PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to the Thorax but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Pneumococcal colonisation density: a new marker for disease severity in HIV-infected adults with pneumonia
AUTHORS	Albrich, Werner; Madhi, Shabir; Adrian, Peter; van Niekerk, Nadia; Telles, Jean-Noel; Ebrahim, Naseem; Messaoudi, Melina; Paranhos-Baccala, Glaucia; Giersdorf, Sven; Vernet, Guy; Mueller, Beat; Klugman, Keith

VERSION 1 – REVIEW

REVIEWER	Nicol, Mark University of Cape Town
REVIEW RETURNED	10-Jun-2014

GENERAL COMMENTS	The authors describe an association, in HIV-infected patients with all-cause pneumonia, between pneumococcal nasopharyngeal colonization density and bacteremia with pneumococci as well as mortality. There was no similar association with other markers of disease severity. These results are interesting, but, given the magnitude of the associations, and the overlap between categories, unlikely to, on their own, have significant implications for clinicians looking for a marker to predict poor outcome. Specific comments: 1. Whilst the manuscript describes a study of HIV-infected patients in South Africa, the introduction refers largely to pneumonia in the USA: perhaps the focus of the introduction could be made more relevant to the population studied, and to whom the results could presumably be generalized. 2. The methods section does not refer to HIV status as an inclusion or exclusion criterion, indeed it is unclear throughout the manuscript what proportion of the patients included were HIV-infected. It appears from the discussion that a few HIV-uninfected patients were included. If this is the case, the results should be stratified by HIV status, or the HIV-uninfected patients excluded. 3. Was current antibiotic therapy (other than TB treatment) an exclusion criterion? If not, did this in any way affect results? 4. It is not clear why quantitative NP density was included in one version of the composite diagnostic for pneumococcal pneumonia: it appears that this version of the diagnostic is used only once in the analysis, and does not change the findings. 5. Further description of the patient cohort is needed. For example, it is not stated how many patients were scored as having

	pneumococcal pneumonia in each of the categories of the composite diagnostic. 6. What proportion of those patients with SP-CAP had nasopharyngeal colonization with pneumococci? 7. Table 1: the headings of each column should be clarified: one needs to read the footnote carefully to figure out what are the differences between the left and right panes. 8. Far fewer patients appear to have been tested for CRP than for the other inflammatory markers. What is the reason for this? Were these patients different in any way? This is likely to have affected the likelihood of detecting an association between CRP and severity – this should be acknowledged. 9. In table 2 pneumococcal etiology is associated with a (non-significant) reduction in mortality, however in the text the proportion of patients with pneumococcal etiology who died was higher (14.5% vs. 11.9%). Is this correct? 10. The meanings of the OR's in Table 2 are unclear for all the risk factors. Do these refer to increases in odds with each single unit increase in risk factor, e.g., is each year increase in age associated with a 1.083 increase in odds of death? Were the risk factors stratified in some way? 11. The discussion refers to detection of organism density at the 'site of infection' line 23 – this is not really what is being tested here. 12. The conclusion deals with issues not tested in this manuscript, e.g., monitoring of response to therapy. Perhaps this could be more focused on whether the findings here can be generalized and how useful they are likely to be in practice for assessing severity of pneumonia.

- This manuscript received two reviews at the Thorax but the other referee had declined to make the reviews public.

VERSION 1 – AUTHOR RESPONSE

Comments to the Author
The authors describe an association, in HIV-infected patients with all-cause pneumonia, between pneumococcal nasopharyngeal colonization density and bacteremia with pneumococci as well as mortality. There was no similar association with other markers of disease severity. These results are interesting, but, given the magnitude of the associations, and the overlap between categories, unlikely to, on their own, have significant implications for clinicians looking for a marker to predict poor outcome.

Specific comments:

1. *Whilst the manuscript describes a study of HIV-infected patients in South Africa, the introduction refers largely to pneumonia in the USA: perhaps the focus of the introduction could be made more relevant to the population studied, and to whom the results could presumably be generalized.*

Response: We have modified the introduction in order to place the study in the African setting and make it more specific to patients with HIV-coinfection.

2. *The methods section does not refer to HIV status as an inclusion or exclusion criterion, indeed it is unclear throughout the manuscript what proportion of the patients included were HIV-infected. It appears from the discussion that a few HIV-uninfected patients were included. If this is the case, the results should be stratified by HIV status, or the HIV-uninfected patients excluded.*

Response: Thank you for pointing this out. We have added this in the methods section ("HIV counselling and testing was offered to all patients with unknown or negative HIV serostatus. It was performed using the Architect HIV Ag/Ab Combo assay (Abbott), and, if positive, was confirmed by Elecsys HIV combi assay (Elecsys 2010 analyzer, Roche). The main study population included only patients who were already known to be HIV-infected or newly diagnosed with HIV infection.") and in the results ("Analyses were restricted to those 280 patients with CAP, in whom HIV-infection was present or newly diagnosed.").

3. *Was current antibiotic therapy (other than TB treatment) an exclusion criterion? If not, did this in any way affect results?*

Response: Thank you for suggesting this additional analysis. We have clarified in the methods that "patients with current or recent antibiotic therapy were not excluded". In the results section we have now added this paragraph: "Mean pneumococcal colonization density was not significantly different between patients with and those without antimicrobial activity in urine (3.36 (95% CI: 2.82-3.89) copies/ml vs. 3.95 (95% CI: 3.30-4.60); p=0.16). There was also no difference in in-hospital mortality between those with and those without antimicrobial activity in urine (13.2% vs. 11.5%; p=0.69)."

4. *It is not clear why quantitative NP density was included in one version of the composite diagnostic for pneumococcal pneumonia: it appears that this version of the diagnostic is used only once in the analysis, and does not change the findings.*

Response: We have clarified in table 1 the definition of the expanded composite diagnostic including the NP density. This confirms that addition of NP density in the definition of pneumococcal pneumonia does not change the association between pneumococcal etiology and prognostic biomarkers. If the editor prefers, we can omit this information from the paper.
The reason that we did not add the NP density to the pneumococcal composite diagnostic in the other analyses was that we tested NP density as a covariate in the univariate and multivariable analyses and the AUC-ROC curve.

5. *Further description of the patient cohort is needed. For example, it is not stated how many patients were scored as having pneumococcal pneumonia in each of the categories of the composite diagnostic.*

Response: This information had been previously published (Albrich et al, Clin Infect Dis 2012;54(5):601-9). We have added a brief summary in the first paragraph of the results section ("Based on the composite diagnostic criteria, SP-CAP was diagnosed in 99 (35.4%) of 280 patients: 75 had either a positive urine ICT, or a blood culture, good quality (i.e. >25 neutrophils and <10 epithelial cells per high-power field) sputum Gram stain or culture with pneumococcus 22; 58 patients had a positive whole blood lytA rtPCR. A lytA rtPCR from NP >8000 copies/ml was present in 126 patients, resulting in a diagnosis of pneumococcal CAP based on the expanded composite diagnostic in 150 (53.6%) of 280 patients.").

6. *What proportion of those patients with SP-CAP had nasopharyngeal colonization with pneumococci?*

Response: We have added this to the results section: "A lytA rtPCR from NP >8000 copies/ml was present in 126 patients".

7. *Table 1: the headings of each column should be clarified: one needs to read the footnote carefully to figure out what are the differences between the left and right panes.*

Response: We have clarified the headings of the columns in table 1 ("Based on composite diagnostic standard[1]" and "Based on expanded composite diagnostic standard[2]") with reference to the footnotes 1 and 2.

8. *Far fewer patients appear to have been tested for CRP than for the other inflammatory markers. What is the reason for this? Were these patients different in any way? This is likely to have affected the likelihood of detecting an association between CRP and severity – this should be acknowledged.*

Response: Thank you for pointing this out. CRP was measured immediately on site only if requested by the treating physicians, while the other biomarkers were collectively measured in batch (added to the manuscript). We have added the following information (footnote to table 1: "Since CRP values were available only when requested by the treating physicians, we compared patients with available values for CRP to patients without available CRP values. There was no difference in mean age, in mean NP colonization density and no difference in pneumococcal diagnosis. However, patients with an available CRP value had a significantly higher (30.6%) in-hospital mortality compared to patients without a CRP value (15.3%) (p=0.02).")

9. *In table 2 pneumococcal etiology is associated with a (non-significant) reduction in mortality, however in the text the proportion of patients with pneumococcal etiology who died was higher (14.5% vs. 11.9%). Is this correct?*

Response: Table 2 is correct. Unfortunately, the previous version of the manuscript had an error. True is (and stated in the new version of the manuscript):" The in-hospital case fatality rate was not different between those with (11.7%) and those without (14.1%; p=0.53) pneumococcal aetiology." Thank you for alerting us to this mistake.

10. *The meanings of the OR's in Table 2 are unclear for all the risk factors. Do these refer to increases in odds with each single unit increase in risk factor, e.g., is each year increase in age associated with a 1.083 increase in odds of death? Were the risk factors stratified in some way?*

Response: Odds ratios are reported as increase per single unit of the respective risk factor, e.g. per year (age), per point (CURB65 score), per cell/µl (CD4 count). This is stated as a footnote to table 2. There was no stratification.

11. *The discussion refers to detection of organism density at the 'site of infection' line 23 – this is not really what is being tested here.*

Response: You are right, this was not tested. We have clarified this in the discussion: "Due to the lack of lung specimens or bronchoalveolar lavage samples representing the direct site of infection, we instead chose to correlate NP colonization density with prognosis."

12. *The conclusion deals with issues not tested in this manuscript, e.g., monitoring of response to therapy. Perhaps this could be more focused on whether the findings here can be generalized and how useful they are likely to be in practice for assessing severity of pneumonia.*

Response: Our previous conclusions, which admittedly dealt with issues not tested in the manuscript, were moved to the discussion section. These questions and possible utilities need to be addressed in future studies. The conclusion now is less speculative and reads: "As previously reported, the quantitative lytA rtPCR from NP swabs is a very promising tool to diagnose pneumococcal pneumonia [22]. In addition, this study shows that the same assay also conveys some prognostic information as it correlated with bacteremia, survival and prognostic biomarkers. How exactly it could be implemented

and how it might change management, such as site of care, antibiotic choices and duration needs to be determined.”