Title: Antifungal Prophylaxis in Chemotherapy-Associated Neutropenia: a Retrospective, Observational Study

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Version: 2 Date: 1 February 2007

Author's response to reviews: see over
February 1, 2007

Dear Editors,

Thank you for your review of our manuscript, Antifungal Prophylaxis in Chemotherapy-Associated Neutropenia: a Retrospective, Observational Study. Please find below our responses to the reviewers’ comments.

Sincerely,

Amy Riedel, Pharm.D.

Reviewer: Dr. De-Silva

1. We strongly encourage you to include an Acknowledgements section between the Authors contributions section and Reference list
   a. An Acknowledgement section has been added to the manuscript.

Reviewer: Leather

2. Correction of multiple periods, commas
   a. The manuscript was edited and corrected for grammar and punctuation

3. Specification of the doses and route of administration of the medications used as prophylaxis
   i. We have added specific doses and route of the medications used as prophylaxis.

4. Suggest reworking the background. Moves from prophylaxis to empiric treatment to prophylaxis. Make a clearer case for rationale and background to study.
a. The background has been revised and hopefully we have made a clearer case for rationale and background.

Reviewer: Basssetti

5. Specify the gravity score of the patients:
   a. Unfortunately, as this was a retrospective study, gravity scores, such as Apache scores were not prospectively collected on the patients. However, in our opinion, this information is unlikely to impact on the outcomes. Our demographics, which are characterized in the manuscript, demonstrate that this was a relatively young, healthy patient population. We believe the comparative demographics between the two patient populations allow for the conclusion that the groups were equal and thus unlikely to be factors for the differences in the observed outcomes.

6. Please specify the patient’s WBC counts:
   a. Please note that all these patients were profoundly neutropenic, as per the eligibility criteria of an absolute neutrophil count less than 500 cells/mm$^3$. We are unable to obtain more specificity than this breakpoint and do not believe this adds to the results. The definition of neutropenic was added to the Patients subsection of the Methods portion of the manuscript for clarification.

7. Specify the number and types of concomitant medications
   a. Information on concomitant medications was not prospectively collected and we are uncertain exactly what medications we would include. Would this information include the chemotherapeutic agents, other antibacterials,
antifungals, pain medications? The numbers of medications administered to these patients is substantial and unlikely to impact on the results. We believe the comparability of the patients in terms of disease state and length of hospitalization is a surrogate marker to the use of comparable medications. Either way, we unfortunately are unable to obtain number and types of concomitant medications at this time.

8. Reasons for discontinuation of antifungals
   
a. This information is included in the Modification of Antifungal therapy section within the Results portion of the manuscript.

Reviewer: Bedogni

9. In statistical analysis, the authors wrote that continuous variables were log-transformed and analyzed using mixed effects logistic regression. The outcome of (binary) logistic regression is dichotomous, so I suppose that these continuous variables were used as predictors. However, I found no reference to them either in the text or in the tables. The same is true for the discrete variables that were analyzed random effects logistic regression. The authors must specify their mixed models because there is no reference to them in the text and many outcomes appear to have been analyzed as “simple” 2 x n Tables. Even more important, they should explain why they preferred mixed to fixed effects models. The reasons that “the bias was statistically minimized” is not tenable because: 1) bias is never modeled adequately post-hoc and 2) mixed models have problems on their own.
a. To take into account multiple hospitalizations for patients, continuous variables (age, length of stay, duration of therapy, costs) were analyzed using mixed effects regression models. Some of these variables (length of stay, duration of therapy, costs) were then log transformed to improve the distribution. These models controlled for the number of hospitalizations and included fixed effects for each medication and for each period.

b. Similarly, dichotomous variables (infection, renal toxicity, hepatic toxicity) were analyzed using random effects logistic regression. These models controlled for the number of hospitalizations and include fixed effects for each medication and for each period.

c. This information is now included in the Statistical Analysis section 10. In Table 1, the authors should give the standard deviation and the range (min-max) of all continuous variables. The SD is a measure of the variability between individuals while 95% CI are obtained from the standard error, which is a measure of uncertainty at the population level. For variables that are not normally distributed, the authors should use the median and interquartile range instead of mean and SD

a. Because some patients were treated multiple times in our study, the simple standard deviation becomes a mix of between patient and within patient variability and does not correctly reflect the variability in the mean. Instead we have fit a statistical model that takes into account the multiple observations of some patients and correctly combines between and within patient variation to a single standard error. In Table 1, reported means are
least squares means from mixed effects regression models that controlled for the number of hospitalizations. The standard errors of those means are now included in Table 1.

11. Which test was used to compare continuous variables between AMB and AZ patients?
   a. The period effect in the mixed effects regression models described above was used for this comparison.

12. Table 1 identifies potential confounders of the relations of interest (age, days from admission to start of anti-fungal therapy, duration of therapy). However, no multivariable analysis is reported in the text. It is vital that the potential confounding effect of these and other clinically relevant variables be taken into account in the evaluation of outcomes.
   a. Multi-variable analyses were conducted for the outcome of total costs using mixed effects regression models that controlled for age, diagnoses, therapy (allogeneic transplant, autologous transplant, chemotherapy, other diagnoses), and duration of antifungal prophylaxis. The cost variables were then log transformed to improve the distribution. These models include fixed effects for each medication and for each period. This information is now in the results sections (under cost analysis).
   b. Multi-variable analyses were also conducted for the outcome of all fungal infection using random effects regression models that controlled for age, diagnoses, therapy (allogeneic transplant, autologous transplant, chemotherapy, other diagnoses), and duration of antifungal prophylaxis.
These models include fixed effects for each medication and for each period. This information is now in the results sections (under breakthrough fungal infections).

13. The kinds of therapy sum to 181 for AMB patients and this equals the total of patients. However, they sum to 235 for AZ patients which is greater than the total of 216 patients. Why this difference?
   a. During the AZ period, there were 216 patients; 19 of these patients were treated with both FLU and VOR during multiple hospitalizations. Therefore, these 19 patients were counted twice in the AZ period (once for receiving FLU and once for receiving VOR). This information is now in the results sections (under patient characteristics).

14. There are 0 AMB patients in the twin-transplant category, making the evaluation of this 2 x 6 table more complicated. Performing an exact Pearson’s chi-square test I obtained a p-value of 0.01051, which is very different from that obtained by the authors (p = 0.09). Why this difference?
   a. The statistical analysis was re-run to compare the odds of having each of the individual therapies in the AZ period relative to the AMB period. These odds ratios are from random effects logistic regression models that controlled for the number of hospitalizations.

15. The authors should add confidence intervals to the estimates of outcomes.
   a. Confidence intervals were added to the estimates of outcomes

16. Why not adding a Table for hepatic toxicity as done for renal toxicity?
a. As one of the comments noted the tables were cumbersome, instead of adding an additional table, the table for renal toxicity was deleted.

Reviewer: AGARWAL

17. The tables are cumbersome. Actually, the results section covers most of the material presented in the tables. So the tables can be shortened and made more precise

a. Table 1 was shortened (baseline renal and hepatic function info was removed as was average number of days from admission to start of anti-fungal). The sub-group data for AZ period was deleted from Table 1 and odds ratios were added. Additionally, the sub-group data was deleted from Table 2 (breakthrough fungal infections). The tables on renal toxicity (previously Table 3) and discontinuation rates (previously Table 4) were deleted. All relevant information from the deleted tables are now included in the text.

18. A reference is made for using logistic regression model. But it is not clear where this is being used. Most of the comparisons between two groups are univariate. If the logistic regression is being used, then the detail of the model (outcome variable, covariates, etc) should be given. Also in that case, the comparison between the “unadjusted” and “adjusted” treatment effect should be made. Only then the real need of the logistic regression model would be justified.

a. To take into account multiple hospitalizations for patients, continuous variables (age, length of stay, duration of therapy, costs) were analyzed using mixed effects regression models. Some of these variables (length of
stay, duration of therapy, costs) were then log transformed to improve the
distribution. These models controlled for the number of hospitalizations
and included fixed effects for each medication and for each period.

b. Similarly, dichotomous variables (infection, renal toxicity, hepatic
toxicity) were analyzed using random effects logistic regression. These
models controlled for the number of hospitalizations and include fixed
effects for each medication and for each period.

c. This information is now included in the Statistical Analysis section
d. Unadjusted odds ratios were run on most of the outcomes. Because the
only covariate that was included in the adjusted odds ratio was the number
of hospitalizations, the unadjusted and adjusted odds ratios were similar.
Therefore only the adjusted odds ratios were reported.

19. In the first para (p. 13), again the reference for “using mixed or random effect
logistic regression” is made. This is being referred for patients who received
multiple courses of antifungals. But it is not clear how are two types of models
being used? In this context, it will be better to do subgroup analysis for patients
receiving multiple courses of antifungals vs. patients receiving single course of
anifungals.

a. The models controlled for the number of hospitalizations and include fixed
effects for each medication and for each period. The clarification was
added to the first para (p. 13). Sub-group analysis was performed on
patients treated in both periods. These patients were determined to have
similar rates of renal dysfunction, hepatic dysfunction, and breakthrough
fungal infections as those patients solely treated in a single study period.

This information is included in the results section.