# Diagnostic accuracy of HIV Viral Load as a marker for Cryptococcal Antigenemia screening at a tertiary hospital in Uganda

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# Abstract

**Background:** Cryptococcal Meningitis (CM), is the second leading cause of HIV-related mortality in Uganda after TB. A CD4 cell count triggered CRAG screening algorithm and pre-emptive therapy is the mainstay of prevention of CM. The recent absolution of routine CD4 monitoring for stable patients on Highly Active Antiretroviral Therapy (HAART) has shifted this trigger to suspected virological failure.

**Objective:** To assess the performance of Viral Load (VL) as a marker for CRAG screening among patients on HAART at an HIV clinic in Mulago National Referral Hospital

**Methods:** This was a cross sectional diagnostic study conducted at the Baylor Uganda Centre of Excellence HIV clinic in Mulago National Referral hospital. Records of 798 HIV positive patients aged 10 years and above, on HAART for at least 6month, with at least one viral load and a corresponding serum CRAG done between January 2017 and December 2018 were extracted from the Electronic Medical Records. The test under evaluation was a VL cut-off of greater or equal to 1000cp/ml (suspected treatment failure) and the Gold standard was Serum CRAG Lateral Flow Assay (LFA). Sensitivity, specificity, positive predictive value, negative predictive value, Likelihood ratio positive, likelihood ratio negative and the Area under the Receiver Operating Characteristic (ROC) curve were then determined using 2x2 contingency tables and ROC curve analysis.

**Results:** Prevalence of CRAG using the gold standard was 0.6% (95%CI: 0.2-1.5). The sensitivity and specificity of VL≥1000cp/ml as a marker for CRAG were 20% (95%CI: 2.1-74.4) and 99.4% (95%CI: 98.4-99.8) respectively. The Area under the ROC curve was 0.56 (95%CI: 0.29-0.82). The likelihood ratio positive and negative were 1.0. The optimal VL cut-off was VL≥49cp/ml with a sensitivity of 60% and specificity of 53%.

**Conclusion:** In populations with low CRAG prevalence, treatment failure (VL≥1000cp/ml) is not a good marker for CRAG screening. A more conservative cut-off of 49cp/ml may be considered were resources allow.


# Introduction

Cryptococcal Meningitis (CM), is the second leading cause of HIV-related mortality in Uganda after Tuberculosis [1] and accounts for 60% of all cases of meningitis in Uganda [2] with a mortality of up to 39% [3]. CM is caused by members of the fungal genus Cryptococcus which was first documented a couple of decades ago and now has at least 36 species described, with only a few known to cause diseases. Two species are known to cause disease in immunocompromised human's namely C. neoformans and C. gattii [4]. CM is the severe form of the Cryptococcal infection involving inflammation of the brain meninges.

In the recent years the prevalence of Cryptococcal infection has seen a decline from 19% in 2012 to 6.5% in 2016 [5, 6] due to increased access to HAART, however the high mortality due to CM has not changed.

A recent study in multiple HIV clinics in Uganda between 2015 and 2017 revealed that over half of the patients with confirmed CM were dead or lost to follow-up after 6months [7]. Death due to CM can be prevented using pre-emptive therapy with high dose fluconazole before symptoms occur. These symptoms include but are not limited to; stiff neck, severe headache, vomiting, photophobia and convulsions and often indicate involvement of the meninges, a point at which preventive therapy is of little value.

Preventive therapy is most effective when CM is identified in its precursor form, prior to the development of symptoms also referred to as Asymptomatic Cryptococcal antigenemia (CRAG) however Identifying patients with Asymptomatic CRAG remains a challenge in many resource limited settings [8]. A quick and cost-effective way to determine whether one has Asymptomatic Cryptococcal antigenemia is by identifying antibodies induced by a substance on the cell wall of the fungus known as a Cryptococcal antigen. The Cryptococcal antigen can be detected in the body weeks to months before symptoms of CM appear using Serum CRAG Lateral Flow Assay (LFA) which is an easy to use bedside rapid test. When this test was validated and approved by WHO it presented a unique opportunity for universal screening of CRAG.

Universal CRAG screening of HIV positive patients was however found not to be cost effective [9] and in 2011, WHO developed an algorithm for targeted CRAG screening that relied on the baseline CD4 counts below 100 cells as a marker for CRAG screening among the HAART naïve and experienced HIV patients. This followed a number of studies that demonstrated that CD4 cell counts below 100cells/UL were highly predictive of Cryptococcal infection [10].

However, with the absolution of CD4 monitoring for the stable patients on ART in the Test and Treat era, the trigger for CRAG screening in Uganda shifted from CD4 cell counts below 100cells/UL to treatment failure (Viral load greater or equal to 1000 copies/ml). In this screening algorithm only patients above this Viral load cut-off and with CD4 cell counts below 100cells/UL are subjected to Serum CRAG Lateral Flow Assay (LFA) test [11]. This has naturally put a spotlight on the validity of viral load cut-offs as markers for opportunistic infection screening in the absence of routine CD4 monitoring and is the motivation for this study. This study therefore sought to assess the diagnostic accuracy of Viral Load as a marker for CRAG screening and determine an optimal cut-off for this purpose.

# Methods

### *Study Design*

This was a cross-sectional diagnostic study utilizing secondary data.

### *Study Setting and Population*

The study was conducted at the Baylor Uganda Centre of Excellence (COE) HIV clinic located at Mulago National Referral Hospital. The Baylor Uganda COE is the largest pediatric clinic in the Africa and it has a

total of 7,302 active clients LHIV of whom 2,102 (29%) are children below the age of 10 years and 3,123 (43%) are adolescents aged 10- 19 years. All children under 15 years of age and 96% of adults enrolled in HIV care are on HAART. VL monitoring is the preferred mode of assessing HAART efficacy and 92% of all the patients in care and on HAART for more than 6 months have had at least one VL test within a 12 months period. Eighty Five Percent of the clients that were monitored in 2018 using viral load achieved viral suppression [12].

The study population was HIV infected patients who had been on ART for at least 6 months at the Baylor Uganda HIV clinic between January 2017 and December 2018. This population was from an ongoing cohort study whose main objective is to determine the long term outcomes of patients initiated on both first and second line HIV treatment. This open cohort consecutively recruits patients aged between zero and twenty years. The patients were recruited into the cohort study as new ART naïve patients or patients switching to the second line. These patients were provided the standard of care with a 6-monthly VL and CD4 monitoring. In addition, all the study participants in the cohort had a serum CRAG test done once a year. The clinical, laboratory and follow-up details of the study participants were stored in a well maintained electronic medical records (EMR) database as well as hardy copy files.

### *Data collection.*

Secondary data was extracted from the existing cohort study's EMR database. This database contained the clinical history and laboratory findings of patients enrolled in the cohort study between January 2017 and December 2018. A data abstraction tool was used to design queries in MS Access database from which the secondary data was being extracted**.** Efforts were made to correct abnormal values and missing values using alternative sources such as the lab register and hard copy files**.** Patients with missing data on key variables such as viral load and CRAG status were excluded from the study.

### *Measurement of variables*

#### Screening test: Current Viral Load

The screening test was current VL which is a measure of the number of HIV RNA copies in a milliliter of plasma. The Viral load was done by the Central Public Health Laboratories (CPHL) that use several viral load measuring machines including: Hologic Panther Fusion system with a low level of detection of 30cpml, Rouche Taqman 1600 series with a detection level of 50 cp/ml, Abbot real-time with a detection level of 40cpml and Rouche COBAS Taqman Version 2.0 with detection level of 20cp/ml. All the above machines have a sensitivity of 100% and a specificity of equal to or greater than 99%. Viral load results presented as Target Not Detected were considered to be zero copies per milliter for the purposes of the study. Suspected treatment failure was determined dichotomising the current Viral load into a group with VL ≥1000 cp/ml considered  screen while patients with a VL <1000 cp/ml were considered screen negative.

#### Gold Standard:

The gold standard was a Serum CRAG Lateral Flow Assay (LFA) test with a binary outcome of positive or negative  The CRAG LFA is a strip filled with monoclonal antibodies that bind with Cryptococcal capsular polysaccharide antigen of all C. Neoformans serotypes.  The CRAG LFA Assay has a sensitivity of 99% and a Specificity of 99%.

### *Data analysis*

Statistical Analysis was done using STATA College Station TX Version 14. Baseline characteristics of the study subjects such as Age, BMI, CD4 count, and VL were summarized using median and interquartile range. Frequencies and proportions were used to summarize categorical variables such as sex. Age, CD4, and VL were then categorized for further analysis. Age was categorized into three levels namely 10-14, 15-19 and ≥20years. CD4 was categorized into three levels namely <100, 100-199 and ≥200 cells/UL. It was further dichotomized into CD4<100 and ≥100 cells/UL. Viral Load was dichotomized into VL≥1000 and <1000cp/ml coded 1 and 0 respectively. The serum CRAG test was the gold standard and was coded as 1 for positive and 0 for negative. Sensitivity, specificity, positive predictive value, negative predictive value, Likelihood ratio positive, and likelihood ratio negative were then determined using 2x2 contingency tables. Cohen's Kappa was used to assess the reliability of VL as a marker for CRAG screening.

The area under the ROC curve (AUC) of a plot of sensitivity versus the false positive rate was calculated to determine the overall accuracy of viral load as a marker for CRAG screening for various VL cut offs. The optimal VL criterion and associated sensitivity and specificity were then determined by obtaining the corresponding coordinates of the ROC curve closest to the upper left corner. The closer the ROC curve is to the upper left corner, the higher the overall accuracy of the cut-off point.

### *Ethical approval*

Ethical approval and exemption from requiring informed consent to collect secondary data from an ongoing cohort study at Baylor Uganda was obtained from Makerere University School of Public Health Higher Degrees, Research and Ethics Committee (IRB/CLEAR/004/19) and Baylor College of Medicine International Pediatrics AIDS Initiative Research Headquarters.

All the methods used in this study were performed in accordance with ICH Good Clinical Practice E6, the Declaration of Helsinki and the Uganda National Guidelines for Research Involving Humans as Research Participants. Where the three guiding documents offered differing guidance, the Uganda National Guidelines for Research Involving Humans as Research Participants took precedence.

Confidentiality was maintained at all times. Routine identifiers such as names of patients were replaced with study numbers. Data protection was ensured by storing the data on a password protected computer. A back up copy of the data was stored on a password protected portable hard drive securely kept by the investigator.

# Results

## Baseline Characterisitcs of study subjects

Records of 838 HAART-experienced HIV positive patients who attended the Baylor Uganda HIV clinic between January 2017 and December 2018 were screened for inclusion into the study. Forty (5%) records of HAART-experienced HIV positive patients were excluded due to missing data on key variables. The final sample size was 798 records which represented 95% of the screened records.

The median age was 18 years (IQR:14-21years). More than half of the study subjects were female, 424 (53.1%). The majority of the patients, 727 (91.1%) had CD4 cell counts that were greater or equal to 200 cells/UL. Only, 44 (6%) of the subjects had a CD4 <100cell/ul. Close to a fifth of the subjects, 155 (19.4%) had suspected treatment failure (≥1000cp/ml). Table 1 below shows the baseline characteristics of the study subjects.

**Table 1: Baseline Characteristics of the study subjects**

| Variables | N=798 |
|---|---|
| **Gender** | |
| Female | 424 (53.1) |
| Male | 374 (46.9) |
| **Age in completed years** | |
| **Median (IQR)** | 18 (14-21) |
| **BMI in kgs/m$^2$** | |
| <18.5 | 303 (39.1) |
| 18.5-24.9 | 430 (55.4) |
| 25.0-29.9 | 33 (4.3) |
| ≥30 | 10 (1.3) |
| **Absolute CD4 count (cells/ul)** | |
| **Median (IQR)** | 728.5 (495-1008) |
| <100 | 44 (5.5) |
| 100-199 | 27 (3.4) |
| ≥200 | 727 (91.1) |
| **Viral Load (cp/ml)** | |
| **Median (IQR)** | 0 (0-280) |
| <1000 | 643 (80.6) |
| ≥1000 | 155 (19.4) |

### Prevalence of cryptococcal antigenemia among art-experienced patients

Only Five patients out of 798 had a positive serum CRAG test, therefore the prevalence of Cryptococcal antigenemia among HAART-experienced patients was 0.6% (95%CI: 0.2-1.5). All the Five patients with a positive serum CRAG had a CD4 cell count <500cells with only Two out of the five patients having CD4 below 100 cells/ul.

### Suspected treatment failure among patients with cryptococcal antigenemia

The median Viral load among patients with a positive CRAG was 49 cp/ml ( IQR: 0 – 119) and was not significantly different from the median viral load among patients with a negative CRAG 0 cp/ml (IQR:0 –

280), p-value: 0.633. Suspected treatment failure was present in only one patient out of the 5 with Cryptococcal antigenemia (Table2).

### Sensitivity and specificity of suspected treatment failure (VL≥1000) as a marker for CRAG screening

Only One out of Five patients with a Positive Serum CRAG was correctly identified by a VL cut off of ≥1000 cp/ml giving a sensitivity of 20% (95% CI: 2.1-74.4) while 639 out of 793 were correctly identified as not having CRAG giving a specificity was 80.6% (95%CI: 77.7-83.2). The Positive Predictive value for VL≥1000 cp/ml was 0.65% (95% CI: 0.09-4.47) while the Negative Predictive value was 99.4% (95%CI: 98.4-99.8) for VL. The likelihood ratio positive was 1.029 while the likelihood negative was 0.992 as shown in table 2.

### Table 2: Sensitivity and specificity of suspected treatment failure (VL≥1000) as a marker for CRAG screening

| | **Disease (Positive CRAG)** | **No Disease (Negative CRAG)** | Total | |
|---|---|---|---|---|
| **Test Positive (>=1000)** | 1 | 154 | 155 | **PPV** 0.65 (0.1-4.5) |
| **Test Negative (<1000)** | 4 | 639 | 643 | **NPV** 99.4 (98.4-99.8) |
| **Total** | 5 | 793 | | |
| | **Sensitivity** | **Specificity** | | |
| | 20 (2.1-74.4) | 80.6 (77.7-83.2) | | |
| | **Likelihood ratio positive** | **Likelihood ratio Negative** | | |
| | 1.029 | 0.992 | | |

### Comparison of suspected treatment failure (VL≥1000cp/ml) and immunological failure (CD4<100cells/UL) as markers for CRAG screening

CD4<100cell/ul had a higher sensitivity 40% (95% CI: 8.2-83.2) and specificity 94.7% (95%CI: 92.9-96.1) than VL≥1000cp/ml. Sequential testing beginning with VL≥1000 cp/ml and then CD4<100cells/ul did not result in an improved sensitivity, 20% (95%CI: 2.1-74.4). Similarly there was a negligable improvement in the net PPV 2.8% (95%CI: 0.1-14.9) and net NPV 99.5% (95%CI: 98.7-99.9) as compared to VL alone. Reliability of VL≥1000 and CD4<100 was less than satisfactory individually and in sequential combination(Cohen's Kappa= 0.0004, 0.0712 and 0.0436 respectively) as shown in table 3.

**Table 3: Comparison of suspected treatment failure (VL≥1000cp/ml) and CD4<100cells/UL as markers for CRAG screening**

| Cut off | Sensitivity | Specificity | PPV | NPV | Cohen's Kappa |
|---|---|---|---|---|---|
| VL≥1000 cp/ml | 20 (2.1-74.4) | 80.6 (77.7-83.2) | 0.65 (0.1-4.5) | 99.4 (98.4-99.8) | 0.0004 |
| CD4<100cells/ul | 40 (8.2-83.2) | 94.7 (92.9-96.1) | 4.5 (1.1-16.7) | 99.6 (98.8-99.9) | 0.0712 |
| VL≥1000 cp/ml and CD4<100cells/ul (Sequential) | 20 (2.1-74.4) | 95.7 (94.1-97.0) | 2.8 (0.1-14.9) | 99.5 (98.7-99.9) | 0.0436 |

**Overall diagnostic accuracy of Viral load for identifying ART-experienced HIV infected patients with cryptococcal antigenemia.**

The ROC curve for VL as a marker for CRAG screening is shown in figure 1 and reveals a slightly above average AUC of 0.557 (0.290-0.824).

CD4 remained superior to VL as a marker for CRAG screening with an AUC=0.912 (0.865, 0.959) as shown in figure 2.

Table 4 below shows the sensitivity and specificity of some selected VL cut off points as markers for CRAG screening.

**Table 4: Sensitivity and specificity of selected cut-off points for VL as a marker for CRAG screening**

| Some selected VL Cut off points(cp/ml) | Sensitivity | Specificity |
|---|---|---|
| ≥ 0 | 100.00% | 0.00% |
| ≥ 19 | 60.00% | 52.46% |
| **≥ 49** | **60.00%** | **53.47%** |
| ≥ 150 | 20.00% | 70.87% |
| ≥ 1,030 | 20.00% | 80.58% |
| ≥ 10,4000 | 20.00% | 95.21% |

Generally, the sensitivity of VL as a marker for CRAG decreased as the cut-off point increased. Conversely, the specificity of VL as a marker for CRAG increased as the cut off increased as shown in figure 3. From the ROC curve in figure 1 and table 4 the optimal cut off VL as a marker for CRAG screening corresponds to a sensitivity of 60% and specificity of 53% giving a VL cut off of ≥49cp/ml. Figure 3 further reveals that there was no change in the sensitivity of VL as a marker for CRAG screening as the cut-off was lowered gradually from over 10,000cp/mls to greater or equal to 150cp/ml.

**Table 5: Comparison of Sensitivity and specificity of VL≥1000cp/ml and VL≥49cp/ml as markers for CRAG screening.**

| | Serum crag | | Likelihood ratio | | |
|---|---|---|---|---|---|
| **VL Cut off** | **Positive (+ve) n=5** | **Negative (-ve) n=793** | **+ve** | **-ve** | **Cohen's Kappa** |
| **≥1000cp/ml** | 1 (SN=20, PPV=0.65) | 639 (SP=80.6, NPV=99.4) | 1.029 | 0.992 | 0.0004 |
| **≥49cp/ml** | 3 (SN=60, PPV=0.81) | 424 (SP=53.5, NPV= 99.5) | 1.289 | 0.748 | 0.0036 |

The VL cut off of ≥49cp/ml identified three out of five patients with a Positive Serum CRAG giving a sensitivity of 60% while the VL cut off of ≥1000cp/ml identified only one out of five patients with Positive serum CRAG giving a sensitivity of 20% as shown in table 5. The PPV for VL≥49cp/ml was 0.81% compared to 0.65% of VL≥1000cp/ml. The NPV of both cut-offs was approximately similar (99.4% vs 99.5%). Both cut-offs had less than satisfactory reliability (Cohen's Kappa <0.7)

# Discussion

In this cross-sectional study that analyzed records of HAART-experienced HIV positive patients who attended the Baylor Uganda HIV clinic between January 2017 and December 2018, the prevalence of CRAG among ART experienced HIV infected patients was 0.6%. This prevalence is lower than the prevalence of CRAG among HAART naïve HIV positive patients in Uganda which ranges from 1.4% to 6% [7, 13]. This could be due to two equally possible phenomena namely; HAART improves the body's immunity making it less susceptible to opportunistic infections such as Cryptococcus or this particular population could simply have a low prevalence of CRAG. In Uganda currently, no published studies have established the prevalence of CRAG among HAART experienced HIV positive patients. However, the prevalence of CRAG in this study is also lower than the prevalence of CRAG among HAART experienced patients in other parts of the world, 2.4% [14], 6% [15] and 14% [16]. The difference between our findings and those in previous studies is likely due to the differences in the study setting and the fact that this study was done among patients in an existing study where the care and treatment provided may be different from the standard of care in other public facilities.

The sensitivity of VL≥1000 cp/ml as a marker for CRAG screening among ART experienced patients in this study is 20%. This low sensitivity is not characteristic of a screening test and indicates that VL≥1000 cp/ml is not an ideal threshold for triggering CRAG screening. R Trevethan [17] agrees with this assertion and notes that a good screening test should have a very high sensitivity. The Positive Predictive value of VL≥1000 cp/ml as a marker for CRAG screening among ART experienced patients in this study is also very low at 0.7%. This low PPV is partly due to the low prevalence of CRAG since PPV is positively associated with prevalence [17]. A screening test with low PPV results in many false positives which has negative psychological impacts on the patients and puts an undue burden on the health facility. Other studies evaluating the predictive value of VL on the risk of HIV/AIDS related short-term outcomes such as death also report low PPV values [18, 19]. Furthermore, in this study, it was determined that a classification of VL≥1000 was 1.029 times more likely to happen in a patient with a positive CRAG than it would in a patient with a negative CRAG. This likelihood ratio of approximately 1 means that a VL≥1000 cut off has no clinical significance in CRAG screening since it does not increase the probability of having a disease [20].

The current CRAG algorithm in Uganda is triggered by a VL cut off of ≥1000 and all the patients with VL≥1000 are then subjected to a CD4 test were only patients with CD4<100 have Serum CRAG done. The net sensitivity of this sequential screening is no different from the sensitivity of VL≥1000 alone and the net PPV shows a negligible increase from 0.6% to 2.8%. There is thus no justification for this sequential testing especially as the world has moved away from CD4 monitoring. Never the less CD4 remains superior to VL as a marker for CRAG screening as this study demonstrates an overall accuracy of 91% as compared to 56% for VL. These findings are supported by previous studies that have found CD4 cell count of <100 to be highly predictive of CRAG positivity [9, 21, 22]. These findings also demonstrate that viral load is not a good proxy for CD4 as a marker for CRAG screening. This could be due to the high prevalence of Immuno-virological discordance [23] or absence of a clinically significant relationship between CRAG and Viral load.

The overall performance of VL as a marker for CRAG screening is just slightly above average as indicated by a 56% accuracy in separating the disease from no disease. This poor performance could be due to a poor relationship between HIV Viral load and CRAG but could also be due to the imbalance of VL in the representation of the binary cut-off classes since more than half of the patients had a VL that was not detected as assigned an arbitrary zero. This could also explain the difference between the current study findings and those of a similar study [19] that assessed the predictive accuracy of current VL in predicting short-term and long-term clinical outcomes. In that study, the AUC of current VL was 0.6. Another similar study [18] reports an even higher AUC of 0.77 for current VL predicting 1 year AIDS related mortality among 489 pregnant women attending Kenya National Hospital. This higher AUC, however, can be explained by the fact that other causes of AIDS related mortality such as TB have a stronger correlation with HIV viral load than Cryptococcal disease [24].

According to the findings in this present study, a VL cut off ≥49cp/ml is, in theory, the ideal cut-off threshold for triggering Cryptococcal antigenemia with a sensitivity of 60% and specificity of 53%.

VL≥49cp/ml correctly identified three out of five patients with Cryptococcal antigenemia compared to VL≥1000cp/ml that only identified one out of five patients with Cryptococcal antigenemia. The statistical implication of these findings is of course severely restricted by the small number of patients with Cryptococcal antigenemia. Similarly, the clinical significance of this cut off is also limited by the very low PPV and Likelihood ratio that is very close to one. In addition, CPHL the VL reference laboratory in Uganda currently uses several VL machines some of which have their detection levels above the 49cp/ml mark. For instance, the Rouche Taqman 1600 series has a detection level of 50 cp/ml. This renders this cut-off unusable among patients whose viral load was measured using such machines. However for purposes of ruling out disease, patients with ≥49 cp/ml and have non-specific signs indicative of Cryptococcal disease such as headache and fever should probably be candidates of CRAG screening despite not being classified under treatment failure. Other factors of course such as the prevalence of the disease, cost effectiveness, purpose of screening, availability of resources should be added to the equation before choosing a cut-off as low as 49cp/ml. It is important to note however that both cut offs do not achieve satisfactory reliability going by the Cohen's Kappa of 0.7 as a threshold for satisfactory reliability [25].

One of the strengths of this study is the missingness rate of only 5%, which was lower than anticipated for secondary data. Secondly the data used in this study was extracted from an ongoing cohort study that not only has its own data quality control measures but also has a conveniently homogeneous population. On the other hand, the Gold standard test in our study, Serum CRAG despite having very high sensitivity and specificity is prone to misclassification due to technical malfunction or human error. The low prevalence of CRAG in this study limits the generalizability of the findings of this study to populations with low CRAG prevalence.

# Conclusion

The prevalence of CRAG among HAART experienced HIV infected patients is low. The VL cut-off of ≥1000cp/ml is a poor marker for CRAG screening, however a conservative cut-off of VL ≥49cp/ml is capable of correctly identifying 60% of HAART experienced HIV infected patients with CRAG. Therefore in populations with low CRAG prevalence, VL should not be considered as a marker for CRAG screening unless a conservative cut-off of 49cp/ml can adopted. A follow-on study in a population with a high CRAG prevalence would provide further information on VL load as a marker for CRAG screening.

# Abbreviations

VL Viral Load; CRAG Cryptococcal antigenemia; CM Cryptococcal Meningitis; HAART Highly active antiretroviral therapy; ART Antiretroviral therapy; HIV Human immunodeficiency virus; IQR: Interquartile range, ROC Receiver operating characteristic; AUC Area under the curve; SN Sensitivity; SP Specificity; PPV Positive Predictive Value; NPV Negative Predictive Value

# Declarations

## Ethical Approval and Consent to Participate

Ethical approval and exemption from requiring informed consent to collect secondary data from an ongoing cohort study at Baylor Uganda was obtained from Makerere University School of Public Health Higher Degrees, Research and Ethics Committee (IRB/CLEAR/004/19) and Baylor College of Medicine International Pediatrics AIDS Initiative Research Headquarters.

All the methods used in this study were performed in accordance with ICH Good Clinical Practice E6, the Declaration of Helsinki and the Uganda National Guidelines for Research Involving Humans as Research Participants. Where the three guiding documents offered differing guidance, the Uganda National Guidelines for Research Involving Humans as Research Participants took precedence.

## Consent for publication

Not applicable.

## Availability of data and materials

The dataset used and analyzed during this study is available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' contributions

NM designed the study, performed data collection and statistical analyses, and wrote the first draft of the manuscript along with RN LN. CN and RO supported data collection and analysis. HK, and DM participated in study conception and interpretation of results. JMS and ENJ actively supervised all stages of the study including manuscript preparation. All authors read and approved the final manuscript.

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The authors report no competing interests.

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# Figures

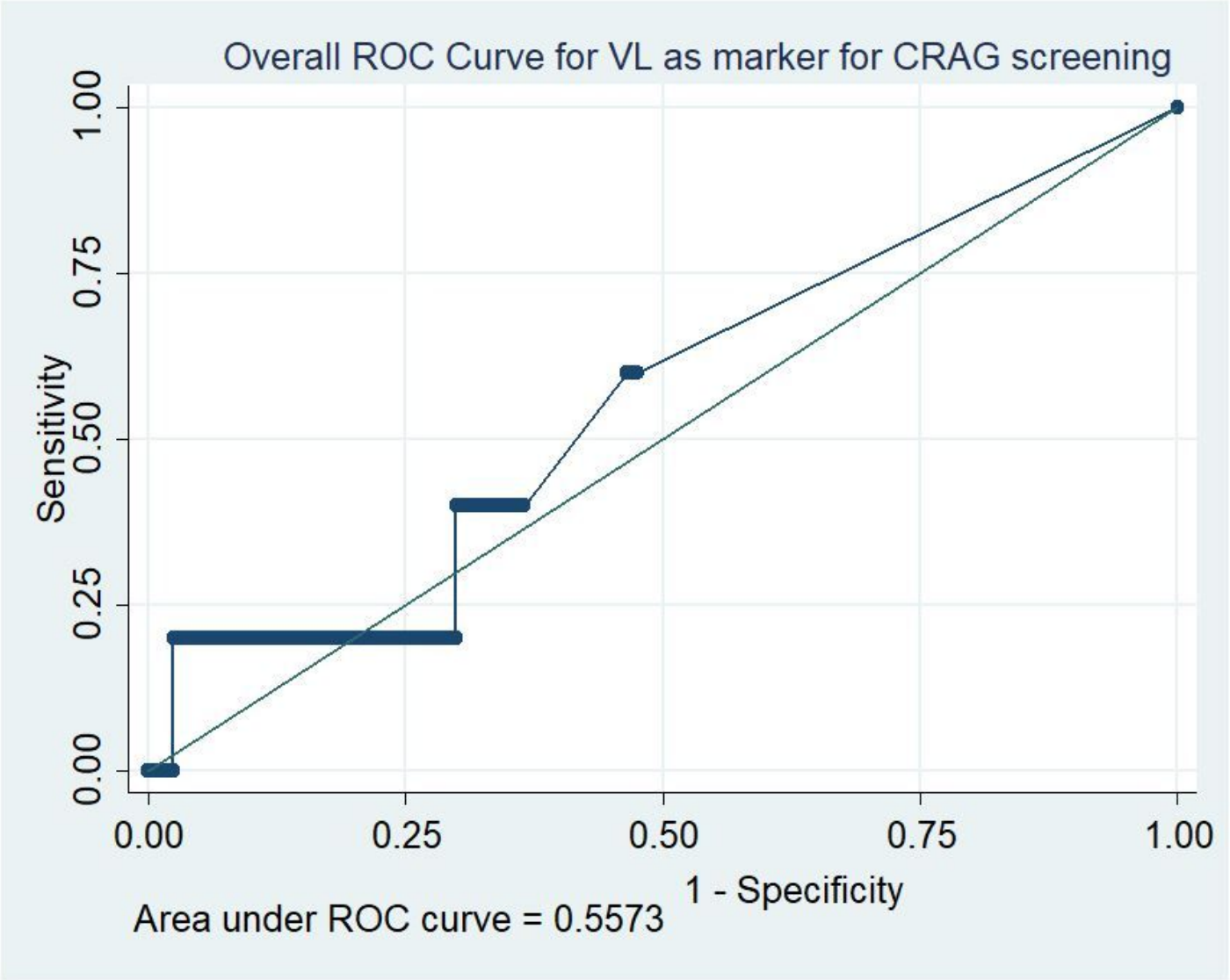



**Figure 1**

ROC curve for VL as a marker for CRAG screening among HAART experienced patients

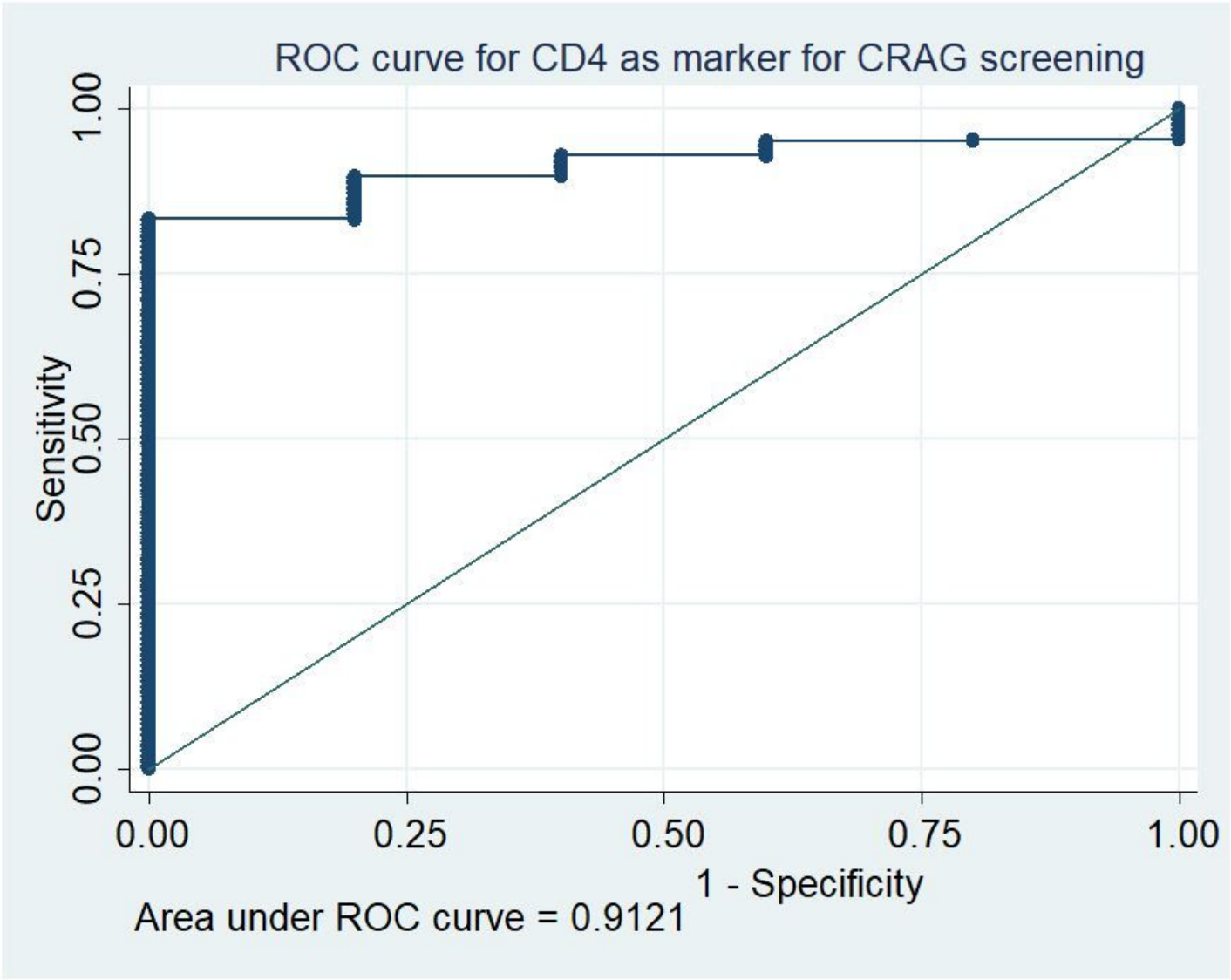
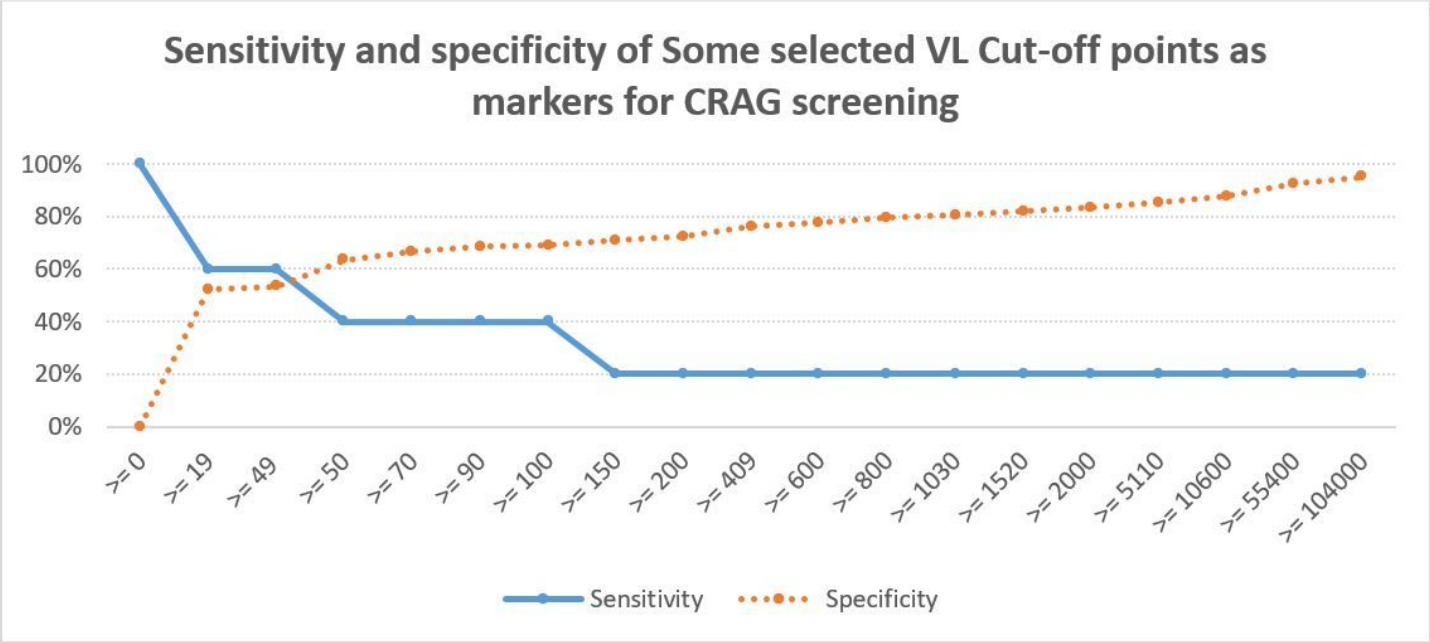



**Figure 2**

ROC curve for CD4 as a marker for CRAG screening among ART experienced patients




## Figure 3

Sensitivity and specificity of some selected VL Cut-off points as markers for CRAG screening