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Prevalence and clinical characteristics of CMV coinfection among HIV infected individuals in Guinea-Bissau: A cross-sectional study

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ABSTRACT

Objectives*:* To describe the prevalence of CMV in a cohort of HIV infected individuals in Guinea-Bissau, West Africa and to evaluate differences in patients' clinical characteristics associated with their CMV status.

Methods*:* Newly diagnosed HIV infected adults were invited to participate in this cross-sectional study, from May until December 2015. Enrolled patients were interviewed and underwent a full physical examination focusing on CMV disease manifestations. Blood samples were analyzed for CMV serology, QuantiFERON-CMV response and CMV DNA. Mortality follow-up were registered for one year after inclusion.

Results*:* In total, 180 patients were enrolled. Anti-CMV IgG positivity was found in 138/138 (100%) and 4/138 (2.8%) were anti-CMV IgM positive. A positive QuantiFERON-CMV response was found in 60/70 (85.7%) of the patients and 83/137 (60.6%) had CMV viremia. QuantiFERON-CMV response and detectable CMV DNA were associated with lower CD4 cell count, older age, and upper gastrointestinal complaints. During one year of follow-up, the IRR for death among CMV DNA

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positive patients was 1.5 (p=0.5).

Conclusions*:* CMV coinfection was detected among all enrolled patients and CMV viremia was highly prevalent. Only age and upper gastrointestinal complaints were associated with the patients' CMV status.

Keywords: Clinical characteristics; Cytomegalovirus; Guinea-Bissau; HIV; Mortality; CMV prevalence

INTRODUCTION

Approximately 37 million people are infected with human immunodeficiency virus (HIV) worldwide (1). The majority of this burden is borne in sub-Saharan Africa, where 74% of HIV-associated deaths occur (2). There are two types of HIV: HIV type 1 (HIV-1) and HIV type 2 (HIV-2), the latter mainly restricted to West Africa (3-5). Compared with HIV-1, HIV-2 is less transmissible and is associated with a lower viral load and a slower rate of CD4 cell count decline (6). The West African country Guinea-Bissau has experienced a stabilization in HIV-1 prevalence (4.0%), and still holds the world´s highest prevalence of HIV-2 (2.8%), although it is decreasing (7).

Cytomegalovirus (CMV) establishes latent infections, mainly in leukocytes and their progenitor cells, and has a suppressive effect on the immune system (8, 9). In immunosuppressed individuals, such as HIV infected individuals with advanced disease progression, the risk of CMV disease is high, with possible symptoms ranging from short-limiting febrile periods to severe end-organ disease (EOD) (10). The degree of immunosuppression correlates with the risk of symptomatic disease, which is particularly increased if the CD4 cell count drops below 100 cells/µl (11). Retinitis and colitis are the most frequent CMV manifestations worldwide (12). In addition to causing EOD, increasing evidence suggests that CMV has an indirect effect on HIV by enhancing HIV transmission and progression with subsequent increased mortality, despite antiretroviral treatment (ART) (13).

CMV seroprevalence is known to range from about 50% in the general adult population in high-income countries (10), to more than 80% in low-income countries (14, 15). Among high risk groups, such as men who have sex with men and individuals infected with HIV, the seroprevalence is more than 80% in both high- and low-income countries (16, 17). However, studies carried out in West Africa have revealed CMV seroprevalences as low as 56% in some HIV infected study populations (13). Considering the varying prevalences of CMV infection reported in West Africa, and the high risk of reactivation among HIV-infected individuals with advanced disease, we aimed to investigate CMV coinfection and associated clinical characteristics in a well-characterized HIV cohort in Guinea-Bissau (18, 19).

METHODS

Setting

Hospital Nacional Simão Mendes (HNSM) is the main hospital in the capital Bissau, and houses the largest HIV outpatient clinic in Guinea-Bissau in terms of patients on follow-up. In 2007, the Bissau HIV Cohort was established, and since then information about the patients' clinical and biochemical status have been registered continuously (19).

Design and Study population

This study was a cross-sectional sample of a prospective cohort. Main data presented are the baseline CMV data retrieved as a cross-sectional study. However, follow-up data on mortality were retrieved as a longitudinal cohort study.

The study was conducted at the HIV outpatient clinic at HNSM from 26th of May until 10th of December 2015. All newly diagnosed HIV infected individuals over 15 years of age were eligible for enrollment. People who did not speak Portuguese Creole and patients who had already started ART elsewhere were excluded from participation.

All included patients were interviewed for symptoms of CMV EOD. Further, a physical examination including full neurological examination and ophthalmoscopy with tropicamide-induced mydriasis (Mydriacyl 1%, Alcon, Fort Worth, Texas, USA) was performed. Before starting ART and within one month of the initial examination, whole blood samples (5 mL) were collected.

One person performed all ophthalmoscopies. The ophtalmoscopist received structured training in the procedure. Abnormal ophthalmoscopy findings were not classified to be CMV retinitis or other due to limited experience.

The main focus of the clinical interview and physical examination were symptoms suggestive of CMV EOD:

- Ophthalmoscopy findings compatible with CMV retinitis +/- vision problems (eye pain, vision loss, scotomas or floaters)
- Palpable hepatomegaly +/- gastrointestinal complaints (odynophagia, dysphagia, retrosternal pain, stomach pain, melena and/or hematochezia)
- Abnormal neurological findings +/- complaints compatible with encephalitis or radiculopathy (headaches, confusion, change in memory function, numbness, pain in legs, change in ability to walk, urine retention and/or reduction of sensibility in legs)

Mortality follow-up on patients was done until death or censoring at 365 days from inclusion. If transferred to other clinics or lost to follow-up (LTFU) they were censored at time of transferal or at last visit at the clinic (if lost). Patients on ART who did not return to the clinic for more than 6 month

were presumed LFTU.

Laboratory methods

HIV screening was done using Alere Determine HIV-1/2 assays (Alere Ltd., Stockport, UK) or SD Bioline HIV Ag/Ab Combo (Standard Diagnostics, Yongin-si, Republic of Korea) at the HIV outpatient clinic. Discrimination of HIV types was done by SD Bioline HIV 1/2 3.0 (Standard Diagnostics, Yongin-si, Korea) or ImmunoComb HIV 1/2 BiSpot (Organics, Yavne, Israel). At times Bioline HIV1/2 3.0 was not available at the clinic, causing a lack in discrimination of HIV type among the patients included in these periods. When the test became available we invited the patients to get tested. However, a large proportion of patients did not return to the clinic. CD4 cell count was measured at the national reference laboratory in Bissau, using the Partec CyFlow SL_3 flowcytometer (CyFlow SL, Partrc, Munster, Germany).

For CMV serology whole blood samples were collected and prepared for storage at the national reference laboratory in Bissau. Plasma samples were frozen until tested for IgG and IgM antibody responses specific to CMV at Department of Clinical Immunology, Aarhus University Hospital, Denmark, using ARCHITECT CMV IgG and IgM chemiluminescence assays (Abbott Laboratories, Abbott Park, IL, USA). Responses were interpreted as reactive, non-reactive or grayzone according to levels set by the manufacturer (20, 21).

Cellular immunity against CMV was determined by detection of IFN-γ following ex-vivo stimulation with CD8 T-cell specific CMV antigens. Whole blood was collected in three specialized QuantiFERON-CMV (QF-CMV) collection tubes (Cellestis, QIAGEN, Chadstone, Australia). IFN-γ levels (IU/mL) were measured using Human IFN gamma ELISA Ready-Set-Go! (eBioscience, Santa Clara, California, USA), at the Institute of Biomedicine, Aarhus University, Denmark. The QF-CMV responses were interpreted as reactive, non-reactive or indeterminate according to IFN-γ levels set by the manufacturer (22).

For CMV virology CMV-DNA in plasma samples was quantified using the Abbott CMV RealTime assay on the Abbott m2000 platform (Abbott Laboratories, Illinois, USA), at Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital (23). The limit of quantification was 20 copies/mL.

In this article 'CMV status' refers to all three biomarkers; CMV DNA, CMV antibodies and CMV INF- γ response. If concerning one specific biomarker, it is specified.

Statistical methods

All data were entered in an Access database (Microsoft Corporation, Redmond, Washington, USA). Statistical analyses were performed using Stata IC 13.0 (StataCorp, College Station, Texas, USA). For categorical variables a Chi-square test was used. Logistic regression analysis was used to evaluate clinical characteristics associated with CMV status in the Chi-squared test for adjustment of sex, age and CD4 cell count. Mortality analysis was done using poisson regressions-analysis. A p-value <0.05 was considered significant. In case of multiple comparisons the Bonferroni correction was used to adjust significance level. When specific data on a patient was missing, the individual was excluded from the specific analysis.

Ethical considerations

The study protocol was reviewed by The National Research Ethics Committee of Denmark, and received a consultative approval in April 2015 (case number 1500122). The study protocol was reviewed and approved by the National Ethical Committee of Guinea-Bissau in May 2015 (0014/CNES/INASA/2015). Written consent, or fingerprint in case of illiteracy, was given by all patients before enrollment in the study.

RESULTS

Study population

We enrolled 180 patients (Figure 1). Of these, 64.4% (116/180) were female, 35.6% (64/180) were male. The age range was 20 to 66 years, with a mean age of 38.6 years. Distribution of HIV types showed 69.4% (125/180) were infected with HIV-1, 9.4% (17/180) with HIV-2, 4.4% (8/180) with HIV-1/2, and 16.7% (30/180) did not have HIV type determined. Of the 180 persons included in this study, 147 patients returned to the clinic for CD4 cell count during the study period. CD4 cell counts ranged from 8 to 1140 cells/µL, with a median of 198 cells/µL (IQR 100-404). An illustration of number of patients included in the different blood analysis is given in Figure 2.

CMV antibody status

Of the 147 patients returning to the clinic, 138 gave a blood sample for CMV antibody testing. We found a 100.0% (138/138) anti-CMV IgG positivity and a 2.8% (4/138) anti-CMV IgM positivity (four patients, one of which was found to be in a grayzone). No significant differences in clinical characteristics (sex, age, CD4 cell count or HIV type), were found between the 42 patients who did not return to the clinic for blood sampling, compared with the patients who had their blood tested by serology.

CMV QuantiFERON status

A limited number of QF-CMV assays (n=71) became available at the clinic during this study, and patients were enrolled for QF-CMV testing consecutively at that point. CMV reactivity was found in 85.7% (60/70) of the patients, and one sample was considered indeterminate. The IFN-γ response ranged from zero to 10,219.8 IU/mL, and among the reactive samples a median of 586.9 IU/mL (IQR 56.1-2,208.3) was found. Compared to patients not examined by QF-CMV, we did not find any significant differences in clinical characteristics.

There were no significant differences in sex, age, HIV-type or CD4 cell count, between QF-CMV reactive and non-reactive HIV patients. Nor was there any significant correlation between QF-CMV response values and CD4 cell count. However, when comparing the reactive QF-CMV response values with age, a significant positive correlation was found (r^2=0.07, regression coefficient (β) =60.7, p=0.05) (Figure 3). When adjusting for CD4 cell count and CMV viral load, the correlation was even more pronounced (r^2=0.11, β=74.1, p=0.02).

CMV viremia status

Of the plasma samples tested for CMV viremia, CMV DNA was detected in 60.6% (83/137) of the samples, with viral load ranging from <20-80.000 copies/mL. A quantitative result (>20 copies/mL) was found in 53 samples, with a median of 120 copies/mL (IQR 35-280) in these 53 patients.

Older age and lower CD4 cell count were both found significantly associated with CMV viremia in patients with detectable CMV DNA compared with patients with undetectable CMV DNA (p=0.02 and p<0.01 respectively) (Table 1). However, the associations did not remain significant when stratifying by CMV viral load, or when using regression analysis. No significant associations between CMV viremia and sex, HIV type or QF-CMV response were found.

Demographic data and clinical characteristics by CMV status

By comparing CMV IFN-γ reactivity and CMV DNA levels with clinical characteristics found at inclusion, and with demographic data, only few associations were found. Self-reported odynophagia and dysphagia were found among 30.0% (21/70) and 31.4% (22/70) of the patients tested by the QF-CMV assay, and these symptoms were twice as frequent among patients with high QF-CMV response and significantly associated with QF-CMV strata (p=0.04 and p=0.02 respectively) (Table 2). Furthermore, 35 patients (n=175) had oral thrush, which were also correlated to odynophagia (p=0.05). No other symptoms, neither self-reported nor found during physical examination, were associated with CMV status.

With regard to CMV retinitis, 41.2% (153/180) of the patients who were examined by ophthalmoscopy claimed to suffer from self-reported floaters and/or scotomas. Yet, only 12.4% (19/153) had unspecified abnormal ophthalmoscopy findings, and only 7.3% (11/150) had vision field deficiencies at the physical examination. However, we did find a significant association between the unspecified abnormal ophthalmoscopy findings and self-reported eye-pain ($p<0.01$), but no associations between ophthalmoscopy findings and QF-CMV or CMV DNA status were found. We did not find any significant differences in clinical characteristics, when comparing the patients who had ophthalmoscopy performed with the patients who declined the examination.

When analyzing a subpopulation, with CD4 cell counts below 100 cell/µL (n=31), self-reported epigastric pain was significantly associated with higher CMV DNA strata ($p=0.05$) (NS Bonferroni). When limiting the population further, to CD4 cell count below 50 cells/µL (n=14), we also found self-reported retrosternal- and umbilical pain to be significantly associated with higher CMV DNA strata ($p=0.05$ and $p=0.02$ respectively, data not shown) (NS Bonferroni).

Follow-up analysis

The CMV DNA tested patients (n=137) was followed one year regarding survival, giving 95.8 person years of follow-up time. During this period 112 (81.8%) started ART. In total, 10 patients died during follow-up, six during ART treatment, four before initiation of ART. The overall incidence rate (IR) for death was 10.4 deaths per 100 person-years. IR for death among CMV DNA positive patients was 13.9 deaths per 100 person-years. Among the CMV DNA negative patients the IR for death was 5.2 deaths per 100 person-years. The incidence rate ratio (IRR) for death was 1.5 among CMV DNA positive patients compared with CMV DNA negative patients, however not significant ($p=0.5$). When only looking at patient with viremia >1000 copies/mL (n=4) compared with patients without CMV viremia (n=54), the IRR for death increased to 3.4, however not significantly ($p=0.3$).

During follow-up eight of the CMV DNA tested patients were transferred to other health centers and 45 patients were LTFU. Regarding the clinical characteristics of patients LTFU, we did not find any significant differences compared with patients not LTFU, except CD4 cell count. We performed a sensitivity analysis and assumed all patients LTFU to be dead. The IRR for death was 1.0 ($p=1.0$) among CMV DNA positive patients compared with CMV DNA negative patients.

DISCUSSION

This study is the first to report CMV prevalence in Guinea-Bissau. Among newly diagnosed HIV infected adults, CMV was highly prevalent with a 100% IgG reactivity specific to CMV, and a 2.8% IgM reactivity specific to CMV. A positive QF-CMV test for IFN-γ response was found in 85.7% of the

patients, and 60.6% of the patients had CMV viremia at time of HIV diagnosis.

This study has certain limitations. Primarily, IgG level measurements were only semi-quantitative, stated by the manufacturer of ARCHITECT (20), causing us not to examine the serology results in relation to any clinical characteristics. Furthermore, a subgroup of the study population did not supply a blood sample. However, their clinical characteristics were not significantly different from the CMV serology-tested patients, which also previously has been shown in the Bissau HIV cohort (24). Thus, it is unlikely that they have caused a selection bias. The low median CD4 cell count in this study population could possibly have caused confounding errors; patients could be suffering from other co- or superinfections. Consequently, the findings from the physical examination or the self-reported symptoms may have had other causes than CMV coinfection. Ideally, this study would have had an HIV negative control group, and additionally a complete diagnostic set-up. However, it was not possible to obtain definite CMV EOD diagnosis in our setting, due to the complexity of the diagnostic procedures including biopsies and fundoscopies. Lastly, it has previously been shown that the discriminatory HIV tests used at the national HIV clinic in Bissau have low discriminatory capacity, causing HIV type misclassifications (25). This may have interfered with our results, giving a falsely high number of HIV-1/2 infected individuals, and it seems mainly to be the HIV-2 infected who is misclassified.

A strength of this study is that we only included ART-naïve HIV-infected individuals, which reduces the risk of ART affecting of our results, as it reduces CMV viremia (11, 26). Also, to our knowledge, the clinical characteristics and prevalence of CMV and HIV coinfection have only been investigated to a limited extent concerning HIV-2 (13). Interestingly, CMV EOD were significantly more frequent among HIV-2 infected individuals than HIV-1 and HIV-1/2 infected individuals in an autopsy study (27), yet we were not able to identify any correlation between HIV type and CMV status in this study. The number of HIV2 and HIV1/2 infected patients included in this study was too small to provide sufficient power to detect any associations with CMV status. Third, we are the first to use the QF-CMV assay in a large HIV population in a resource-limited setting. Our exhaustive examination of symptoms suggestive of CMV EOD is also unpreceded, with previous studies mainly focusing on CMV biomarkers and mortality rates. Eight studies across Africa have investigated HIV and CMV coinfection associated mortality (13). All found similar results: HIV-1-infected patients with CMV viremia had higher mortality risk. In this study, the IRR for death among CMV DNA positive patients was 1.5, however not significant (p=0.5). This may be due to a relatively small number of included patients, few registered deaths, and a high frequency of LTFU. Also, a CMV viral load of 120 copies/mL is considered a low level of viremia (28, 29), and it is possible that the lower CMV viral loads have diluted any increased risk of death in patients with higher viremia. The fact that most

patients started ART shortly after inclusion probably even further diminished the chance of finding any increased risk of death, as ART is known to reduce CMV viral loads.

Due to previous studies showing a high mortality rate among patients LTFU (30, 31) we performed a sensitivity analysis assuming all patients LTFU to be dead. However, no significant IRR for death appeared. We only found the patients LTFU to significantly differ in CD4 cell count comparing clinical characteristics with patients not LTFU, which is in consistency with previously findings (32).

CMV is known to be highly prevalent in resource-limited settings worldwide (33), consistent with our findings. Using the QF-CMV assay, we found a significant correlation with age, which indicates a higher ability of CMV IFN-γ production with age, independent of CD4 cell count and CMV viral load, perhaps due to longer CMV exposure time. Also, self-reported odynophagia and dysphagia were associated with patients' QF-CMV responses. This may indicate an association between infection in the upper gastrointestinal tract and CMV-specific IFN- γ production. However, the main reason for upper gastrointestinal complaints in HIV infected individuals is known to be esophageal candidiasis (34), which is consistent with our findings of a significant correlation between oral thrush and odynophagia. There are only three other studies using QF-CMV assay on HIV infected individuals (35-37), none of which report on clinical symptoms, hence we could not compare these results with other studies.

We showed CMV viremia to be highly prevalent and significantly associated to both age and CD4 strata. Yet, the findings did not remain significant in a regression analysis. Other studies in African settings have found CMV viremia in HIV infected adults less prevalent, with detection of CMV viremia among 2-23% (13). This may be an expression of an unexplained high prevalence of active CMV infection in our cohort, possibly combined with a high quality PCR analysis conducted in Denmark. The relatively low CD4 cell counts possibly also affect the prevalence of CMV viremia in our population. However, the Tanzanian study describing the second highest prevalence of CMV viremia at 22.6% (14), was conducted on a population with a median CD4 cell count comparable to ours at 205 (IQR 79-403) cell/µL.

Self-reported symptoms were often not backed by objective findings during the physical examination, and only few correlations to CMV status were found. Comparison of unspecified abnormal ophthalmoscopy findings and self-reported eye pain did show a significant association, but there was no association between ophthalmoscopy findings and CMV status. The lack of associations between clinical characteristics and CMV status in this study may be due to relatively low CMV viral loads, and CD4 cell counts above 100 cells/µL, which reduce the risk of CMV EOD development. Using subgroups with CD4 cell counts below 100 and 50 cells/µL revealed a few more associations,

between CMV DNA and gastrointestinal complaints. A Danish study has shown CMV DNA detection to be predictive of the development of CMV disease with an odds ratio at 30, in an HIV-infected population with a median CD4 cell count of 34 cells/μL at inclusion (38). Thus one could expect a high frequency of CMV disease development in our study population. However, the high mortality rate previously described in the Bissau HIV Cohort (39), may cause patients not to survive their illness long enough to develop CMV EOD, as suggested in other CMV studies (40). With regard to diagnosis of CMV EOD in Africa, underdiagnosing is likely to be common, as multiple autopsy studies have found pre-mortem undiagnosed CMV EOD (41, 42). Also, the positive predictive value of CMV serology and virology have been found to be low (43, 44), but are still the most accessible diagnostic tool in many resource-limited settings. Finally, it should be mentioned that for unknown reasons, CMV retinitis is less commonly found in Africa than other parts of the world (40).

In conclusion, CMV coinfection was highly prevalent among newly diagnosed adult HIV-infected patients in Guinea-Bissau. This CMV coinfected HIV study population had multiple symptoms compatible with CMV EODs and low CD4 cell count at time of HIV diagnosis. However, only few correlations between clinical characteristics and CMV status were found. New and more accessible diagnostic tools or indicators of CMV EOD are needed, as evaluation of CMV EOD in resource-limited settings currently is difficult.

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REFERENCES

1. UNAIDS F, statistics 2015. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf 2016 [FactSheet 2016, statistiscs 5].
2. UNAIDS. The Gab Report, http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf 2014 [
3. Arien KK, Abraha A, Quinones-Mateu ME, Kestens L, Vanham G, Arts EJ. The replicative fitness of primary human immunodeficiency virus type 1 (HIV-1) group M, HIV-1 group O, and HIV-2

isolates. Journal of virology. 2005;79(14):8979-90.
4. http://www.hivguidelines.org NYSDoHAI. http://www.hivguidelines.org/wp-content/uploads/2014/04/human-immunodeficiency-virus-type-2-hiv-2.pdf 2012 [Human Immunodeficiency Virus Type 2 (HIV-)].
5. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. Trends Microbiol. 2008;16(12):588-95.
6. Rowland-Jones SL, Whittle HC. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? Nature immunology. 2007;8(4):329-31.
7. Olesen JS, Jespersen S, Da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, et al. HIV-2 continues to decrease, while HIV-1 is stabilizing in Guinea-Bissau. Aids. 2018.
8. Mayaphi SH, Brauer M, Morobadi DM, Mazanderani AH, Mafuyeka RT, Olorunju SA, et al. Cytomegalovirus viral load kinetics in patients with HIV/AIDS admitted to a medical intensive care unit: a case for pre-emptive therapy. PLoS One. 2014;9(4):e93702.
9. Rouse BT, Horohov DW. Immunosuppression in viral infections. Reviews of infectious diseases. 1986;8(6):850-73.
10. Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. The Journal of pathology. 2015;235(2):288-97.
11. Steininger C, Puchhammer-Stockl E, Popow-Kraupp T. Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2006;37(1):1-9.
12. Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58(Rr-4):1-207; quiz CE1-4.
13. Gronborg HL, Jespersen S, Honge BL, Jensen-Fangel S, Wejse C. Review of cytomegalovirus coinfection in HIV-infected individuals in Africa. Rev Med Virol. 2017;27(1).
14. Brantsaeter AB, Johannessen A, Holberg-Petersen M, Sandvik L, Naman E, Kivuyo SL, et al. Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2012;16(12):e879-85.
15. Rabenau HF, Lennemann T, Kircher C, Gurtler L, Staszewski S, Preiser W, et al. Prevalence- and gender-specific immune response to opportunistic infections in HIV-infected patients in Lesotho. Sexually transmitted diseases. 2010;37(7):454-9.

16. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology. 6. ed. ed. Philadelphia: Mosby/Elsevier; 2009. 947 p.
17. Human Cytomegaloviruses, Methods and Protocols: Humana Press; 2014.
18. Jespersen S, Honge BL, Oliveira I, Medina C, da Silva Te D, Correia FG, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. Bull World Health Organ. 2014;92(12):909-14.
19. Jespersen S, Honge BL, Oliveira I, Medina C, da Silva Te D, Correira FG, et al. Cohort Profile: The Bissau HIV Cohort-a cohort of HIV-1, HIV-2 and co-infected patients. Int J Epidemiol. 2015;44(3):756-63.
20. ARCHITECT System ADD. http://www.ilexmedical.com/files/PDF/CMVIgG.pdf Retrieved July 2016 [CMV IgG ARCHITECT manual].
21. ARCHITECT System ADD. http://www.ilexmedical.com/files/PDF/CMVIgM_ARC.pdf Retrieved Feb 2016 [CMV IgM ARCHITECT manual].
22. Cellestis aQC, level 2, Office Tower 2, Chadstone Centre, 1341 Dandenong Road, Chadstone, Victoria, 3148, Australia. http://www.quantiferon.com/irm/content/package-inserts.aspx?RID=346&RedirectCount=1 Retrieved July 2016 [QuantiFERON-CMV ELISA Package Insert 02/2015].
23. Schnepf N, Scieux C, Resche-Riggon M, Feghoul L, Xhaard A, Gallien S, et al. Fully automated quantification of cytomegalovirus (CMV) in whole blood with the new sensitive Abbott RealTime CMV assay in the era of the CMV international standard. J Clin Microbiol. 2013;51(7):2096-102.
24. Hønge B, Jespersen S, Aunsborg J, Mendes D, Medina C, da Silva Té D, et al. High prevalence and excess mortality of late presenters among HIV-1, HIV-2 and HIV-1/2 dually infected patients in Guinea-Bissau - a cohort study from West Africa2016 doi:10.11604/pamj.2016.25.40.8329; 25:40.
25. Honge BL, Bjarnason Obinah MP, Jespersen S, Medina C, Te Dda S, da Silva ZJ, et al. Performance of 3 rapid tests for discrimination between HIV-1 and HIV-2 in Guinea-Bissau, West Africa. J Acquir Immune Defic Syndr. 2014;65(1):87-90.
26. Deayton J, Mocroft A, Wilson P, Emery VC, Johnson MA, Griffiths PD. Loss of cytomegalovirus (CMV) viraemia following highly active antiretroviral therapy in the absence of specific anti-CMV therapy. Aids. 1999;13(10):1203-6.
27. Lucas SB, Hounnou A, Peacock C, Beaumel A, Djomand G, N'Gbichi JM, et al. The mortality and pathology of HIV infection in a west African city. Aids. 1993;7(12):1569-79.
28. Benmarzouk-Hidalgo OJ, Cordero E, Martin-Pena A, Garcia-Prado E, Gentil MA, Gomez-Bravo MA, et al. Prevention of cytomegalovirus disease using pre-emptive treatment after solid organ

transplant in patients at high risk for cytomegalovirus infection. Antivir Ther. 2009;14(5):641-7.
29. Levitsky J, Freifeld AG, Puumala S, Bargenquast K, Hardiman P, Gebhart C, et al. Cytomegalovirus viremia in solid organ transplantation: does the initial viral load correlate with risk factors and outcomes? Clin Transplant. 2008;22(2):222-8.
30. Nordentoft PB, Engell-Sorensen T, Jespersen S, Correia FG, Medina C, da Silva Te D, et al. Assessing factors for loss to follow-up of HIV infected patients in Guinea-Bissau. Infection. 2017;45(2):187-97.
31. Brinkhof MW, Pujades-Rodriguez M, Egger M. Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. PLoS One. 2009;4(6):e5790.
32. Honge BL, Jespersen S, Nordentoft PB, Medina C, da Silva D, da Silva ZJ, et al. Loss to follow-up occurs at all stages in the diagnostic and follow-up period among HIV-infected patients in Guinea-Bissau: a 7-year retrospective cohort study. BMJ Open. 2013;3(10):e003499.
33. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. 2010;20(4):202-13.
34. Raufman JP. Declining gastrointestinal opportunistic infections in HIV-infected persons: a triumph of science and a challenge for our HAARTs and minds. Am J Gastroenterol. 100. United States2005. p. 1455-8.
35. Wittkop L, Bitard J, Lazaro E, Neau D, Bonnet F, Mercie P, et al. Effect of cytomegalovirus-induced immune response, self antigen-induced immune response, and microbial translocation on chronic immune activation in successfully treated HIV type 1-infected patients: the ANRS CO3 Aquitaine Cohort. J Infect Dis. 2013;207(4):622-7.
36. Singh KP, Howard JL, Wild SP, Jones SL, Hoy J, Lewin SR. Human cytomegalovirus (CMV)-specific CD8+ T cell responses are reduced in HIV-infected individuals with a history of CMV disease despite CD4+ T cell recovery. Clin Immunol. 2007;124(2):200-6.
37. Aichelburg MC, Weseslindtner L, Mandorfer M, Strassl R, Rieger A, Reiberger T, et al. Association of CMV-Specific T Cell-Mediated Immunity with CMV DNAemia and Development of CMV Disease in HIV-1-Infected Individuals. PLoS One. 2015;10(8):e0137096.
38. Dodt KK, Jacobsen PH, Hofmann B, Meyer C, Kolmos HJ, Skinhoj P, et al. Development of cytomegalovirus (CMV) disease may be predicted in HIV-infected patients by CMV polymerase chain reaction and the antigenemia test. Aids. 1997;11(3):F21-8.
39. Oliveira I, Andersen A, Furtado A, Medina C, da Silva D, da Silva ZJ, et al. Assessment of simple risk markers for early mortality among HIV-infected patients in Guinea-Bissau: a cohort study. BMJ Open. 2012;2(6).

40. Ford N, Shubber Z, Saranchuk P, Pathai S, Durier N, O'Brien DP, et al. Burden of HIV-related cytomegalovirus retinitis in resource-limited settings: a systematic review. Clin Infect Dis. 2013;57(9):1351-61.
41. Martinson NA, Karstaedt A, Venter WD, Omar T, King P, Mbengo T, et al. Causes of death in hospitalized adults with a premortem diagnosis of tuberculosis: an autopsy study. Aids. 2007;21(15):2043-50.
42. Rennert WP, Kilner D, Hale M, Stevens G, Stevens W, Crewe-Brown H. Tuberculosis in children dying with HIV-related lung disease: clinical-pathological correlations. Int J Tuberc Lung Dis. 2002;6(9):806-13.
43. Hardie DR, Korsman SN, Hsiao NY. Cytomegalovirus load in whole blood is more reliable for predicting and assessing CMV disease than pp65 antigenaemia. J Virol Methods. 2013;193(1):166-8.
44. Mhiri L, Kaabi B, Houimel M, Arrouji Z, Slim A. Comparison of pp65 antigenemia, quantitative PCR and DNA hybrid capture for detection of cytomegalovirus in transplant recipients and AIDS patients. J Virol Methods. 2007;143(1):23-8.

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TABLE 1: Clinical characteristics of included HIV infected patients, stratified according to CMV DNA status, using chi-square test for comparison of groups.

	CMV DNA undetectable	**CMV DNA detectable**	
	n (%)	n (%)	p-value
Sex, n	54	83	0.85
Female	36 (66.7)	54 (65.1)	
Male	18 (33.3)	29 (34.9)	
Age (years), n	54	83	0.02
18-30	12 (22.2)	21 (25.3)	
31-45	35 (64.8)	34 (41.0)	
46-60	7 (13.0)	26 (31.3)	
>61	0 (0.0)	2 (2.4)	
HIV type, n	46	74	0.83
HIV-1	39 (84.8)	60 (81.1)	
HIV-2	5 (10.9)	9 (12.2)	
HIV-1/2	2 (4.4)	5 (6.8)	
CD4 cell count (cell/µL), n	50	83	<0.01
0-200	22 (44.0)	44 (53.0)	
201-350	2 (4.0)	19 (22.9)	
351-500	10 (20.0)	10 (12.1)	
500-max	16 (32.0)	10 (12.1)	

TABLE 2: Clinical characteristics of patients, stratified according to QuantiFERON-CMV response.

	Low[1] QF-CMV response n (%)	**High[2] QF-CMV response** n (%)	p-value
Odynophagia*, n	29	31	0.04
No	23 (79.3)	17 (54.8)	
Yes	6 (20.7)	14 (45.3)	
Dysphagia*, n	29	31	0.02
No	23 (79.3)	16 (51.6)	
Yes	6 (20.7)	15 (48.4)	

*Self-reported, [1]Low response: 0.2-500 IU/mL, [2]High response: 501-max IU/mL

FIGURE 1: Flowchart of patient inclusion in the CMV prevalence study.

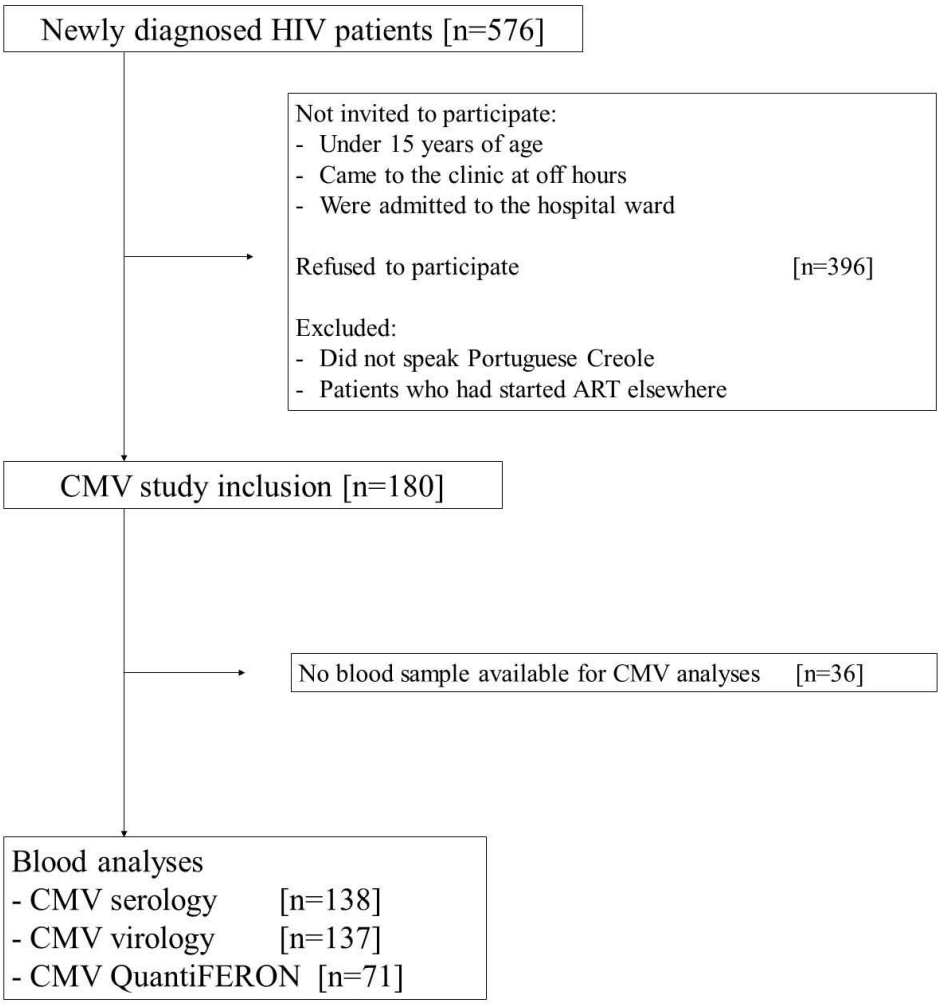
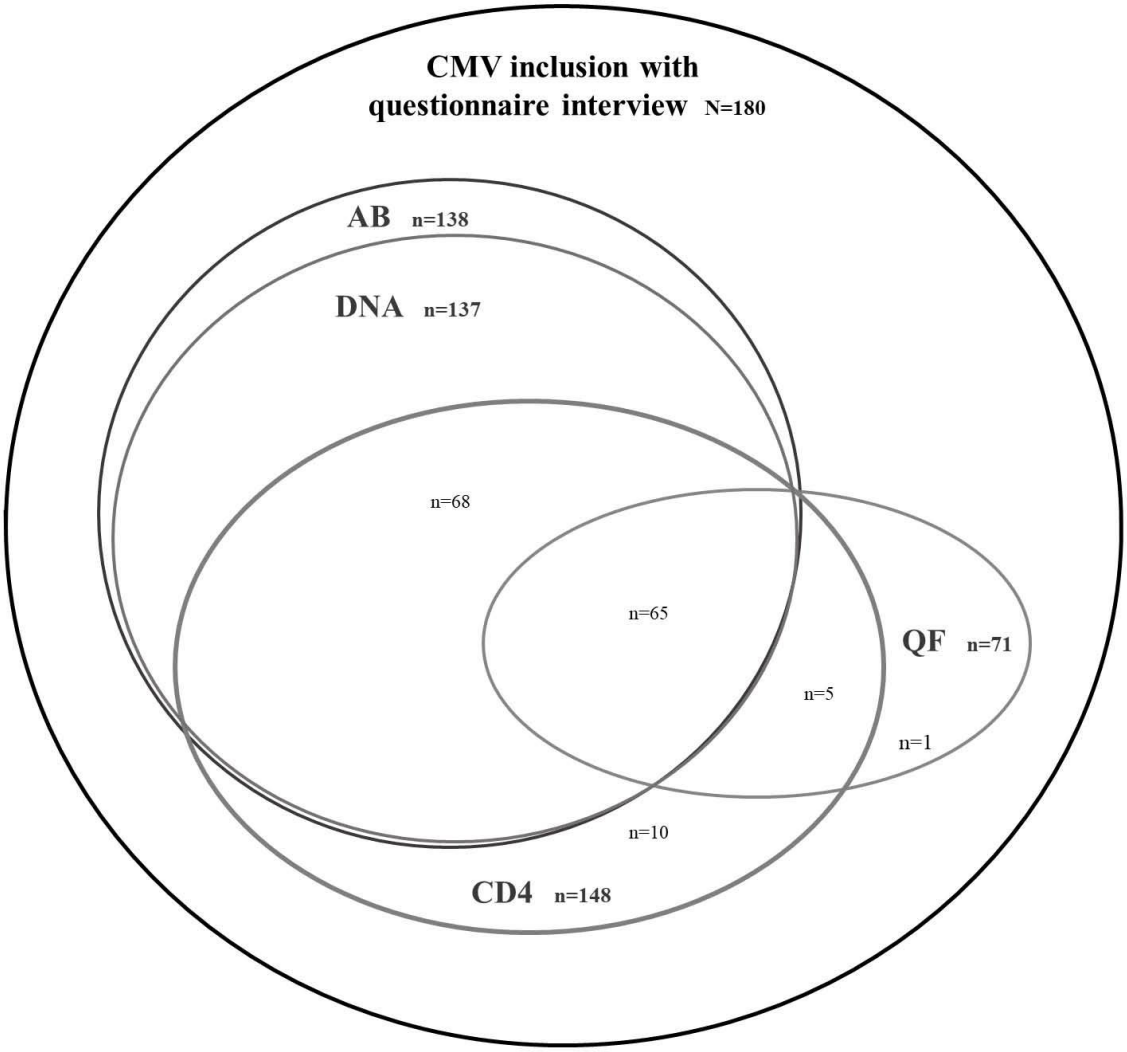


FIGURE 2: Illustration of number of patients included in the different blood analyses during the study.



(**CD4:** CD4 cell count: **DNA:** CMV DNA analysis; **QF:** CMV QuantiFERON analysis; **AB:** CMV antibody analysis)

FIGURE 3: Illustration of the association between the reactive QuantiFERON-CMV response values and age, using logistic regression analysis.