Reviewer Response

Dear Dr. Orenstein,

Thank you for sending review reports for our manuscript. The reviewer's comments were very useful, and we have implemented the majority of them.

We enclose a revised version of the manuscript (clean and with tracked changes), which addreses the reviewer's comments and suggestions. The reviewer's comments are shown below with our response to each comment in italics.

Yours sincerely,

Sam Abbott

Authors' response to Reviewer #1:

Summary

The manuscript "Exploring the effects of BCG vaccination in patients diagnosed with tuberculosis: observational study using the Enhanced Tuberculosis Surveillance system" by Abbott et al. is a retrospective analysis that examines the association between prior BCG vaccination and all-cause mortality and TB-related mortality in reported TB cases in England. Although BCG has been shown to reduce the incidence of TB disease and disease severity in children, there is less data as to its effect on mortality and data on the length of protection are mixed. In this study, prior BCG vaccination was associated with lower all-cause and TB-related mortality (though the latter did not reach statistical significance) in patients diagnosed with TB. While these findings are interesting, I think there are significant methodologic concerns. The data set used has a very large amount of missing data that has high potential to introduce bias into the results. About 1/3 of people studied had unknown BCG vaccination status and those with unknown vaccination status had much higher mortality than people in whom vaccination status was known (11% vs 3%). Of those that died and had known vaccination status, a further 38% of people did not have a known cause of death. Because this study only analyzed cases with complete data, conclusions are based on a subset of TB cases that may not be representative of the population as a whole.

Thank you for your detailed comments on our manuscript. We have addressed your concerns below.

We agree that missing data was an issue in this study, particularly missing BCG vaccination status. To address this we conducted all analyses in this study using multiple imputation (imputing all outcomes and exposures). We also discussed results from the complete analysis in the context of the results from the analysis with multiply imputed data. However, we were unable to fully account for the impact of a missing not at random mechanism for BCG vaccination. This was therefore listed as a limitation in the discussion:

"Variable data completeness changed with time, with both BCG vaccination status and year of vaccination having a high percentage of missing data, which may not be missing completely at random. We therefore checked the robustness of our results with multiple imputation including regional variability, however an unknown missing not at random mechanism, or unmeasured confounding may still have introduced bias. We found a greatly increased risk of all-cause mortality for those vaccinated more than 10 years ago in the analysis with multiply imputed data, compared to the complete case analysis. This is likely to be driven by a missing not at random mechanism for years since vaccination, with older cases being both more likely to have been vaccinated more than 10 years previously and to also have an unknown year of vaccination."

Highlights

It would be more helpful if this section listed exactly what was found in the study and how it contributes to the literature rather than make general statements about what was explored.

We agree that this section should focus on the results from this study. Based on the requirments laid out on the Vaccine help page (https://www.elsevier.com/authors/journal-authors/highlights), we have adapted the final bullet point as follows:

There was weak evidence of associations between TB outcomes and age at, or years since, vaccination.

Introduction

I suggest the introduction section discuss in more precise terms exactly what is known about the efficacy of BCG vaccination and what questions remain unanswered that this study is trying to address.

We acknowledge that information known about the efficacy of BCG vaccination is important to include as context for our study. We did not give precise estimates for the efficacy of the BCG vaccine as efficacy has been shown to be context specific. However, We updated paragraph two of the introduction to explicitly state that the BCG vaccine has been shown to be highly effective in England, and that its effectivness may be correlated with distance from the equator. This now reads as follows.

"BCG's primary mode of action is to directly prevent the development of active, symptomatic disease. Its efficacy in adults is context specific, with estimates ranging between 0% and 78%.[3] It has been shown to highly efficacious in England and there is some evidence that efficacy increases with distance from the equator.[7] Efficacy has been shown to be dependent on previous exposure, with unexposed individuals receiving the greatest benefit.[4] Unlike in adults, BCG has consistently been shown to be highly protective against TB and TB meningitis in children.[5,6] For this reason the majority of countries that use BCG vaccinate at birth.[7,8]"

We feel that the final paragraph of the introduction details which questions remain unanswered - this is included below in response to your next comment.

There is frequent mention of in the introduction (and throughout this manuscript) of the "additional effects" of BCG aside from reducing the incidence of active TB. However, this term is very imprecise, and it makes it difficult to interpret exactly which additional effects the authors are referring to. There are numerous studies showing BCG has many such additional effects, particularly reducing the development of TB meningitis and disseminated TB in young children. The authors need to more clearly describe which "additional effects" of BCG vaccination are not known and how this study can help us understand them better.

We agree that this needed clarifiction. As part of our response to your comment below lines 18-19 we have added an explanation of additional effects in the context of TB outcomes. We feel that the following sentences (page 6, lines 19-23 and page 7, lines 1-3) complete this definition:

"There is some evidence that BCG vaccination induces innate immune responses which may provide non-specific protection,[11] TB patients with BCG scars were found to respond better to treatment with earlier sputum smear conversion,[12] and there is evidence to suggest that BCG vaccination is associated with reduced all-cause neonatal mortality[13,14] and both reduced TB[15] and all-cause[16] mortality in the general population. Given that the immunology behind TB immunity is not well understood these findings suggest that BCG may play a more important role in improving TB outcomes than previously thought."

This was the only use of this phrase in the manuscript.

The statement on line 7-9 that the emergence of drug-resistant TB has made BCG the best available vaccine needs further justification. I am unaware of any vaccine candidates that have differential vaccine efficacy based on MDR status.

We agree that this sentence was unclear. We have reworded it to the following:

"However, the lack of a more effective vaccine and the emergence of drug-resistant TB strains means that BCG vaccination remains an important tool for reducing TB incidence and mortality rates."

I would suggest revising the statement on lines 18-19 that "Vaccination policy has been primarily based on reducing the incidence of active TB and 19 little attention has been given to any additional effects of BCG" be revised. Vaccination policy is based just as much on the attenuation of disease severity (less meningitis and disseminated TB in kids) as it is reducing the overall incidence of TB.

We agree that this was an omission. We have amended this sentence to the following:

"Vaccination policy has been primarily based on reducing the incidence of TB disease, and mitigating disease severity, with little attention having been given to any additional effects of BCG vaccination on TB outcomes."

I would recommend the introduction section end with a clear statement of the study question.

The primary aim of the study is detailed in the penultimate sentence of the introduction:

"We aimed to quantify the effects of BCG vaccination on outcomes for individuals with notified TB in England using routinely collected surveillance data to provide evidence for appropriate public health action and provision."

The final sentence details the secondary aim of the study:

"Where we found an association, we additionally explored the role of years since vaccination, and age at vaccination."

Methods

A much more detailed discussion is needed to understand how the outcome variables differ from one another and how the information was collected. Specifically, a clearer description of how "all-cause mortality" and "TB-related mortality" were collected is needed. How long were most people followed to assess for all-cause mortality? The table indicates people were "followed for 12, 24, and 36 months (or until treatment completion)". Given patients with drug susceptible TB are usually only treated for 6 months, it seems few people would have been followed for this amount of time. It is also unclear what criteria were used to determine whether the death was due to TB and who made the assessment.

We agree that futher detail was required to help differentiate outcomes. We also agree that the expplanation of the approach used to define mortality needed reworking. We made these changes below at the same time as moving from a table to describing the outcomes in the text.

There is no information included as to how information about BCG vaccination was collected. Was this entirely by self-report? If so, was there any effort made to ensure the information provided was accurate (e.g. cross-referencing vaccination records), particularly the age at vaccination?

We agree that this should be included in the methods section. We have amended the explanation of BCG vaccination status to the following:

"BCG status was collected during the assessment of TB cases and then entered in the ETS. When available BCG vaccination records are used, although often these are not available and so patient recall or the presence of a scare is relied on. When cases are uncertain, and there is no evidence of a scar, no BCG status is given. Year of vaccination is collected similarly."

We acknowledge that this is a limitation of our study for which ideally a validation study would be carried out. To highlight this we included the following in the discussion (amended to include year of vaccination).

"Finally, BCG vaccination status, and year of vaccination, may be subject to misclassification due to recall bias; validation studies of the recording of BCG status in the ETS would be required to assess this."

For the multiple imputation procedure, which variables were imputed? Was BCG vaccination status imputed for this model when missing?

We updated the methods section to highlight that all outcome and explanatory variables were included in the multple imputation approach:

"To mitigate the impact of missing data we used multiple imputation, with the MICE package.[27] We imputed 50 data sets (for 20 iterations) using all outcome and explanatory variables included in the analysis as predictors along with Public Health England centre. The model results were pooled using the small sample method,[28] and effect sizes compared with those from the main analysis."

I would consider discussing the outcome variables in the text rather than listing them in table format. As above, the information in the tables does not provide much clarity about the outcome variables.

We have moved the outcome variable definitions from a table into the main text of the manuscript. Including the modifications from comments 1., 2., and 3. above this now reads as follows:

"The outcomes we considered were: all-cause mortality, death due to TB (in those who died), recurrent TB, pulmonary disease, and sputum smear status. These outcomes were selected based on: their availability in the ETS; evidence from the literature of prior associations with BCG vaccination; associations with increased case infectiousness; or severe outcomes for patients."

"All-cause mortality was defined using the overall outcome recorded in ETS, this is based on follow to 36 months starting from date of starting treatment. Where the treatment start date was not available the notification date was used if appropriate. The date of death was validated against Office for National Statistics (ONS) data. Those that were lost to follow up, or not evaluated were treated as missing. In cases with a known cause of death, death due to TB was defined as those that died from TB, or where TB had contributed to their death. This was assessed using the cases death certificates. TB cases who had recurrent episodes were identified in the dataset using their NHS number. Positive sputum smear status was given to cases that had a sputum sample shown to contain Acid-Fast Bacilli. A positive sputum smear status indicates that cases are more likely to be infectious. Cases were defined as having pulmonary TB if a positive sputum smear sample was recorded or if a positive culture was grown from a pulmonary laboratory specimen. People with laryngeal TB were defined as having pulmonary TB, and people with miliary TB were not excluded."

Results

This manuscript contains a large number of tables and many of them seem to be redundant. I would suggest combining some of the tables so more information can be included in the main manuscript rather than the supplement. For example, Tables S1 and S2 contain information that would normally be a part of "Table 1". I would consider combining these tables to make a new Table 1 so readers can more clearly see how outcomes and covariates differed based on BCG vaccine status.

We have moved both S1 and S2 into the main body of the text. The tables are presented seperately to make it clear that not all outcomes were considered in the same model. Whilst this is our prefered method of presenting this data we would be happy to combine these tables into a single table if the reviewer felt this would improve interpretability.

I suggest including the total n used for each analysis in all tables.

We have included both the univariate and multivariate sample size for all analyses that use complete case analysis. These can be found at the bottom of each table as a footnote. For analyses that use mutltiple imputation, all samples with a given outcome were used. For BCG vaccination this is all samples. For age at vaccination and years at vaccination the sample size varied between multiply imputed datasets as the proportion of the population with imputed BCG vaccination changed - this means we cannot give a definite sample size for these analyses. For the sensitivity analysis the sample size was presented in the table caption. This has been highlighted by using n = prior to the sample size.

In supplemental table S9, the post-hoc power calculation is not necessary. This is simply re-stating the primary analysis. By definition those analyses that did not reach statistical significance were not adequately powered to detect statistical significance given the observed effect size.

We agree that this is not required and have removed it from the supplementary information.

Since there were an unusually high number of deaths determined to not be due to TB in this population, it would be helpful to include the other most common causes of death.

Unfortunately causes of death that were not due to TB, or related to TB were not recorded in our dataset, so we are unable to add this information.

Discussion

Much of the discussion section is a re-statement of the results. I would suggest the authors focus more on interpreting the results from the study and describing how they relate to the published literature.

We agree that the first paragraph of the discussion summarises the results of this study. We feel that this is required as this is the only summary of our findings in the paper, with results presented by outcome in the results section. A large part of the relates to the limitations of the study, and the approaches that we took to mitigate them, that we felt needed to be discussed in order to put our results into context. We feel that paragraphs four and five of the discussion interpret the results from this study and relate to the published literature. However, we would be very happy to add any suggested literature references to the paper, as additional context can only improve the interpretation of our findings.

This study had a large amount of missing data for the exposure and outcome variables of interest and this could be a source of significant bias. This is a major limitation of the study and warrants a thorough discussion (usually the 2nd to last paragraph).

We agree that the proportion of missing data is a significant limitation of this study (as with many studies using routine surveillance data). As outlined above, to explore the impact of this we repeated all analyses using multiple imputation. This is discussed, along with the remaining limitations in the following sections:

"Variable data completeness changed with time, with both BCG vaccination status and year of vaccination having a high percentage of missing data, which may not be missing completely at random. We therefore checked the robustness of our results with multiple imputation including regional variability, however an unknown missing not at random mechanism, or unmeasured confounding may still have introduced bias. We found a greatly increased risk of all-cause mortality for those vaccinated more than 10 years ago in the analysis with multiply imputed data, compared to the complete case analysis. This is likely to be driven by a missing not at random mechanism for years since vaccination, with older cases being both more likely to have been vaccinated more than 10 years previously and to also have an unknown year of vaccination. The high percentage of missing data also means that we were likely to be underpowered to detect an effect of BCG vaccination on sputum smear status and deaths due to TB (in those who died), with years since vaccination, and age at vaccination likely to be underpowered for all outcomes"

Authors' response to Reviewer #2:

There is renewed interest in BCG vaccination. For example, recently published data suggests that BCG revaccination in adolescence may prevent Mtb infection. More data are emerging about effects of different strains, doses and routes of vaccination: all variables that may ultimately significantly impact TB outcomes. This manuscript is therefore very timely and contributes to the knowledge through a thorough retrospective look at specific outcomes of TB disease, and of mortality. There are shortcomings, but the authors have spelled these out. The suggestion that BCG may reduce all-come mortality in older age groups, and that recurrent TB may be prevented by BCG, is intriguing and contributes to our knowledge of this vaccine.

Thank you for your review of our manuscript. We have addressed your comments below.

1. Given the recent revaccination results, it would be good to see a breakdown of the effect of primary vaccination and revaccination on outcome. It is not clear from S4, for example, whether the non-neonatal persons were revaccinated.

We agree that it would have been interesting to explore the impact of revaccination on our findings. Unfortunately, we were not able to investigate this as we did not have data on revaccination. Given that the rate of revaccination in England is thought to be low we feel that any analyses looking at this would likely be underpowered.

2. I am a bit baffled by examining the duration of effect data according to a discrete variable: more or less than 10 year since vaccination... why was age since vaccination not used as a continuous variable in analysis?

We initially considered using a continuous varibale but ultimately decided to use a binary categorical variable for years since vaccination. This was primarily motivated by concerns over the linearity of any relationship between TB outcomes and years since vaccination. Whilst BCG effectiveness appears to decrease in the first 10-15 years after vaccination with a near linear relationship it is unknown whether this continues beyond 15 years. The use of a linear relationship in this scenario may have led to a reduction in the effect size. 10 years was chosen as a breakpoint as this is a conservative estimate of when BCG effectiveness has reduced to near parity with no vaccination. We hypothesised that any protection from negative outcomes would also decrease over the same timescale. We were also concerned the recall bias of year of vaccination would increase with years since vaccination, categorising reduces the impact of this bias. We have now highlighted this in the manuscript with the following statement in the methods secton:

"Years since BCG vaccination was defined as year of notification minus year of vaccination and categorised into two groups (0 to 10 and 11+ years). This was based on: evidence that the average duration of BCG protection is 10-15 years;[15] increasing recall bias with time since vaccination, and any association between years since vaccination and TB outcomes may be non-linear."

To highlight that understanding the functional form for the decay of the effectiveness of the BCG vaccine at preventing negative outcomes we have amended future work section of the discussion as follows:

"Further work is required to determine whether years since vaccination and age at vaccination are associated with TB outcomes as this study was limited by low sample size, missing data for year of vaccination, and the relative rarity of some TB outcomes. However, due to the continuous collection of the surveillance data used in this analysis, this study could be repeated once additional data have been collected. If this study were to repeated with a larger sample size particular attention should be given to the functional form of any decay in protection from negative TB outcomes. The results from this study require validation in independent datasets and the analysis should be reproducible in other low incidence countries that have similarly developed surveillance systems. If validated in low incidence countries, similar studies in medium to high incidence countries should be conducted because any effect would have a greater impact in these settings."

3. The outcome measures used for this study appear randomly chosen - there could be many additional outcomes of TB disease. Such as extent of pulmonary disease on chest roentgenograms.

We agree that there could be additional outcome variables included in this study. However, we could only include outcomes that were included in the routine surveliance dataset on which this study was based. Given the availability of outcome data, we selected outcomes for further evaluation based on evidence from the literature of prior associations with BCG vaccination, associations with TB infectiousness, or poor consequences for patients. We have now highlighted this in the manuscript with the following statement in the methods section:

"The outcomes we considered were: all-cause mortality, death due to TB (in those who died), recurrent TB, pulmonary disease, and sputum smear status. These outcomes were selected based on: their availability in the ETS; evidence from the literature of prior associations with BCG vaccination; associations with increased case infectiousness; or severe outcomes for patients"

4. Similarly, was a specific analysis done to look at extrapulmonary, disseminated or tuberculous disease?

This would be an interesting follow up study as it is plausible that some of the effects we have identified would differ by disease type. However, we did not pre-specify this in our analysis plan and therefore did not explore this in the current study. Additionally, given the low sample size for some outcomes, analyses on disease subset are likely to be underpowered. We have added the following to the final paragraph of our discussion highlighting that this is a logical next step.

"Additionally, a larger sample size would allow investigation of the associations identified between TB outcomes and BCG vaccination stratified by pulmonary, extrapulmonary, and disseminated TB disease"