BCG vaccination status may predict sputum conversion in patients with pulmonary tuberculosis: a new consideration for an old vaccine?

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Received 6 January 2010
Accepted 25 June 2010
Published Online First
1 October 2010

ABSTRACT

Background Failure to convert (persistent sputum and/or culture positivity) while on antituberculosis (anti-TB) treatment at the end of the second month of anti-TB therapy has been reported to be a predictor of treatment failure. Factors that could be associated with persistent bacillary positivity at the end of the second month after initiation of anti-TB treatment were assessed.

Methods A prospective cohort study was conducted in 754 patients with sputum culture positive pulmonary TB in Mwanza, Tanzania. Information on social demographic characteristics, anthropometric measurements, BCG scar status, HIV status, CD4+ count, white blood cell count, haemoglobin and sputum culture status was obtained.

Results Factors associated with sputum culture non-conversion at the end of the second month of anti-TB treatment were initial acid-fast bacilli (AFB) culture grading of 3+ (OR 5.70, 95% CI 1.34 to 24.31, p=0.02) and absence of a BCG scar (OR 3.35, 95% CI 1.48 to 7.58, p=0.004).

Conclusion Patients with pulmonary TB with no BCG scar and high initial AFB sputum intensity are at risk of remaining sputum culture positive at the end of the second month of anti-TB treatment. These findings reflect a beneficial role for BCG vaccination on sputum conversion which should also be examined in large studies in other areas. The finding of a beneficial role for BCG vaccination on the treatment of pulmonary TB is important for TB control and vaccination programmes.

INTRODUCTION

Tuberculosis (TB) remains one of the major public health problems worldwide and represents a major challenge to health systems in the 21st century. The total numbers of new TB cases is rising, especially in Africa, South-East Asia and the Western Pacific region. These regions contributed 83% of the total TB cases notified globally in 2006.[1] Tanzania ranks 14th among the 22 countries, with the highest TB burden worldwide and sixth in Africa.[1] Bacillus Calmette-Guérin (BCG) is still the only available vaccine for combating TB worldwide. The BCG vaccine was initially used in humans in 1921 as a disease-modifying agent before the era of antibiotics and was introduced for protection against TB disease.[2] In 2002 the BCG vaccine was estimated to have been administered to more than 100 million infants worldwide, covering 76% of the total 130 million infants globally.[3] In sub-Saharan Africa, BCG is given as a routine to all infants at birth or at first contact with health facilities.[4] The role of BCG vaccine in protecting against TB has been reported to vary, being more effective in children with severe forms of TB (meningitis or miliary disease).[3] Meta-analysis studies show that the BCG vaccine confers 73% protection against TB meningitis and 77% against miliary forms of TB.[5 6] However, the extent to which BCG vaccination confers protection against adolescent and adult TB is under debate.[3] Failure to convert while on anti-TB treatment at the end of the second month has been reported to be a predictor of treatment failure.[7 8] Previous studies have identified factors such as pretreatment high sputum acid-fast bacilli (AFB) smear and sputum culture grading, gender, smoking and age as potential predictors of sputum conversion.[8–10] However, little is known about factors that may be associated with persistent sputum AFB positivity in Tanzania. Thus, factors that could be associated with sputum non-conversion at the end of the 2 months after initiation of anti-TB treatment were assessed.

MATERIALS AND METHODS

Study population

The study was conducted in Tanzania in the city of Mwanza which comprises two districts, Ilemera and Nyamagana. Mwanza city is divided by the National Tuberculosis and Leprosy Programme (NTLP) into three operating TB districts. Four health facilities (two hospitals and two health centres) served as recruitment centres. All patients diagnosed with sputum smear positive pulmonary TB (PTB) recruited from the three NTLP districts and attending the four major health facilities from April 2006 to October 2008 in Mwanza city were considered for recruitment.

Inclusion and exclusion criteria

Patients with sputum smear positive PTB aged ≥15 years recruited at the local health facilities and confirmed as sputum culture positive at the Zonal Reference Laboratory were included in the study. Pregnant women, patients with terminal illness (from TB or any other serious disease unlikely to survive more than 48 h), non-residents (patients who would not stay in the study area for the entire period of 6 months of anti-TB treatment) and patients not willing to participate were not considered for enrolment.

Patient enrolment

Patients diagnosed as sputum smear positive by microscopy using Ziehl–Neelsen (ZN) staining at

the first visit to a diagnostic health facility were referred to any of the four main recruitment health facilities. Patients were provided with information on the study and those who were eligible were asked for oral and written consent. Those who consented and for whom one or both spot and next day early morning sputum samples were ZN smear microscopy positive were requested to provide an additional early morning sputum specimen. Smear microscopy examination was performed using Auramine O staining and culture using Lowenstein–Jensen (LJ) solid media. The LJ tubes were incubated for 8 weeks before concluding that the samples were culture negative. Sputum results for AFB were graded according to the NTLP recommendations.[11]

Data collection and laboratory procedures

Information on the participants was collected using structured questionnaires at the first visit before initiation of anti-TB treatment. Information on social demographics, medical history, smoking history and anthropometric measurements (height and weight) was ascertained at baseline. Patient weight and height were measured using a digital weighing scale and height board, respectively. Documentation on the presence or absence of BCG scars for each patient was performed by a directly observed treatment nurse (DOT) at the TB clinic. Confirmation of the presence of a BCG scar was done by a study team member by counterchecking on the patient's TB clinic card in order to minimise the possibility of an observation bias. The presence of a BCG scar was regarded as evidence of BCG vaccination. If the presence of a scar was in doubt it was recorded as absent. HIV counselling and testing was undertaken for all recruited participants. Venous blood (15ml) was drawn from each participant for HIV testing, total lymphocyte count and CD4 cell counts. Ethylene diaminetetra-acetic acid tubes were used to collect blood for haemoglobin, white blood cell counts and CD4 counts. HIV testing and other blood parameters were performed on the same day as collection at the National Institute for Medical Research Laboratory, Mwanza, Tanzania. HIV testing was done using two different rapid antibody tests: Determine HIV-1/2 (Inverness Medical Japan, Abbot, Japan) and Capillus HIV-1/2 (Trinity Biotech, Ireland). Discordant samples were tested using Uniform II Vironostika-HIV Ag/Ab Micro-Elisa system (Biomerieux BV, The Netherlands). CD4+ counts were performed using a Partec Cyflow counter machine (Partec GmbH, Münster, Germany). Total lymphocyte counts were determined manually.

Follow-up

All patients with PTB who were diagnosed as sputum smear positive and/or culture positive were initiated on anti-TB treatment that was provided under the directly observed treatment short course strategy for a duration of 6 months. Drugs were given in a fixed dose combination of four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) during the first 2 months of intensive phase and two drugs in a fixed dose combination (rifampicin and isoniazid) during the 4 months of the continuation phase.[11] Patients were then followed up by sputum culture examination at the end of 2 months after anti-TB treatment.

Statistical analysis

The data were analysed using Stata IC Version 10.1 (StataCorp, College Station, Texas, USA). The outcome of interest was culture non-conversion at the end of the second month of treatment. To identify predictors of non-conversion, the proportions of non-converters were compared between categories of potential predictor variables using the χ^2 test. Potential predictors considered were age, sex, smoking, initial culture grading, presence of BCG scar, HIV status, CD4 count, anaemia and body mass index (BMI). Logistic regression analysis was done to estimate the relationship between potential predictors and culture non-conversion, and crude odds ratios as well as age- and sex-adjusted odds ratios were estimated. A final multivariable logistic regression analysis was performed with all the predictors with $p<0.10$ in the age- and sex-adjusted analyses and with adjustment for other confounders. Continuous variables were categorised using conventional cut-off points. Goodness of fit comparisons were done to ensure that the categorisations of the continuous variables were appropriate. Tests for interaction between all variables in the final model and HIV status on sputum non-conversion were carried out. p Values <0.05 were considered to indicate statistical significance.

RESULTS

A total of 842 patients with smear positive PTB met the study criteria for this cohort from April 2006 to October 2008, of which 754 were enrolled based on a positive culture at baseline. Of these, 329 (43.6%) were HIV positive. The culture contamination rate at baseline was 4.3%. The majority of recruited patients were new TB cases (92.3%). Of the 754 patients who were sputum culture positive at baseline, 208 were excluded (174 failed to return at the end of the intensive phase or came much later than the scheduled 2-month visit, 7 died and 27 had contaminated sputum culture results, figure 1). When compared with those patients who remained in the study, the drop-outs did not differ in their BCG scar status.

Table 1 summarises the background characteristics of the recruited patients. Their mean (SD) age was 34.5 (11.7) years. Of the 754 patients recruited, 546 (72%) presented at the end of the second month of the intensive phase of treatment. The sputum culture non-conversion rate at the end of the second month after anti-TB treatment for these 546 patients was 5.5% (table 2). A total of 3.5% of the women and 6.7% of the men (p=0.12) remained sputum culture positive at the end of the second month of anti-TB treatment. Of the 546 patients who were AFB culture positive at baseline and followed up at the end of the

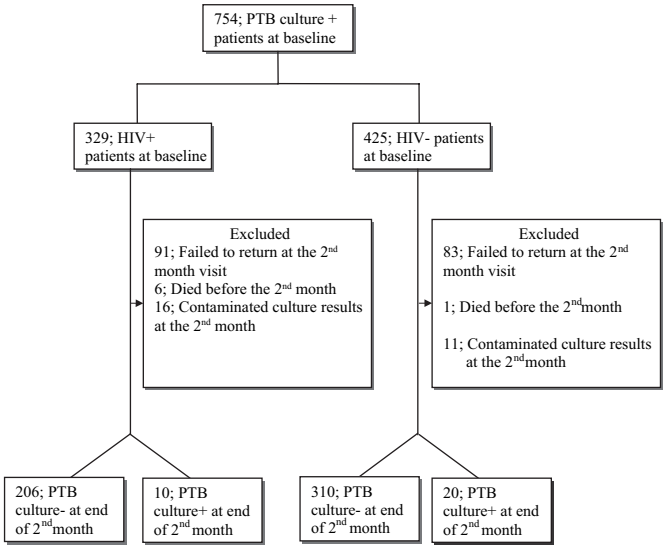

Figure 1 Flow chart for patients with culture positive pulmonary tuberculosis (PTB) at baseline followed for sputum culture conversion at the end of the second month of the intensive phase of treatment.

Table 1 Background characteristics for 754 patients with sputum culture positive pulmonary tuberculosis*

Age (N=754)	34.6 (11.7)
15–24 years	18.4 (139)
25–34 years	38.1 (287)
35–44 years	25.1 (189)
≥45 years	18.4 (139)
Sex (N=754)	
Female	37.5 (283)
Male	62.5 (471)
Smoking (N=751)	
Never smoker	66.8 (502)
Past smoker	9.9 (74)
Current smoker	23.3 (175)
Culture grading (N=754)†	
1+	17.1 (129)
2+	14.3 (108)
3+	68.6 (517)
BCG scar (N=747)	
Present	81.4 (608)
Not present	18.6 (139)
CD4 count (N=753)	354 (210; 572)
<200 cells/μl	23.0 (173)
201–350 cells/μl	26.6 (200)
>351 cells/μl	50.4 (380)
HIV status (N=754)	
Negative	56.4 (425)
Positive	43.6 (329)
Haemoglobin (g/dl) (N=753)‡	10.6 (2.4)
No anaemia	19.3 (145)
Anaemia	80.7 (608)
Body mass index (N=753)	18.4 (2.7)
≥18.5 kg/m^2	41.4 (312)
17.0–18.5 kg/m^2	26.4 (199)
<17.0 kg/m^2	32.1 (242)

*Data are mean (SD), median (IQR) or % (n).
†Culture grading defined as follows: 1+, 1–100 colonies; 2+, >100 colonies, 3+, confluent colonies.
‡Anaemia defined as haemoglobin <12.0 g/dl for women and <13.0 g/dl for men.
BCG, Bacillus Calmette-Guérin.

second month of the intensive phase, there was no observed difference between sputum culture non-conversion among patients categorised by age, smoking, CD4 count, HIV status, haemoglobin levels and BMI (table 2). However, a higher proportion of patients who had a pretreatment AFB culture grading of 3+ failed to convert compared with patients with a baseline sputum culture grading of ≤2+ (7.2% and 1.3%, respectively; p=0.006). For patients who did not have a BCG scar, 11.6% remained sputum culture positive compared with 4.1% of those who had a BCG scar (p=0.003, table 2).

Logistic regression was performed to identify factors that were independently associated with sputum culture non-conversion at the end of the second month after anti-TB treatment. The absence of a BCG scar (OR 3.35, 95% CI 1.48 to 7.58, p=0.004) and initial AFB culture grading of 3+ (OR 5.70, 95% CI 1.34 to 24.31, p=0.02) remained significantly associated with persistent sputum culture positivity (table 2).

In the multivariate regression model, factors that remained independently associated with culture non-conversion at 2 months after being adjusted for age and sex included an initial AFB culture grading of 3+, which was associated with an almost fivefold increased risk of sputum culture conversion failure (OR 5.24, 95% CI 1.22 to 22.48, p=0.03) and absence of a BCG scar, which was associated with a threefold increased risk (OR 3.18, 95% CI 1.39 to 7.26, p=0.006) of sputum culture conversion failure. There was no confounding or interaction for the factors that remained significant.

DISCUSSION

Sputum smear/culture conversion at the end of the second month of anti-TB treatment has been considered as an important indicator for the success of TB treatment.[7 8 12] In the present study we observed an overall non-conversion rate (2 months after anti-TB treatment) of 5.5% for the 546 patients who were initially sputum culture positive. For the 30 patients who did not convert at 2 months, 29 were sputum smear negative (ie, they were cured by standard anti-TB treatment) while one patient remained sputum smear positive at 5 months after anti-TB treatment (data not shown). Patients with TB who had a high initial (pretreatment) culture grading and an absence of a BCG scar were more likely to have persistent sputum culture positivity at the end of the second month of anti-TB treatment. Previous studies have shown that sputum non-conversion at the end of the intensive phase of anti-TB treatment has been associated with high initial smear/culture grading, male gender and low BMI.[7–9 12 13] Patients with numerous AFB in the sputum reflect the high numbers of bacilli in the lung, with a corresponding increase in cavity formation. Thus, delayed sputum conversion in patients with high initial bacillary loads in the sputum is not surprising.[13 14] Interaction between malnutrition and TB is associated with complex mechanisms. BMI is an indicator of nutritional status. Both micronutrient and macronutrient deficiency can influence susceptibility to TB. Severe malnutrition has a profound effect on cell-mediated immunity.[15] In this study patients with PTB with a BMI <17.0 kg/m^2 and 17.0–18.5 kg/m^2 had a sputum culture non-conversion rate of 5.4% and 8.2%, respectively, at the end of the second month of the intensive phase of treatment. Patients with a BMI of <18.5 kg/m^2 were more likely to remain sputum culture positive than those with a BMI of >18.5 kg/m^2, although the results were not significant.

Our study shows that there was no significant difference in conversion rates among patients with TB co-infected with HIV and those not infected with HIV. The sputum culture conversion rate for HIV negative patients was 93.9% compared with 95.4% for those who were HIV positive. CD4+ counts, despite playing a role in immunity, did not significantly influence the sputum conversion rate. In agreement with previous studies,[15] there was no difference in sputum conversion rates with different CD4+ counts. Patients with CD4+ counts of ≤200, 201–350 and >350 had sputum culture conversion rates of 95.9%, 92.7% and 94.8%, respectively. At the 2-month observation period the mean number of days in anti-TB drug compliance among converters was 57 days compared with 59 days for non-converters (p=0.16), indicating that there was no difference in treatment compliance between the two groups (data not shown).

Interestingly and, to our knowledge, reported for the first time, the presence of a BCG scar was significantly associated with sputum culture conversion (p=0.006) at the end of the second month of the intensive phase of anti-TB treatment. The dose of BCG and the route of administration are important for scar development. In Tanzania, BCG vaccine is given to all infants at birth intradermally in the upper part of the right arm at a dose of 0.05 ml and is included in the expanded programme of immunisation. The presence of a scar on the appropriate area of the arm has been considered evidence of prior BCG vaccination. However, BCG scars may be difficult to assess. Moreover,

Table 2 Predictors of sputum culture non-conversion for 546 patients with pulmonary tuberculosis who were sputum culture positive at baseline and followed up at the end of the second month of intensive phase treatment

	All (N=546)	Converted (N=516)	Not converted (N=30)	p Value	Crude OR (95% CI) for non-conversion	p Value	Adjusted* OR (95% CI) for non-conversion	p Value
Age (years) (N=546)								
15–24	103	95 (92.2%)	8 (7.8%)	0.31	2.13 (0.77 to 5.84)	0.14	2.09 (0.76 to 5.77)	0.15
25–34	210	202 (96.2%)	8 (3.8%)		1.0		1.0	
35–44	234	124 (92.5%)	10 (7.5%)		2.04 (0.78 to 5.30)	0.15	1.83 (0.70 to 4.80)	0.22
≥45	99	95 (96.0%)	4 (4.0%)		1.06 (0.31 to 3.62)	0.92	2.00 (0.83 to 4.82)	0.87
Sex (N=546)								
Female	201	194 (96.5%)	7 (3.5%)	0.12	1.0		1.0	
Male	345	322 (93.3%)	23 (6.7%)		1.98 (0.83 to 4.70)	0.12	2.00 (0.83 to 4.82)	0.12
Smoking (N=543)								
Never	351	336 (95.7%)	15 (4.3%)	0.20	1.0		1.0	
Past	55	50 (90.9%)	5 (9.1%)		2.24 (0.78 to 6.43)	0.13	2.18 (0.69 to 6.82)	0.18
Current	137	127 (92.7%)	10 (7.3%)		1.76 (0.77 to 4.03)	0.18	1.69 (0.66 to 4.32)	0.27
Culture grading (N=546)†								
≤2+	157	155 (98.7%)	2 (1.3%)	0.006	1.0		1.0	
3+	389	361 (92.8%)	28 (7.2%)		6.01 (1.41 to 25.54)	0.02	5.70 (1.34 to 24.31)	0.02
BCG scar (N=539)								
Present	444	426 (96.0%)	18 (4.1%)	0.003	1.0		1.0	
Not present	95	84 (88.4%)	11 (11.6%)		3.10 (1.41 to 6.80)	0.005	3.35 (1.48 to 7.58)	0.004
CD4 (cells/μl) (N=546)								
<200	122	117 (95.9%)	5 (4.1%)		1.0		1.0	
201–350	136	126 (92.7%)	10 (7.4%)	0.50	1.86 (0.62 to 5.59)	0.27	1.86 (0.61 to 5.68)	0.28
>351	288	273 (94.8%)	15 (5.2%)		1.29 (0.46 to 3.62)	0.63	1.16 (0.40 to 3.36)	0.79
HIV status (N=546)								
Negative	330	310 (93.9%)	20 (6.1%)	0.47	1.0		1.0	
Positive	216	206 (95.4%)	10 (4.6%)		0.75 (0.35 to 1.64)	0.47	0.87 (0.39 to 1.96)	0.74
Anaemia (N=546)‡								
Not present	117	109 (93.2%)	8 (6.8%)	0.47	1.0		1.0	0.61
Present	429	407 (94.9%)	22 (5.1%)		0.74 (0.32 to 1.70)	0.47	0.80 (0.34 to 1.87)	
BMI (kg/m^2) (N=546)								
≥18.5	227	218 (96.0%)	9 (4.0%)	0.24	1.0		1.0	
17.0–18.5	135	124 (91.9%)	11 (8.2%)		1.39 (0.55 to 3.50)	0.48	1.32 (0.52 to 3.37)	0.56
<17.0	184	174 (94.6%)	10 (5.4%)		2.15 (0.87 to 5.33)	0.10	1.86 (0.73 to 4.70)	0.19

*Adjusted for age and sex.
†Culture grading defined as: ≤2+, 1–>100 non-confluent colonies; 3+, confluent colonies.
‡Anaemia defined as haemoglobin <12.0 g/dl for women and <13.0 g/dl for men.
BCG, Bacillus Calmette-Guérin; BMI, body mass index.

as shown by Fine *et al*,[16] the less clear the mark or scar, the greater the potential for subjective bias in observing or interpreting a scar. In this study the presence of a BCG scar was regarded as evidence of being vaccinated and was considered a reliable way of identifying whether patients received the vaccine or not.[17] Those with doubtful scar information were considered as lacking a BCG scar. Our decision to consider patients with doubtful scar information as having no scar did not influence the outcome, as analysis with or without inclusion of such patients did not change the significant association of the presence of a BCG scar with sputum culture conversion.

The BCG vaccine is still the only vaccine in use against TB, but efforts to develop new vaccines are in progress.[18] Despite the BCG vaccine being in use for over 80 years, its mechanism of protection in humans is not well understood although, in the mouse model of TB, BCG vaccination tends to reduce the bacillary burden and dissemination.[19] Apart from the protection that BCG confers against severe forms of childhood TB, it has been suggested that BCG vaccination may have a non-specific beneficial effect on childhood survival.[20 21] In BCG-vaccinated children in Guinea-Bissau a BCG scar was related to better child survival and in a hospital study from Malawi a BCG scar was related to fewer skin infections and less sepsis.[18 22] A meta-analysis of BCG trials has reported that the protective efficacy of the vaccine varies across populations, ranging from 0% to 80%.[6 23] A meta-analysis of BCG trial data also suggests that the protective efficacy of BCG may persist for ≥10 years after infant vaccination.[24] A study by Weir *et al* showed that BCG vaccination during infancy and adolescence can induce immunological memory to mycobacterial antigens that is measurable up to 14 years.[25] Additionally, recent studies of the long-term efficacy of BCG vaccination show that, among American Indians and Alaska natives, protection could last up to 60 years[26] and for up to 20 years in Brazilians vaccinated as neonates.[27] Studies in BCG-vaccinated and unvaccinated individuals who develop TB followed up to the 2-month observation point are needed to identify potential biomarkers associated with early sputum conversion.

In conclusion, this study shows that initial (pretreatment) high culture grading is a risk factor for sputum culture non-conversion. Interestingly, the presence of a BCG scar is significantly associated with culture conversion at the end of the intensive phase of anti-TB treatment. This novel finding needs to be confirmed in larger studies in Tanzania as well as in other geographical locations and populations. While waiting for a new effective TB vaccine, we should not forget the benefit that the existing vaccine (BCG) may convey.

Acknowledgements The authors would like to acknowledge the TB-Nutrition study team for their contribution and the patients and their treatment supervisors who participated in this study. We thank Dr A C Hesseling for valuable comments and Mr O Kaswamila at the National Institute of Medical Research Mwanza Centre and Mr Appolinary Mgomela at the Zonal Reference Laboratory for excellent laboratory assistance.

Funding The Danish Medical Research Council, Danish Council for Development Research, the Norwegian Quota Programme and the Norwegian Centre for International Cooperation in Higher Education (NUFUPRO-2007/10183).

Competing interests None.

Ethics approval Permission to conduct the study was granted by the medical research coordinating committee from the National Institute for Medical Research in Tanzania.

Provenance and peer review Not commissioned; externally peer reviewed.

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