The incidence of tuberculosis in infants, Siaya District, Western Kenya

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Background | Infants are a target population for new tuberculosis (TB) vaccines. TB incidence estimates are needed to guide the design of trials. Objectives: To determine the TB incidence and cohort retention among young children using comprehensive diagnostic methods in a high burden area.

Methods | Infants 0-42 days were enrolled. Through four monthly follow up and unscheduled (sick) visits up to the age of two years, infants with Presumptive TB based on a history of contact, TB symptoms or pre-determined hospitalization criteria were admitted to a case verification ward. Two induced sputa and gastric aspirates were collected for culture and GeneXpert. Mantoux and HIV tests were done. Clinical management was based on the Keith Edwards Score (KE Score). Cases were classified into microbiologically confirmed or radiological**,** diagnosed by blinded expert assessment. Cox regression was used to identify risk factors for incident TB and study retention.

Results | Of 2900 infants enrolled, 927 (32%) developed presumptive TB. Sixty-nine TB cases were diagnosed (bacteriological and radiological). All TB incidence was 2/100 person-years of observation (pyo) (95% CI 1.65, 2.65). Nine were bacteriological cases, incidence 0.3/100 pyo. Radiological TB incidence was 1.82/100 pyo. Bacteriological TB was associated with infant HIV infection and higher KE scores. Completeness of 4 month vaccinations and HIV infection were positively associated with retention.

Conclusion | TB incidence was high. An all TB endpoint would require a sample size of a few thousand children, but tens of thousands, when limited to bacteriological TB.

INTRODUCTION

Pulmonary tuberculosis (TB) is an important public health problem worldwide. With 10 million new cases of TB and 1.3 million deaths in 2017 (1). In Kenya, a recent national prevalence survey, utilizing newer tools such as the GeneXpert showed higher rates than previously reported (2). The End TB Strategy of WHO emphasizes diagnosis and treatment of TB patients, as well as the need for research on methods to prevent TB, including new vaccines for high risk populations such as adolescents (3) and infants (4, 5). Childhood tuberculosis, while not easily transmissible, has higher morbidity and mortality risk (6, 7) for both HIV infected and uninfected individuals (8). It is also harder to accurately detect (7, 9). Young children are unable to expectorate adequately, and more invasive methods are required that include nasopharyngeal and gastric aspirates, or induced sputa. The necessary skills and infrastructure are hardly available in most primary care settings (10, 11). When sputum culture or GeneXpert are available, sensitivity is somewhat low as young children often have paucibacillary disease (7).

In many settings, clinical and radiological criteria form the backbone of diagnosis. Clinical criteria include a composite score chart (12, 13) ranking for history of contact or evidence of exposure to TB by Mantoux tests or their equivalent, protracted classical TB symptoms, failure to thrive with or without a suggestive chest radiograph. The criteria have poor specificity for TB in HIV infected infants who will tend to have prolonged cough, night sweats or weight loss due to other co-morbidities. (11, 13, 14). Also, children with respiratory illnesses frequently have multiple infections, chiefly bacterial, complicating the clinical picture (8, 11, 15). Chest Computed tomography (CT), the gold standard for detection of mediastinal lymphadenopathy, the radiological hallmark of primary tuberculosis (16), is unscalable, costly and associated with high radiation exposure. Chest radiographs (CXR) are more readily available in TB endemic settings. It has been noted that CXR readings have poor specificity among non-experts or clinicians with basic training (17) and classical diagnostic features are less frequently observed than among adults (18). Having a chest radiograph compatible with TB, doubled the odds of culture positivity among children (19). The CXR therefore seems a valuable addition to the paediatric TB detection armoury.

In most low and middle income countries where TB is endemic, extensive neonatal vaccination programs using Bacille Calmette-Guérin (BCG), have reportedly reduced the incidence of severe childhood TB including TB meningitis, and miliary TB (20) (21). Nevertheless, several trials for new infant TB vaccines have advanced in the last decade (22-24) in recognition of BCG's variable efficacy against pulmonary TB (25) and evidence that protection wanes (26). We sought to conduct a large cohort study, utilizing more comprehensive case finding and diagnostic methods to determine TB incidence among infants in Western Kenya, in order to inform sample size calculations, mortality patterns and estimates ahead of a trial of new TB vaccine candidates. A large portion of the study area is covered by a Health and Demographic Surveillance System (HDSS), tracking births, deaths, and migration. The majority of the deliveries occur at home (27). The study area has a high morbidity burden from respiratory diseases (28-30), acute and chronic undernutrition (31, 32) Birth rates and infant mortality are high but declining (31).

METHODS

The Infant Cohort Study was conducted from 2009 to 2011, in the Karemo, Gem and Boro Divisions in Siaya County, Western Kenya.

Recruitment

Home births were notified via traditional birth attendants, while health facility births were notified by the respective staff to the recruitment supervisor. Due to the health and demographic surveillance system, all home births were notified within 6 weeks of birth to the study staff. Infants aged zero to 42 days, and weighing ≥1700g were eligible, if they were expected to remain in the study area for more than two years and had been in the area since birth or for at least one month. Low birth weight babies were excluded due to their higher risk of mortality (would take away potential TB disease endpoints). Families planning to out migrate from the study area would make it impossible to ascertain TB disease endpoints and increase loss to follow up. Following notification, a study nurse was dispatched to review the infant for eligibility, obtain informed consent, take anthropometric measures, and provide BCG vaccination (Danish Strain, Staten's Serum Institute).

Study follow up visits took place at health facilities closest to the parents/guardians as follows; at six weeks of age for HIV DNA PCR testing (AMPLICOR COBAS), thereafter four monthly for one to two years depending on time of enrolment. During follow up visits, parents were asked about history of TB contact, TB symptoms in their infants and history of hospitalization. Participants who were unable to come to health facilities had home visits. Loss to follow up (LTFU) was defined as unknown status after three unsuccessful tracing attempts by study close out. Free ancillary care was provided at the study clinic, with hospitalization at the Siaya County Hospital. HIV infected participants were referred for care and anti-retroviral therapy (ART) at the HIV comprehensive care clinic.

Identification of presumptive TB.

Due to the non-specific presentation of infant TB, a broad criteria for presumptive TB was defined in the protocol for study purposes. At scheduled or ancillary unscheduled visits infants meeting the following criteria were considered to have presumptive TB: parental report of household TB contact or TB symptoms (cough for two weeks or more, night sweats for two weeks or more, fever for two weeks or more or undernutrition (underweight for age)) or a history of hospitalization with severe lower respiratory tract infections, meningitis, HIV/AIDS, or malnutrition. Health record surveillance of TB registers and the laboratories in the region was conducted to identify if notified TB cases were contacts of study participants. This was operationalized by searching the HDSS database using the case name and location and matching that to our study participants' HDSS address.

TB investigations

Participants with presumptive TB were admitted to a case verification ward (CVW) for collection of two serial sputum induction and gastric lavage specimens, Mantoux (tuberculin skin test-TST) testing

using 2 Tuberculin Units PPD RT23 (Staten Serum, Denmark), DNA PCR or HIV Antibody testing, and antero-posterior and lateral digital radiographs (CXR). Clinicians evaluated the radiographs during admission. Anthropometric measurements (middle upper arm circumference and weight for age) and clinical ranking using the Keith Edward (KE) Score chart for TB was done. TB treatment was initiated if the KE score was ≥7 or if the CXR was consistent with TB or if TB was microbiologically confirmed. Participants who started TB treatment with a KE score ≥7 but a negative CXR for TB or not microbiologically confirmed were not considered cases for this study.

Sputum samples underwent liquid and solid culture by Mycobacterial Growth Indicator Tube (MGIT) and Lowenstein Jensen (LJ) media. Thereafter, speciation for positive cultures was done using Capilia (FIND and Tauns co. Ltd) or GenoType assay (Hain Diagnostika, Nehren, Germany). Later, when GeneXpert MTB/RIF became available, additional sputum testing was performed from frozen stored sputum pellets. No drug susceptibility testing was done.

Case Definitions

TB cases were classified into bacteriologically confirmed and radiographically diagnosed cases. Bacteriologically confirmed were microbiologically confirmed. An expert panel comprising a paediatric pulmonologist and radiologist performed blinded reviews of CXRs using a standard form developed for TB vaccine trials sites through consensus (17). Any radiograph classified as consistent with TB was defined as radiographically diagnosed TB. Due to subjective assessment of clinical criteria, radiological and bacteriological criteria are presented in this paper to provide a more objective assessment of actual cases that would be end-points in a future TB vaccine trial and that are more stringent. Clinical criteria are non-specific due to other prevalent co morbidities like HIV, malnutrition and failure to thrive. A positive Mantoux test was an induration of ≥ 10mm (or ≥5mm in the presence of severe acute malnutrition or HIV infection).

Statistical Methods

Electronic case report forms were used. Data was analysed using STATA 13 (STATA corp California). We defined enrolment weight as low if it was <2500g and normal at ≥2500g. Nutritional status was classified based on mid-upper arm circumference for infants older than 6 months or weight for age WHO Z scores for those less than 6 months. Person time was calculated from enrolment to the last study contact in a scheduled or unscheduled visit or death whichever was last or TB disease diagnosis. Radiographically cases diagnosed by experts were censored at the date of CXR. Incident TB was computed for bacteriologically confirmed and all TB by dividing the number of cases by the total person time. Cox proportional hazards was used to compare those who became TB cases versus the rest of the study population, identify risk factors for TB and study retention. Vaccination status at four months of follow up, when Pentavalent III has been given was tested as a potential predictor of retention.

Factors that were statistically significant during univariate analysis were further included in a multivariate Cox regression model to adjust for multiple factors to determine those remaining significant risk factors for TB and study retention. Logistic regression was used to compare the clinical characteristics

of bacteriologically confirmed and radiographically diagnosed TB. These independent variables were included: TST, KE Score, nutritional status, history of household contact, infant HIV and reason for TB investigations.

Frequency of ancillary care visits (sick visits) and hospitalization between zero and four months were considered potential risk factors for incident TB at follow up.

RESULTS

TB incidence

Of 2900 infants enrolled, 196 (6.8%), moved out of the study area upon enrolment. A total of 2704 infants were followed up for TB incidence, with 3298 person years of follow up (pyo). (Figure 1).

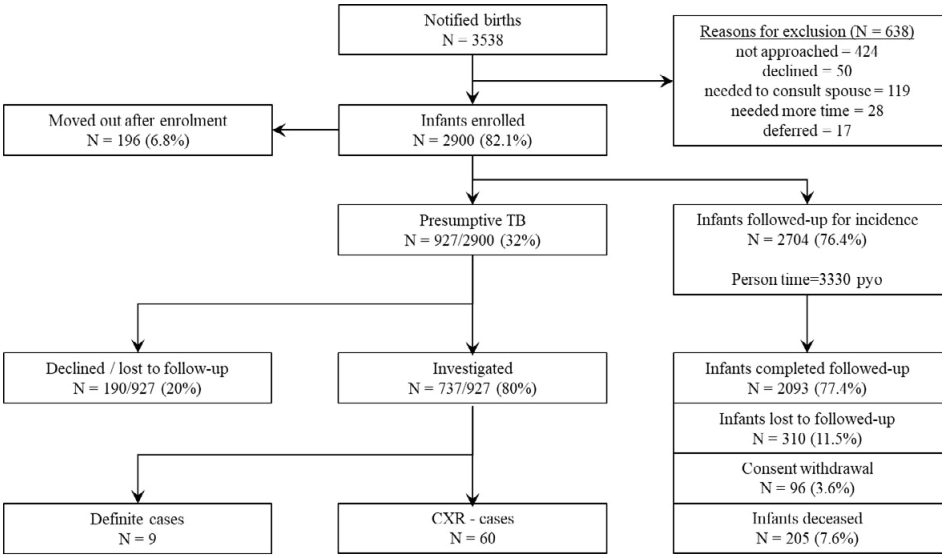

Figure 1 | Flow diagram of the selection of infants for incidence follow-up and the number, who are deceased, withdrew or were lost to follow-up.

Mean follow up time was 1.14 pyo (median 1.3 pyo). There were 205 (7%) total deaths, of whom 26/205 (13%) were known to be HIV infected. About a third of the deaths were in the neonatal period, thereafter most identifiable immediate causes of death were due to severe dehydration and acute respiratory ailments (33).

We identified 927/2900 (32%) as presumptive TB cases, of these we investigated 737/927 (80%) and found 69 individuals to have TB based on microbiological confirmation or radiological criteria. The incidence rate of all TB was 2.0/100 pyo (95% CI 1.65, 2.65). There were nine bacteriologically confirmed TB cases, incidence rate was 0.3 per 100 pyo (95% CI 0.1, 0.5) and the incidence of radiological TB was 1.82/100 pyo. In addition, sixty cases had CXRs consistent with TB. The experts

identified 64/69 all TB cases, of which they agreed on only seven. One of the experts identified 43/60 radiographically diagnosed cases and 4/9 bacteriologically confirmed on CXR. The other expert identified 21/60 radiographically diagnosed cases and 3/9 bacteriologically confirmed on CXR.

Sociodemographic predictors of TB

There were no differences in demographic (sex, maternal age) socioeconomic indicators (maternal occupation) between TB cases and the rest of the cohort at baseline. There were also no significant differences in infant enrolment weight and maternal HIV status between the groups. However, the incidence of TB was higher in those with HIV (p=0.0004) and those reporting a household contact at baseline (p=0.04). TB incidence was lower among mothers who attended antenatal care visits (p=0.04). (Table 1).

Table 1 | Baseline characteristics of TB cases versus study population and univariate HR for incident TB

Baseline Variable		TB case n=69	pyrs	HR (95% CI)
Gender	Male	38	1610	1.17 (0.73, 1.88)
	Female	31	1688	1*
Place of Birth	Home	49	2147	1.29 (0.77, 2.18)
	Health Facility	20	1131	1*
Maternal HIV	Positive	9	2766	0.87 (0.43, 1.75)
	Negative	60	474	1*
Infant HIV status	Negative	62	3228	1*
	Positive	7	69	5.81 (2.65, 12.7)
Maternal age (years)	<19	16	635	1*
	20-29	39	1722	0.86 (0.48, 1.55)
	>29	14	941	0.56 (0.27, 1.15)
ANC attendance	Yes	57	2911	0.53 (0.28, 0.98)
	No	12	328	1*
Enrolment weight (grams)	1700-<2500	4	239	0.79 (0.29, 2.16)
	>=2500	65	3058	1*
Household contact	Yes	9	203	2.11 (1.05, 4.29)
	No	60	3095	1*
Maternal occupation	Unemployed/farmer	63	2872	1*
	Business/Salaried	6	426	0.61 (0.26, 1.42)

* 1 = set as the reference

Comparative clinical characteristics of TB cases

We compared the clinical characteristics of bacteriologically confirmed and radiological cases. Compared with cases identified with radiology, bacteriologically confirmed TB patients had more often a KE score>=7 (OR 17.0, 95% CI 2.78, 104), were more often TST positive (OR 10.8, 95% CI 2.16, 54.4), and reported more frequently a history of TB contact (OR 6.13, 95% CI 1.10, 34.2). There were no other significant differences in clinical characteristics. (Table 2)

Table 2 | Comparing clinical characteristics of definite and chest radiograph TB cases

Characteristic		Definite TB N=9	CXR TB N=60	HR (95% CI)
TST	Negative	5	52	1*
	Positive	3	8	10.8 (2.16, 54.4)
	Missing	1		
KE Score⊥ category	<7	4	51	1*
	≥7	4	3	17.0 (2.78, 104)
	Missing	1	6	
Nutrition	Normal	2	7	1*
	At risk	2	10	0.70 (0.08, 6.22)
	Moderate malnutrition	4	33	0.42 (0.07, 2.79)
	Severe malnutrition	1	10	0.35 (0.03, 4.65)
History of TB contact	No	4	49	1*
	Yes	3	6	6.13 (1.10, 34.2)
	Missing	2	5	
Reason for investigation	Contact	1	4	1*
	Hospitalization	3	36	0.33 (0.03, 4.01)
	Symptoms	3	14	0.86 (0.07, 10.7)
	Missing	2	6	
Infant HIV status	Negative	6	50	1*
	Negative (exposed)	2	4	4.17 (0.63, 27.8)
	Positive	1	6	1.39 (0.14, 13.6)

* 1 = set as the reference

⊥ KE score (Keith Edward Score)

Risk factors for incident TB

In univariate comparisons, infant HIV infection, two or more hospitalisations, one or more sick visits and household TB contact at baseline were risk factors for incident TB. We adjusted for these variables and found infant HIV infection increased the risk of incident TB, adjusted Hazards Ratio (aHR) 4.71 (95% CI 2.13, 10.4)]. Two or more hospitalisations by 4 months of age also increased risk, adjusted Hazard Ratio (aHR) 2.10 (95% CI 1.09, 4.03)] as did multiple sick visits, adjusted Hazard Ratio (aHR) 2.17 (95% CI 1.12, 4.22)]. Household TB contact was not a significant predictor in the adjusted model. (Table 3)

Loss to Follow up

One year loss to follow-up was lower with increasing maternal age in both the univariate and multivariate analysis; adjusted Hazard Ratio (aHR) 0.89 (95% CI 0.79, 1.00). Complete vaccination status measured by proportion who had received all required vaccinations by four months per the Kenya Expanded Immunization Program (KEPI) was associated with lower loss to follow-up; adjusted Hazard Ratio (aHR) 0.44 (95% CI 0.39, 0.49). Employed mothers or those in business had lower loss to follow up compared to unemployed; adjusted Hazard Ratio (aHR) 0.73 (95% CI 0.64, 0.84)]. (Table 4)

Table 3 | Prospective risk factors for TB based on baseline and follow up characteristics of whole study population

Characteristic		TB cases N=69	Study Population	Person-years	HR (95% CI)	Adjusted HR (95% CI)
HIV status	Negative	56	2409	2793	1*	1*
	Negative (exposed)	6	356	436	0.68 (0.29, 1.57)	0.62 (0.27, 1.45)
	Positive	7	66	69	5.56 (2.53, 12.2)	4.71 (2.13, 10.4)
Nutrition	Normal	9	376	442	1*	
	At risk	12	656	828	0.71 (0.30, 1.68)	
	Moderate malnutrition	37	1300	1672	1.09 (0.53, 2.25)	
	Severe malnutrition	11	290	343	1.60 (0.66, 3.86)	
	Missing		209			
Hospitalization	0	52	2521	2902	1*	1*
	1	5	137	160	1.80 (0.72, 4.50)	1.43 (0.57, 3.63)
	≥2	12	173	237	2.80 (1.49, 5.24)	2.10 (1.09, 4.03)
Sick visits by 4 months	0 visits	13	1311	1269	1*	1*
	1 visits	20	663	821	2.37 (1.18, 4.76)	2.05 (1.01, 4.16)
	≥2 visits	36	926	1208	2.82 (1.49, 5.32)	2.17 (1.12, 4.22)
History of TB contact	No	60	2698	3095	1*	1*
	Yes	9	133	203	2.12 (1.05, 4.29)	1.93 (0.95, 3.94)
Maternal age	<19	16	619	635	1*	
	20-29	39	1494	1722	0.86 (0.48, 1.54)	
	>29	14	718	941	0.56 (0.27, 1.15)	
Maternal occupation	Unemployed / farmer	63	2479	2872	1*	
	Business / salaried	6	352	426	0.61 2(0.26, 1.42)	
Retained	No	2	628	170	1*	
	Yes	67	2202	3127	1.10 (0.26, 4.62)	

* 1 = set as the reference

Table 4 | One year loss to follow up (LTFU) and factors associated with LTFU of prospectively followed up infants

Baseline Variable		LTFU* (n)	Person years	HR (95% CI)	Adjusted HR (95% CI)
Gender	Male	220	1610	1*	
	Female	215	1688	1.01 (0.94, 1.11)	
Place of Birth	Home	181	1131	1*	1*
	Health Facility	252	2147	1.04 (0.96, 1.14)	0.80 (0.60, 1.09)
Infant HIV status	Negative	395	2793	1*	1*
	Negative exposed	32	436	0.98 (0.87, 1.10)	0.98 (0.86, 1.12)
	Positive	8	69	1.53 (1.20, 1.96)	1.41 (1.06, 1.89)
Maternal Age	<19	149	635	1*	1*
	20-29	232	1722	0.86 (0.77, 0.96)	0.89 (0.79, 1.00)
	>29	54	941	0.85 (0.75, 0.96)	0.89 (0.78, 1.02)
ANC attendance	Yes	43	328	1*	
	No	387	2911	1.02 (0.89, 1.17)	
Vaccination status	incomplete	78	510	1*	1*
	complete	97	2477	0.44 (0.39, 0.49)	0.44 (0.39, 0.49)
Maternal occupation	Unemployed/ Farmer	378	2872	1*	1*
	Business/Salaried	57	426	0.71 (0.62, 0.81)	0.73 (0.64, 0.84)

* 1 = set as the reference

Sample size requirements for vaccine trials in this population

We estimate based on our bacteriologically confirmed incidence rate a total of 24,321 infants in a 1:1 randomization would need to be enrolled for both arms combined at an incidence rate of 0.3/ 100 pyo to demonstrate a 50% vaccine efficacy with 91 TB cases in the placebo arm and a 20% loss to follow up over 3 years. About half that number would be required with a vaccine of 70% efficacy. Conversely a total of one thousand four hundred and four infants would be needed for both arms combined to demonstrate a 50% vaccine efficacy given an all TB rate in the order of 2/ 100 pyo. (Table 5)

Table 5 | TB vaccine sample sizes

	Presumed vaccine efficacy								
	50%			60%			70%		
Incidence rate of placebo culture confirmed	Total size of sample*	placebo	vaccine	Total size of sample*	placebo	vaccine	Total size of sample*	placebo	vaccine
0.3/100	24.321	88	44	16.318	59	23	11.554	42	12
2.0/100	1.404	34	17	942	23	9	667	16	5

* Lost to follow-up percentage set to 20%

DISCUSSION

Incidence of TB and implications

We observed a high incidence of all TB among infants in Western Kenya in the order of of 2/100pyo. A much lower incidence of bacteriologically confirmed TB (0.28 per 100 pyo) was found. This is consistent with infant disease which is classically paucibacillary. It is higher than reported definite TB incidence in Mozambique, (0.14 per 100 pyo (34)) but lower than in the Western Cape, (1.2 per 100 pyo (5)). A large number of participants were identified as having presumptive TB indicating a high morbidity burden that lead to TB suspicion. TB vaccine trials will need to formulate strategies to deal with this high morbidity burden in infant vaccine trial cohorts and provide the staff capacity needed to cope with the large number of TB investigations required. In addition, 20% of participants with presumptive TB were not investigated. The requirement for a 48 hour admission for TB investigations might have led some parents to decline investigations. Future studies need to offer flexibility including exploring day admissions and discharges for infants whose parents are unwilling to have overnight admissions. The missed investigations might have underestimated the burden of TB in this cohort.

Case definitions

Our study utilized a chest radiographs (CXR) to define non-microbiologically confirmed TB. To be clear, these infants met clinical criteria for TB suspicion, therefore the CXR was a confirmatory tool, in the absence of microbiological confirmation. There are challenges related to the diagnosis of TB in children in regard to the use of CXRs which include; Identification and interpretation of CXR abnormalities are variable and often inconsistent (35), CXR parenchymal abnormalities in infants and young children with TB are not specific for TB but overlap with CXR abnormalities due to other lower respiratory tract infections (36) and CXR is less sensitive for detecting TB-related intrathoracic abnormalities than other imaging modalities (37). Despite the low agreement between the expert readers, 3 out of 7 CXRs defined as consistent with TB were also culture confirmed indicating having more than one reader is key in identifying TB. One way to improve on the poor agreement between experts would be to require an additional criteria like latent TB infection, for definition of TB in addition to a CXR consistent with TB where the experts don't agree. The study area has a high morbidity burden due to HIV, undernutrition and acute respiratory diseases (19, 27, 30, 31, 33). The limitations of a clinical score chart, namely low specificity and high sensitivity, leading to over-treatment have been documented (13, 14). The chest radiograph therefore was used for a viable case definition, in light of low sensitivity of microbiological methods. It has been shown that chest radiographs, are a significant correlate of culture confirmed TB in settings endemic for HIV and other co-morbidities (19, 38). Unfortunately, an intrinsic risk of this approach is the lack of specificity which would underestimate vaccine efficacy, given that new candidates are not geared toward prevention of non-TB respiratory ailments. As this study utilized blinded experts expected to have higher specificity compared to non-expert clinicians (17), misclassification was minimized to the extent possible. This approach also permitted evaluation of risk factors for incident TB, as they were not part of the diagnostic criteria.

Infant TB diagnostics and alternative samples

There are currently limited options for TB diagnosis in young infants. Nasopharyngeal and stool samples could be non-invasive alternatives to induced sputum and gastric lavage. Nevertheless, when analysed using Gene Xpert, both have shown lower sensitivity in children compared to sputum (39-41). The WHO has recommended the use of Xpert MTB/RIF over conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB, while acknowledging the resource implications. IGRAs and commercial serology diagnostics tests are not recommended for use in infants regardless of their HIV status (42, 43). New diagnostic tools are needed. These must then be validated in very young children.

In an autopsy study of children with lung disease, undiagnosed TB was among the top three causes of death among HIV infected and uninfected children, supplementing data showing that definite TB alone could be an insensitive indicator of disease (8). Missed TB cases are found at autopsies (44, 45), particularly among children (45, 46) in both low and high TB incidence settings. About 7% of the cohort died during the study; obtaining ethical approval and informed consent to include limited but targeted post mortem exams, would increase the number of end-points in a TB vaccine trial. One necropsy study showed an additional 25% of respiratory related deaths among hospitalized children were attributable to undiagnosed TB. This potential end-point has not been included in previous trials.

Risk factors

HIV infected infants had a two to ten-fold increased risk of TB disease. Unfortunately, most TB vaccine trials exclude this population for safety reasons. This is the population that most urgently needs a more efficacious TB vaccine. Frequent hospitalization and out-patient sick visits can be considered a marker of chronic morbidity. Patients with one or more sick visits and hospitalizations had more than one to four fold increased risk of TB. While it is possible that the sick visits increased the probability of TB detection, the higher frequency may also be an indication of underlying vulnerability to TB.

TB vaccine trial sample sizes

Our study sought to determine incidence estimates for sample size calculations of hypothetical clinical trials. Due to reliability and validity concerns around TB diagnosis in young children; relying narrowly on bacteriologic confirmation of cases would necessitate exposure of tens of thousands of trial participants to an investigational product, depending on vaccine efficacy. Unfortunately, broad criteria that include radiographic and clinical cases might mean some non-cases are included, leading to underestimates of the efficacy of a vaccine candidate.

Including HIV infected infants who are more likely to develop TB, and also most likely to benefit from the protection of an efficacious vaccine, would minimize sample sizes considerably. However, protective efficacy among HIV individuals may be harder to achieve due to challenges with achieving adequate immunological response.

Retention

Critical to the success of any vaccine trial is retention of participants for end-point determination. A considerable proportion of study participants, exited the study area after enrolment (7%). Future studies must consider cultural norms where women migrate to other areas to give birth and return to their marital homes shortly after. This should be taken into consideration on deciding on inclusion and thus reduce loss to follow-up. Thereafter, the loss to follow-up was about 12% and this would need to be factored into sample size considerations. Factors of health seeking behaviour, particularly completeness of infant vaccination predicted retention. This suggests possible selection bias in case detection, as those who are lost to follow up, may have less favourable health seeking behaviour and therefore are less likely to be diagnosed with TB.

Increasing maternal age was positively associated with retention. Vaccine trials, can possibly deploy better strategies to retain younger mothers including targeting them for phone/home follow up to increase their retention. Our study population largely comprised children of peasant farmers and unemployed women, whereas those who were employed or running businesses were more likely to be retained. In consideration of this skew, such mothers might require off-site follow up such as home visits or provisions for comprehensive tracing when they out-migrate.

Limitations

As the majority of TB is not microbiologically confirmed, alternative case definitions that use radiological criteria were used. We diagnosed TB when any of the blinded experts considered the radiograph to be consistent with TB, however most clinical trials would only consider the radiographs where experts agreed to be a TB case. Low expert inter-rater agreement has been demonstrated (17). Since experts agreed on only seven infants as having TB, the low yield would be an additional limitation to overcome. With all cohort studies, loss to follow up may occur and this may have underestimated our TB incidence estimates. In addition, sputum samples were not tested further to exclude other viral or bacterial respiratory pathogens that could have similar radiological presentation, this could potentially overestimate TB incidence.

Strengths

Our study is one of the first to reliably document TB rates among infants in Western Kenya. We deployed expert readers to adjudicate on cases, as would be the case in a vaccine trial. We deployed CXRs to estimating the TB incidence, in consideration of the disease patterns in the study area as well as the diagnostic challenges. Therefore we consider the study findings to be generalizable to similar settings.

CONCLUSIONS

High TB incidence among young children in Western Kenya particularly among the HIV infected demonstrates the need for novel infant TB vaccines, suited for these children. In light of this, this

population is suitable for new TB vaccine trials. However, large sample sizes would be needed for phase III trials, given the low definite TB incidence. Inclusion of necropsy studies and radiological TB may increase the number of study end-points and reduce the sample sizes. Retention of young women and unvaccinated infants who may have a different risk of TB profile, requires deliberate design of such studies.

Ethical Approval

The protocol and informed consent forms were reviewed and approved by the KEMRI local and national scientific steering committees and the KEMRI national ethical review committee (SSC 1465).

REFERENCES

1. WHO. Global tuberculosis report 2019.
2. Masini Enos JS, Jane Ong'ang'o, et al. Kenya tuberculosis prevalence survey 2016: Challenges and opportunities of ending TB in Kenya. PLOS ONE. 2018.
3. Nduba V, Van't Hoog, A. H, Mitchell, E. M. H, Borgdorff, M, Laserson, K. F. Incidence of Active Tuberculosis and Cohort Retention Among Adolescents in Western Kenya. Pediatr Infect Dis J. 2018;37(1):10-5.
4. Abraham J Herbst a GSCa, Till Bärnighausen a, Angelique KanyKany a, Frank Tanser a & Marie-Louise Newell. Adult mortality and antiretroviral treatment roll-out in rural KwaZulu-Natal, South Africa. Bulletin of the World Health Organization. 2009;87.
5. Hawkridge A HM, Little F, Goetz MA, Barker L, Mahomed H, Sadoff J, Hanekom W, Geiter L, Hussey G; South African BCG trial team. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. BMJ. 2008.
6. Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. Lancet Glob Health. 2017;5(9):e898-e906.
7. Atherton RR CF, Ellis J, Kitaka SB, Boulware DR. Xpert MTB/RIF Ultra for Tuberculosis Testing in Children: A Mini-Review and Commentary. Front Pediatr. 2019.
8. Chintu C MV, Lucas S, Nunn A, Lishimpi K, Maswahu D, Kasolo F, Mwaba P, Bhat G, Terunuma H, Zumla A; UNZA-UCLMS Project Paediatric Post-mortem Study Group. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet. 2002.
9. World Health Organisation. Global Tuberculosis Report 2012.
10. Amber Kunkel PAzW, Ruvandhi R. Nathavitharana, Florian M. Marx, Helen E. Jenkins, and Ted Cohen. Smear positivity in paediatric and adult tuberculosis: systematic review and meta-analysis. BMC Infect Dis. 2016.
11. Osborne CM. The challenge of diagnosing childhood tuberculosis in a developing country. Arch Dis Child. 1995;72(4):369-74.
12. Clemax Couto Santanna MA, R. C. Santos, Rosana Franco. Diagnosis of pulmonary tuberculosis by score system in children and adolescents: a trial in a reference center in Bahia, Brazil. Braz J Infect Dis. 2004;8.
13. Rheenen Pv. The use of the paediatric tuberculosis score chart in an HIV-endemic area. Tropical Medicine and International Health. 2002;7.
14. Supriya Sarkar DKP, Sudipta Chakrabarti, Nirmal Kumar Mandal, A.G. Ghoshal. The Keith Edward scoring system: A case control study. Lung India. 2009;26.
15. Jacquie N Oliwa JMK, Ben J Marais, Shabir A Madhi, Stephen M Graham. Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review. Lancet Respir Med. 2015.
16. Andronikou S BB, Galpin J, Brachmeyer S, Lucas S, Joseph E, Dutoit G, Swingler G. Interobserver variability in the detection of mediastinal and hilar lymph nodes on CT in children with suspected pulmonary tuberculosis. Pediatr Radiol. 2005;35.
17. Kaguthi G NV, Nyokabi J, Onchiri F, Gie R, Borgdorff M. Chest Radiographs for Pediatric TB Diagnosis: Interrater Agreement and Utility. Interdiscip Perspect Infect Dis. 2014.
18. García-Basteiro AL L-VE, Augusto OJ, Gondo K, Muñoz J, Sacarlal J, Marais B, Alonso PL, Ribó JL. Radiological findings in young children investigated for tuberculosis in Mozambique. PLOS ONE. 2015.
19. Luabeya KK MH, Moyo S, Tameris M, Sikhondze W, Geldenhuys H, Mohamed H, Hanekom W, Hussey G, Hatherill M. Diagnostic features associated with culture of Mycobacterium tuberculosis among young children in a vaccine trial setting. Pediatr Infect Dis J. 2012.

20. Alice Zwerling MAB, Aman Verma, Timothy F. Brewer, Dick Menzies, Madhukar Pai The BCG World Atlas: A Database of Global BCG Vaccination Policies and Practices. PLOS ONE. 2011.
21. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. Lancet. 2006;367(9517):1173-80.
22. Tameris M HD, Nduba V, Sacarlal J, Laher F, Kiringa G, Gondo K, Lazarus EM, Gray GE, Nachman S, Mahomed H, Downing K, Abel B, Scriba TJ, McClain JB, Pau MG, Hendriks J, Dheenadhayalan V2, Ishmukhamedov S, Luabeya AK, Geldenhuys H, Shepherd B, Blatner G, Cardenas V, Walker R, Hanekom WA, Sadoff J, Douoguih M, Barker L, Hatherill M. A double-blind, randomised, placebo-controlled, dose-finding trial of the novel tuberculosis vaccine AERAS-402, an adenovirus-vectored fusion protein, in healthy, BCG-vaccinated infants. Vaccine. 2015.
23. Nieuwenhuizen NE KP, Shaligram U, Cotton MF, Rentsch CA, Eisele B, Grode L, Kaufmann SHE. The Recombinant Bacille Calmette-Guérin Vaccine VPM1002: Ready for Clinical Efficacy Testing. Front Immunol. 2017.
24. Tameris MD HM, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea JE, McClain JB, Hussey GD, Hanekom WA, Mahomed H, McShane H; MVA85A 020 Trial Study Team. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet. 2013.
25. Punam Mangtani IA, Cono Ariti, Rebecca Beynon, Laura Pimpin, Paul E. M. Fine, Laura C. Rodrigues, Peter G. Smith, Marc Lipman, Penny F. Whiting, and Jonathan A. Sterne. Protection by BCG Vaccine Against Tuberculosis: A Systematic Review of Randomized Controlled Trials. Clinical Infectious Diseases. 2013;58.
26. Abubakar I PL, Ariti C, Beynon R, Mangtani P, Sterne JA, Fine PE, Smith PG, Lipman M, Elliman D, Watson JM, Drumright LN, Whiting PF, Vynnycky E, Rodrigues LC. Systematic review and meta-analysis of the current evidence on the duration of protection by Bacille Calmette-Guérin vaccination against tuberculosis. Health Technol Assess. 2013.
27. Odhiambo FO, Sewe M, Hamel MJ, Feikin DR, Adazu K, Ogwang S, Obor D, Amek N, Bayoh N, Ombok M, Lindblade K, Desai M, ter Kuile F, Phillips-Howard P, van Eijk AM, Rosen D, Hightower A, Ofware P, Muttai H, Nahlen B, DeCock K, Slutsker L, Breiman RF, Vulule JM. Profile: the KEMRI/CDC Health and Demographic Surveillance System - Western Kenya. Int J Epidemiol. 2012.
28. Emukule GO SP, Chaves SS, Mott JA, Tempia S, Bigogo G, Nyawanda B, Nyaguara A, Widdowson MA, van der Velden K, Paget JW. Estimating influenza and respiratory syncytial virus-associated mortality in Western Kenya using health and demographic surveillance system data, 2007-2013. PLOS ONE. 2017.
29. Nduba V HA, Mitchell E, Onyango P, Laserson K, Borgdorff M. Prevalence of tuberculosis in adolescents, Western Kenya: implications for control programs. Int J Infect Dis. 2015;35.
30. Katz MA LE, Emukule G, Njuguna HN, Aura B, Cosmas L, Audi A, Junghae M, Waiboci LW, Olack B, Bigogo G, Njenga MK, Feikin DR, Breiman RF. Epidemiology, seasonality, and burden of influenza and influenza-like illness in urban and rural Kenya, 2007-2010. J Infect Dis. 2012.
31. Grace Kaguthi, Martien W. Borgdorff, Suzanne Verver. Predictors of post neonatal mortality in Western Kenya: a cohort study. Pan African Medical Journal. 2018.
32. Patricia B. Pavlinac JMN, Grace C. John-Stewart, Frankline M. Onchiri, Albert O. Okumu, Ruth R. Sitati, Lisa M. Cranmer, Erica M. Lokken, Benson O. Singa, Judd L. Walson. Mycobacterium tuberculosis Bacteremia among Acutely Febrile Children in Western Kenya. Am J Trop Med Hyg. 2015.
33. Kaguthi G, Nduba V, Borgdorff MW, Verver S. Predictors of post neonatal mortality in Western Kenya: a cohort study. Pan Afr Med J. 2018;31:114.

34. López-Varela E1 AO, Gondo K, García-Basteiro AL, Fraile O, Ira T, Ribó Aristizabal JL, Bulo H, Muñoz Gutierrez J, Aponte J, Macete E, Sacarlal J, Alonso PL. Incidence of Tuberculosis Among Young Children in Rural Mozambique. Pediatr Infect Dis J. 2015.
35. Du Toit G, Swingler G, Iloni K. Observer variation in detecting lymphadenopathy on chest radiography. Int J Tuberc Lung Dis. 2002;6(9):814-7.
36. Marais BJ, Gie, R. P, Hesseling, A. C., Schaaf HS, Enarson, D. A, Beyers, N. Radiographic signs and symptoms in children treated for tuberculosis: possible implications for symptom-based screening in resource-limited settings. Pediatr Infect Dis J. 2006;25(3):237-40.
37. Swingler GH, du Toit, G, Andronikou, S, van der Merwe, L, Zar, H. J. Diagnostic accuracy of chest radiography in detecting mediastinal lymphadenopathy in suspected pulmonary tuberculosis. Arch Dis Child. 2005;90(11):1153-6.
38. Frigati L MM, Workman L, Munro J, Andronikou S, Nicol MP, Zar HJ. Clinical Predictors of Culture-confirmed Pulmonary Tuberculosis in Children in a High Tuberculosis and HIV Prevalence Area. Pediatr Infect Dis J. 2015.
39. Cakir E, Ozdemir, A, Daskaya, H, Umutoglu, T, Yuksel, M. The value of nasopharyngeal aspirate, gastric aspirate and bronchoalveolar lavage fluid in the diagnosis of childhood tuberculosis. Turk J Pediatr. 2018;60(1):10-3.
40. Orikiriza P, Nansumba, M, Nyehangane, D, Bastard, M, Mugisha, I. T, Nansera, D, Mwanga-Amumpaire, J., Boum Y, Kumbakumba, E, Bonnet, M. Xpert MTB/RIF diagnosis of childhood tuberculosis from sputum and stool samples in a high TB-HIV-prevalent setting. Eur J Clin Microbiol Infect Dis. 2018;37(8):1465-73.
41. LaCourse SM, Pavlinac, P. B., Cranmer LM, Njuguna, I. N, Mugo, C., Gatimu J, Stern, J, Walson, J. L., Maleche-Obimbo E, Oyugi, J, Wamalwa, D, John-Stewart, G. Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children. AIDS. 2018;32(1):69-78.
42. WHO. Commercial Serodiagnostic Tests for Diagnosis of Tuberculosis: Policy Statement. Geneva: World Health Organization 2011.
43. Achkar JM, Ziegenbalg, A. Antibody responses to mycobacterial antigens in children with tuberculosis: challenges and potential diagnostic value. Clin Vaccine Immunol. 2012;19(12):1898-906.
44. R J Flavin NG, D S O'Briain. Mycobacterium tuberculosis at autopsy—exposure and protection: an old adversary revisited. J Clin Pathol. 2007.
45. Monika Garg ADA, Sneh Singh, Sant Prakash Kataria. Tuberculous Lesions at Autopsy. J Indian Acad Forensic Med. 2011.
46. Matthew Bates AS, Victor Mudenda, Charles Chimoga, John Tembo, Mwila Kabwe, Moses Chilufya, Michael Hoelscher, Markus Maeurer, Sylvester Sinyangwe, Peter Mwaba, Nathan Kapata and Alimuddin Zumla. Burden of respiratory tract infections at post mortem in Zambian children. BMC Medicine. 2016.