

One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical and CXR findings. One of these children was HIV-infected and was enrolled in an antiretroviral (ARV) program.

TB exposure and mortality

Two children died during IPT. For a 6-month old boy, hospital records stated the cause of death as severe malaria and anaemia. The mother of a 2-year old girl, who died two months after the initiation of the IPT program, reported that the child had had diarrhoea, cough and fever prior to death. Antibiotics and other medications had been prescribed. The research clinician had requested a CXR, but the result was never received. No further IPT children died during the follow-up period.

In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT contributed with 1023 PYO and the controls contributed with 30713 PYO.

Though not statistically significant, the exposed children receiving IPT had a lower mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-1.2) (Table 1). This estimate changed little when controlled for background factors (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and some exposed children did not receive IPT (Figure 1). In this group of TB-exposed children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7) (Table 1).

There were 68 children on IPT who were not formally exposed to TB because the child was born or registered after exposure occurred in the house. Excluding these from the IPT group we observed one death from 642 person years of observation giving a MRR of 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

Comparison of mortality among TB-exposed children in the absence and presence of IPT

We previously found that the excess mortality after exposure to TB only started 6 months after exposure[19]. We made a similar analysis for the period 2005-2008; table 3 shows the comparison of exposed children without IPT and unexposed community control children, stratified by time since exposure and age at exposure.

Restricted to the period after 6 months, the mortality rate relative to community controls in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

It was furthermore examined whether the effect in the exposed house was caused by lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among children in the houses which later had TB cases was the same as the mortality in the control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

In a previous study in the same community, exposure to TB at home was associated with an MRR of 1.66 (1.2-2.3) compared to community control children[19]. The present study assessed whether the excess mortality could be reduced by implementing an IPT program. Both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction, p=0.01 and p=0.004). It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

Discussion

In the present study we have shown an impact of the IPT program on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed community control children. The mortality rate ratio was inverted in 2005-2008 with markedly (though not significantly) lower mortality among exposed children on IPT compared to the community control children. It should be emphasized that comparing data from different time-periods is not straight-forward, as the conditions

have changed in many ways that cannot be completely deduced, and the results can be biased. In our situation, the child mortality has dropped markedly between these two time-periods. However, the excess mortality from 1996-1998 has changed to a marked trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child mortality, and our data suggest that this is partly due to the introduction of IPT.

Unexpected observations

Mortality in the study area declined dramatically between the two study periods and mortality declined more than expected among both exposed children receiving IPT and exposed children not receiving IPT. Based on the experience from the 1996-1998 period, TB exposure at home in the absence of IPT should have been associated with a 19/1000 person-years excess mortality.

Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa. However, despite the recent HIV epidemic, mortality rates have decreased drastically over the last decade following the same pattern observed in the other sub-Saharan African countries[25-27]. The reasons for the mortality decline are not fully understood but systematic annual vitamin A campaigns and the marked decrease in malaria incidence are likely to have contributed.

The strong trend toward less mortality among IPT-treated children could possibly be linked to increased attention to these children including easier access to other forms of treatment and more attention from the parents. However, this would be unlikely to explain why mortality also declined more than expected among the children who did not receive IPT. This may suggest that all TB-associated mortality is not directly due to

clinical TB disease, but may be due to interactions with other infections. If the incidence or severity of these other infections goes down, as has happened in Bissau, the mortality associated with TB exposure would also decline.

There may be a slight trend towards better outcome in the contacts receiving IPT, although not significant. Travelling or absence was the main reason why exposed children were not enrolled in the IPT program. These children may therefore have had little contract with the TB case. Another possible reason may be selection bias so that the group of children going through 9 months of treatment had mothers who were better able to take care of them (although no differences in education level or socioeconomic index was seen), or simply that the children at home at the time of inclusion were not exposed to the possible dangers of travelling.

Table 3 showed no difference in overall mortality between unexposed controls and exposed who did not get IPT, but did indeed show an effect among the oldest children with +12 months since exposure. With the limited number of events available we were not able to show a overall difference in mortality which would be expected. Yet despite the limited number of events, it was possible to show a significant effect of exposure in a subgroup of the un-treated children, namely among those most likely affected, ie the mobile children + 3 years of age with the longest observation time since exposure.

Interpretation and consistency with previous studies

Children exposed to TB at home had excess mortality from around 6 months after exposure in the present study as well as in the previous study from 1996-1998. In both studies, we showed that the TB houses 3 years earlier had exactly the same mortality as community controls. It therefore seems unlikely that general social conditions in houses

with TB cases explain the higher mortality of TB-exposed children. Furthermore, children enrolled on IPT in the IPT cohort (compared with community controls) had significantly lower mortality than the TB exposed children not receiving IPT in the pre-IPT cohort (compared with community controls). Hence, our results suggest that use of isoniazid plays an important role in decreasing mortality in children exposed to TB. Studies conducted in South Africa and Zambia have reported isoniazid to be highly effective in reducing the mortality and incidence of TB in HIV-infected children and adults living in an area with a high prevalence of TB[14;28;29]. Our findings support those studies, but also observations made by Dr. Lincoln in the early 1950s showing that isoniazid chemotherapy reduced the case fatality from primary TB among children[30].

Implications and conclusions

In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was reduced in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance. When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. Furthermore, in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT. All our data indicate that IPT should be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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Author's contributions

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

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Data Sharing: No additional unpublished data from the study

Table 1: The effect of exposure on mortality according to age

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR[#1] IPT/Unexposed	MRR[#2] IPT/Unexposed	MRR[#1] No IPT/Unexposed	MRR[#2] No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

* Include unexposed children on IPT

[#1] Mortality Rate Ratio from a model with age as underlying time

[#2] Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.

	Age at exposure (Months)			
Months since exposure	0-11	12-35	36-59	Total
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	**2005-2008**
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

FIGURE LEGENDS

Figure 1: Illustration of the inclusion process

Figure 2: Flow chart of inclusion

Figure 3: Person years of observation (PYO)

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Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

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ABSTRACT

Background and objective: In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

Setting: This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

Participants: All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT program~~me~~.

Main outcome measures (end points): The all-cause mortality rate ratio (MRR) between exposed children on IPT, exposed without IPT and unexposed community control children.

Results: A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT program~~me~~. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction, p=0.01).

Conclusion: In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control children, the mortality among TB-exposed children on IPT in 2005-2008 was significantly lower than the mortality among TB-exposed children not on IPT in 1996-1998.

Article summary

This article focuses on:

- Impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home: comparing 1) exposed children who received IPT to unexposed children and 2) exposed children who did not received IPT to unexposed children
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study. Due to the current World Health Organisation (WHO) recommendation it was decided not to conduct a randomised study, hence, there are a number of theoretical biases. Furthermore, the previous study was conducted 10 years earlier than the present study and many things changed in the meantime. Another limitation was that the children were not HIV tested, it might bias the results if there were more HIV--infected children in the IPT group compared to the no IPT group. However, we would then expect higher mortality in the no IPT group compared with~~to~~ the community controls in the present IPT cohort.

Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic[1;2]. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality[2]. In 2011 TB incidence among children was estimate at 490,000, equivalent to about 6% of the total number 8.7 million incident cases ~~Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were children~~[3], and the proportion of children in the high-burden countries is estimated to be higher[4;5].

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home[6] and reliable medical information on causes of death is therefore lacking[7]. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries[7;8]. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas[9].

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages[10]. After all this time, isoniazid continues to be the drug of choice[11], and WHO recommends that all TB contacts under

the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded that there was not enough evidence for general recommendation of IPT for HIV--infected children[12]. The use of isoniazid in low income countries is limited by difficulties of ruling out TB disease before initiation[13], liver toxicity[14-16], and poor adherence[17]. ~~The use of isoniazid in low income countries is, however, limited by several circumstances: difficulties of ruling out TB disease in children, mainly those infected with HIV, before initiation of IPT[13], liver toxicity[14-16], and poor adherence[17]. These circumstances may limit the widespread use of IPT in the resource-constrained settings, where provision of TB care often falls short of internationally recommended standards[13].~~ IPT has been shown to be effective in recent tuberculin skin test (TST) converters and recent contacts of identified cases of TB disease[17;18]. In one study of IPT in HIV-~~positive~~ infected subjects, a 20% reduction of mortality was found in those with positive TST[18]. The effect of such preventive therapy in children, however, is not well established.

The present study examined the impact of IPT on mortality in children less than 5 years of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous study in the same community, we found that exposure to TB at home was associated with 66% excess mortality compared to community control children not exposed to TB at home[19]. The aim of the present study was to compare mortality between exposed children on IPT and community control children, and to compare this relative mortality to the previously observed excess mortality.

Materials and methods

Setting

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau, where the prevalence of HIV1 and HIV2 among adults was 4.6% and 4.4%, respectively[20]. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. Information about children older than 3 years of age and adults is obtained from general censuses carried out approximately every 3rd year. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years[21~~0~~].

Due to difficulties in obtaining specific causes of deaths in our setting the ~~all-cause~~all-cause mortality was used as the main outcome measurement of the effect of IPT. The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

Houses and household contacts

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families

or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

Recruitment of participants and patients

Identification of adult TB index cases

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital ("Hospital Raoul Follereau"), has identified adult (≥15 years) intrathoracic TB cases using passive and active case finding[2~~10~~]. As previously described in more detail[2~~10~~], an intrathoracic TB case was defined as an adult with symptoms of TB with sputum smear microscopy positive or negative for acid-fast bacilli, presenting abnormalities in the chest X-ray (CXR) with no improvement on treatment with broad spectrum antibiotics for two weeks.~~As previously described in more detail[20], a TB case was defined as an adult with symptoms of TB disease with sputum smear positive or negative for AFB, presenting abnormalities in CX ray with no improvement under treatment with broad spectrum antibiotics for two weeks.~~

Enrolment in the IPT cohort

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score[22~~21~~]. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and CXR~~chest x ray~~. Broad spectrum antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically after 2 weeks of broad spectrum antibiotics, and without other explanatory disease were given a full TB treatment regimen according to the national protocol. Antiretroviral treatment was not available at the time of the study and HIV testing was therefore not generally performed. It was only performed when the investigation suggested TB. Children who developed signs and symptoms suggestive of TB disease while on IPT were evaluated and treated in a similar way.

Children with TB disease, those who did not give consent, who were absent from first, second or third visit or at enrolment consultation were excluded from the IPT cohort.

There were several steps in the enrolment procedure as depicted in Figure 1. Once an adult TB case from the study area was identified a project assistant went to the patient's house to update the census for the families living in the house, and socio-economic and demographic information was noted on the questionnaire. Following the census-update, a field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house was visited by the nurse who read the TST and referred potential TB cases for further clinical examination. Children without TB disease were eligible for enrolment in the IPT cohort regardless of the TST result and were invited to attend the enrolment visit at the local health centre. Eligible children who did not show up at inclusion were traced again.

If not found they were considered absent, but still followed up using basic census information. Due to limited time frame, logistic reasons and limited funding they were not included later.

For children enrolled in the IPT program~~me~~, isoniazid tablets were administered at 5 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children receiving >100 mg of isoniazid [2~~32~~]. The medicine was provided at the house every two weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT. The follow-up visits at 1 and 7 months were performed by the research clinician at the local health centre and at 4 and 9 months by a field assistant at the child´s home. Evaluation at follow-up visits included questions about side effects and a physical assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study routines are described in detail elsewhere[2~~43~~]. The initially intended IPT enrolment period was September 2005 to October 2007 with 9 months of follow-up to June 2008. However, children continued to be enrolled on IPT until the end of the study period in June 2008.

Pre-IPT cohort

As previously described; children less than 5 years of age living in the same house as an adult index TB case at the time of initiation of treatment during the period May 1996 to July 1998 were retrieved from the BHP register[19]. To assess the impact of TB exposure at home in the absence of IPT, their mortality was compared with the mortality of children living in the study area who had not been exposed to TB at home, during the same period.

Groups in the study

In the Pre-IPT cohort we had two groups: TB-exposed children and community control children. In the IPT cohort we intended also to have two groups: TB-exposed children on IPT and community control children. As we failed to include all exposed children in the IPT program~~me~~, a third group arose: TB-exposed children not on IPT.

Effect-size calculation

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

Ethical approval

The legal guardians (parents) or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before enrolment. The study protocol was approved by the Guinea-Bissau National Research Coordination and Ethics Committee.

Statistical analysis

Data regarding adult TB cases were obtained from the general TB identification system in the study area while demographic information was taken from the basic surveillance system of the BHP. Statistical analyses were conducted in STATA version 10.

Similar to the analysis of the impact of TB exposure in the absence of IPT, the average delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months. Hence, children registered in the same house as an adult index TB case 0-3 months before treatment were considered exposed. The effect of exposure on mortality was evaluated by rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in Guinea-Bissau is very high. To be exposed a child had to be born and registered at the time of exposure. Consequently only few children were exposed before 3 months of age. Therefore we have chosen to commence the analyses at 3 months of age.

IPT treatment was initiated in September 2005. To allow exposure time before IPT, follow-up started in July 2005. IPT enrolment for the present study ended in October 2007 with treatment ending in June 2008. Thus, the study period for the present study is July 2005 to June 2008. Exposed children counted as unexposed controls until the time of exposure. Exposed children never receiving IPT counted as exposed without IPT from the start of exposure to the end of follow-up. Children subsequently enrolled on IPT counted as exposed without IPT from the start of exposure until the start of IPT and then as exposed with IPT to the end of follow-up. However, enrolment into the IPT program continued after the enrolment period of the present study. Children enrolled on IPT after October 2007 were also counted as exposed on IPT even though they did not finish the treatment within the study period. Censoring the children enrolled on IPT after October 2007 at the time of IPT had little impact on the results. Some children who were present when the TB team visited the TB case house (figure 1) were enrolled on IPT even though they were not born or registered in the house before the TB case initiated treatment. According to the epidemiological definitions these children were not exposed and they have counted as unexposed with IPT. A separate analysis was conducted excluding these subjects.

An adjusted analysis was conducted including possible confounders related to child mortality: gender, ethnicity, district, socio-economic status, schooling of the mother, child crowding (<5 years) and crowding among older individuals (>5 years). A score for socio-economic status was calculated adding house indicators (yes=1; no/missing=0): corrugated iron roof, electricity, television and in-door toilet. A separate "Missing" category was constructed when information was missing on all four variables. Crowding

was defined as the number of individuals in the house of the TB case on January 1, 2007, the mid-point of the examined period. Crowding was included in the analysis as a linear predictor.

It was further examined whether differential mortality not related to TB exposure may have existed in the exposed houses. Mortality was compared between children living in the house of the adult TB case three years before TB exposure began and children living in the remaining houses. As for the main study[19], the comparison was made over a 3-year period from July 2002 to June 2005. The period was chosen not to overlap the study period.

Results:

Index TB cases and included children

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from "case houses with no inclusion" and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

TB among exposed children

One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical and ~~chest X ray~~CXR findings. One of these children ~~tested~~ was HIV-~~positive~~ infected and was enrolled in an antiretroviral (ARV) program~~me~~.

TB exposure and mortality

Two children died during IPT. For a 6-month old boy, hospital records stated the cause of death as severe malaria and anaemia. The mother of a 2-year old girl, who died two months after the initiation of the IPT program, reported that the child had had diarrhoea, cough and fever prior to death. Antibiotics and other medications had been prescribed. The research clinician had requested a ~~chest X ray~~CXR, but the result was never received. No further IPT children died during the follow-up period.

In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT contributed with 1023 PYO and the controls contributed with 30713 PYO.

Though not statistically significant, the exposed children receiving IPT had a lower mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-1.2) (Table 1). This estimate changed little when controlled for background factors (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and some exposed children did not receive IPT (Figure 1). In this group of TB-exposed children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7) (Table 1).

There were 68 children on IPT who were not formally exposed to TB because the child was born or registered after exposure occurred in the house. Excluding these from the IPT group we observed one death from 642 person years of observation giving a MRR of 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

Comparison of mortality among TB-exposed children in the absence and presence of IPT

We previously found that the excess mortality after exposure to TB only started 6 months after exposure[19]. We made a similar analysis for the period 2005-2008; table 3 shows the comparison of exposed children without IPT and unexposed community control children, stratified by time since exposure and age at exposure.

Restricted to the period after 6 months, the mortality rate relative to community controls in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

It was furthermore examined whether the effect in the exposed house was caused by lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among children in the houses which later had TB cases was the same as the mortality in the control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

In a previous study in the same community, exposure to TB at home was associated with an MRR of 1.66 (1.2-2.3) compared to community control children[19]. The present study assessed whether the excess mortality could be reduced by implementing an IPT programme. Both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction, p=0.01 and p=0.004).

It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

Discussion

In the present study we have shown an impact of the IPT programme on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed community control children. The mortality rate ratio was inverted in 2005-2008 with markedly (though not significantly) lower mortality among exposed children on IPT compared to the community control children. It should be emphasized

that comparing data from different time-periods is not straight-forward, as the conditions have changed in many ways that cannot be completely deduced, and the results can be biased. In our situation, the child mortality has dropped markedly between these two time-periods. However, the excess mortality from 1996-1998 has changed to a marked trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child mortality, and our data suggest that this is partly due to the introduction of IPT.

Unexpected observations

Mortality in the study area declined dramatically between the two study periods and mortality declined more than expected among both exposed children receiving IPT and exposed children not receiving IPT. Based on the experience from the 1996-1998 period, TB exposure at home in the absence of IPT should have been associated with a 19/1000 person-years excess mortality.

Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa. However, despite the recent HIV epidemic, mortality rates have decreased drastically over the last decade following the same pattern observed in the other sub-Saharan African countries[25,26]. The reasons for the mortality decline are not fully understood but systematic annual vitamin A campaigns and the marked decrease in malaria incidence are likely to have contributed.

The strong trend toward less mortality among IPT-treated children could possibly be linked to increased attention to these children including easier access to other forms of treatment and more attention from the parents. However, this would be unlikely to explain why mortality also declined more than expected among the children who did not

receive IPT. This may suggest that all TB-associated mortality is not directly due to clinical TB disease, but may be due to interactions with other infections. If the incidence or severity of these other infections goes down, as has happened in Bissau, the mortality associated with TB exposure would also decline.

There may be a slight trend towards better outcome in the contacts receiving IPT, although not significant. Travelling or absence was the main reason why exposed children were not enrolled in the IPT program. These children may therefore have had little contract with the TB case. Another possible reason may be selection bias so that the group of children going through 9 months of treatment had mothers who were better able to take care of them (although no differences in education level or socioeconomic index was seen), or simply that the children at home at the time of inclusion were not exposed to the possible dangers of travelling.

Table 3 showed no difference in overall mortality between unexposed controls and exposed who did not get IPT, but did indeed show an effect among the oldest children with +12 months since exposure. With the limited number of events available we were not able to show a overall difference in mortality which would be expected. Yet despite the limited number of events, it was possible to show a significant effect of exposure in a subgroup of the un-treated children, namely among those most likely affected, ie the mobile children + 3 years of age with the longest observation time since exposure.

Interpretation and consistency with previous studies

Children exposed to TB at home had excess mortality from around 6 months after exposure in the present study as well as in the previous study from 1996-1998. In both

studies, we showed that the TB houses 3 years earlier had exactly the same mortality as community controls. It therefore seems unlikely that general social conditions in houses with TB cases explain the higher mortality of TB-exposed children. Furthermore, children enrolled on IPT in the IPT cohort (compared with community controls) had significantly lower mortality than the TB exposed children not receiving IPT in the pre-IPT cohort (compared with community controls). Hence, our results suggest that use of isoniazid plays an important role in decreasing mortality in children exposed to TB. Studies conducted in South Africa and Zambia have reported isoniazid to be highly effective in reducing the mortality and incidence of TB in HIV-infected children and adults living in an area with a high prevalence of TB[14;28;29]. Our findings support those studies, but also observations made by Dr. Lincoln in the early 1950s showing that isoniazid chemotherapy reduced the case fatality from primary TB among children[30].

Implications and conclusions

In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was reduced in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance. When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. Furthermore, in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT. All our data indicate that IPT should

be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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Author's contributions

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

Conflict of interest: There are no competing or conflicting interests.

Table 1: The effect of exposure on mortality according to age

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR[#1] IPT/Unexposed	MRR[#2] IPT/Unexposed	MRR[#1] No IPT/Unexposed	MRR[#2] No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

* Include unexposed children on IPT

[#1] Mortality Rate Ratio from a model with age as underlying time

[#2] Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.

	Age at exposure (Months)			
Months since exposure	0-11	12-35	36-59	Total
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	**2005-2008**
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

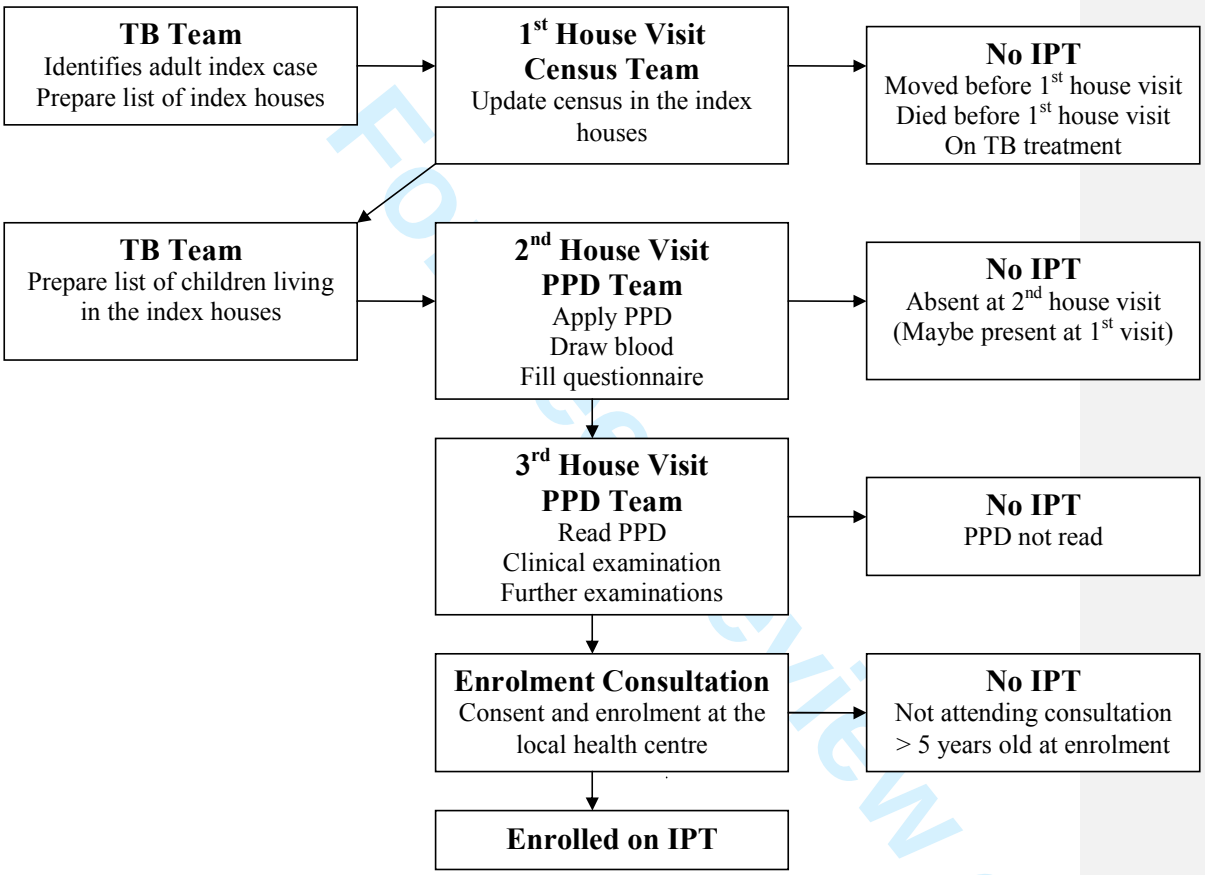

Figure 2: Flow chart of inclusion

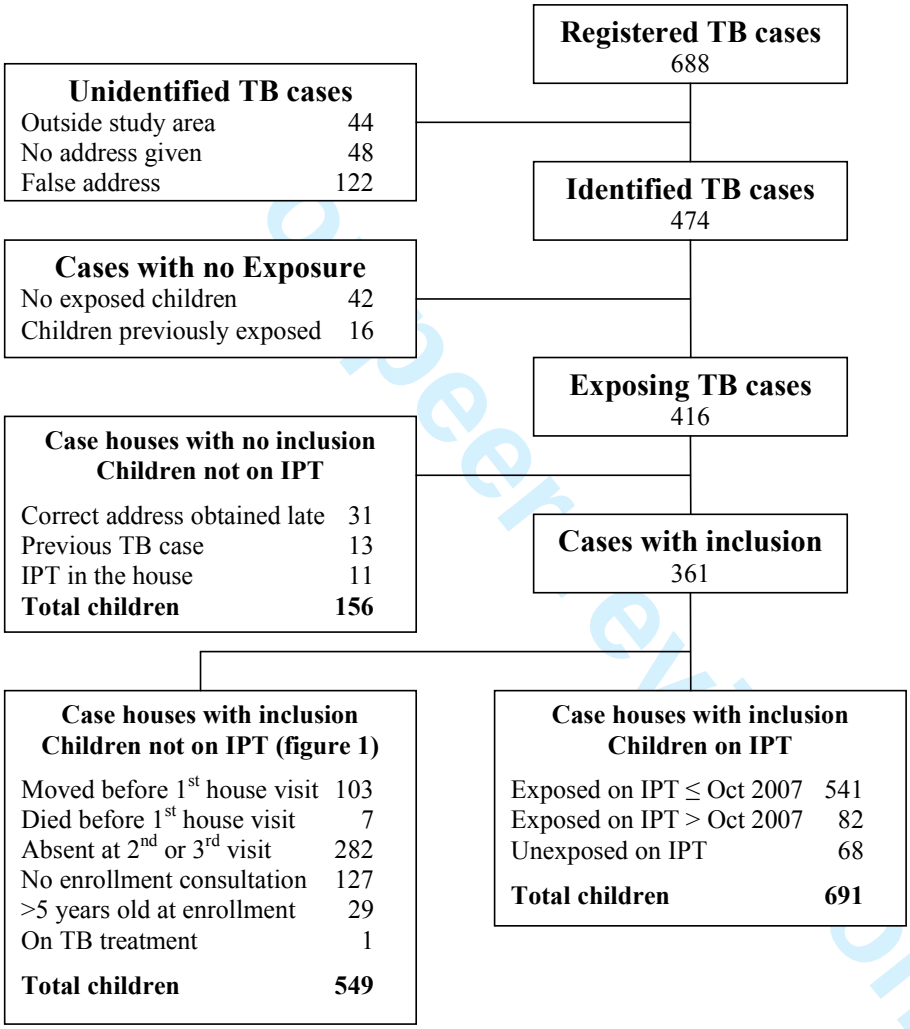

Figure 3: Person years of observation (PYO)

Children not on IPT		Children on IPT	
Exposed		**Exposed**	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		**Unexposed**	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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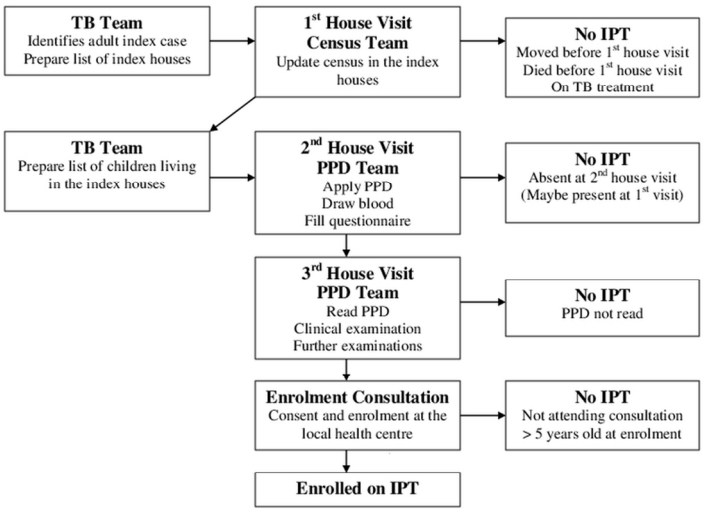

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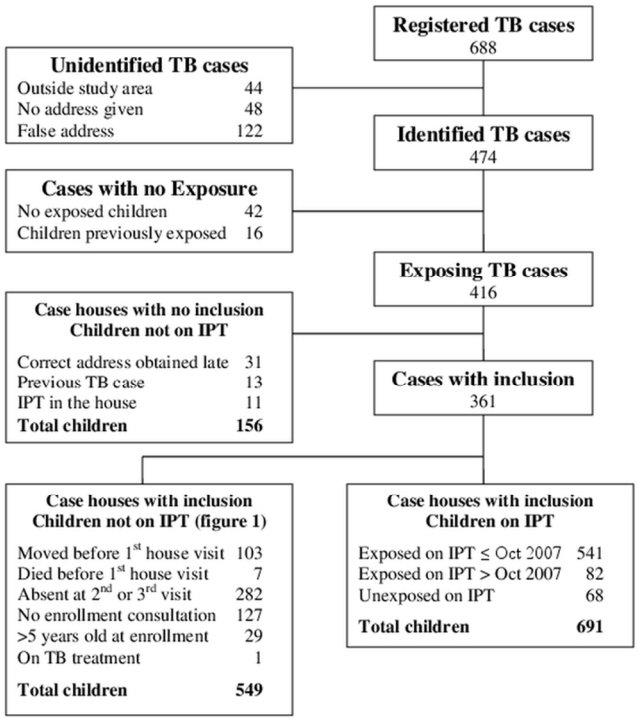

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