Assessment of simple risk markers for early mortality among HIV-infected patients in Guinea-Bissau: a cohort study

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ABSTRACT

Background: Decisions about when to start antiretroviral therapy (ART) are normally based on CD4 cell counts and viral load (VL). However, these measurements require equipment beyond the capacity of most laboratories in low-income and middle-income settings. Thus, there is an urgent need to identify and test simple markers to guide the optimal time for starting and for monitoring the effect of ART in developing countries.

Objectives: (1) To evaluate anthropometric measurements and measurement of plasma-soluble form of the urokinase plasminogen activator receptor (suPAR) levels as potential risk factors for early mortality among HIV-infected patients; (2) to assess whether these markers could help identify patients to whom ART should be prioritised and (3) to determine if these markers may add information to CD4 cell count when VL is not available.

Design: An observational study.

Setting: The largest ART centre in Bissau, Guinea-Bissau.

Participants: 1083 ART-naïve HIV-infected patients.

Outcome measures: Associations between baseline anthropometric measurements, CD4 cell counts, plasma suPAR levels and survival were examined using Cox proportional hazards models.

Results: Low body mass index (BMI≤18.5 kg/m²), low mid-upper-arm-circumference (MUAC≤250 mm), low CD4 cell count (≤350 cells/μl) and high suPAR plasma levels (>5.3 ng/ml) were independent predictors of death. Furthermore, mortality among patients with low CD4 cell count, low MUAC or low BMI was concentrated in the highest suPAR quartile.

Conclusions: Irrespective of ART initiation and baseline CD4 count, MUAC and suPAR plasma levels were independent predictors of early mortality in this urban cohort. These markers could be useful in identifying patients at the highest risk of short-term mortality and may aid triage for ART when CD4 cell count is not available or when there is shortness of antiretroviral drugs.

INTRODUCTION

Since its introduction in the mid-1990s, antiretroviral therapy (ART) has reduced the morbidity and mortality of HIV-infected patients worldwide.1–3 Nevertheless, despite progress, access to these lifesaving drugs remains limited where the need is the
Risk markers for early mortality among HIV-infected patients in Guinea-Bissau

ARTICLE SUMMARY

Strengths and limitations of this study
- Data presented could help health providers to identify patients with the highest risk of short-term mortality and to prioritise treatment among eligible patients in a context of poor healthcare infrastructure.
- There are several limitations to this study. First, information on all four investigated risk factors (CD4, suPAR, BMI and MUAC) was only available in 58% (628/1083) of our population. Second, WHO-stage, pregnancy status and comorbidities presented at the time of inclusion were not recorded in the database. Third, contact information for some patients was not sufficient to allow active tracing in case of missed appointments. Thus, it is likely that patients lost to follow-up may include unascertained deaths and that the effects of the risk factors are underestimated in our work.

easily measured on all patients, including bedridden ones, and does not require further calculations. Nevertheless, studies assessing this marker in HIV adults in LMICs are scarce.

SuPAR is a risk biomarker protein that reflects the level of immune activation and inflammation. It is known that immune activation plays an important role in the pathogenesis of HIV infection. High blood levels of suPAR have been associated with poor clinical outcome and independently predict mortality in non-ART-treated HIV infected patients. Furthermore, plasma concentrations of suPAR can be measured using a simple ELISA or lateral flow quick tests and thus require less-sophisticated laboratory infrastructure than that needed for routine measurements of CD4 count or plasma VL.

The rollout of ART in Guinea-Bissau started in 2005. All treatments are free of charge in the country, however during the later years frequent shortages of ARV drugs and reagents for CD4 count equipment, have hampered the management of HIV patients in the country and has enforced clinicians to prioritise treatment among eligible patients.

The objectives of the present study were: (1) to evaluate anthropometric measurements, other than weight loss and measurement of suPAR levels as potential risk factors for early mortality in an urban cohort of HIV-1, HIV-2 and HIV-double-infected patients; (2) to examine whether these markers could help in identifying patients to whom ART should be prioritised when CD4 cell count is not available or in situations of shortness of ARV drugs and (3) to assess whether these markers may add information to CD4 cell count and aid in selection of patients most in need of ART among eligible patients when VL is not available.

METHODS

Setting
The Republic of Guinea-Bissau has an estimated population of 1 600 000 inhabitants and an economy based primarily on farming and fishing activities. More than 2/5 of the population lives below the poverty line. Ranking 176 out of 187 countries on the United Nations Human Development Index 2011, it is one of the poorest countries in the world. The HIV National Programme was implemented by the Ministry of Health (MINSAP) in 2005. The combined ART is free of charge in the country, the cost being fully subsidised by the Global Fund to Fight AIDS, Tuberculosis (TB) and Malaria, the Brazilian Government and other international partners.

At the beginning of 2010, there were 26 ART centres in Guinea-Bissau, with more than 7000 patients on a follow-up and 2764 patients on treatment.

Study population
The National Simão Mendes Hospital (NSMH), located in the capital, Bissau, is the reference hospital in the...
country. The outpatient ART centre of NSMH (ART-NSMH) is the largest ART centre in Guinea-Bissau in terms of patients on a follow-up. At the end of 2009, one-third of the patients on ART in the country were followed in this centre.32

The present study population includes all HIV-infected patients, aged 15 years and older, included in the Bissau-HIV cohort between July 2007 and December 2009. The aims and characteristics of the cohort have been described elsewhere.32 During the study period, National ART guidelines were based on the 2002 WHO recommendations.33 The standard first-line ART for HIV-1 patients comprised Zidovudine (AZT), Lamivudine (3TC) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) (Efavirenz (EFV) or Nevirapine (NVP)). Regarding HIV-2 and dually infected patients (HIV-1+2), the standard first-line comprised AZT, 3TC plus Indinavir/ritonavir (IND/rtv). ABC was offered as an alternative to the protease inhibitor (PI) when CD4 count was over the 200 cells/μl threshold. Stavudine (d4t) was the alternative to AZT for patients with anaemia (haemoglobin ≤8 g/dl) in all groups.

Study description
Information about the study was provided by experienced assistants at the time of inclusion in the Bissau-HIV cohort. After signing the consent form, an interview was carried out using a structured questionnaire and a venous blood sample of 8 ml was collected at the clinic when quick medical decisions were needed. A rapid test, Standard Diagnostics, Inc, Korea) was used in the clinic while samples were collected. SD Bioline (HIV 1+2 SD Bioline HIV 1/2 3.0 rapid test, Standard Diagnostics, Inc, Korea) was used. Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) was used for HIV screening while HIV-1 and HIV-2 were assessed by Immunocomb II HIV-1&2 Bispot (Orgenics, Yavne, Israel) were the tests used. A normal BMI was defined as BMI<18.5 kg/m² and malnutrition was defined as BMI<18.5 kg/m² according to the United Nations FAO criteria.35 Low MUAC was defined as MUAC ≤250 mm.

A forward selection procedure for selecting predictive markers with p<0.05 was used to create a multivariate Cox model. Mortality according to CD4≤350 cells/μl, BMI≤18.5 kg/m² and MUAC≤250 mm were further assessed. Receiver operating characteristic (ROC) curves were applied to assess the prognostic capacity of each marker as well as the area under the curve (AUC) was presented. The Youden Index36 was used to calculate the cut-off yielding the maximal joint sensitivity and specificity. Measurements of MUAC, BMI and especially suPAR were not available for all patients (see online Supplementary data file 1). Some patients were too sick to stand to measure height and weight. Consequently, mortality was very high in the group with the BMI information missing. The predictive capacity of BMI has therefore been penalised in the analyses where these missing observations have been excluded. Many suPAR measurements were missing because samples were lost during transportation from Guinea Bissau to Denmark.

Laboratory methods
HIV testing
HIV screening was carried out using a rapid test (Determine HIV-1/2 assay (Abbott Laboratories, Abbott Park, Illinois, USA). Patients testing positive were sent to the National Public Health Laboratory (NPHL), the reference laboratory in the country, for confirmation and discrimination. Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland) as well as Immunocomb II HIV-1&2 Bispot (Orgenics, Yavne, Israel) were the tests used. SD Bioline (HIV 1+2 SD Bioline HIV 1/2 3.0 rapid test, Standard Diagnostics, Inc, Korea) was used in the clinic when quick medical decisions were needed or when there was a rupture of stock of reagents in the NPHL.

CD4 cell count
CD4 cell counts were obtained using the Partec CyFlow SL_3 (Cyflow SL, Partec, Munster, Germany) at the NPHL.

Plasma suPAR measurement
Plasma samples were stored at—20°C at the NPHL and sent to Denmark and measured using the suPARnostic ELISA kit (ViroGates, Copenhagen, Denmark) according to the manufacturer’s instructions. One kit served to process 82 samples in <2 h. The interassay variation of a control sample run on all plates was 12%.

Statistical methods and analysis
Data were entered into an Access database by trained data entry staff and the analysis was carried out using STATA V.11. Patients who did not attend the clinic for two consecutive months (missed two scheduled visits) and for whom vital status was unknown were registered as lost to follow-up (LTFU).34 Patients LTFU were contacted by the staff whenever a telephone number was available. In case a patient died the time of death was obtained from hospital records or relatives.

Follow-up was restricted to 6 months. Person-time was calculated from inclusion date until death, permanent deferral or the end of the study, whichever came first. Patients LTFU and transferred patients were included in the survival analysis until the last day they were known to be alive.

We estimated the overall incidence of mortality and studied risk factors associated with death using Cox proportional hazard models using age as the underlying time variable. Baseline explanatory variables were age, sex, HIV type, BMI, MUAC, suPAR plasma levels and baseline CD4 cell count (with two cut-off points tested: ≤200 and ≤350 cells/μl). A normal BMI was defined as 18.5–24.9 kg/m² and malnutrition was defined as a BMI<18.5 kg/m² according to the United Nations FAO criteria.35 Low MUAC was defined as MUAC ≤250 mm.

Risk markers for early mortality among HIV-infected patients in Guinea-Bissau
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- **Population A**: Patients included with MUAC, BMI and CD4 available (n=1,054). Out of them, 1,049 contributed with pre-ART risk time.
- **Population B**: Patients included with MUAC, BMI, CD4 and suPAR available (n=628). Out of them, 625 contributed with pre-ART risk time.

**Ethical considerations**
The study was approved by the National Ethics Committee of Guinea-Bissau. All participants were counselled and provided with informed written consent before their inclusion in the study.

**RESULTS**

**Study population**
Between July 2007 and December 2009, 1,562 adults were enrolled in the Bissau-HIV cohort. A total of 1,402 patients (90%) were seen at least once after recruitment. Those who were on ART at enrolment (n=228) or without CD4 cell count available at inclusion (n=91) were excluded from analysis. Among the remaining 1,083 ART-naïve patients (70% women), the median age was 35 years (IQR 29 to 45), women being younger than men (34 vs 40 years, p<0.001). The majority of patients were HIV-1-seropositive (67%), whereas 20% were HIV-2-seropositive and 13% tested positive for both viruses. After screening, 567 patients (52%) started ART within the first 6 months of follow-up.

**Baseline CD4 cell count, BMI, MUAC and suPAR**
The median baseline CD4 cell count for the entire cohort was 192 cells/μl (IQR 82–353, n=1083). There were slight differences in CD4 cell count according to HIV types: HIV-1 patients had the lowest CD4 count with a median of 176 cells/μl (IQR 77–334) followed by double-infected patients (median 190 cells/μl (IQR 91–337) and HIV-2-infected patients (median 219 cells/μl (IQR 110–439), p<0.001). Patients who died had a significantly lower baseline CD4 counts than those who survived the 6-month follow-up period (median 67 vs 208 cells/μl, respectively).

Regarding the anthropometric measurements, median BMI at inclusion was 19.7 kg/m² (IQR 17.4–22.4, n=1057) and 256 mm (IQR 232–286, n=1064) for MUAC. Both measurements were lower for patients who died during follow-up (Median BMI 17.5 vs 20 kg/m² in survivors, p<0.001, and median MUAC 230 mm in patients who died vs 262 mm in survivors, p<0.001). The baseline median of suPAR plasma level was 3.5 ng/ml (IQR 2.6–5.3, N=646), being 3.4 ng/ml for patients who survived vs 7.6 ng/ml in patients who died, p<0.001. There were no statistically significant differences in BMI, MUAC or suPAR according to sex or HIV type. Baseline characteristics of the cohort are summarised in the online supplementary data file 1.

The parameters of organ function creatinine, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured at inclusion. The median concentration was 0.8 mg/dl (IQR 0.6–1) for creatinine (n=534), 30 μI/L (IQR 20.3–43) for AST (n=436) and 17 μI/L (IQR 10–27.3) for ALT (n=450). The correlation with suPAR was 0.18 for creatinine (p=0.002), 0.15 for AST (p=0.011) and 0.12 for ALT (p=0.04).

**Overall risk factors for mortality**
During follow-up there were 120 deaths, 178 were LTFU and 23 patients were transferred to other centres (study profile of the study available in online supplementary data file 2). Forty-one patients returned to the clinic after being considered LTFU. With 426 person-years of observation (PYO) in the study, the overall death rate was 28 per 100 PYO.

Including the time before and after the initiation of treatment, the overall mortality HR was 4.98 (3.05 to 8.15) for patients with CD4≤200 cells/μl and 4.73 (2.29 to 9.75) for patients with CD4≤350 cells/μl. The HR was 2.66 (1.77 to 3.99) for patients with BMI≤18.5 kg/m² (n=1056), 4.70 (2.97 to 7.45) for patients with MUAC≤250 mm (n=1063) and 11.8 (6.40 to 21.6) for patients in the highest suPAR quartile (suPAR>5.35 ng/ml, n=646).

Starting ART was associated with an HR of 1.17 (0.78 to 1.76). Adjusted for CD4, MUAC and BMI, the HR for ART became 0.66 (0.42 to 1.03). HIV-1 and double-infected patients had an HR of 2.14 (1.19 to 3.85) and 1.92 (0.91 to 4.02), respectively, compared with HIV-2.

**Marker sensitivity and specificity: cut-offs leading to the highest combined value**
The optimal combination of sensitivity and specificity to predict mortality within 6 months was obtained at the following cut-offs: CD4 91.8 cells/μl (data not shown), BMI 18.9 kg/m² (n=1056), 4.70 (2.97 to 7.45) for patients with MUAC≤250 mm (n=1063) and 11.8 (6.40 to 21.6) for patients in the highest suPAR quartile (suPAR>5.35 ng/ml, n=646).

**Evaluation of prognostic markers: MUAC and BMI**
In table 1 are given the results of the assessment of the strengths of the prognostic markers in a situation where suPAR was not available. The analysis included population A with 1054 patients with available MUAC, BMI and CD4 information. It was seen that only CD4≤350 cells/μl and MUAC≤250 mm had significant capacity to predict pre-ART mortality in the multivariate model. A similar result was observed using CD4≤200 cells/μl instead of CD4≤350 cells/μl (data not shown). This information suggests that MUAC adds predictive information to the CD4 cell count. Table 2 further gives the pre-ART predictive capacities of CD4, BMI and MUAC measured by the area under the ROC curve (AUC), where it is observed that MUAC measurement has a capacity close to CD4 count.
Prognostic value of suPAR

In table 3, are provided the results of the assessment of the strength of the prognostic markers in population B with suPAR, MUAC and BMI information available (n=628). It was observed that only CD4 ≤ 350 cells/μl and suPAR had a significant capacity to predict pre-ART mortality in the multivariate model. Table 2 gives the predictive capacities of CD4, BMI, MUAC and suPAR measured by AUC. It is noted that suPAR had the largest AUC; it was significantly larger than that for BMI (p=0.02) but not for CD4 (p=0.75) and MUAC (p=0.15). ROC curves corresponding to this analysis are illustrated in online supplementary data file 3.

We further analysed whether baseline suPAR added information on 6-month survival in high-risk patients defined as: patients with CD4 ≤ 350 cells/μl, BMI ≤ 18.5 kg/m² or MUAC ≤ 250 mm. Mortality was again concentrated in the highest suPAR quartile (figure 1). The HR was respectively, 8.76 (3.44 to 22.3), 8.49 (1.79 to 40.4) and 6.76 (2.18 to 21.0) in the three groups.

When CD4 count is not available

We also assessed if suPAR, MUAC and BMI could help prioritise ART initiation if CD4 is unavailable. We created high-risk groups based on different combinations of the lowest CD4 quartile (CD4≤92 cells/μl), the lowest MUAC quartile (MUAC≤231 mm) and the highest suPAR quartile (suPAR>5.31 ng/ml). We compared sensitivity and specificity in these groups to those of CD4≤200 and ≤ 350 cells/μl. We compared sensitivity and specificity in these groups to those of CD4≤200 and ≤ 350 cells/μl. In addition, we present the performance of MUAC≤250 mm corresponding to situations when no other measures were available. The results in table 4 show that the highest sensitivity was obtained by using both CD4 and suPAR. Adding MUAC just seemed to decrease specificity. Combining CD4 and

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**Table 1** Univariate and multivariate mortality HR from a model without suPAR

<table>
<thead>
<tr>
<th>Clinical and laboratory variables</th>
<th>Pre-ART</th>
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<th>Post-ART</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted HR</td>
<td>Adjusted HR*</td>
<td>Unadjusted HR</td>
<td>Adjusted HR*</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
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<td>Sex</td>
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<td>Female</td>
<td>1</td>
<td></td>
<td>1</td>
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</tr>
<tr>
<td>Male</td>
<td>1.25 (0.71 to 2.19)</td>
<td>1.17 (0.63 to 2.18)</td>
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<tr>
<td>Type of HIV</td>
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<tr>
<td>HIV-2</td>
<td>1</td>
<td></td>
<td>1</td>
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<tr>
<td>HIV-1</td>
<td>3.86 (1.50 to 9.93)</td>
<td>0.97 (0.43 to 2.17)</td>
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<tr>
<td>Double infected</td>
<td>2.35 (0.70 to 7.92)</td>
<td>1.38 (0.50 to 3.83)</td>
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<tr>
<td>Baseline CD4 (cells/μl) &lt;200</td>
<td>13.9 (6.63 to 29.0)</td>
<td>1.38 (0.65 to 2.92)</td>
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<tr>
<td>&gt;200</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>≤350</td>
<td>8.00 (3.16 to 20.3)</td>
<td>5.96 (2.32 to 15.3)</td>
<td>0.25 (0.05 to 1.30)</td>
<td>0.35 (0.07 to 1.85)</td>
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<tr>
<td>&gt;350</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²) ≤18.5</td>
<td>3.33 (1.91 to 5.78)</td>
<td>1.95 (1.07 to 3.54)</td>
<td></td>
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<tr>
<td>&gt;18.5</td>
<td>1</td>
<td></td>
<td>1</td>
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<tr>
<td>MUAC (mm) First quartile (116–231)</td>
<td>15.5 (5.36 to 44.7)</td>
<td>5.90 (1.74 to 20.0)</td>
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<tr>
<td>Second quartile (232–255)</td>
<td>6.86 (2.21 to 21.3)</td>
<td>4.29 (1.22 to 15.2)</td>
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<tr>
<td>Third quartile (256–285)</td>
<td>2.42 (0.72 to 8.12)</td>
<td>1.97 (0.50 to 7.79)</td>
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<tr>
<td>Fourth quartile (286–390)</td>
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<td></td>
<td>1</td>
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<tr>
<td>≤250</td>
<td>7.17 (3.71 to 13.9)</td>
<td>5.60 (2.88 to 10.9)</td>
<td>3.46 (1.69 to 7.06)</td>
<td>3.43 (1.68 to 7.02)</td>
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<tr>
<td>&gt;250</td>
<td>1</td>
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</table>

The analysis includes population A with 1054 patients with available CD4 count information, MUAC and BMI. Of the 1054 contributed 1049 with pre-ART risk time.

*Model with variables significant at the 5% level (CD4<350 cells/μl and MUAC<250 mm) in a forward selection including sex, HIV, CD4<350 cells/μl, BMI≤18.5 kg/m² and MUAC<250 mm.

**Table 2** Predictive capacity of the different markers: CD4, suPAR, BMI and MUAC measured by area under the ROC curve (with 95% CI)

<table>
<thead>
<tr>
<th>Population A</th>
<th>N</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>1049</td>
<td>0.760</td>
<td>0.696 to 0.823</td>
</tr>
<tr>
<td>BMI</td>
<td>1049</td>
<td>0.699</td>
<td>0.628 to 0.771</td>
</tr>
<tr>
<td>MUAC</td>
<td>1049</td>
<td>0.747</td>
<td>0.681 to 0.813</td>
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</table>

<table>
<thead>
<tr>
<th>Population B</th>
<th>N</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>625</td>
<td>0.786</td>
<td>0.709 to 0.862</td>
</tr>
<tr>
<td>BMI</td>
<td>625</td>
<td>0.667</td>
<td>0.569 to 0.765</td>
</tr>
<tr>
<td>MUAC</td>
<td>625</td>
<td>0.717</td>
<td>0.626 to 0.808</td>
</tr>
<tr>
<td>suPAR</td>
<td>625</td>
<td>0.812</td>
<td>0.731 to 0.893</td>
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</table>

AUC, area under the curve; BMI, body mass index; CD4, CD4 T cell; MUAC, middle-up-arm circumference; ROC, receiver operating characteristic; suPAR, soluble urokinase-type plasminogen activator receptor.
suPAR gave higher sensitivity and specificity compared with both CD4 ≤ 350 and ≤ 200 cells/μl. Combining either CD4 or suPAR with MUAC gave similar specificity but smaller sensitivity compared to CD4 and suPAR. These combinations gave similar sensitivity as CD4 ≤ 200 cells/μl, but higher specificity. For reference, we observed that a sensitivity of 72% could be obtained using only MUAC ≤ 250 mm in situations where nothing else is available.

**DISCUSSION**

Our data suggest that BMI, MUAC and suPAR levels are independent risk factors for early mortality in HIV patients in the Bissau-HIV cohort. Other studies in Africa have assessed the short-term prognostic value of nutritional indicators at HIV diagnosis on survival. The disagreement with previous studies may in part be explained by the fact that BMI was missing in patients too weak to stand in our cohort, and thus, with poor prognosis. Measurement of MUAC only needs minimal resources and does not require sophisticated clinical skills. Moreover, MUAC has the advantage that it can be measured in bedridden patients and does not need further calculations. However, studies using this anthropometric marker in adults are scarce in LMICs.

In a previous study done among Ethiopian HIV patients, the MUAC did not have a prognostic value on survival while BMI had. Nevertheless, due to the good prognostic strength observed in this study and the simplicity of the MUAC measurement, future studies could easily include and test this marker.

With regard to suPAR, studies conducted in pre-ART HIV patients have demonstrated that the circulating levels of this marker are linked to immune activation and independently predict mortality and disease progression. This observation was confirmed in the present study and extended to patients that start ART.

### Table 3

<table>
<thead>
<tr>
<th>Clinical and Laboratory Variables</th>
<th>Pre-ART Unadjusted HR (95% CI)</th>
<th>Pre-ART Adjusted HR (95% CI)</th>
<th>Post-ART Unadjusted HR (95% CI)</th>
<th>Post-ART Adjusted HR (95% CI)</th>
</tr>
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<td>Sex</td>
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<tr>
<td>Male</td>
<td>2.07 (0.98 to 4.35)</td>
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<td>Type of HIV</td>
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<td>HIV-1</td>
<td>3.61 (1.05 to 12.4)</td>
<td>0.81 (0.31 to 2.13)</td>
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<tr>
<td>Double infected</td>
<td>2.53 (0.54 to 11.8)</td>
<td>1.31 (0.38 to 4.49)</td>
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<td>Baseline CD4 (cells/μl)</td>
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<tr>
<td>≤ 200</td>
<td>18.3 (6.12 to 54.7)</td>
<td>1.71 (0.62 to 4.67)</td>
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<tr>
<td>&gt; 200</td>
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<td>1</td>
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<tr>
<td>≤ 350</td>
<td>12.6 (2.95 to 53.8)</td>
<td>5.33 (1.15 to 24.8)</td>
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<td>–</td>
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<td>&gt; 350</td>
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<td>SuPAR (ng/ml)</td>
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<td>First quartile (0.22–2.54)</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Second quartile (2.54–3.53)</td>
<td>0.48 (0.04 to 5.62)</td>
<td>0.45 (0.04 to 5.09)</td>
<td>–</td>
<td>0.00</td>
</tr>
<tr>
<td>Third quartile (3.53–5.31)</td>
<td>1.89 (0.32 to 11.1)</td>
<td>1.24 (0.20 to 7.65)</td>
<td>1.24 (0.29 to 5.29)</td>
<td>1.29 (0.30 to 5.54)</td>
</tr>
<tr>
<td>Fourth quartile (5.31–38.7)</td>
<td>19.0 (4.38 to 82.7)</td>
<td>10.7 (2.38 to 48.4)</td>
<td>5.32 (1.52 to 18.7)</td>
<td>5.09 (1.45 to 17.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
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<tr>
<td>≤ 18.5</td>
<td>2.30 (1.10 to 4.82)</td>
<td>1.87 (0.88 to 3.98)</td>
<td></td>
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</tr>
<tr>
<td>&gt; 18.5</td>
<td>1</td>
<td></td>
<td>1</td>
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<tr>
<td>MUAC (mm)</td>
<td></td>
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</tr>
<tr>
<td>First quartile (116–231)</td>
<td>19.3 (4.26 to 87.6)</td>
<td>5.32 (1.16 to 24.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second quartile (232–261)</td>
<td>8.24 (1.76 to 38.6)</td>
<td>3.85 (0.82 to 18.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third quartile (262–285)</td>
<td>4.63 (0.88 to 24.5)</td>
<td>1.53 (0.24 to 9.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth quartile (286–390)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≤ 250</td>
<td>5.27 (2.34 to 11.9)</td>
<td>2.36 (1.01 to 5.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 250</td>
<td>1</td>
<td></td>
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</tbody>
</table>

The analysis includes 628 patients with available MUAC, BMI and suPAR. Of the 628 contributed 625 with pre-ART risk time. ART, antiretroviral therapy; BMI, body mass index; CD4, CD4+ T-cell; MUAC, middle-up-arm circumference; suPAR, soluble urokinase-type plasminogen activator receptor.
It should be noted that although a high suPAR level at inclusion was still significantly associated with an increased risk of mortality post-ART initiation, the HR post-ART decreased almost fourfold in the highest suPAR quartile (table 3). Thus, initiation of ART reduces the risk of mortality in patients with high inclusion suPAR. Nevertheless, patients with high inclusion suPAR should receive further clinical and diagnostic examination to identify the reasons for an increased risk of mortality.

A recent study by Haupt et al.\(^\text{38}\) showed that suPAR was strongly correlated to the Charlson Score, with increasing number of comorbidities with increasing suPAR. While comorbidities or coinfections were not determined in all patients in the present cohort due to limited resources at the time of the study, subsequent studies in the Bissau-HIV cohort have found an HBV coinfection rate of 11.5%\(^\text{39}\) while antibodies against HCV (anti-HCV) were positive in 4% of subjects tested and most frequent among patients >50 years.\(^\text{40}\) In this study, no significant difference in death rate was observed between HBV-positive and HBV-negative HIV patients.\(^\text{39}\) With regard to other coinfections, TB is a common disease in Guinea-Bissau, where a community study estimated the incidence of this disease to 471 per

| Table 4 | Comparing sensitivity and specificity of CD4≤200 cells/μl and CD4≤350 cells/μl to groups consisting of the lowest quartile of CD4 and MUAC and the highest quartile of suPAR |
|-----------------|-----------------|-------------------|-----------------|-----------------|
| CD4≤92 cells/μl, MUAC≤231 mm and suPAR >5.31 ng/ml | 96.9 | 57.5 | 154.4 | 283/625 (45%) |
| CD4≤92 cells/μl and suPAR >5.31 ng/ml | 96.9 | 65.1 | 162.0 | 238/625 (38%) |
| MUAC≤231 mm and suPAR >5.31 ng/ml | 87.5 | 67.1 | 154.6 | 223/625 (36%) |
| CD4≤92 cells/μl and MUAC≤231 mm | 87.5 | 65.6 | 153.1 | 232/625 (37%) |
| MUAC≤250 mm | 71.9 | 57.8 | 129.7 | 273/625 (44%) |
| CD4≤200 cells/μl | 87.5 | 50.6 | 138.1 | 321/625 (51%) |
| CD4≤350 cells/μl | 93.8 | 28.0 | 121.7 | 457/625 (73%) |

The analysis includes patients from population B with available MUAC, BMI and suPAR who contribute with pre-ART risk time (n=625). ART, antiretroviral therapy; BMI, body mass index; CD4, CD4+ T-cell; MUAC, middle-up-arm circumference; suPAR, soluble urokinase-type plasminogen activator receptor.

Risk markers for early mortality among HIV-infected patients in Guinea-Bissau

100 000 persons-years. We have recently shown that an elevated suPAR is associated with an increased risk of mortality for TB patients on treatment in Guinea-Bissau. In addition, resent work has shown that suPAR's prognostic value might be related to its ability to display organ dysfunction. Future studies should register comorbidities, organ biomarkers and test for coinfections to determine whether elevated suPAR is associated with higher prevalence of these conditions. If so, this knowledge could help designing decision trees on tests that can be carried out on HIV-patients with high suPAR levels.

Moreover, our results suggest the plasma level of suPAR in HIV-infected patients adds significant information to that provided by CD4 count. In this study plasma VL was not measured, however previous studies have found only a weak or inexistnt relation between suPAR levels and VL, and it was seen that changes in plasma suPAR were independent of changes in VL and CD4 cell count, but were strongly correlated with plasma levels of the soluble tumour necrosis factor receptor II. Therefore, it should be noted that suPAR is not a replacement for VL or CD4 count. SuPAR seems to have a potential as a risk status marker while VL is a marker of treatment efficacy. Whether suPAR also has potential as a marker of treatment efficacy still remains to be investigated. Thus CD4, VL, and suPAR, all add independent information to the prognosis of the patient.

Additionally, our data show that the HR associated with the risk markers decreased following ART enrolment. Opposed to CD4, we observed that BMI, MUAC and suPAR continued to carry prognostic information after ART initiation. This indicates that those patients with a high baseline risk should receive further clinical and diagnostic examination to identify the reasons for increased risk of mortality.

Lastly, according to the new WHO ART guidelines, ART should be offered to all patients with CD4 count below 350 cells/μl irrespective of the WHO clinical stage. Lack of health infrastructure or shortness of ARV drugs will hamper the implementation of these guidelines in many developing countries. When many patients access HIV care with advanced HIV infection it is difficult to start treatment to all patients that fulfil ART eligibility. Furthermore, drug shortages have been previously identified as one of the greatest logistic challenge to ART scale-up in subSaharan Africa. In the context of limited resources, prioritising who should start ART first is not a choice, but a practical necessity. In the present study, we observed that MUAC and especially suPAR add prognostic information on early mortality to the CD4 cell count. Also, we observed that none of the 197 patients with suPAR below median and CD4 above 200 died during the follow-up period. Interestingly, patients with low suPAR show very little mortality even in the groups with CD4<350 cells/μl, or low MUAC or low BMI (figure 1). We are currently investigating whether HIV patients enrolled into ART treatment and characterised as good responders (adherent and CD4>350 cells/μl) and with low suPAR can have reduced monitoring thereby reducing costs of the ART programme.

Furthermore, a combination of CD4 and suPAR gave better sensitivity and specificity compared to CD4≤350 or ≤200 cells/μl alone. Using the CD4/suPAR combination, we would reduce the need to start ART immediately to 38% of the patients compared with 73% using only the 350 cells/μl CD4 count threshold. This information may have important public health implications in settings with limited resources and ARV drug shortages. Health providers could combine CD4 with suPAR to identify patients in immediate need of treatment when scarce drug resources have to be prioritised. Besides suPAR, a combination of CD4 and MUAC could give the same sensitivity as CD4≤200 cells/μl but a better specificity. Actually, this combination could give a sensitivity which is only slightly smaller than CD4≤350 cells/μl.

Finally, we observed that suPAR and MUAC carried the same prognostic information as CD4 and MUAC. The combination of suPAR and MUAC gave same sensitivity and higher specificity compared to CD4≤ 200 cells/μl. To summarise, these markers could help identifying patients to whom ART should be prioritised when the CD4 cell count is not available. Finally, we found that MUAC alone can be used as a very simple prioritising tool if nothing else is available.

There are several limitations to this study. First, complete information about risk factors (CD4, suPAR, BMI and MUAC) was only available in 58% (628/1083) of our population. This was mainly due to lack of suPAR levels that were not measured in 40% of the patients. The reasons for not having this information available were due to logistic reasons not related to healthcare or mortality, thus we think that it should not affect the general trends presented. Second, WHO-stage, pregnancy status and comorbidities presented at the time of inclusion were not recorded in the database. Furthermore, while a high suPAR was strongly related to mortality, specific causes of mortality were not registered. In a recently published systematic review describing relative causes of early mortality post-ART initiation in different regions, the most common causes of death reported were TB, advanced HIV, wasting, chronic diarrhoea and others. It is unclear what proportion of wasting in HIV patients could be attributed to TB or other opportunistic infections, but it is likely that TB is playing an important role as described in an autopsy study in South Africa where TB was identified as the most common cause of death in patients on ART. With the high TB incidence described in Guinea-Bissau, it is plausible that TB has an important contribution to the mortality in the Bissau-HIV cohort, however only future studies describing causes of death in this setting could help corroborate or reject this hypothesis. Third, contact information for some patients was not sufficient to allow active tracing in case of missed appointments.
An analysis comparing alive patients versus dead and alive patients versus LTFU subjects showed higher suPAR, lower CD4 and thinness among LTFU than survivors, although not as much as those who we know died (see online supplementary data file 4). Thus, it is likely that patients LTFU may include unascertained deaths and that the effects of the risk factors are underestimated in our work.

CONCLUSIONS
Assessing the nutritional status by measuring MUAC or BMI should be part of the initial clinical evaluation of HIV patients enrolled in HIV care. Our findings suggest that these anthropometrics markers, specifically MUAC, could be helpful in identifying patients with a high risk of dying early. Further studies are needed to evaluate whether nutritional supplements to ART, in these high-risk groups, could be useful for decreasing an early mortality. In case of limited ARV drug resources suPAR plasma levels and MUAC could help health providers to prioritise ART initiation among people with low CD4 count. In situations where CD4 is unavailable or infeasible, suPAR and MUAC could also be used as relatively simple alternatives for ART prioritisation.

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Contributors
The study was planned by JE-O, CW and IO, and executed by IO. AA was responsible for the statistical analyses. PA and ALL were involved in the interpretation of data. CM and DdaS carried out the clinical follow-up of the patients. ZJ daS was responsible for the laboratory HIV test strategies. AF and Mra were responsible for the logistic work in the outpatient ART-SMNH Center. IO wrote the first draft and all authors contributed to the final version of the paper.

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Competing interests
Jesper Eugen-Olsen is co-founder and shareholder in ViroGates AS, the company that produces the suPARnostic assay. No other authors have any conflict of interest.

Patient Consent
Obtained.

REFERENCES

Ethics approval
National Ethics Committee of Guinea-Bissau.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data sharing statement
Technical appendix, statistical code and dataset are available from the corresponding author at jespereugenolsen@gmail.com. Consent was not obtained by data sharing but the presented data are anonymised and risk of identification is low.


9.36
Risk markers for early mortality among HIV-infected patients in Guinea-Bissau


Assessment of simple risk markers for early mortality among HIV-infected patients in Guinea-Bissau: a cohort study

Inés Oliveira, Andreas Andersen, Alcino Furtado, Candida Medina, David da Silva, Zacarias J da Silva, Peter Aaby, Alex Lund Laursen, Christian Wejse, Jesper Eugen-Olsen and for the Bissau HIV cohort study group

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