Author’s response to reviews

Title: Malaria prevention reduces in-hospital mortality among severely ill Tuberculosis patients: a three step intervention in Bissau, Guine-Bissau

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Author’s response to reviews: see over
Dear Sir
The Editors of BMC Infectious Disease,

Thank you for taking our paper into consideration.
We write in response to the critique you sent regarding the above-indicated manuscript. We believe that by following the comments and suggestions that were provided by the reviewers, we have strengthened the manuscript considerably.
Below, please find a point-by-point response to the comments raised by the reviewers.

Reviewer Elizabeth Ashley

Major Compulsory revisions:

1. Please clarify if the study was designed prospectively, why this design was chosen and include a statement on sample size calculation if performed

The study was designed prospectively and the decision was made mainly due to clinical reasons. During 2004, when the hospital re-opened to the public after the civil war, mortality was really high, with peaks of 60% during the rainy season and 30% during the dry season. According to the clinical reports made by the hospital’s physicians, high fever with Plasmodium Falciparum positive blood film was the main reason of death. In agreement with the hospital’s staff, we therefore thought to reduce mortality with a malaria prevention program.
We added the above information, requested by the reviewer, at the end of the Background: “In the Hospital Raoul Follereau (HRF), National Reference Hospital for Tuberculosis and Lung Disease in Bissau, Guiné-Bissau, mortality during 2004 rainy season reached peaks of 60%. According to the clinical reports made by the hospital’s physicians, high fever with Plasmodium Falciparum positive blood film was the main reason of death. In order to reduce mortality during the rainy season in admitted TB patients, since January 2005 we performed a prospective malaria prevention program in the Hospital. Our hypothesis was that the successive combination of personal protection, vector control, and cotrimoxazole prophylaxis could be progressively more effective in reducing mortality in severely affected TB patients, who are admitted for long periods of time for specialized care.”
Sample size calculation was not performed and so a statement on this subject was not included in the text.

2. The objective of the study was stated as reducing mortality. Was there any evidence that malaria was an important cause of death in the rainy season?

Yes, according to reports made after 2004 rainy season by the hospital’s physicians, many deaths occurred in patients with fever and positive blood films (for malaria). No registered data are available, but reports of clinical meetings and grand-rounds routinely performed at the hospital clearly demonstrate malaria burden. (see above answer and information included in the Background).
3. Some more explanation and justification for the choice of the death/discharge ratio as the outcome for comparison rather than mortality rates would be welcome. Please include raw data for deaths and discharges as well as the ratios to aid data interpretation. Can it be assumed that all patients who did not die were discharged? Can a comparison of mortality rates with 95% confidence intervals each year as well as the ratios be presented?

- Raw data have been added. As requested by both reviewers, data have been better presented: Figures 2a and 2b have been replaced with Table 2 and Table 3 which include raw data (admissions, deaths, discharges), mortality (deaths/admissions) with 95% intervals, death/discharge ratio and p-values for rainy season and dry season respectively.
- Death/discharge ratio and mortality have been both used as outcomes for comparison (see Table 2 and 3) and both of them show progressive and significant reduction during rainy season. Nevertheless, we believe that, due to the long Length Of Stay (almost three months) for TB patients, it is more correct to compare the outcomes (death or discharge) independently from when a patient was admitted. In fact, a patient could be admitted in June (at the end of the dry season) but be discharged three months later, in September (during the rainy season).
- All patients who did not die were discharged, since no drop-outs were observed, as stated in the result section
- Comparison of mortality rates with 95% intervals each year has been presented in Table 2 and Table 3.

4. Outcomes: ideally malaria would have been a secondary outcome. If this was not possible please explain why. Was malaria diagnosis clinical or laboratory confirmed?

The following sentence has been added in the Material and Methods section: “A clinical episode of malaria was defined as an axillary temperature ≥37.5 °C together with the presence of malaria parasitemia at any density”

It is true that malaria could ideally have been a secondary outcome. The limited availability of personnel working in the hospital raised several constrains regarding the capacity to register too many data. The National Center for Tuberculosis had been closed for 4 years after the war and therefore, when it re-opened to the public, daily clinical activity was overwhelming for the personnel that had to be re-trained and re-motivated after a long “stop”. Together with the hospital staff we thought it would be better to concentrate on those tasks that they could clearly afford to perform: daily patients care, the three step malaria prevention program and registration of limited amounts of data.

We added a comment in the Discussion section, when considering the limits of the study (see below answer 5)

5. More discussion on the limitations of the design would be worthwhile
This has been done. In the Discussion we added the following observations: “Our study has several limits. Firstly, it is an observational study and therefore does not have the power of a randomized study. Nevertheless, even if it doesn’t compare the three different strategies in a randomized fashion, the three step intervention testes them subsequently and suggests the additive benefit of each successive one (death/discharge ratio dropped from 0.79 to 0.55 to 0.26). Secondly, malaria was not evaluated as a secondary outcome and therefore there is no direct demonstration that the
reduction in mortality was due to the reduction in the number of both malaria episodes and malaria related mortality. Even so, while all treatment protocols and main clinical characteristic of patient population remained fairly unchanged across the three years, the only modifying interventions were related to malaria prevention. A reduction in positive blood films was indeed observed from the period August-November 2005 to August-November 2006 and 2007 (23.65% vs. 15.7% vs. 8.4% respectively, p-value 0.001), and even if the results were not registered on a regular basis and therefore are not completely reliable, they contribute to the hypothesis that malaria reduction was a determinant of reduced mortality.”

Minor Essential Revisions:
1. Results- is it possible to compare outcomes stratified by HIV status?

With the data that were collected during this study it is not possible to compare outcomes stratified by HIV status. Nevertheless, since the number of HIV+ patients was evaluated and was similar across the three years ((43%, 45% and 48% in 2005, 2006 and 2007 respectively) and the number of HIV+ patients receiving ART was also similar across the three years (78%, 75%, 79% in 2005, 2006 and 2007 respectively), we believe that these data are sufficient to allow a comparison between the outcomes of the three years.

2. Please clarify how patients admitted towards the end of a year were dealt with in the analysis and whether admission rates across the months of the 3 years were broadly comparable.

Admission rates were comparable across the rainy season of the three years (39.5, 32.5, 43.25 mean admissions/month in 2005, 2006 and 2007 respectively) while there was a slight reduction in admission rates during the dry season of 2006, due to financial constrains in the hospital that brought to a reduction in admissions during the month of December 2006 (33.62, 27, 43 mean admissions/month in 2005, 2006, 2007 dry season respectively). The data have been added in Table 2 and Table 3. Since the interventions were carried out during the rainy season and admission rates were similar during the rainy season we believe that the slight reduction in admission rates in December 2006 doesn’t affect mortality and death/discharge ratio.

Due to the long LOS both mortality (deaths/admissions) and death/discharge ratio were used to compare the effect of the intervention (see above point 3 Major Compulsory revisions). Therefore patients admitted towards the end of the year were considered as patients admitted during the rest of the year.

3. Can the relative benefits of cotrimoxazole versus the other interventions be explored in more detail?

In the discussion session we stated that “The benefit of cotrimoxazole in our study may have been enhanced by the potential reduction of gastrointestinal illnesses, sepsis [34-35], pneumonia and toxoplasmosis [36-37], often leading causes of death in immunocompromised patients. These factors have not been directly investigated by our study and a prospective randomized study could have enough power to address the specific correlations”.

Cotrimoxazole may have indeed contributed to reduce mortality through multiple effects: reduction of severe malaria episodes and reduction of other severe infections. Our study did not register the number of other infections during the admission, therefore we cannot compare frequency of gastrointestinal illness, pneumonia, toxoplasmosis during the three years of the intervention. This is
a limit of the study (as reported in the discussion), but in Guine Bissau there wasn’t, at the time of the study, the possibility to perform stool, blood or liquor cultures. Clinical episodes of gastrointestinal illness, pneumonia, cerebral disease (toxoplasmosis) were not registered due to limited availability of the personnel (see above answer 4 to Major compulsory revision). We are aware that the relative benefits could have been explored in more details, but it was not a primary purpose of the study. Mentioning the relative adjunctive benefits of cotrimoxazole in the discussion is indeed an incentive to investigate its global effects in a randomized study.

4. It is likely that most malaria in G-B at the time of the study was sensitive to sulfadoxine-pyrimethamine (SP). The findings may not be generalisable to other African countries with high rates of antifol/sulfa drug resistance in bacteria and malarial parasites. The rapid evolution of antifol resistance observed in malaria elsewhere also raises questions about the durability of this strategy. This could be mentioned

We agree with the reviewer and have added a comment in the Discussion: “The rapid evolution of antifolate resistance observed in malaria in sub-Saharan African countries where bacterial resistance to cotrimoxazole is higher and cross-resistance between cotrimoxazole and sulfadoxine-pyrimethamine (SP) may impair SP efficacy for malaria treatment, raises questions about the durability of this strategy. A careful resistance analysis to evaluate the emergence of resistance to the drug should be performed to tailor malaria prevention”.

**Discretionary Revisions:**

1. Introduction- possible interactions between TB & malaria are mentioned and referenced but with no details given. It would be interesting and highly relevant to have any hypotheses summarized

We thank the reviewer for this suggestion. Possible interactions between TB and Malaria have been detailed in the Background section as follows and five more references have been added to the paper (references 11-15):

“In fact, in pulmonary TB there is a transient systemic immunosuppression due to overexpression of transforming growth factor beta and interleukin-10 [11]. Interactions between TB and malaria have been demonstrated both in vitro and in vivo: Plasmodium Falciparum modulates Mycobacterium Tuberculosis infection [12] and malaria has been shown to exacerbate mycobacterial infection [13]. The reasons for this are not completely explored but seem to involve parasite-parasite interaction and host-parasite interaction [12-14]: malaria causes a further depression in immunity through a qualitative and quantitative defect in T lymphocytes, mainly the CD8+ that are necessary for anti-Mycobacterial response, and through a deregulation of the cytokine cascade. Moreover, the respiratory distress frequent during acute malaria both in children (due to metabolic acidosis) and adults (due to pulmonary edema and Acute Respiratory Distress Syndrome) [15], can worsen the respiratory effort related to TB”.

2. Introduction mentions ‘TB seronegative’ patients twice. Presumably this means seronegative for HIV? Should be stated.

The statement is correct and the correction has been made in the Background section
3. Did the same proportion of patients receive antiretroviral therapy each year? These data would be of interest if available

Yes, the same proportion of patients received the Antiretroviral Therapy each year. The information has been added in the Result section (“HIV prevalence was similar during the three years (43%, 45% and 48% in 2005, 2006 and 2007 respectively) as well as the proportion of patients receiving Antiretroviral Therapy every year (78%, 75%, 79% in 2005, 2006 and 2007 respectively)). The protocol was also the same across the years and this has been specified in the Method section (Methods, Diagnosis of TB and Malaria and standard treatment in the following sentence “The hospital’s treatment protocols for TB, Malaria, HIV/AIDS and other diseases were the same in 2005, 2006, 2007)

4. It is likely that the efficacy of chloroquine, even though national policy, was poor. This raises the possibility that patients may have died partly as a result of ineffective treatment for their malaria. Are there data on chloroquine efficacy in Guinea Bissau? I believe national policy has changed to artemether-lumefantrine? This could be added.

This is an interesting point. There are limited data available for Guine Bissau. Nevertheless, Chloroquine efficacy in Guine Bissau doesn’t seem to be reduced by resistance patterns. Several studies suggested that the higher dosages of chloroquine routinely used in Guine Bissau might result in the persistent efficacy of chloroquine in the country (Ursing J, Kofoed PE, Rodrigues A, Rombo L. No seasonal accumulation of resistant P. falciparum when high-dose chloroquine is used. PLoS One. 2009 Aug 31;4(8):e6866. and Ursing J, Schmidt BA, Lebbad M, Kofoed PE, Dias F, Gil JP, Rombo L. Chloroquine resistant P. falciparum prevalence is low and unchanged between 1990 and 2005 in Guinea-Bissau: an effect of high chloroquine dosage? Infect Genet Evol. 2007 Sep;7(5):555-61).

Although National policy officially changed to artemether-lumefantrine in December 2006, due to a low availability of the drug in the country (and a higher price compared to chloroquine), the general attitude both in hospitals and health centers is to prescribe chloroquine. During our study period (2007), artemether-lumefantrine was therefore officially approved but not routinely available.

The above information has been added in the text in the Discussion: “It is unlikely that patients died partly as a result of ineffective treatment for their malaria because chloroquine resistance is scarce in the country probably due to the higher chloroquine dosages routinely used”. A reference have also been added (41).

Reviewer Stephen Graham

1. The introduction is too long and much is repeated in discussion.

As suggested, the introduction has been shortened. As pointed out by the reviewer, comments on the three different approaches were already present in the discussion and therefore have been removed from the Background section. Following the suggestions made by the previous reviewer (see discretionary revisions 1), an hypothesis on TB-malaria interactions has been added to the Background section.

2. Data could be better presented. What are the raw numbers used for “death: discharge ratio”. It is hard to guage accuracy of this ratio but as it is written, it does not seem to match with mortality data.
We agree with both reviewers and have presented the data according to their suggestions, modifying the Result section as following:
- Figures 2a and 2b have been replaced with Table 2 and Table 3 which include raw data (admissions, deaths, discharges), mortality (deaths/admissions), death/discharge ratio and p-values for rainy season and dry season respectively.
- Death/(death+discharge) ratio has been removed since it reflected the same data as death/discharge ratio.

3. Is it possible to get a clear idea of admission policy for TB cases i.e. severity of disease?

Patients coming directly to the outpatient clinic of the Hospital or referred to the outpatient clinic by a TB health center were admitted to the hospital ward according to the attending physician’s clinical judgment: if patients were in poor clinical conditions (important wasting and weakness) or with severe disease (respiratory distress) they were considered unable to perform home treatment and considered needing admission and more comprehensive treatment.

According to the reviewer request we modified the Method/Setting as follows:
“The Hospital Raoul Follereau (HRF) is the National Reference Hospital for Tuberculosis and Lung Disease in Bissau, capital of Guiné-Bissau. TB patients in poor clinical conditions or with severe disease (i.e. important wasting or respiratory distress) are admitted after referrals from regional hospitals or from TB health centers across the entire country.”

4. The first line of abstract and introduction refers to adult disease patterns - pneumonia is a more common cause of death in children than TB, HIV or malaria.

This is true and we agree with the reviewer. We have modified the sentence as follows: “Malaria and Tuberculosis are important causes of morbidity and mortality in Africa.”

5. Did or how did ART usage change with time? Use of ART for TB/HIV changes in practice could be one confounder.

ART usage did not change over time and criteria to begin or withdraw treatment were the same across the three years. See above answer to Discretionary revisions point 3.

6. Was there an effect on mortality by age? Susceptibility to severe malaria is affected by age.

No, there was no effect on mortality by age in our group of patients, even if susceptibility to severe malaria is affected by age. The majority of our patients were adults ranging from 15 to 59 years of age and the numbers of children could be too small to document a possible difference in mortality. The information has been, nevertheless, added to the text in the following sentence “There was no effect on mortality by age in our group of patients, even if susceptibility to severe malaria is affected by age, but this could be due to the relative small proportion of children in our group.” All the changes have been highlighted in the text in green.

The quality of written English has been reviewed by a professional copyediting service as suggested by both reviewers.

We have obtained the trial registration number ISRCTN83944306, which has been added at the end of the abstract. The abstract and the Manuscript have been modified according to journal style.
The Authors’ email have been added to the title page, as requested. Thank you again for taking our paper into consideration and we hope you find it suitable for publication in BMC Infectious Diseases.

Sincerely,

Dr. Raffaella Colombatti