Author's response to reviews

Title: Dynamics of c-reactive protein and white blood cell count in critically ill patients with nosocomial gram-positive vs. gram-negative bacteremia: a historical cohort study

Authors:

Dominique M Vandijck (Dominique.Vandijck@UGent.be)
Eric A Hoste (Eric.Hoste@UGent.be)
Stijn I Blot (Stijn.Blot@UGent.be)
Pieter O Depuydt (Pieter.Depuydt@UGent.be)
Renaat A Peleman (Renaat.Peleman@UGent.be)
Johan M Decruyenaere (Johan.Decruyenaere@UGent.be)

Version: 3 Date: 2 May 2007

Author's response to reviews: see over
Dear Editor / Dear Referee

We have addressed each of the reviewers’ comments to the above-mentioned manuscript, and numbered our responses sequentially according to the reviewers’ questions.

**REFEREE:**

**Major Compulsory Revisions**

**Question 1:**
My great concern relates to the way statistical analysis was performed as well as the BSI episodes were included. In my opinion the statistical analysis is not entirely correct as I had already pointed out and in addition it is not in accordance with the
authors reply. In response 4 they clearly stated that “only the first episode of a patient was taken into account”, however in the manuscript the authors included successive BSI episodes of the same patient as 105 BSI were analysed from 84 patients. That is to say that at least 21 BSI were used repeated. It is essential to know that this is a major violation of the statistical methods used. According to the SPSS user's guide as an example, the assumption to use the Mann-Whitney U test is to test two independent random samples. And independent samples mean that the subjects should be randomly assigned to two groups, so that any difference is due to the treatment (or lack of treatment) and not to any other factors. Consequently, each patient is measured only once and belongs to one group. Presently the authors have repeated measures of the same patient and they used tests that are no robust to deal simultaneously with within and between patient variations. As a result and as the authors stated in Response 4, only one episode of BSI per patient can be included in the final analysis, to be precise 84 that is the number of patients.

- **Response 1.** The reviewer is correct. After critically rereading and reconsidering our methodology and results section, we have to admit some essential issues are not clear enough for the reader and therefore needs further explanation. As such we made some complementary changes to our manuscript i.e. methods section, results section, and figure 1, aiming to elaborate these issues.

According to the reviewers’ comment, in the previous ‘cover letter – revision 1’ we indeed stated in Response 4 that for analysis only the first episode may be considered. Particularly when interpreting/looking at some parts of the results section, as well as figure 1, they may have been somewhat confusing. First, when looking at figure 1, one can interpret this as more than one episode was taking into account for some patients, which, however, is incorrect. Therefore, we added extra information to figure 1 (two boxes including the respective number of patients with nosocomial BSI caused by either Gram positive or Gram negative bacteria)
aiming to better illustrate the exclusion criteria and the way patients were selected for analysis. In the manuscript figure 1 was adapted as follows:

**Figure 1: Flow chart of exclusion criteria of episodes of nosocomial bacteremia (2003–2004)**

*ICU, intensive care unit; BSI, bloodstream infection; GPB, Gram positive bacteremia; GNB, Gram negative bacteremia*
Second, we want to take the opportunity to stress that the results described in our manuscript were obtained after analysing only the first episode of BSI per patient. Nevertheless, taking into account the above mentioned comment of the reviewer, to be absolutely sure, we performed all statistical analyses once again, and found one sentence in our results section that was incorrect. Regarding the cutt-offs of both, CRP (increase of 5 mg/dL from day -2 to d+1) and WCC levels (increase of 5,000 x 10³ cells/mm³ from day -2 to day+1), the numbers given in the manuscript concerned the total number of episodes of BSI (n=105), and not only the first episode of BSI (n=84). For the sake of clearness, the latter is the one and only correct number to take into account for analysis. Hereby, we thank the reviewer for making us attentively to this fault. Now we have, correctly, calculated these numbers based on the data of the 84 critically ill patients with either a Gram positive (n=36) vs. Gram negative bacteremia (n=48), respectively.

In the text this was changed as follows:

“Both cutt-offs were exceeded in 9/36 (25.0%) and 8/36 (22.2%) of patients with GPB, compared to 35/48 (72.9%) and 36/48 (75.0%) in patients with GNB (P=0.011 and P=0.035, respectively).”

Additionally, in the beginning of the results section, to be clearer, we changed the text a little, including the following information:

“Ninety-three episodes were excluded because of missing laboratory variables (n=29), when polymicrobial (n=40), or when fungal (n=20) or anaerobic pathogens involved (n=4). For 105 episodes (occurring in 84 patients) all data were available. Of these episodes, 42 were classified as Gram positive bacteremia (GPB) (43%) (occurring in 36 patients) and 63 as Gram negative bacteremia (GNB) (57%) (occurring in 48 patients) (Figure 1).”
Finally, at the end of the paragraph ‘study design’ (Methods section) we included an additional sentence stating that per patient only the first episode of BSI was retained for further analysis.

The text was changed as follows:

“For analysis, only the first episode of BSI was considered.”

Discretionary Revisions

Question 1.

1. Response 1b – according to the authors new version of the manuscript 90.5% of the patients with Gram negative BSI as well as with Gram positive BSI were adequately treated. Does this mean that 9.5% of the patients were not adequately treated even after the results from the microbiology lab? The way the text is written can give such an interpretation that for sure is not correct.

- Response 1. We agree with the reviewer. The above mentioned sentence (results) may absolutely not be interpreted as should 9.5% of patients did not receive adequate antibiotic treatment even after culture results, including antibiogram, were available from the microbiology lab. Antibiotic therapy was defined as ‘appropriate’ when the drug administered had in-vitro activity against the isolated strain “and” when initiated within the first 48-hours after sampling the positive blood culture. The latter “detail” was not written down in the ‘definitions’ (Methods section) of our manuscript. Accordingly, it is impossible for the reader to correctly interpret the results and to make appropriate conclusions. Our results reporting that patients with GPB and GNB both received 90.5% adequate therapy should be interpreted taking into account this additional information which is now included in our definition of
‘appropriate therapy’. For the other 9.5% of patients, appropriate antibiotics were initiated with longer time delay (i.e. >48-hours after sampling the positive blood culture, respectively) or some of these patients did not received adequate antibiotics because they died before microbiology results were available.

In the ‘definitions’ (Methods section) we completed the definition of appropriate antimicrobial therapy as follows:

“Antibiotic therapy was defined as ‘appropriate’ when the drug administered had in-vitro and clinical activity against the isolated strain and when initiated within 48-hours after sampling the positive blood culture. Therapy was considered ‘inappropriate’ when there was no activity both, in-vitro as well as clinical against the isolated strains or when no drug was administered.”

Question 2.

2. Response 8 – the APACHE II score has only been validated as a severity score to assess the ICU case-mix and is collected at admission. In the methods the authors should state if this was the case or if it was assessed daily as a surrogate marker of organ dysfunction as it seems from their response; besides it is also important to comment on the discussion concerning an unexpected finding that the APACHE II score seems to have a protective effect on Gram negative BSI (OR,0.87; 95%CI,0.79-0.96; P=0.006) whereas that is not the case on Gram positive BSI (OR,1.10; 95%CI,1.00-1.20; P=0.0044).

- Response 2. The reviewer is correct and we fully agree with both comments made. Regarding the reviewers’ first and second comment, we (i) included the information of daily APACHE II assessment, aiming to evaluate a patients’ clinical evolution, and (ii) further comment on this in the discussion section, as requested.
The text (Methods section) was completed as follows:

“Severity of illness was assessed by means of the Acute Physiology and Chronic Health Evaluation (APACHE) II score and determined daily as a surrogate marker of organ dysfunction aiming to evaluate a patients’ clinical evolution.”

In the results and discussion section the text was adapted to:

“Multivariate logistic regression analysis identified GNB (OR,5.06; 95% CI,1.52-16.91; P=0.008) to be independently associated with a CRP level increase of 5 mg/dL from d_2 to d_{+1}, whereas APACHE II score was not (OR,0.87; 95% CI,0.79-0.96; P=0.006) (Goodness-of-fit; chi-square,2.46; df,8; P=0.963). Gram negative aetiology of bacteremia (OR,3.31; 95% CI,0.96-11.41; P=0.040) and APACHE II score (OR,1.10; 95% CI,1.00-1.20; P=0.044) (Goodness-of-fit; chi-square,6.45; df,8; P=0.587) were both independently associated with an increase of WCC levels from d_2 to d_{+1} of 5,000 x 10^3 cells/mm^3.”

“Using ∆ CRP and ∆ WCC levels in order to evaluate their respective evolution over time, multivariate analysis showed that Gram negative aetiology of bacteremia was independently associated with (i) an increase of CRP levels of 5 mg/dL, and (ii) an increase of WCC of 5,000 x 10^3 cells/mm^3 from two days prior until one day after onset of bacteremia, respectively. Remarkably, APACHE II score was, however, not independently associated with the first scenario (CRP increase), whereas it was independently associated with the latter (WCC increase).”