PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | Assessment of simple risk markers for early mortality among HIV infected patients in Guinea-Bissau: A cohort study |
|---------------------|-----------------------------------------------------------------------------------------------------------|
| AUTHORS             | Oliveira, Ines ; Andersen, Andreas; FURTADO, Alcino; MEDINA, Candida; DA SILVA, David; DA SILVA, Zacarias; Aaby, Peter; LAURSEN, Alex Lund; Wejse, Christian; EUGEN-OLSEN, Jesper |

VERSION 1 - REVIEW

| REVIEWER            | Frank Tacke
|---------------------|------------------------------------------|
|                     | University Hospital Aachen, Germany      |
| REVIEW RETURNED     | 01-Jul-2012                               |

| GENERAL COMMENTS    | The group by Eugen-Olsen aimed at developing easier risk stratification strategies for HIV infected individuals in low income countries. Their study is interesting and valuable. However, I have some comments. |
|---------------------|-----------------------------------------------------------------------------------------------------------|
|                     | 1. In order to justify any new score for further prospective validation (which should be the aim of future studies), it is important that the patients are characterized more precisely. Especially: how many of the patients were HCV-positive? HBV-positive? How many were in the status of AIDS, defined by which illnesses? |
|                     | 2. suPAR is truly an interesting marker in this situation. Many recent publications nowadays indicate that suPAR's prognostic value might be related to its ability to display organ dysfunction. Thus, it is important to present data on liver and renal dysfunction and discuss these pathophysiological associations in more detail. |
|                     | 3. To me, the causes of death are not clear in the patients, but likely very important if the new prognostic parameters are intended to be used to prioritize HAART therapy. Thus, detailed information needs to be given, and subgroup analyses for the major types of mortality would be appreciated. |
|                     | 4. The authors suggest that suPAR and other markers might be helpful to identify patients in need for urgent therapy. It would then be very important to see how these marker change during therapy. Are they just an epiphenomenon of CD4 counts and HIV viral load? Thus, I think the authors should at least for a small cohort of patients present data during (successful or non-successful) therapy. |

| REVIEWER            | Eugène Messou, MD PhD
|---------------------|------------------------------------------|
|                     | CePReF- Aconda Côte D'Ivoire, Université de Cocody, Abidjan |
| REVIEW RETURNED     | 22-Jul-2012                               |
# RESULTS & CONCLUSIONS

The definition of the lost to follow up appeared very restrictive. How many patients returned in the follow-up after being considered lost to follow-up? It is necessary to describe patients who are lost to follow-up, compare them to those which are followed in the same way it has been done with the death because the two phenomena are closely linked. 11% of patients were the lost to follow-up could increase the number of deaths in the case where some patients among these lost to follow-up were already dead in fact. Thus analyses looking for risk of death shown in tables 1 and 3 should include a sensibility analysis by considering that the lost to follow-up are dead in order to see if the results did not change.

# GENERAL COMMENTS

**General idea**

We read with attention the work on the topic: Review of article assessment of simple risk markers for early mortality among HIV infected patients in Guinea-Bissau: A cohort study of Inês OLIVEIRA. The article deals with a subject that although a little outdated remains topical for countries with limited resources. To this day it is not desirable to make choices among HIV-infected subjects based on criteria such as the risk of death. However, the program are not necessarily equipped to implement the recommendations of the international institutions such as the WHO and donors. It is in our view the case in Guinea Bissau where ARVS are not sufficient for all patients seeking treatment. This situation therefore gives this work an importance and interest. Finding patients may not be placed under treatment according to international recommendations based on the measurement of the CD4. Frequent stock out of input of laboratory does not allow practitioners to have the results of CD4 measurement to begin deciding the eligibility of patients on the one hand. On the other hand if the patients are eligible with the rate of CD4, ARV drugs stocks do not initiate treatment for all. Anthropometric measurements if they can provide proof to the risk of death will help the practitioner to decide who will have to receive ARV treatment.

**Minor criticisms**

If there is no possibility to make routine CD4 counts, how will be the monitoring of the efficacy of ARV in Guinea-Bissau treatments as it is shown in the work of Messou and collaborators (bull who) that these settings are not valid or reliable to identify patients in failure or in success in the first months of ARV treatment.

The changes in population of analysis make difficult understanding.

Last column of table 3: need to adjust the scope of the boxes for figures

**Major criticisms**

The selection of the population does not give the proportion of women in pregnancy. The effect of pregnancies in the amendment...
of the weight was also a weakness described in other studies. If no woman did pregnancy, then the question is: can we consider that the pregnancy was a criterion for exclusion in your analyses?

The definition of the lost to follow up appeared very restrictive. How many patients returned in the follow-up after been considered lost to follow-up? It is necessary to describe patients who are lost to follow-up, compare them to those which are followed in the same way it has been done with the death because the two phenomena are closely linked. 11% of patients were the lost to follow-up could increase the number of deaths in the case where some patients among these lost to follow-up were already dead in fact. Thus analyses looking for risk of death shown in tables 1 and 3 should include a sensibility analysis by considering that the lost to follow-up are dead in order to see if the results did not change.

The use of the suPAR which is a laboratory test requires machines and inputs that can be stock out in the program of poor country. on the basis of limited means. If the result with the BMI, the MUAC and CD4 appear interesting, it is not the same for the suPAR which the measure will require inputs.

Conclusion

If the sensibilities analyses can be add to tables, the article would be complete. However this work has shown to us reality in the theoretical procedures dictated by international organizations. I wish a publication of this article to allow more people to read and then they will continue research on anthropometric parameters because in the hope that all people infected with HIV are treated by ARVs, we will have to redouble their ingenuity for the monitoring of programs of support for PLHA everywhere in the world

RESPONSE TO REVIEWER 1: Frank Tacke, University Hospital Aachen, Germany

Dear Dr Tacke,

Thank you very much for reviewing this paper and for your detailed comments and suggestions.

We have tried to revise the manuscript in line with your suggestions and comments. The specific changes and response to the different points raised include:
R1-1. In order to justify any new score for further prospective validation (which should be the aim of future studies), it is important that the patients are characterized more precisely. Especially: how many of the patients were HCV-positive? HBV-positive? How many were in the status of AIDS, defined by which illnesses?

Comment 1-1:
We agree it would be of interest to determine co-infections and co-morbidities. A recent study (Haupt, crit care Aug 2012) found that suPAR was strongly correlated to the Charlson Score, with increasing number of co-morbidities with increasing suPAR. Thus, knowledge of co-infections may aid in designing decision trees on diagnostic tests that should be carried out on HIV-patients with high suPAR levels. Unfortunately, resources are limited in Guinea-Bissau and knowledge of co-infections limited. However, we have the following knowledge regarding co-infections:

HBV-positive:
Even though international guidelines recommend to test for HCV and HBV when enrolling HIV patients in HIV care, this practice was not common in Guinea-Bissau at the time of the study due to lack of funding. However, during the periods when the rapid HBV test [detecting the hepatitis B surface Antigen (HBsAg)] was available thanks to donations, the patients were tested. Unfortunately we only have this information in 155 out of 1,083 patients included in the present cohort and the prevalence of positive HBsAg test was 11% (17/155). Although it is a small subgroup, we have included the HBV prevalence in the table describing baseline information of patients provided in supplementary data file 1. Now it is routine to test for HBV in the clinic and we have in stead in the discussion provided data on HBV in the cohort on lines 432-434 page 21, in a larger sample of 1,450 tested in the HIV cohort the prevalence of HBsAg positive was also 11%.

HCV-positive:
Unfortunately we do not have any information available regarding HCV prevalence from the cohort presented in this paper, however there is some information from the subsequent study done in the Bissau-HIV cohort. This data has been included in the manuscript in page 21, from 434 to 436 lines.

WHO-stage:
This variable was included in the database once the study was already running and for this reason the information is only available in 51.7% (560/1,083) of the patients included. However, after the
introduction of the new variable the staff had difficulties identifying the WHO-stage of the patients at inclusion. For this reason several training courses were needed during the study in order to improve performance. Considering the questionable quality of this variable during the initial months of the study, we have decided not include this information in the analysis.

The lack of this information has now been included in limitations of the study (page 24, lines 505-506).

**Status of AIDS and defined illnesses**

Unfortunately we have not recorded the main defining diseases in the database. However, physicians who have been working in the clinic for years refer the following diseases as the most commonly seen in the clinic:

*For Who-stage 1*, asymptomatic patients sent from the blood bank of the hospital, asymptomatic young people that came to the clinic for screening or asymptomatic partners of the patients.

*For Who-stage 2*, mainly patients with Herpes Zoster.

*For Who-Stage 3*: Unexplained severe weight loss, unexplained chronic diarrhoea, unexplained persistent fever for >1 month and current pulmonary tuberculosis.

*For Who-stage 4*, HIV wasting syndrome and extrapulmonary tuberculosis. Only few cases of kaposi Sarcoma are seen.

Nevertheless, we don’t have this information systematized recorded for being able to add this data to the tables of the paper. The lack of this information has been included in limitations of the study (page 24, lines 505-506).

To make clear all the issues discussed so far, we have added a clarifying section following sentences to the discussion (pages 20-21, lines 430-446).

**R1-2. suPAR is truly an interesting marker in this situation. Many recent publications nowadays indicate that suPAR’s prognostic value might be related to its ability to display organ dysfunction. Thus, it is important to present data on liver and renal dysfunction and discuss these pathophysiological associations in more detail.**
C1-2:

It would be very interesting to discuss the association between organ dysfunction and suPAR levels. However, even though biochemistry measurement is routine now in the clinic, it was not at the time of this study was carried out. For this reason there is only information available of liver and renal function values in a subgroup of the patients.

Please note that we have rewritten the discussion to accommodate this relevant question (please see answer to question 1) and the new section in the discussion page 21, lines 437 to 453.

Furthermore, we provide below a description of these values by gender (due to the different thresholds) and 3 scatter plots from linear regressions of AST, ALT and creatinine on suPAR where we can observe weak but significant positive correlations between suPAR and creatinine, suPAR and Aspartate aminotransferase and suPAR and Alanine aminotransferase. We have included this information in page 13, lines 279-283.

Information divided by gender:

Median of AST and of ALT at inclusion for men was 30.8 UI/L [IQR: 23.9-50.8. Reference value: ≤ 37 UI/L] and 21 UI/L [IQR: 14-34. Reference value: ≤ 42 UI/L] respectively."

Median of AST and of ALT at inclusion for women was 29.3 UI/L [IQR: 19.2-41.5 Reference value ≤ 31 UI/L] and 15.6 UI/L [IQR: 10-25.9. Reference value:≤ 32 UI/L] respectively."
R1-3. To me, the causes of death are not clear in the patients, but likely very important if the new prognostic parameters are intended to be used to prioritize HAART therapy. Thus, detailed information needs to be given, and subgroup analyses for the major types of mortality would be appreciated.

C1-3:

When HIV treatment was initiated, many of our patients had progressed disease (AIDS). Despite treatment initiation, many were too weak to recover. We have not reported specific symptoms of death, but we are currently working on establishing a verbal autopsy routine.
To clarify this issue, we have added this as a limitation of the study in the discussion: lines 506-517, page 24.

R1-4. The authors suggest that suPAR and other markers might be helpful to identify patients in need for urgent therapy. It would then be very important to see how these markers change during therapy. Are they just an epiphenomenon of CD4 counts and HIV viral load? Thus, I think the authors should at least for a small cohort of patients present data during (successful or non-successful) therapy.

C1-4:

It would be very interesting to see how the suPAR levels change during follow-up, and we do collect blood samples during follow-up in order to be able to address this question in the future. Yet, it has been impossible to be able to measure follow-up samples within the current deadline for response to reviewer comments. Furthermore the scope of this study was to show how high levels of SUPAR are associated with short term mortality and how this information could help physicians to choose which patients should be prioritized for starting ART without delay.

Furthermore, suPAR and viral replication are not intimately associated in untreated ART-HIV patients. Previous studies have shown that suPAR and VL only correlate weakly or do not correlate at all. Changes in plasma suPAR were independent of changes in VL and CD4 cell count but were strongly correlated with plasma levels of the soluble TNF receptor II. Therefore, it should be noted that suPAR is not a replacement for VL or CD4 count.

We have included a clarification in the manuscript discussing this issue. Pages 21-22, lines 449-457.

RESPONSE TO REVIEWER 2: Eugène Messou, MD PhD

CePReF- Aconda Côte D'Ivoire, Université de Cocody, Abidjan
Dear Dr Messou,

Thank you very much for reviewing this paper and for your comprehensive comments and suggestions.

We have tried to revise the manuscript in line with your suggestions. The specific changes and response to the different points raised are explained below.

R2-1. The definition of the lost to follow up appeared very restrictive.

C2-1:

The definitions of lost to follow-up vary substantially among different studies. Missed 1 or 2 scheduled visits, or missed last scheduled visit by 2-12 weeks are the most common used.

In our manuscript we have used the definition of 2 missing appointments because it was the definition of LTFU used in the country for statistical purposes.

We have added a clarification about LTFU in the Statistical methods and analysis, pages 9-10 from 202 to 206 lines and provided a reference in support of our choice of definition.

R2-2. How many patients returned in the follow-up after been considered lost to follow-up?

C2-2:

During the study period 41 patients of the cohort returned to the clinic after been considered lost to follow-up. We have added this information on page 13, line 288.

R2-3. It is necessary to describe patients who are lost to follow-up, compare them to those which are followed in the same way it has been done with the death because the two phenomena are closely linked. 11% of patients were the lost to follow-up could increase the number of deaths in the case where some patients among these lost to follow-up were already dead in fact. Thus analyses looking for risk of death shown in tables 1 and 3 should include a sensibility analysis by considering that the lost to follow-up are dead in order to see if the results did not change.
Please find in supplementary data file 4 a table where they are compared alive patients vs. dead and alive patients vs. LTFU subjects. In this analysis we observe that patients LTFU have higher suPAR, lower CD4 and are thinner than patients still alive and attending clinic at end of follow-up, although not as much as those who we know died. This table has been explained in discussion page 24, lines 518-521. In the view of this analysis it is likely that the effects of suPAR, CD4, BMI, MUAC, sex and HIV are underestimated and this fact has been included as a limitation in pages 24-25 (lines 521-523).

R2-4. If there is no possibility to make routine CD4 counts, how will be the monitoring of the efficacy of ARV in Guinea-Bissau treatments as it is shown in the work of Messou and collaborators (bull who) that these settings are not valid or reliable to identify patients in failure or in success in the first months of ARV treatment.

Regarding the assessment of the response to ART, we are aware that this is a critical step in settings without access to plasma viral-load measurement, which is the case in Guinea-Bissau and in many HIV-clinics in Sub-Saharan Africa.

In the study carried out by Messou et al [Bulletin of WHO. June 2008, 86(6)] it was assessed the gain or loss in BMI, alone or in combination with the gain or loss in CD4 as a tool for predicting the response to ART. The conclusion of the study was that a high BMI gain does not reflect virological success, even when combined with a high CD4 gain.

It would be interesting to see if these results are confirmed in other populations and to assess if the suPAR measurement (in combination with the MUAC, BMI and CD4 gain) could help to distinguish the patients with detectable viral-load from the ones who reach viral suppression. Our group is working in follow-up samples and we hope to be able to contribute to knowledge in this area in the near future.

We have included a more detailed description of this in the discussion (pages 21-22, lines 450-457).
R2-5. The changes in population of analysis make difficult understanding.

C2-5:

We thank the reviewer for pointing this out. In order to make the manuscript more readable and understandable we have added explanatory cohort descriptions in Statistical methods and analysis section. Page: 11, from 235 to 241 lines.

R2-6. Last column of table 3: need to adjust the scope of the boxes for figures

C2-6: We have now revised the tables so that they are in accordance with the format requested by BMJ Open.

R2-7. The selection of the population does not give the proportion of women in pregnancy. The effect of pregnancies in the amendment of the weight was also a weakness described in other studies. If no woman did pregnancy, then the question is: can we consider that the pregnancy was a criterion for exclusion in your analyses?

C2-7:

The information about pregnancy was only available in 373/764 (48.8%) of the women included in the study. In this subgroup there were 8 pregnancies recorded.

We have included a sentence in the discussion (page 24 lines 505-506) in order to point out this limitation.

R2-8. The use of the suPAR which is a laboratory test requires machines and inputs that can be stock out in the program of poor country on the basis of limited means. If the result with the BMI, the MUAC and CD4 appear interesting, it is not the same for the suPAR which the measure will require inputs.

C2-8:
We agree with the reviewer in this issue. One aspect of why suPAR is a potential monitoring tool to test in developing countries such as Guinea-Bissau is the simplicity of the assay (an ELISA). However, although ELISA is simpler to measure than viral load or CD4 count, it still requires a laboratory infrastructure and skilled personal. As suPAR is expressed in high amounts in blood (Nanograms/ml), it should be possible to measure using lateral flow assay (sticks) similar to those used in e.g. pregnancy tests. Such a test has recently been developed (www.suparnostic.com) and it is thus appropriate to test whether this suPAR quick test has the potential as a risk marker monitoring tool.

As an example of the kind of constraints that can be suffered in the ground, between January 2007 and December 2009 we have recorded in the database that CD4 count was not available in the country during 14 out of 36 months of follow-up. The main causes were problems with power supply or due to lack of reagents for the CD4 count equipment in the National Public Health Laboratory.

Our knowledge about this reality was one of the main reasons why alternative ways to select patients to whom ART must be prioritized in situations with laboratory constrains or scarce resources are explored.

Interestingly we observed that suPAR and MUAC gave same sensitivity and higher specificity compared to CD4 ≤ 200 cells/μL and could be used when CD4 count is not available. Furthermore, we found that MUAC alone can be used as a very simple prioritizing tool if nothing else is available. This information is relevant for countries such as Guinea-Bissau with an unstable drug supply system and a developing laboratory but it is also applicable for ART-clinics located in rural areas of any LMICs.

**VERSION 2 – REVIEW**

| REVIEWER           | Frank Tacke  
|--------------------|---------------|
|                    | University Hospital Aachen, Germany |
|                    | I declare no competing interests. |

| REVIEW RETURNED    | 26-Sep-2012 |

| THE STUDY          | For the “limitations of the study” the authors should not state that complete information was available in 58% of the patients, because this is misleading. Only very limited data are available for the patients, due to restrictions in the third-world-country-setting. The study results cannot be translated into typical patients of Western countries. |

| GENERAL COMMENTS   | This is an interesting and relevant manuscript that has been further improved in the revision process. However, I would suggest to openly point out the limitations in the appropriate section (following the abstract). |
