The prognostic value of HIV viral load in predicting viraemic outcomes of post-partum women on antiretroviral therapy: a secondary analysis of two randomised controlled trials from Gugulethu, Cape Town

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PREAMBLE

Declaration

I, Daniel James Stadler Egan (EGNDAN001), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 06 September 2022

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This dissertation is dedicated to my parents, John and Josie.

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Dissertation Abstract

Background: Vertical transmission of HIV continues to be a significant health concern for HIV-infected women in pregnancy and postpartum, especially in Southern Africa, despite decreasing rates of infection. While better access to effective and acceptable antiretroviral therapy (ART) regimens has helped to improve control of viraemia in this group, ongoing vertical transmission continues despite being entirely preventable. The dynamics of transient viraemia, even in previously well-controlled individuals on ART, are not fully understood and may contribute to ongoing mother-to-child transmission (MTCT) despite therapy that is otherwise optimal.

Methodology: Two randomised controlled trials (RCTs), the MCH-ART and PACART trials, were conducted in an antenatal clinic in Cape Town, South Africa, between 2013-2016 and 2016-2019 to investigate improvements to postnatal HIV care through use of integrated postnatal maternal and child clinics and adherence clubs, respectively. The present study conducted a pooled secondary analysis of data from these two trials to investigate the dynamics of HIV viraemia in these women and to estimate the prognostic strength of viral load (VL) measurements during the postpartum period. Sensitivity, specificity, predictive values, likelihood ratios and odds ratios for VL measurements were calculated at different cut-offs to predict later VL >1000 copies/ml. Cox proportional hazards models were used to estimate the risk of two consecutive measurements of VL >1000 copies/ml over the study period, based on starting viral load.

Results: 883 HIV-infected pregnant women were followed-up for a median of 22.1 months (IQR: 17.7-24.1), with a longer median length of follow-up in the PACART cohort (24.0 months) compared to the MCH-ART group (17.9 months). 826 (93.5%) of participants recorded at least one episode of VL <50 copies/ml, with 694 (78.6%) demonstrating VL <50 at the first study visit postpartum. 306 women (37%) demonstrated at least one episode of viraemia in the range of 50-999, while 307 (37.2%) experienced one or more episodes of VL >1000.

As a predictor of future VL >1000, measurable viraemia >50 copies/ml was a specific (87.5%) but not sensitive (68%) test, with a clinical odds ratio of 14.8 (95% CI: 12.4-17.8). Similarly, current VL >200 was a good predictor of VL >1000 at the next visit, with an odds ratio of 26.1 (95% CI: 21.4-31.8), a sensitivity of 64%, and specificity of 94%. Cox proportional hazards analysis found that viraemia in the range of 50-999 copies/ml at the first study visit post-partum was associated with an increased hazard

of two consecutive VL >1000 over the study period, when compared to participants with VL <50 at the same timepoint (HR: 1.75; 95% CI: 1.3-2.4; p<0.001).

Conclusion: Episodes of viraemia in HIV-infected postpartum women remain common despite improving ART regimens and public HIV services. These represent windows of increased risk during which vertical transmission is more likely to occur. VL testing remains a useful tool in monitoring viraemia and may be helpful for identifying those who are more likely to develop substantial and prolonged viraemic episodes. These findings highlight one of the remaining problems perpetuating vertical transmission of HIV: unmeasured, untreated viraemia.

Part A. The protocol for the present study, submitted for departmental and institutional ethics review. This section includes background and rationale, study aims and objectives, and the methodology used.

Part B. A journal-ready manuscript, prepared according to the guidelines for the Journal of the International AIDS Society.

Part C. Appendices, including relevant ethics approval documents and journal submission guidelines.

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List Of Abbreviations

3TC	Lamivudine
ANC	Antenatal Care
ART	Antiretroviral Therapy
ARV	Antiretroviral
CI	Confidence interval
EFZ	Efavirenz
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
IQR	Interquartile range
LLV	Low-level viraemia
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
MCH-ART	Maternal and Child Health Antiretroviral Therapy
MOU	Midwife Obstetric Unit
MTCT	Mother-to-child transmission
NPV	Negative predictive value
PACART	Postpartum Adherence Clubs for Antiretroviral Therapy
PMTCT	Prevention of mother-to-child transmission
PPV	Positive predictive value
ROC	Receiver-operating characteristic
SA	South Africa
SES	Socioeconomic status
TDF	Tenofovir
UCT-HREC	University of Cape Town Human Research Ethics Committee
VF	Virological failure
VL	Viral load
VS	Virological suppression
WHO	World Health Organization

PART A: RESEARCH PROTOCOL

Background and Rationale

In 2021, South Africa (SA) accounted for approximately 15% of all new Human Immunodeficiency Virus (HIV) infections globally – about 230 000 people [1]. While substantial, this is a 45% decrease in the incidence of HIV in SA compared to 2010, highlighting some of the gains made through the national treatment programmes [1]. Similarly, South Africa accounts for 24% of the global prevalence of HIV-positive pregnant women, while approximately 30% of all women presenting for antenatal care in SA are HIV-infected [1,2]. This illustrates a disproportionately large burden on this group of vulnerable women who represent a substantial number of preventable HIV transmissions: those that occur during the peri-partum period.

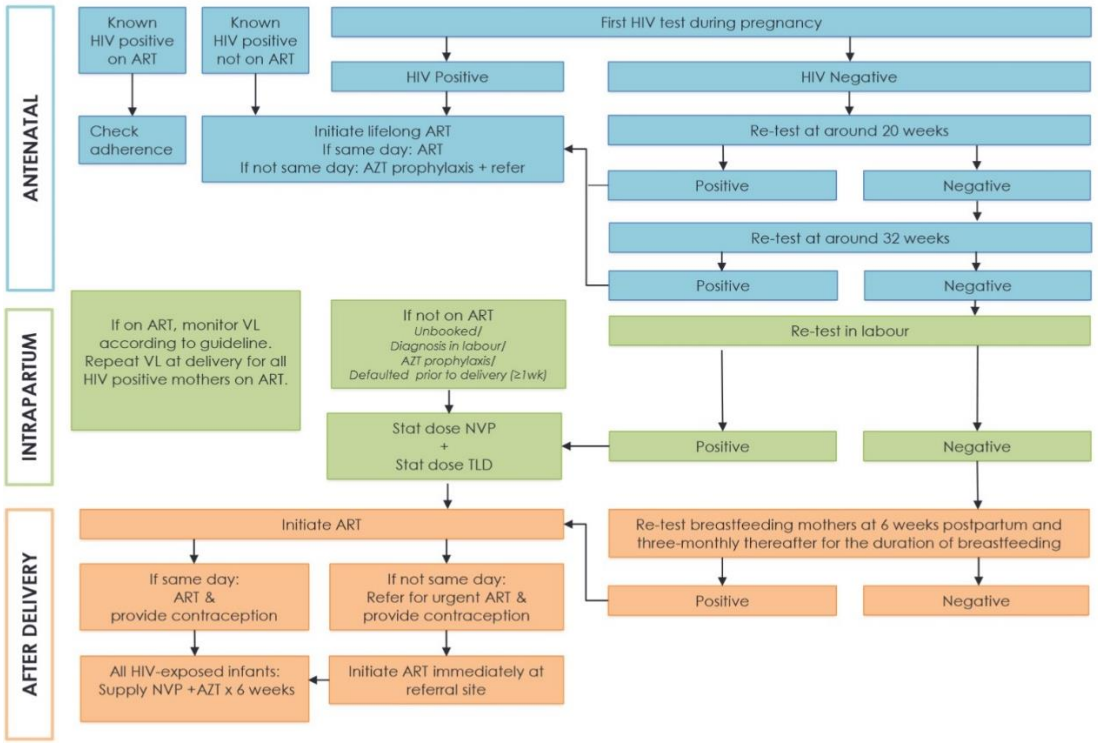
Antiretroviral therapy (ART) is known to be highly effective in preventing mother-to-child transmission (PMTCT), in addition to improving overall maternal health outcomes [2,3]. Over the past decade, the standard of care for women living with HIV has become lifelong triple-drug ART, regardless of disease status, in accordance with the World Health Organisation's (WHO) 'Option B+' approach [4,5]. South Africa's coverage for pregnant women who receive ART for PMTCT increased from 71% in 2010, to 97% in 2020 [1]. Parallel to this, the mother-to-child-transmission (MTCT) rate has steadily declined from 15% to 4% over the same period [1], illustrating the success of this campaign. Despite clear benefits to both mother and child, adherence to ART throughout the antenatal and postpartum periods often presents challenges, with loss to follow-up and poor adherence being common issues [6–8].

Central to the prevention of MTCT is the targeted reduction of transmission during the highest-risk periods of pregnancy, namely, childbirth and breastfeeding [9]. There are multiple benefits to reducing maternal HIV viral load (VL) during these periods, including: reduced risk of perinatal HIV transmission, improved maternal outcomes, and reduced horizontal transmission [2,3,9]. The importance of post-partum HIV viral suppression is further emphasised in SA, where exclusive breastfeeding is the preferred option regardless of maternal HIV status, in line with WHO recommendations [10]. In the context of encouraging mothers to breastfeed for prolonged periods, virological suppression (VS: VL below viral assay detection threshold) becomes even more important to reduce MTCT during this extended phase that the infant is at risk [4,5,11,12]. The policy to initiate lifelong ART regardless of

CD4 count (Option B+) supports the reduction of the risk of MTCT through increasing the proportion of HIV-infected women who maintain persistent VS.

HIV-positive mothers are prioritised as an important target group for long-term therapy as they represent a substantial proportion of the HIV-infected population that is most readily accessible to healthcare interventions [13]. As the subset of HIV infected people that may go on to transmit the infection vertically, they are an important target for interventions to reduce ongoing spread of HIV[14,15]. Prioritisation of this approach is illustrated in the Western Cape PMTCT management algorithm (Figure 1), which has multiple instances of re-testing for HIV throughout the antenatal period in an effort to identify infections as early as possible, and to initiate ART rapidly when appropriate [12]. It is noteworthy that the emphasis of this algorithm is on the antenatal, intrapartum, and immediate postpartum periods. The current norm in SA is for HIV positive women to return to general ART clinics after this time [16]. This separation between PMTCT and ART services occurs at a time when there is substantial ongoing risk of MTCT, and has been identified as a source of attrition from ART services [7].

Figure 1. PMTCT algorithm for the Western Cape, South Africa. Adapted from Western Cape Department of Health HIV Guidelines [12].



Both the Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) [17] and Maternal and Child Health ART (MCH-ART)[18] trials sought ways to bridge this gap through improving retention on ART for postpartum mothers through adherence support strategies. The PACART trial investigated the use of postpartum community-based adherence clubs to achieve this, while MCH-ART looked at an integrated postnatal service for mothers and their infants.

Virological suppression in pregnant and postpartum women living with HIV through universal access to ART is a major focus of the PMTCT programme in South Africa [12,19]. Current guidelines are unclear as to the optimal course of treatment for women with low-level viraemia (LLV) in the range of 50-1000 copies/ml. SA guidelines, mirroring recommendations from the WHO, advise clinical staff to assess the patient's adherence, drug dosing and the possibility of drug interactions, as these have been associated with persistent low-level viraemia in previous studies [4,12,19,20]. While this is an operationally sound decision given the resource constraints in this setting, these guidelines may prolong time spent on failing first-line treatment in patients that have selected for resistance to ART regimens with a low genetic barrier to resistance [21,22]. For this reason, high-income countries tend to initiate further clinical investigation and intervention when a VL >50 copies/ml is detected [23].

The clinical importance of LLV, as well as transient episodic viraemia, has been demonstrated in multiple different contexts. One large multicentre cohort study conducted in HIV-infected South African patients receiving ART showed an increased risk of future virological failure (VF; two consecutive VL >1000 copies/ml) and switch to second-line ART in patients with LLV compared to patients with VS [22,24,25]. Study investigators found high rates of viraemia, with 79% of patients on first-line ART recording a single episode of LLV, while 21% had an episode of VF, over the study period.

Another study conducted in a cohort of HIV-infected individuals in the high-income setting found that LLV in the range of 50-199 copies/ml was associated with the development of VF in ART-experienced, but not ART-naïve patients [22]. This association was found to be independent of ART regimen or cumulative duration of viraemia. Similarly, a study in newly-diagnosed HIV-infected patients with sustained VS on ART found that patients with isolated episodes of viraemia of VL 50-200 copies/ml, or three consecutive episodes of detectable viraemia <20 copies/ml, were at increased risk of VF within 24 and 60 months [25].

There is comparatively less literature exploring the same phenomenon in the pregnant and postpartum population although there is a demonstrated, substantial ongoing risk of viraemia in the postpartum, as compared to pre-pregnancy or non-pregnant comparison groups [26–33]. One SA-based rural cohort found a similar risk of VF during the antenatal and post-natal periods up to 24

months post-delivery, while a UK-based study found an increased risk of viral rebound postpartum, particularly in the first 3 months after delivery [26,34]. A cross sectional study of a large rural district in South Africa further demonstrated a rebound of viraemia post-partum, following improved VL around the date of delivery [35]. The issues of continued postpartum support, counselling, linkage between antenatal and postnatal care, and community-based care are highlighted as a potential areas for improvement of HIV care in this group [26,34,35].

One large study conducted in Malawi identified relatively low rates of MTCT and mortality in a nationally-representative cohort of breastfeeding infants followed up to 24 months postpartum [36]. Important factors associated with increased risk of HIV transmission or infant death included maternal ART initiation post-conception and maternal viral load >1000 copies/ml at enrolment[36]. Further, adverse outcomes for infants by 24 months were associated with maternal primiparity, non-disclosure of HIV status to their partner, and poor ART adherence [36].

Another study in the Malawian context found that low-detectable VL in the range of 40-1000 copies/ml in postpartum women on ART was associated with a ninefold increased risk of MTCT when compared to those mothers who had a VL <40 copies/ml [37]. Key factors associated with detectable viraemia in this cohort included sub-optimal adherence, adolescence, on ART for <6 months, and fewer years of education [37].

In a South African cohort of women initiating ART during pregnancy, elevated VL in the 12 months following initial suppression was strongly associated with poor adherence, with a minority (<10%) of all instances of viraemia likely to be due to pre-ART drug resistance mutations [38]. The authors further highlight the need for effective strategies to improve support and adherence in this group. A large study using national laboratory surveillance data identified high rates of viraemia (37%) at the time of delivery despite high ART coverage [39]. An ecological study in the South African setting found that population-level predictors of MTCT include maternal postpartum viraemia, geographical areas with higher levels of maternal HIV, late ART initiation in pregnancy and incident HIV infection during pregnancy [40].

The use of VL measurements as a predictive tool could aid in the prioritisation of care for women at a higher risk of VF, and to target these women to improve adherence, support, monitoring, and treatment to prevent VF. Additionally, by using HIV viral load, a routine test in this context, this tool makes use of existing management pathways and structures, incurring no additional burden on medical staff. By identifying these patients, this study hopes to improve the outcomes of both mothers and their infants through improved ART treatment programmes.

In light of this gap in the understanding of the importance of VL measurements in pregnant women living with HIV, this study will investigate the predictive ability of VL measurements as a prognostic tool to estimate future HIV VL.

Study Aim

To investigate the dynamics of HIV viraemia in the postpartum and to determine the ability of HIV viral load measurements to predict future viral load outcomes amongst HIV-infected post-partum women on antiretroviral therapy in Gugulethu, Cape Town.

Objectives

Primary objectives

1. To measure the value of post-partum HIV viral load measurements in predicting later viral load >1000 copies/ml in post-partum HIV positive women
    - Hypothesis: post-partum HIV viral load is a good predictor of future viral load >1000 copies/ml in the subsequent months

Secondary objectives

1. To describe the frequency of HIV viraemia in this population.
    - Hypothesis: None – descriptive

2. To determine if sub-strata with different virological prognoses exist within the range of low-level viraemia
    - Hypothesis: Specific strata exist within the range of 50-1000 copies/ml with differing risks of developing later viral load >1000 copies/ml

3. To describe the demographic and clinical characteristics of this population of HIV-infected post-partum women in South Africa
    - Hypothesis: None – observational/exploratory

Methodology

Study design

This study will consist of a secondary analysis of pooled data from two randomised controlled trials conducted in Gugulethu, Cape Town – the MCH-ART and PACART trials. These studies investigated the role of focused postnatal ART clinics (MCH-ART) and adherence clubs (PACART) as improvements on the current standard-of-care in general ART clinics. These interventions were specifically directed towards HIV-positive women in the postpartum period with the aim of improving ART adherence and maternal HIV viral suppression, thus reducing peri-partum HIV transmission from mother to child. These studies were conducted at the primary care level by investigators from the Division of Epidemiology and Biostatistics in the School of Public Health and Family Medicine at the University of Cape Town. The two studies used sufficiently homogenous study designs, settings, and cohort characteristics for their data to be pooled for the purposes of this analysis.

Setting

The data to be used for this analysis will be obtained from the existing study databases from the MCH-ART and PACART studies. These parent studies obtained data for 471 and 412 participants, respectively, who attended the Midwife-Obstetric Unit (MOU) at Gugulethu Community Health Centre in Cape Town, South Africa. The study periods ranged from 2013-2016 (MCH-ART) and 2016-2019 (PACART). This health centre functions as a primary care antenatal, obstetric and postpartum clinic in the public sector, with an estimated patient load of >4000 women annually[18] . The MOU is primarily operated by nurses with midwifery training, with twice-weekly obstetrician input. Patients requiring a higher level of care are referred to nearby secondary-level obstetric units. The prevalence of HIV in the local antenatal population is high at approximately 30% [16], with a mother-to-child transmission rate of 5.1% in those women who initiate ART during their pregnancy [15]. This MOU has been able to offer PMTCT services since 2001, with integration of ART into the service since 2011 [16,41].

Study population and sampling

Participants enrolled in the initial phase in the MCH-ART study were consecutively sampled, consenting HIV-positive women who sought antenatal care at Gugulethu MOU. Of these women, the subset who were ART-eligible advanced to Phase 2. The further subset of these women who entered Phase 3 of the study consisted of those who elected to breastfeed their infant and who consented to being randomised to one of two postpartum HIV care strategies. This group from Phase 3 forms the MCH-ART contribution to the present sub-study.

Similarly, participants for the PACART study were recruited at the Gugulethu MOU either at the postnatal clinic within 7 days post-delivery, or at the ART clinic if they initiated ART during the current pregnancy. Women were sampled consecutively from these clinics, where they were approached by study staff. These participants form the PACART contribution to this sub-analysis.

The combination of these two groups of post-natal HIV-positive women forms the study cohort to be analysed in this dissertation. A summary of the inclusion and exclusion criteria of these two studies, as well as their key similarities and differences, is presented in *Table 1*.

Sample size calculation

This secondary data analysis will use the entire sample available from the parent studies. Sample sizes for these studies was based on their primary objectives and can be found in the relevant study protocols. The current study will not make use of any post-hoc power analyses.

Recruitment and enrolment

No recruitment of new participants will take place as part of this sub-study – all required data has been collected through MCH-ART and PACART studies which have concluded. Enrolment in these studies was conducted as follows: HIV-positive women at the Gugulethu MOU attending their first antenatal visit during their current pregnancy were informed about the study by the MOU staff. Women who expressed interest at this point were either introduced directly to the study staff for screening or were requested for permission for study staff to approach them directly for screening. These women were provided with basic information about the study, and if they were both interested in participating and passed the screening inclusion/exclusion criteria, provided written informed consent for study enrolment. Participants were informed of their ability to refuse or withdraw from the study at any point, with no negative consequences on their clinical care.

Table 1. Summary of key eligibility criteria and operational similarities between MCH-ART and PACART studies.

	MCH-ART (Phase 3)	PACART
Study intervention	Integrated maternal and child postnatal health services vs standard of care	Post-partum adherence clubs for ART vs standard of care
Primary study outcome measure	Composite endpoint of woman's retention in ART care and viral load <50 copies/ml at 12 months postpartum	Time to viral load >1000 copies/ml
Inclusion criteria	Women (>18 years old) seeking postnatal care at Gugulethu MOU	Women (>18 years old) seeking postnatal care at Gugulethu MOU
	HIV positive, with ART initiation during the antenatal period	HIV positive, with ART initiation in preceding antenatal period
	Able and willing to be randomised by treatment group, return for visits, provide informed consent	Able and willing to be randomised by treatment group, return for visits, provide informed consent
	Currently breastfeeding within <7 days postpartum	Viral suppression documented in pregnancy, with most recent VL <400 copies/ml
	Eligible participant who has completed Phase 1 & 2	
Exclusion criteria	Intention to relocate out of Cape Town during study period	Intention to relocate out of Cape Town during study period
	Any medical, psychiatric, or social condition deemed by the study investigators to impair the participant's ability to consent and/or participate in the study	Any medical, psychiatric, or social condition deemed by the study investigators to impair the participant's ability to consent and/or participate in the study
	Loss of pregnancy/neonate at time of eligibility consideration	Loss of pregnancy/neonate at time of eligibility consideration
	Ineligible from Phase 1 & 2	Current comorbidity that is not controlled or stable and requires additional medical attention
Follow-up schedule	<7 days, 6 weeks and 3, 6, 9, 12 and 18 months postpartum	<70 days and 3, 6, 9, 12, 18, and 24 months postpartum

Variable definitions

Table 2. List and definitions of study variables.

Variable	Type	Definition
Age at date of delivery	Numerical – continuous	Mother's age at time of delivery
Time of HIV diagnosis	Categorical – binary	Whether participant was diagnosed with HIV during current pregnancy, or prior to the current pregnancy
HIV viral load	Numerical – continuous	Number of copies of HIV per millilitre of peripheral blood, represented on the integer and log10 scales
HIV viral load	Categorical – ordinal	Classification of patient's viral load by pre-determined categories, representing different risk groups
Months post-partum	Numerical – continuous	Number of months post-partum at which follow-up visits occurred
Treatment arm	Categorical – binary	Whether participant received standard of care or intervention according to original PACART/MCH-ART studies
Original trial enrolment	Categorical – binary	Whether the participant was enrolled into MCH-ART or PACART trial originally
Visit number	Numerical – discrete	Count variable representing the current visit number post-partum
Level of education	Categorical – binary	Whether the participant has completed secondary/tertiary education or not
Employment status	Categorical – binary	Whether participant was employed directly prior to current pregnancy
ART regimen	Categorical – binary	Whether the participant was using EFZ/TDF/3TC regimen or not

Definition of outcome variables

1. Performance characteristics of postnatal VL testing to predict peripartum viraemia: sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, odds ratio
2. Maternal HIV virological failure, classified as two consecutive VL measurements of >1000 copies/ml, quantified by polymerase chain reaction

The study period for each woman will commence at her first postnatal visit within 6 weeks of delivery, with some leeway given as per original study protocols, and end at the final study visit at 18-24 months postpartum. Viral load is quantified as HIV viral copies per millilitre of blood, and will be measured on both the linear and $\log_{10}$ and scales, as well as in constructed categories which represent important clinical cut-offs such as virological suppression, virological failure, and low-level viraemia.

Procedures and data collection

The data for this secondary analysis will be obtained from the databases of the MCH-ART and PACART studies and combined into a single database. All data for these studies has been collected to date.

Data Management

Data for this analysis will be extracted from the combined databases of the MCH-ART and PACART trials, obtained electronically from a secure and encrypted cloud-storage platform. Data analysis conducted on personal computers will ensure that the dataset, study information and analysis will be run on a password-protected hard drive. Data will be cleaned by filtering the dataset by inclusion and exclusion criteria; identifying and removing unwanted duplicate data entries; and resolution of logic errors and important missing data through corroboration with electronic study records where possible, if required. All data quality queries, responses and edits will be recorded in appropriate log files. Once the PACART and MCH-ART databases are merged, all further data manipulation and analysis will be conducted on the merged data set. Identifying information is absent from the original trials' databases used for analysis, robustly protecting participants' anonymity.

Data Analysis

Anonymised data will be analysed using R statistical software version 4.2.0 [42] using both base R and various open-source packages in the R environment. Normality of sample distributions will be assessed by Shapiro-Wilk tests and histograms, with these data summarised as either mean and standard deviation (normally distributed), or median and interquartile range (non-normally distributed). Categorical variables will be compared and described using frequency tables, proportions and/or graphical illustrations. Differences between groups are assessed using t-tests (normal distribution) or Wilcoxon rank-sum test (non-normal distribution) for numerical variables, while categorical data are compared using X^2 or Fischer's Exact test.

Diagnostic test evaluation will be conducted through use of contingency tables to calculate appropriate test statistics, such as sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. The characteristics of these tests will be further explored through receiver operating characteristic curves. Simple odds ratios calculated from these tables will be corroborated with logistic regression models to assess the probability of different viral load outcomes based on prior viral load test results. Model outputs will be communicated as odds ratios (OR) with 95% confidence intervals (95% CI)

Cox proportional hazards models will be used to examine the risk of progression to virological failure based on the viral load as measured at the first study visit. The primary endpoint for patients for these models is virological failure, as defined by two consecutive VL measurements of >1000 copies/ml. Kaplan-Meier survival curves will be plotted to illustrate the proportion of participants who have progressed to virological failure (primary outcome) according to their starting viral load (grouping variable). Model outputs will be displayed as hazard ratios (HR) with 95% CI.

Ethical Considerations

Both the MCH-ART and PACART studies acquired appropriate ethics committee approval. MCH-ART (REF 451/2012; 567/2014) acquired ethics approval through the University of Cape Town Human Research Ethics Committee (UCT-HREC) and the Columbia University Medical Center Institutional Review Board, while PACART (REF 194/2015) was approved by the UCT-REC. These ethics approvals included scope for later sub-analyses such as this.

Risks and benefits

The main risk to study participants following this secondary analysis is breach of confidentiality, given that personal and identifying data was collected in the parent study. Significant precautions, as mentioned prior, will be in place to prevent this occurrence, while identifying information is absent from the databases accessed, rendering this a minor risk. The potential benefits of this study have a direct bearing on the study population and may provide insights to improve local HIV monitoring and treatment guidelines. The minimal risks posed by this study are outweighed by the potential benefits.

Informed consent

Data collection for this analysis was completed under the MCH-ART and PACART studies, where informed consent for the parent study and multiple sub-studies such as this were obtained at the time of recruitment. No additional consent or participant recruitment will be required for this sub-analysis.

Privacy and confidentiality

Participants' identifying data will not be present in the original databases accessed for analysis, and thus are not at increased risk through any actions of this study. All data and analyses will be stored on a secure, password-protected hard drive.

Dissemination of study results

Results of this study will be submitted to the University of Cape Town in partial fulfilment of the Master of Public Health degree and will be accessible on the OpenUCT platform after it is submitted to the University. A manuscript of these findings will also be submitted for publication in an appropriate peer-reviewed journal.

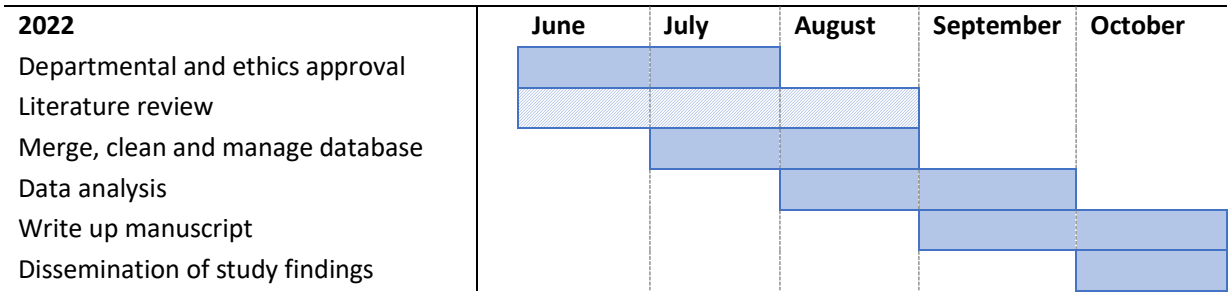
Reimbursement

This study is a secondary data analysis and therefore no further compensation will be granted to study participants.

Budget and logistics

Data for the proposed study has already been collected, thus no further expenditure will be required. All data analysis will be conducted using the open-source R statistical software, on personal devices.

Figure 2. Gantt chart of proposed study timetable for completion. Partially shaded cells represent background focus, while fully shaded cells represent main focus.



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PART B: JOURNAL MANUSCRIPT

The prognostic value of HIV viral load in predicting viraemic outcomes of postpartum women on antiretroviral therapy: a secondary analysis of two randomised controlled trials from Gugulethu, Cape Town

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Abstract

Background: Vertical HIV transmission is a significant health concern for HIV-infected women during pregnancy and postpartum. While better access to effective and acceptable antiretroviral therapy regimens have helped to improve viraemic control in this group, vertical transmission continues to occur despite improvements in care and treatment options. The dynamics of episodic transient viraemia, and their contribution to mother-to-child transmission, are not fully understood.

Methodology: A secondary analysis was performed on pooled data from two randomised controlled trials conducted between 2013-2019 in an antenatal clinic in Cape Town, South Africa, investigating improvements to postnatal HIV care through targeted postnatal services. The present study used this data to investigate the dynamics of HIV viraemia and estimate the prognostic strength of viral load (VL) measurements in postpartum women. Test characteristics of VL as a predictor of later VL >1000 copies/ml were calculated for different cut-offs. Cox proportional hazards models were used to estimate the risk of two consecutive VL >1000 copies/ml over the study period.

Results: 883 HIV-infected postpartum women were followed-up for a median of 22.1 months (IQR: 17.7-24.1). 826 (94%) participants recorded at least one episode of VL <50 copies/ml, 306 (37%), demonstrated at least one episode of viraemia in the range of 50-999 copies/ml, while 307 (37%) had one or more episodes of VL >1000 copies/ml. As a predictor of future VL >1000 copies/ml, viraemia >50 copies/ml was a specific (87.5%) but not sensitive (68%) test, with an odds ratio (OR) of 14.8 (95% CI: 12.4-17.8), while current VL >200 copies/ml had an OR of 26.1 (95% CI: 21.4-31.8), sensitivity of 64%, and specificity of 94%. Cox proportional hazards analysis found that viraemia in the range of 50-999 copies/ml at the first study visit postpartum was associated with an increased hazard ratio of two consecutive VL >1000 copies/ml over the study period, when compared to participants with VL <50 copies/ml at the same timepoint (HR: 1.75; 95% CI: 1.3-2.4; p<0.001).

Conclusion: Episodic transient viraemia in HIV-infected postpartum women occurs frequently despite improvements in HIV-related services and treatments. VL testing remains a useful monitoring tool and may help to identify those at risk of prolonged, elevated VL.

Introduction

Despite efforts to improve access to HIV testing and anti-retroviral therapy (ART), vertical transmission of HIV is ongoing, with a mother-to-child-transmission (MTCT) rate of 4% in South Africa (SA) in 2020 [1]. Maternal HIV viral load (VL) during the peripartum is a strong predictor of MTCT, with a proportional increase in risk of vertical transmission as maternal VL rises [2–4]. One review identifies vertical HIV transmission rates of 0.22% in mothers with VL <1000 copies/ml, compared to 5.8% in those with VL >1000 copies/ml [5]. With the promotion of exclusive breastfeeding of infants, it becomes even more important that mothers have access to effective HIV services and ART [6]. Pregnant and breastfeeding women are recommended to commence ART at the time of diagnosis, regardless of WHO clinical stage or CD4 count, with viral suppression being the major goal of prevention of MTCT [5,7].

Current guidelines lack clarity on the optimal course of action for women with viraemia of 50-999 copies/ml (low-level viraemia; LLV), opting for a conservative approach centred on adherence counselling, followed by a repeat assay 8 weeks, rather than the usual 3 months, thereafter [5,6]. Episodes of LLV following previous viral suppression have been documented in multiple studies to date. This area of clinical uncertainty occurs frequently in practice, and viraemia may only be detected several months after it has developed [3,8–11].

Few studies have examined transient viraemia in postpartum women. There is a continued risk of unsuppressed viraemia in postpartum women, with an SA-based study identifying an ongoing risk of VL >1000 copies/ml in the postpartum similar to that in the antenatal period [11]. This effect persists up to 24 months postpartum, highlighting the importance of improving HIV services for a prolonged period after delivery. One study in the Malawian context found an association between unsuppressed or low-detectable VL and MTCT in women on ART, likely linked to sub-optimal ART adherence [12].

Multiple studies have demonstrated the increased risk of progressing to uncontrolled viraemia in the postpartum compared to pre-pregnancy, with issues of postpartum support, linkage between antenatal and postnatal care, and poor adherence identified as major contributors to this phenomenon [12–19]. In the South African context, viraemia in the postpartum period has been strongly associated to poor adherence, with few cases due to background ART drug resistance, and high rates of viraemia despite reported good ART coverage [17,20]. Other key issues relate to level of education, late ART initiation in pregnancy, incident HIV infection during pregnancy, primiparity, and non-disclosure of HIV status to their partner [21,22].

An estimated 38 million people currently live with HIV globally with 1.5 million new infections each year, while SA accounts for about 20% of these despite vast improvements in HIV-related care [1]. Suppression of HIV replication through lifelong ART is essential for long-term health of HIV-infected individuals, as well as for prevention of further viral transmission [5,23] . Adherence to ART is important for individuals to realise the benefits of treatment, while sub-optimal adherence increases the risks of antiretroviral (ARV) drug resistance, treatment failure, and transmission of HIV to others [24]. The preferred approach for monitoring adherence and treatment response is through serum VL measurements [5].

Studies in patients with transient viraemic episodes of 50-1000 copies/ml are unable to state the significance of these episodes conclusively, nor the optimal clinical response. One study of HIV-infected individuals demonstrates an association between viraemia 50-199 copies/ml and the development of virological failure, defined as two consecutive VL measurements >1000 copies/ml [25]. This association is noted in ART-experienced, but not ART-naïve patients, and is independent of ART regimen or cumulative duration of viraemia. Similarly, a study in newly-diagnosed HIV-infected patients on ART with sustained VL <50 copies/ml (viral suppression; VS), identifies patients with isolated episodes of viraemia of 50-200 copies/ml as being at increased risk of persistent VL >1000 copies/ml in the subsequent 24 months [8]. In SA, results from a large multicentre observational cohort of HIV-infected patients on ART demonstrate high rates of viraemia, with 79% of patients on first-line ART recording an episode of LLV, and 21% recording VL >1000 copies/ml over the study period [9]. Further, they show that viraemia in this range is associated with an elevated risk of future VL >1000 copies/ml, as well as later switch to second-line ART.

A recent WHO review of 31 studies identifies LLV as being predictive of later VL >1000 copies/ml, with VL 200-500 copies/ml being a significant predictor of later VL >1000 copies/ml [5]. This report further identifies multiple studies that demonstrate an association between LLV and the development of ARV drug resistance mutations, primarily in non-nucleoside reverse-transcriptase inhibitor-based regimens [5].

This study aims to describe the virological outcomes and dynamics of HIV viraemia in a group of postpartum, breastfeeding, HIV-positive women in SA. Using repeat VL measurements, it aims to identify the predictive ability of VL as a tool to identify likely progression to later VL >1000 copies/ml.

Methods

Study design

A retrospective secondary analysis was conducted on maternal postpartum HIV VL testing data that was initially collected prospectively as part of two separate randomised controlled trials, the MCH-ART and PACART studies, described separately [26,27]. These studies investigated the role of interventions targeted at behavioural and adherence aspects of postpartum care in the participants, testing whether these interventions improved retention of mothers in HIV services and control of HIV infection. The MCH-ART intervention focused on integrated mother and child postnatal services while PACART looked at postpartum community-based adherence clubs. Both studies were conducted in women presenting to Gugulethu midwife obstetric unit (MOU) in Cape Town, SA. The MCH-ART study ran from 2013-2016, while PACART was run from 2016-2019.

Study setting

At the time of these studies, Gugulethu MOU provided primary level healthcare for >4000 pregnant women per year amongst a population of approximately 350 000 people [27,28]. The prevalence of HIV in this predominantly low socioeconomic status (SES) community was estimated at 30% in 2013, with >95% uptake of antenatal care [28].

Study participants

All women in the study received their clinical care, ART initiation and infant follow-up through the public health service based on standard treatment guidelines and were initiated on the recommended once-daily fixed-dose combination first-line ART regimen at the time: tenofovir (300mg), emtricitabine (200mg), and efavirenz (600mg). The first postnatal follow-up occurred within seven days of delivery for routine maternal and infant support and monitoring, with a subsequent MOU ART clinic visit within the next month for counselling, health check and dispensing of ART. Following this, mothers were referred to the general adult ART services at their local community health centre, while infants were seen at separate 'well-baby' services for routine growth monitoring and vaccinations. Routine infant HIV testing was conducted at 6-10 weeks of age using standard nucleic acid amplification tests [6,26,27].

The MCH-ART study consisted of 3 separate phases, with patients recruited into the initial phase being consecutively-sampled HIV positive women who both sought antenatal care at Gugulethu MOU and provided consent for the study. Of these participants, those who were ART eligible (phase 2) and who chose to breastfeed their infant were randomised to postpartum intervention (phase 3). The data for

this secondary analysis came only from those women who completed phase 3 of the MCH-ART trial. Study visits occurred at the following time-points postpartum: <7 days, 6 weeks, and 3, 6, 9, 12 and 18 months.

PACART study participants were similarly consecutively enrolled at Gugulethu MOU, either at the postnatal clinic within 7 days of delivery, or at the ART clinic if they initiated ART antenatally. All women who participated in the PACART trial were included in this secondary analysis. Study visits occurred at the following time-points postpartum: <70 days, and 3, 6, 9, 12, 18 and 24 months.

The pooled data from these two groups of participants formed the cohort for this study (Supplementary figure 1). Study visits were separate from postnatal care, and all samples and measurements were taken independent of the clinical services.

Measurement

Trained study personnel administered structured questionnaires and collected biological samples at study visits. VL measurements were taken separately to routine clinical monitoring and batch processed at the South African National Health Laboratory Services using the Abbott RealTime HIV-1 assay.

Statistical analysis

All statistical analyses were performed using R statistical software version 4.2.0 [29]. Categorical variables were presented as number of cases and proportion, while continuous variables were presented as the either mean and standard deviation (SD), or median and interquartile range (IQR). Contingency tables were used to assess the diagnostic test characteristics of present VL as a predictor of subsequent VL, and receiver-operator curves were used to further demonstrate this. Test sensitivity, specificity, predictive values, likelihood ratios, odds ratios and 95% confidence intervals were calculated from these tables. Cox proportional hazards models were used to estimate the risk of recording two consecutive VL measurements >1000 copies/ml (primary outcome) during the follow-up period as measured by the hazard ratio (HR) and 95% confidence intervals. Kaplan-Meier curves were used to demonstrate the probability of recording two consecutive VL >1000 copies/ml over the study period. A p-value of <0.05 was considered statistically significant for all tests.

Ethical considerations

Both the MCH-ART (REF 451/2012; 567/2014) and PACART (REF 194/2015) studies received ethical clearance from the University of Cape Town Health Sciences Research Ethics Committee (UCT HREC).

Both prior ethics approvals included scope for secondary data analysis, and this was included in the informed consent for the original studies. No further participant recruitment was required for this study, and all study data was anonymised prior to creation of a joint database for analysis. The current study was approved by the UCT HREC (REF 554/2022).

Results

Data were obtained from 883 HIV-infected pregnant women who attended Gugulethu MOU Clinic in Cape Town, Western Cape, between 2013-2019 and who were enrolled in either the MCHART (n=471) or PACART (n=412) study. 24 participants were excluded from analysis of VL outcomes, due to completing only a single study visit. A further 17 observations were excluded based on a missing VL measurement, resulting in 5186 observational timepoints and 4326 timepoint pairs for analysis.

The median duration of follow-up was 22.1 months (IQR: 18-24), with a longer average follow-up time amongst participants in the PACART cohort (17.9 vs 24.0 months) (Table 1), in keeping with its longer study duration. The pooled cohort had a mean age of 28.9 years (SD 24-34), with low rates of higher education (27%) and employment (36%), which was similar between studies. Approximately 36% of women were first diagnosed with HIV during the current pregnancy, while 96% of women were on the standard first-line ART regimen of efavirenz, tenofovir and emtricitabine.

Table 1. Study population demographics, key characteristics, and virological outcomes within parent studies, and as a pooled cohort.

Variable	MCH-ART (n = 471)	PACART (n = 412)	Pooled (n = 883)
Demographics and cohort characteristics			
Age (years)	28.5 (23-34)	29.2 (24-34)	28.9 (24-34)
Follow-up time (months)	17.9 (11-25)	24.0 (23-24)	22.1 (18-24)
Married/cohabiting with partner	193 (41%)	174 (42%)	367 (42%)
Completed secondary/tertiary education	117 (25%)	119 (29%)	236 (27%)
Employed	184 (39%)	132 (32%)	316 (36%)
HIV diagnosed in this pregnancy	268 (57%)	50 (12%)	318 (36%)
EFZ/TDF/3TC ART regimen	444 (94%)	400 (97%)	844 (96%)
Virological outcomes of participants			
VL < 50 copies/ml			
Any episode[†]	435 (92%)	391 (95%)	826 (94%)
At starting visit[†]	346 (73%)	348 (84%)	694 (79%)
Single episode	37 (8%)	23 (6%)	60 (7%)
2 consecutive measurements	41 (9%)	40 (10%)	81 (9%)
>2 consecutive measurements	357 (76%)	328 (80%)	685 (78%)
Total number of episodes[‡]	2130 (41%)	1787 (34%)	3917 (76%)
VL 50 – 999 copies/ml			
Any episodes[†]	179 (38%)	127 (31%)	306 (35%)
At starting visit[†]	85 (18%)	48 (12%)	133 (15%)
Single episode	116 (25%)	79 (19%)	195 (22%)
2 consecutive measurements	39 (8%)	35 (8%)	74 (8%)
>2 consecutive measurements	24 (5%)	13 (3%)	37 (4%)
Total number of episodes[‡]	281 (%)	191 (4%)	472 (9%)
VL > 1000 copies/ml			
Any episodes[†]	181 (38%)	126 (31%)	307 (35%)
At starting visit[†]	29 (6%)	2 (1%)	31 (4%)
Single episode	70 (15%)	33 (8%)	103 (12%)
2 consecutive measurements	111 (24%)	93 (23%)	65 (7%)
>2 consecutive measurements	76 (16%)	63 (15%)	139 (16%)
Total number of episodes[‡]	470 (9%)	327 (7%)	797 (15%)

Data shown are n (%), mean (SD), or median (IQR). Percentages are rounded to the nearest whole number. Abbreviations: EFZ, efavirenz; TDF, tenofovir; 3TC, lamivudine. [†]Pooled percentages calculated relative to entire cohort (n=883). [‡]Pooled percentages calculated relative to all VL observational timepoints (n=5186). All other percentages calculated relative to relevant 'Any episodes' category.

826 out of 883 (94%) participants had at least one episode of VS over the entire study period, with 694 (79%) of all participants demonstrating VS immediately postpartum. There was a similar distribution of instances of VS between MCH-ART (73%) and PACART (84%) groups at the initial study visit. At least one episode of LLV was recorded in 306 (35%) patients, while 195 of these participants

had exactly one episode in this range, 74 recorded two consecutive measurements, and 37 demonstrated more than two consecutive episodes.

VL measurements of >1000 copies/ml occurred in 307 (35%) patients on at least one occasion during the study. There were low rates of viraemia in this range at the start of the study, with 31 (4%) participants recording VL >1000 copies/ml at their initial visit postpartum. Close to a third of all participants (103) that recorded a VL >1000 copies/ml experienced only a single episode, while roughly two thirds (204) recorded two or more consecutive episodes of VL >1000 copies/ml (persistent viraemia).

Of visits where VS was measured, 7% progressed to VL >1000 copies/ml by the following visit (Table 2). Visits with a VL in the range of 200-999 copies/ml showed a much higher proportion of progression to VL >1000 copies/ml than any other group, at 31%, although this represents a much smaller subgroup of all women on treatment (3%). Additionally, of all visits at which a VL >1000 copies/ml was recorded, VL in the same range was recorded at the subsequent visit in 78% of cases. We saw that participants' VL fluctuated frequently, but also that patients tended to progress into the VL >1000 copies/ml group, while far fewer progressed out of this group, proportionally, over the study period (Supplementary figures 2-4).

Table 2. Dynamics of viral load progression to >1000 copies/ml between consecutive study visits.

Current VL	Subsequent VL		Total
	> 1000	**< 1000**	
< 50	245 (7%)	3115 (93%)	3360 (78 %)
50 – 199	31 (12%)	220 (88%)	251 (6%)
200 – 999	43 (31%)	98 (70%)	141 (3%)
> 1000	447 (78%)	129 (22%)	576 (13%)

Data shown are n (%), rounded to the nearest whole number where appropriate. VL < 1000 copies/ml includes undetectable VL.

Following from this, we assessed the ability of VL at the current time point to predict VL at the following study visit. Contingency tables were constructed to evaluate the diagnostic characteristics of these tests (Table 3*)*.

Table 3. Contingency table of VL measurements between subsequent time points.

		Subsequent VL			
		< 50	**50 – 199**	**200 – 999**	**> 1000**
Current VL	**< 50**	2948	106	61	245
	50 – 199	169	37	14	31
	200 – 999	39	28	31	43
	> 1000	67	25	37	447

Data shown are numbers of VL measurements, with n=5186.

Exploration of the diagnostic characteristics of current VL as a predictor of future VL >1000 copies/ml (Table 4) found that this test was highly specific, but poorly sensitive, across a variety of cut-off points. VS was a poor predictor of future VL >1000 copies/ml, while the presence of viraemia in each of the ranges of 50-199, 200-999, and >1000 copies/ml was a specific, but not sensitive, test of future VL >1000 copies/ml. At the stratum of VL 50-199 copies/ml, test sensitivity was 4.1%, specificity was 93.8%, with a positive predictive value (PPV) of 7.3% and a negative predictive value (NPV) of 81.9% in this study population.

Using test cut-off points rather than specific strata, we found that all levels of viraemia >50 copies/ml were good predictors of later VL >1000 copies/ml. This effect increased the higher the cut-off was set (Table 5*)*, with VL >50 copies/ml having a sensitivity of 68%, specificity of 87.5%, PPV of 5.4% and NPV of 92.7%. Thus, VL in this setting represents a test which has potential application in identifying patients at risk of progressing to future VL >1000. The receiver-operating characteristic (ROC) curve for this test demonstrates its modest performance, with an AUC of 0.793 (Figure 1). The inflection point of this curve is noted at a VL cut-off of 148 copies/ml, where the test has a sensitivity of 58.9% and a specificity of 93.7%.

Table 4. Performance characteristics of postnatal VL testing to predict later VL > 1000 copies/ml by different VL strata.

Test stratum (copies/ml)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	OR (95% CI)
<50	31.9% (29-35)	12.6% (12-14)	7.3% (7-8)	46.2 (44-49)	0.37 (0.3-0.4)	5.4 (4.9-5.9)	0.07 (0.06-0.08)
50 – 199	4.1% (3-6)	93.8% (93-95)	12.4% (9-17)	81.9% (81-82)	0.66 (0.4-0.9)	1.02 (1.01-1.04)	0.64 (0.4-0.9)
200 – 999	5.6% (4-8)	97.3% (97-98)	30.5% (24-38)	82.7% (82-83)	2.04 (1.4-2.9)	0.97 (0.95-0.99)	2.1 (1-3)
> 1000	58.4% (55-62)	96.4% (96-97)	77.6% (74-81)	91.5% (91-92)	16.1 (14-19)	0.43 (0.40-0.47)	37.3 (30-47)

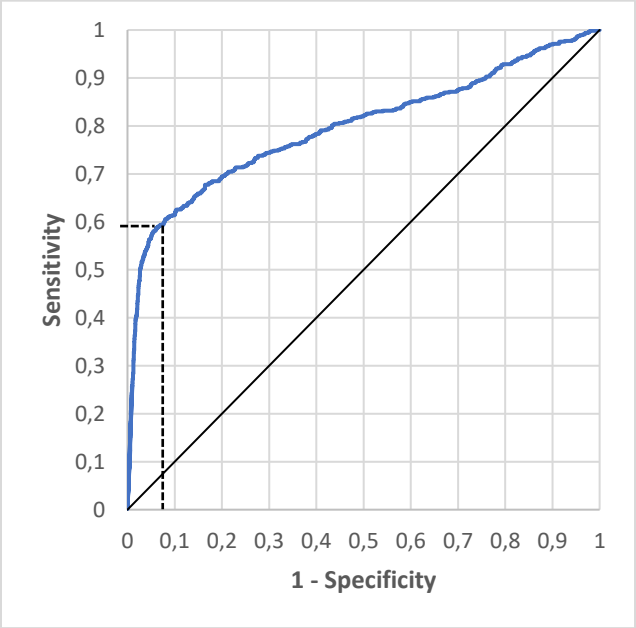
Data shown are percentages or ratios with 95% confidence intervals, rounded to the nearest whole number where appropriate. Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; OR, odds ratio; 95% CI, 95% confidence interval.

Table 5. Performance characteristics of postnatal VL testing to predict later VL > 1000 copies/ml using different test cut-off points.

Test cut-off (copies/ml)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	OR (95% CI)
> 50	68% (65-71)	87.5% (86-89)	5.4 (5-6)	92.7 (92-93)	5.4 (5-6)	0.37 (0.33-0.41)	14.8 (12-18)
> 200	63.9% (61-67)	93.6% (93-94)	68.3 (65-71)	92.4 (92-93)	10.04 (8-12)	0.38 (0.35-0.42)	26.1 (21-32)
> 1000	58.4 (55-62)	96.4% (96-97)	77.6 (74-81)	91.5 (91-92)	16.1 (14-19)	0.43 (0.4-0.47)	37.3 (30-47)

Data shown are percentages or ratios with 95% confidence intervals, rounded to the nearest whole number where appropriate.

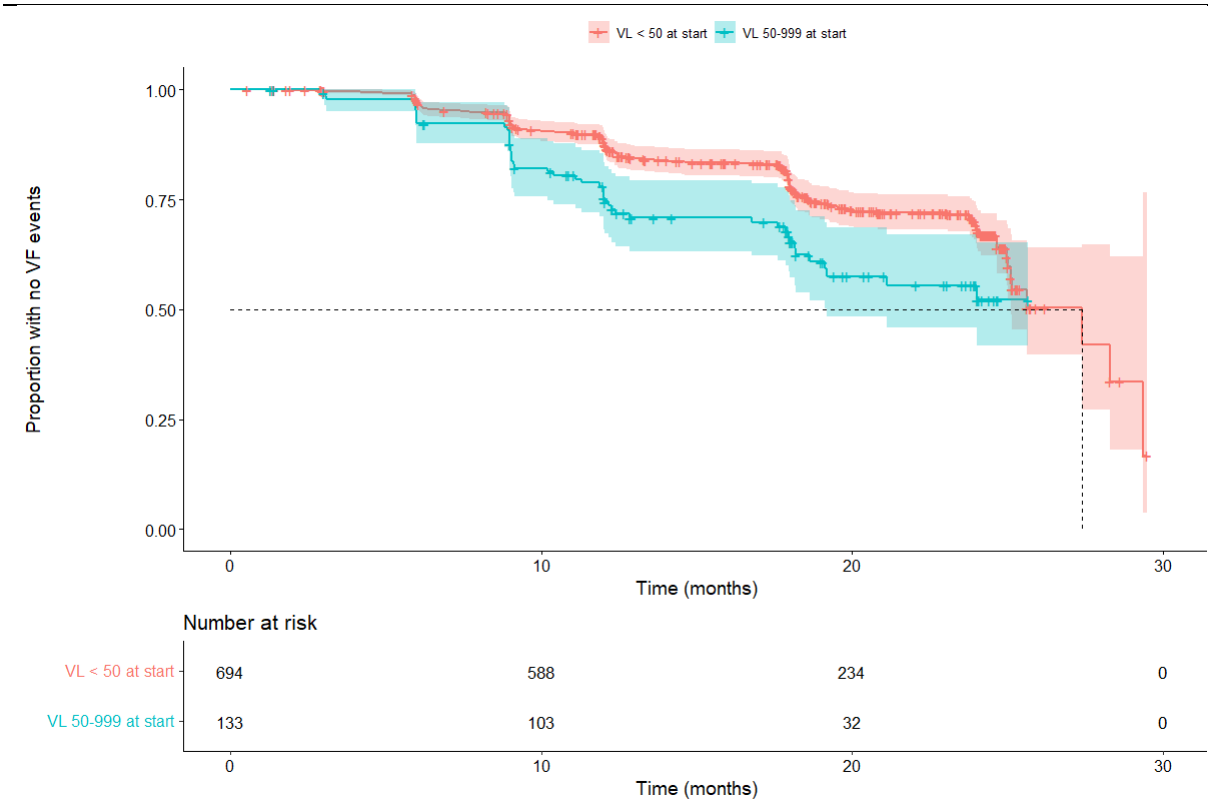
Figure 1. ROC curve for VL testing as a predictor of future VL >1000 copies/ml. Inflection point is noted at VL of 148, with a sensitivity of 58.9% and specificity of 93.7%.



Kaplan-Meier curves were used to explore the time to first event of two consecutive VL >1000 copies/ml in all women with VL <1000 copies/ml at their initial study visit, including those who were VS at this time point. 31 participants were excluded from this analysis due to VL >1000 copies/ml at baseline. We found that a substantial proportion, almost 50%, went on to experience the primary outcome by 24 months postpartum (Figure 2). Grouping this analysis by VL at first study visit, there was a delineation between patients that had VS immediately postpartum compared to those who had LLV at this time. The primary outcome occurred at a higher rate early in the postpartum in the LLV group, although there was a similar median time-to-event for both groups (18.0 vs 17.9 months). Cox proportional hazards models showed that LLV at the initial study visit was significantly associated with an increased hazard of recording two consecutive VL measurements >1000 copies/ml (persistent viraemia) over the study period, with an HR of 1.75 (95% CI: 1.3-2.4, p <0.001), when compared to those with VS at the same time point.

Subgroup analysis of different strata of HIV viraemia found that episodes of viraemia in the range VL 50-199 copies/ml were the main contributors to this pooled HR (Supplementary figure 5), with stratum-specific HR of 1.53 (95% CI: 1.11-2.13, p = 0.01). VL measures in the range of 200-999 copies/ml were too scarce in this cohort to be a reliable predictor, with a wide confidence interval crossing 1 (HR: 1.54, 95% CI: 0.92 – 2.53).

Figure 2. Kaplan-Meier curve comparing time to first episode of two consecutive VL >1000 copies/ml, according to VL category immediately postpartum.



Discussion

This study assessed the ability of early postpartum VL measurements to predict future virological outcomes in HIV-positive women in the months following delivery. In this secondary analysis of two randomised-controlled trials from the same location in SA, close to half of all women with starting VL <1000 copies/ml progressed to two consecutive VL >1000 copies/ml within 24 months of delivery. This occurred at a significantly higher rate for women who had LLV at delivery compared to those with VS. This finding highlights the importance of early identification of these women, providing an opportunity to prevent treatment failure. This is preferable to reacting later to already-established treatment failure. Additionally, we demonstrated the high frequency with which episodes of persistent viraemia occur in this group of patients even under optimal conditions, despite the benefits of the original trials' interventions [26,27,30]. It is likely that real-world rates of persistently elevated VL are even higher, with longer periods of sustained viraemia than those observed here.

The findings of this study identified women with detectable viraemia <1000 copies/ml immediately postpartum as being at a substantially higher risk of progression to subsequent VL >1000 and later

virological failure, compared to women with suppressed VL at the same time point. This was further shown for later time-points in the postpartum period, with almost a third of all women with VL in the range of 200-999 copies/ml progressing to VL >1000 at the very next visit. This highlights the clinical importance of acting decisively during this window to prevent progressive and prolonged viraemic periods in this group.

Following from this, we evaluated the test characteristics of VL measurements in the postpartum as a predictor of VL at the next study visit. We showed that viraemia >50 copies/ml is a highly specific, but non-sensitive test for VL >1000 copies/ml at the next clinic visit, as was VL 200-999 copies/ml. These findings suggest that postpartum women recording VL >50 copies/ml should be followed up more closely to detect and proactively manage viraemia to prevent virological failure. As VL measurement is already central to treatment guidelines, these findings may be incorporated into clinical decision-making without further expense. The utility of such guidelines does however fall down in the setting of infrequent VL testing, which is known to be a common issue in postpartum women [21]**,** although the current predictive tool was developed in this setting of variable and infrequent VL testing, thus reflecting real-world practices. Additionally, this measure does not estimate the degree to which current VL as a predictor of future VL may change over time.

Multiple other studies of low-level viraemia have concluded that the presence of low-level viraemia in the peripartum is associated with increased risk of HIV transmission, and that higher levels of viraemia convey higher levels of risk [8,12–14,19,24,31,32]. Many of these studies were conducted in high-income countries and may have underappreciated the significance of low-level viraemia in the low- and middle-income setting where treatment protocols may differ, and where virological monitoring may occur less frequently. Multiple studies have shown that women in the postpartum period are at substantial risk of persistent viraemia, with important implications for MTCT [12,13,19,21,30,32]. Additionally, the importance of the complex and interwoven social factors underlying this issue have been difficult to address across various contexts [11,14,21,32].

It is currently unclear precisely how the dynamics of low-level viraemia are affected by newer treatment regimens incorporating dolutegravir and other newer ARVs. Some evidence suggests comparatively low rates of detectable viraemia <1000 copies/ml in dolutegravir-containing regimens, similar to those seen in protease inhibitor-based ART [33,34]. These newer regimens tend to have a higher genetic barrier to the development of drug resistance, as well as more favourable side-effect profiles, which may alter the dynamics of treatment adherence. The implications of detectable viraemia <1000 copies/ml in patients on these newer regimens is unclear, and there is a dearth of research on the appropriate course of action to take in this setting.

Despite a relatively large cohort for the purposes of the pooled analyses in this study, the size of some subgroups within the sample limited a more granular statistical analysis, such as stratification by different VL subcategories <1000. Patient attrition resulted in incomplete follow-up data, although there were sufficient participants retained up to 18 months postpartum, the period during which mothers were encouraged to continue breastfeeding and which is most important for PMTCT [6]. This was the period of highest risk of transmission from mother to child following delivery, and thus the area of primary interest for the purposes of this analysis [3].

In settings with good access to VL monitoring, it may be appropriate to conduct follow-up VL testing more frequently than current local and international guidelines suggest. Point-of-care VL testing could also play an important role in this setting, facilitating rapid response to low-level viraemic episodes as well as overt failure [5,35,36]. Implementation of more frequent VL testing may have particular utility for peripartum women as they are a more accessible population, and the benefits of better care extend to both mother and child.

The results of this study were notable for their reliance on measurements that were taken in parallel to standard care that participants received at their community clinic, with samples and measurements taken at timepoints separate to regular clinic visits. This reduced the chance of bias related to receiving care at normal scheduled clinic visits and blinded healthcare workers to these measurements which were taken more frequently than would have occurred under standard care guidelines.

As the MCH-ART and PACART studies were conducted under similar conditions in the same location and population, it was deemed acceptable to pool their results for this analysis. However, this doesn't remove the possibility that the study groups had substantial relevant differences that weren't measured. Additionally, as these studies were conducted serially, it is possible that there were differences in factors related to medical care, prevalent social and political events, clinic staff, as well as HIV testing and treatment, that may have affected the studies differentially. Evidence of this was not measured nor found to be relevant to the data collected here, however, this does not remove the possibility of insidious unmeasured effects.

Both trials were conducted in Gugulethu, Cape Town. This area's high prevalence of HIV, relatively low SES, and high population density make this a useful comparison to other populations in low- and middle-income settings. However, these factors may limit the generalisability of these findings to other populations. Further, standard ARV treatment guidelines have changed since these studies were conducted, with a shift towards first-line dolutegravir-based ART regimens which have a higher barrier to viral resistance mechanisms in addition to better side-effect profiles than many previous regimens [37–41].

Further studies in different postpartum populations are required to better understand the role that detectable viraemia <1000 copies/ml has in determining perinatal HIV transmission and long-term maternal outcomes, particularly in women on dolutegravir-containing ART regimens. Comparison of the risk of treatment failure across both antenatal and postnatal periods, and investigation of differences between ART-naïve and ART-experienced women would provide important insights to help guide clinical and policy decisions.

Conclusion

Low-level viraemia <1000 copies/ml is an important risk factor for subsequent VL >1000 copies/ml. Detection of this is represents a window of opportunity for intervention to improve the management of HIV in postpartum mothers and reduce the risk of transmission to their infants. We have shown here that persistent viraemia >1000 copies/ml occurs frequently in HIV-positive women in the postpartum period, despite optimal medical care and prior virological control.

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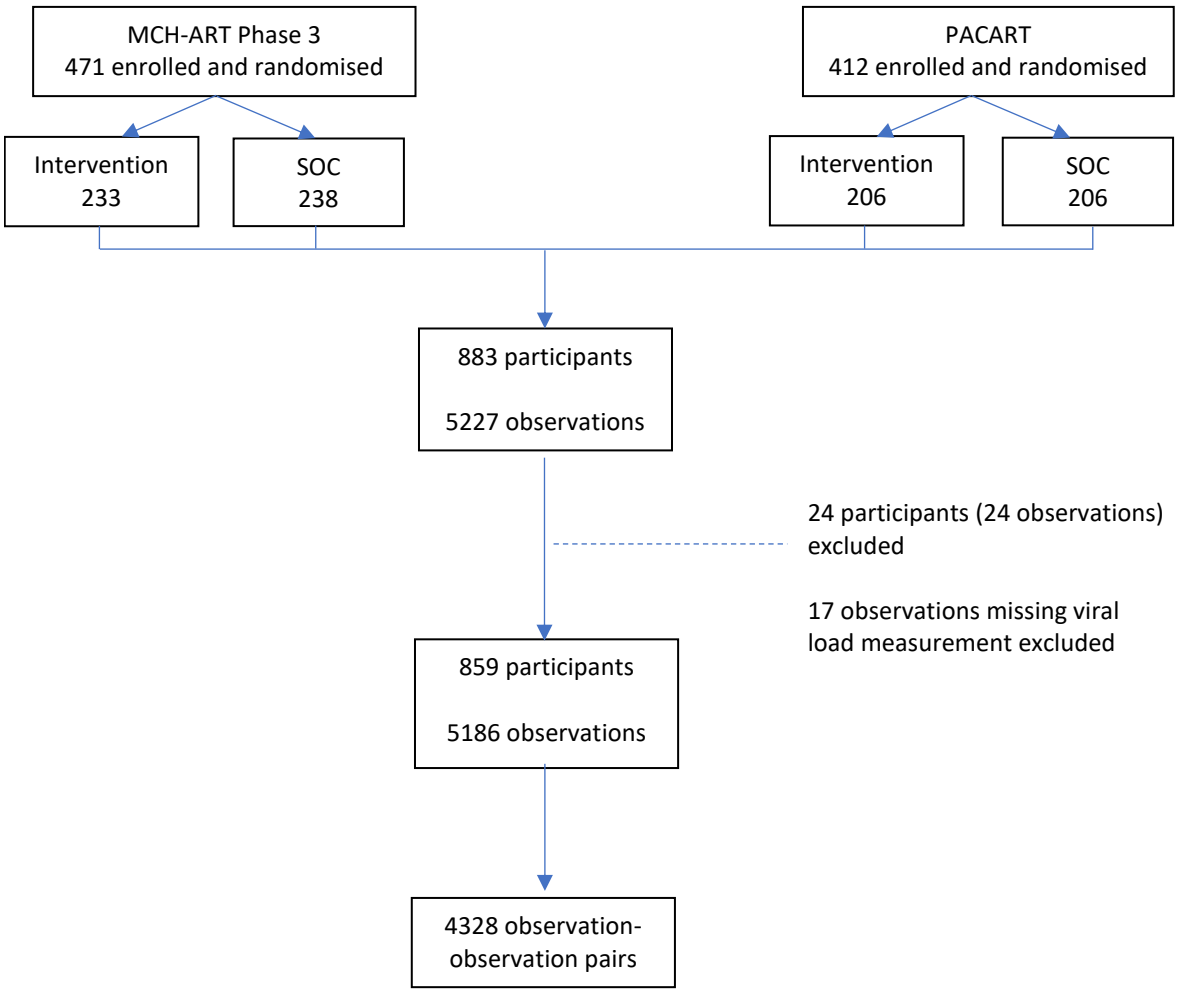
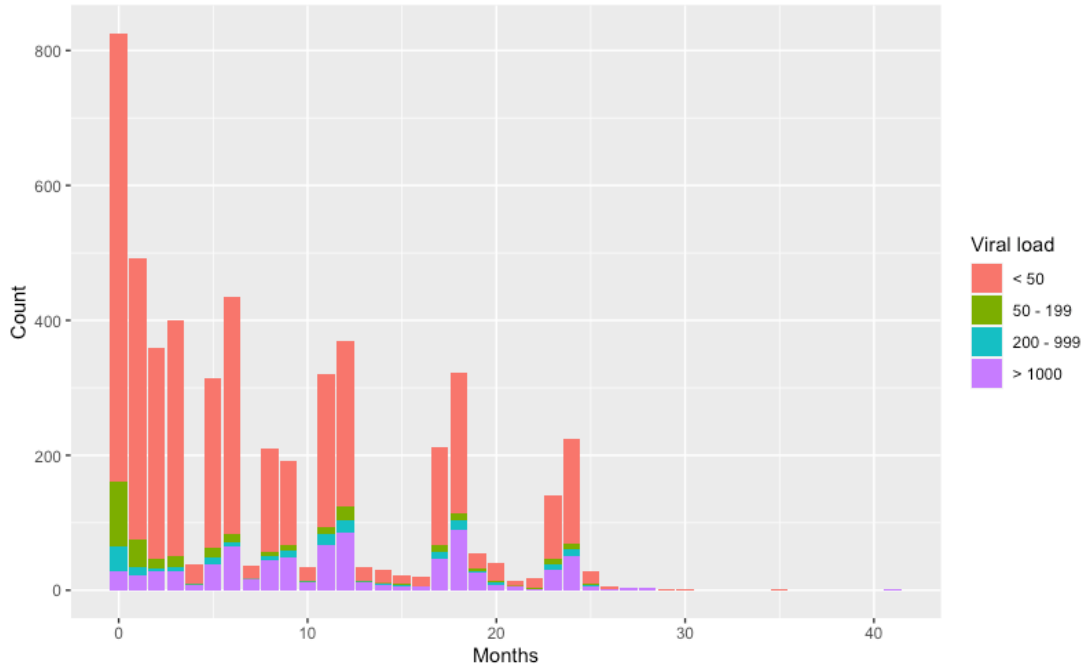
Supplementary Materials

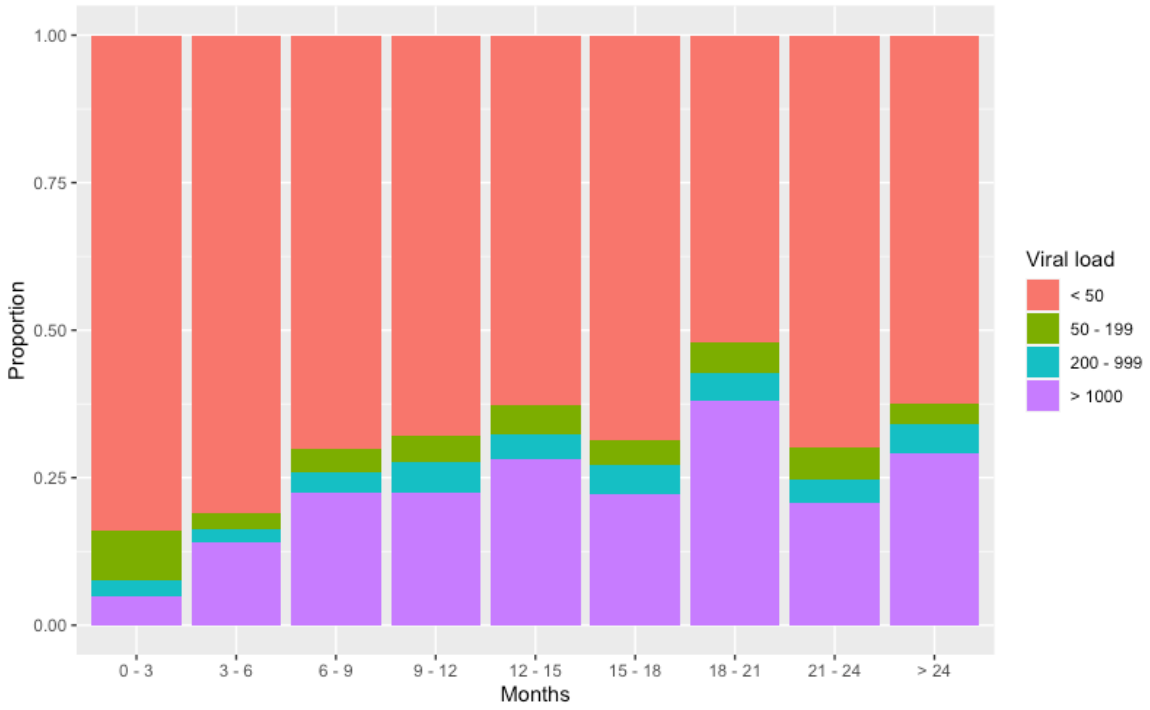
Supplementary figure 1. Flow chart illustrating breakdown of data contribution from parent studies (MCH-ART and PACART).



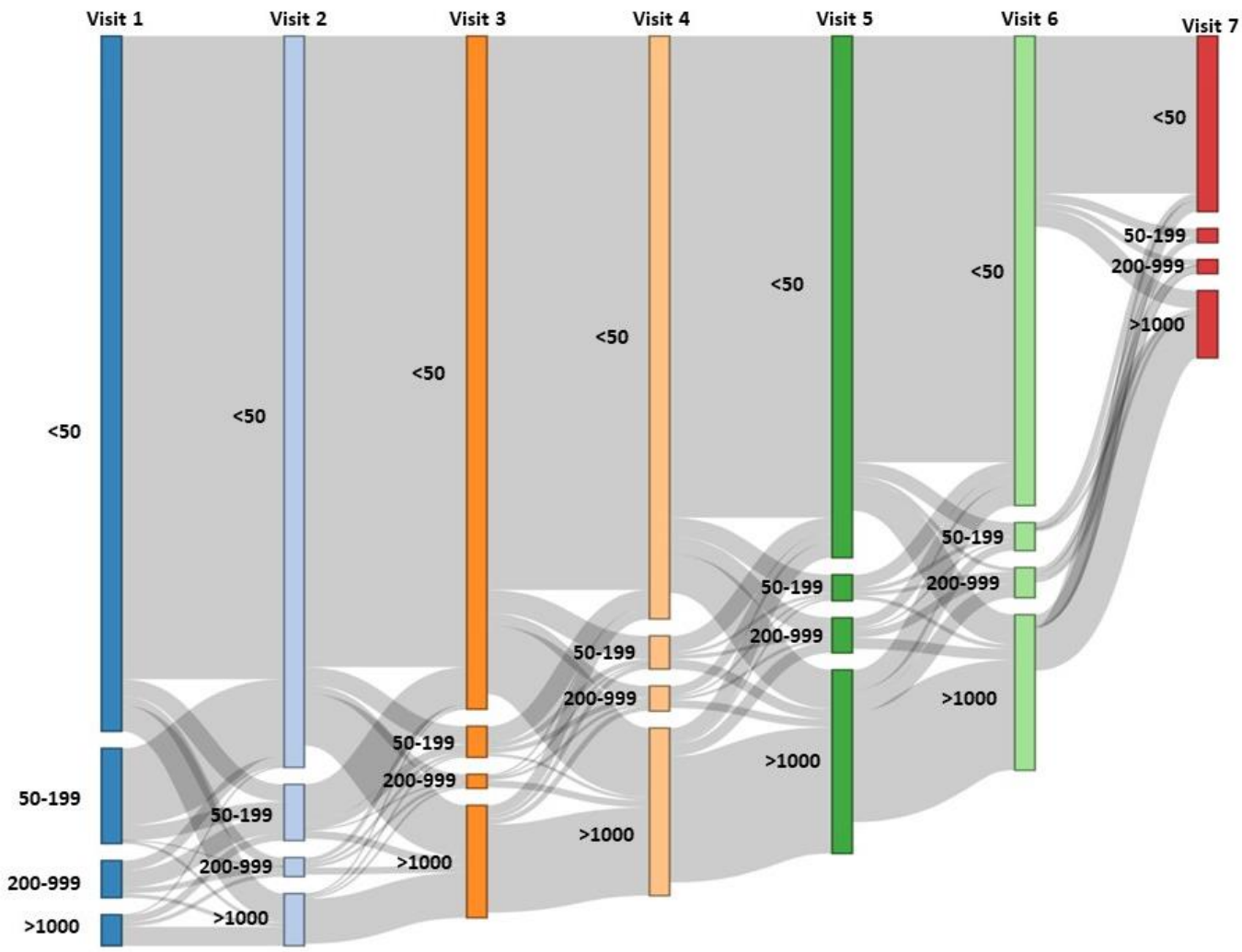
Supplementary figure 2. Stacked bar graph of frequency of each VL category across study period.



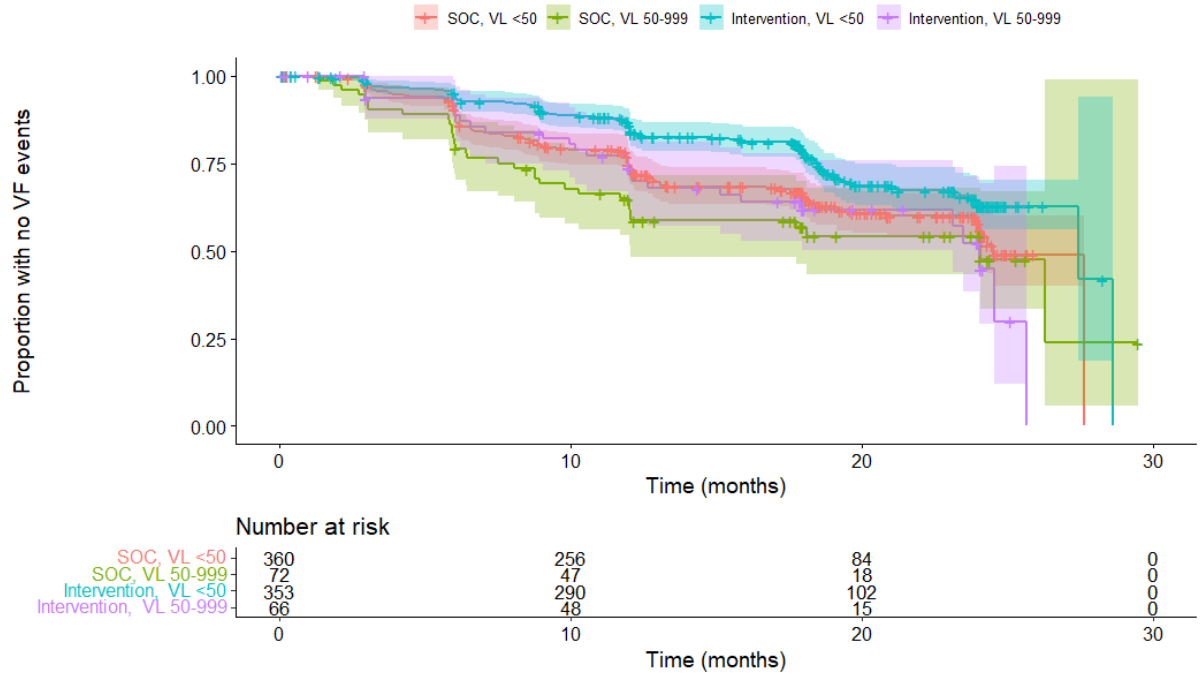
Supplementary figure 3. Stacked bar graph of proportions of each VL category across study period.



Supplementary figure 4. Sankey plot of all VL measurements by category across first seven study visits, illustrating fluctuations of VL across the study period. Size of vertical bars represent number of participants in labelled VL category. Bar colours represent sequential study visits.



Supplementary figure 5. Kaplan-Meier curve comparing time to first episode of two consecutive VL >1000 copies/ml, according to intervention group and VL category immediately postpartum.



PART C: APPENDICES

Appendix A: Ethics Approval Forms

ETHICS APPROVAL FOR THE PROPOSED STUDY

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
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30 August 2022

HREC REF:554/2022

Prof L Myer
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Dear Prof Myer

PROJECT TITLE : THE PROGNOSTIC VALUE OF HIV VIRAL LOAD IN PREDICTING VIRAEMIC OUTCOMES OF POST-PARTUM WOMEN ON ANTIRETROVIRAL THERAPY: A SECONDARY ANALYSIS OF TWO RANDOMISED CONTROL TRIALS FROM GUGULETHU, CAPE TOWN- (MASTERS CANDIDATE-DR DANIEL EGAN-SUB-STUDY LINKEND TO 451/2012)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 August 2023.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Daniel Egan will also be involved in this study.

Please quote the HREC REF 554/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC.REF 554.2022

MCH-ART ETHICS APPROVAL

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Faculty of Health Sciences Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za

24 October 2012

HREC REF: 451/2012

A/Prof L Myer
CIDER
School of Public Health & Family Medicine
FHS

Dear A/Prof Myer

PROJECT TITLE: STRATEGIES TO OPTIMIZE ANTIRETROVIRAL THERAPY SERVICES FOR MATERNAL & CHILD HEALTH: THE MCH-ART STUDY.

Thank you for addressing the issues raised by the Human Research Ethics Committee.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study including the following documentation:-

- Study protocol MCH-ART study: Version 1.2 (FINAL DRAFT, dated 08Oct2012)
- Phase 1 Informed Consent form: Version 2.0, 18 October 2012
- Phase 2 Informed Consent Form: Version 2.0 18 October 2012
- Phase 3 Informed Consent Form: Version 2.0 18 Oct 2012

Approval is granted for one year till the 28 October 2013.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

sAriefdien

PACART ETHICS APPROVAL

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 April 2015

HREC REF: 194/2015

Prof L Myer
Epidemiology & Biostatistics
Public Health & Family Medicine
Falmouth Building

Dear Prof Myer

PROJECT TITLE: POSTPARTUM ADHERENCE CLUBS FOR ANTIRETROVIRAL THERAPY (PACART)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

pp TBurgess

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki guidelines.

Appendix B: Journal Submission Guidelines

Sections

1. SUBMISSION

Please carefully read through the Instructions for Authors and prepare your manuscript according to the guidelines, including structuring it manuscript based on the chosen article category. Manuscripts that do not follow the instructions may be returned to the authors for corrections.

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at https://mc.manuscriptcentral.com/jias. The submission system will prompt authors to use an ORCID iD (a unique author identifier) to help distinguish their work from that of other researchers. Click here to find out more.

You will be asked to suggest potential peer reviewers for your manuscript: they should be experts in the field and be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same institution. Suggested reviewers will be considered alongside potential reviewers identified by the Editorial team.

Click here for more details on how to use ScholarOne.

2. AIMS AND SCOPE

The *JIAS* welcomes submissions on HIV-related topics from across all scientific disciplines, including but not limited to:

• Basic and biomedical sciences
• Behavioural sciences
• Epidemiology

• Clinical sciences
• Health economics and health policy
• Operations research and implementation sciences
• Social sciences and humanities, including political sciences and media

The *JIAS* prioritizes submissions from operational research and implementation science as publication of such material can provide valuable information on various algorithms for monitoring and providing support for comprehensive, yet affordable and sustainable treatment, prevention and care programmes in different contexts.

Submission of HIV research carried out in low- and middle-income countries is strongly encouraged.

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

The *JIAS* accepts submissions in the following categories:

• **Research**
• **Short report**
• **Review**
• **Debate**
• **Commentary**
• **Letter to the Editor**
• **Viewpoint**
• **Field notes**

Research - full reports of data from original research studies

Abstract:
Headings: Introduction, Methods, Results, Conclusions
Word limit: 350 words

Main text:
Headings: Introduction, Methods, Results, Discussion, Conclusions
Word limit (quantitative): 3500 words; Tables do not contribute to the word count
Word limit (qualitative and mixed methods): 5000 words; tables with quotes contribute to the word count
Numbers of figures and tables: Unlimited
Additional files: Yes

Download the manuscript template

Short report - brief reports of data from original research, such as follow-up or confirmatory studies, case series and negative results

Abstract:
Headings: Introduction, Methods, Results and discussion, Conclusions
Word limit: 350 words

Main text:

Headings: Introduction, Methods, Results and discussion, Conclusions
Word limit: 2000 words
Numbers of figures and tables: 3
Additional files: No

Download the manuscript template

Review - comprehensive, authoritative descriptions and summaries of a specific subject area providing a systematic and substantial overview of the field

Abstract:
Headings: Introduction, Methods, Results and discussion (if applicable, otherwise Discussion only), Conclusions
Word limit: 350 words

Main text:
Headings: Introduction, Methods, Results and discussion (if applicable, otherwise Discussion only), Conclusions
Word limit: 5000 words
Numbers of figures and tables: Unlimited
Additional files: Yes

Download the manuscript template

Debate - presentation of an evidence-based argument

Abstract:
Headings: Introduction, Discussion, Conclusions
Word limit: 350 words

Main text:
Headings: Introduction, Discussion, Conclusions
Word limit: 3500 words
Numbers of figures and tables: 4
Additional files: No

Download the manuscript template

Commentary - focused and opinionated articles on important and timely issues, no original data

Abstract:
Headings: Introduction, Discussion, Conclusions
Word limit: 350 words

Main text:
Headings: Introduction, Discussion, Conclusions
Word limit: 2500 words
Numbers of figures and tables: 1
Additional files: No

Download the manuscript template

Letter to the Editor - comments on and responses to published articles

Abstract:
None

Main text:
Headings: None
Word limit: 500 words
Numbers of figures and tables: None
Additional files: No

Download the manuscript template

Viewpoint - constructive, stand-alone views on current topics

Abstract:
None

Main text:
Headings: None
Word limit: 1000 words
Numbers of figures and tables: 1
Maximum number of references: 15
Maximum number of authors: 5
Additional files: No

Download the manuscript template

Field Notes – focused articles providing timely insights regarding contemporary challenges in HIV public health practice. This includes observations about the mechanisms of change, contextual influences, and findings that emerge from implementation of evidence-based interventions and programmes. Field Notes can inform others, spread best practices and promote innovative ideas.

Contributors should focus on one issue or question that arose during their intervention or strategy, summarize the novelty of their approach, the lessons learned, and how their experiences and insights could inform local and/or global practice.

Contributions written in the form of programme reports are not appropriate for this article category.

Field Notes are distinct from Viewpoints or Commentaries. They should describe direct experiences and lessons learned rather than opinions or other first-person reflections. Manuscripts should succinctly describe the context, issue, and the innovative aspects of the implementation strategy adopted, and report implementation outcomes, lessons learned, and implications for practice and/or policy. Attention to describing the strategy or approach will be key in making the piece understandable to the audience.

Preliminary data can be included, and explanations of how a programme succeeded or failed will strengthen the submission. A clear rationale for the programmatic approach will help others understand whether the experience described might work in their setting as well, with the objective to provide timely insights and advice for planning, implementing, or evaluating HIV service delivery programmes.

Field Notes will be internally assessed by Section Editors based on their novelty, relevance and implications for practice and/or policy. Manuscripts will not be externally peer-reviewed.

Abstract:

None

Main text:

Headings: None

Word limit: 1200 words

Maximum number of figures or tables: 1

Maximum number of references: 10

Maximum number of authors: 8

Additional files: At the discretion of the Section Editors

4. PREPARING THE SUBMISSION

Cover letter
In the cover letter, please explain why your manuscript should be published in the journal. If necessary, address any issues relating to our editorial policies .and declare any competing interests (see **Editorial Policies and Ethical Considerations**)

Parts of the Manuscript
The manuscript should be submitted as a main text file including tables and figures. Appendices and supporting information should be submitted as separate files.

Main Text File
The text file should be presented in the following order:

1. Title page;
2. Keywords;
3. Abstract;
4. Main text;
5. Conflict of Interest Statement;
6. Authorship;
7. Acknowledgments;

8. References;
9. Tables;
10. Figures;

Title page
The title should not contain abbreviations, except commonly used abbreviations such as HIV or AIDS (see **Wiley's best practice SEO tips**).

On the title page, you should mention the title of the manuscript, list all authors' names in full, and list any study groups if applicable. Each authors' affiliation should be numbered in superscript consecutively and listed underneath, including department, institution, city and country.

The corresponding author should be marked with the symbol § in superscript and full contact details should be provided, including a telephone number with country code. Authors who have contributed equally to the work should be marked with the symbol * in superscript. Deceased authors should be marked with the symbol ^ in superscript. The email addresses of all authors should be listed by their initials.

Keywords
Please provide six keywords. Keywords should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser list at **https://www.nlm.nih.gov/mesh/**. Preferably alternate words to those found in the abstract in order to improve search hits for the article in repositories.

Abstract
The Abstract should not exceed 350 words and should be structured according to the headings of the selected article category (see above), excluding the heading “Discussion” for Research articles. Avoid using abbreviations and do not cite references in the Abstract. If you are reporting results from a controlled health care intervention, please include your trial registry, together with your unique identifying number at the end of the Abstract. For randomized controlled trials, follow the **CONSORT extension for abstracts** .

Main Text

Article sections
Introduction
The Introduction section should introduce the topic to readers without specialist knowledge in that area and must clearly outline the current state of knowledge in this field, the motivation and the aim of the study or the article.

Methods
The Methods section should include all information necessary to repeat the study, in particular, the study design, how data was collected and analyzed, clarifying the choice of methods that were made. If applicable, you should describe the setting of the study, the dates the study were conducted, and the sample or participants, as well as necessary power calculations and materials, including statistical packages, used. Interventions and programmes should be described in detail. Generic names for drugs or any molecules should be used.

All studies involving humans or animals require a statement on ethical approval, and for the former, the consent procedure that was followed. Please include the names of the ethics review

board(s) that approved the study. If the research study was specific to one sex/gender, the reasons for this should be clearly stated.

Results
This section should include only data and findings from the authors' study. Presentation of statistical results should mention confidence intervals and levels of significance where appropriate. Quotes from qualitative study participants of less than three lines should be quoted in the text using quotation marks. For quotes longer than three lines, place the quote in a separate, indented paragraph and introduce it with a colon. No quotation marks are needed in this case. Details of the participant can be added in round brackets following the quote, but should not contain identifiable information to ensure confidentiality. Clarifications within the quotation should be placed in square brackets. In some cases, it may also be appropriate to organize quotes within a table. For details on the use of tables to display qualitative quotes, please see guidance below, under *Tables*.

Submitting authors should include data disaggregated by sex (and, whenever possible, by race or ethnicity) and provide a comprehensive analysis of gender and racial or ethnic differences. The authors should include the number and percentage of men, women and, if appropriate, transgender persons who participated in the research study. Anatomical and physiological differences between men and women (height, weight, body fat-to-muscle ratios, cell counts, hormonal cycles, etc.), as well as social and cultural variables (socio-economic, education, access to care, etc.), should be taken into consideration in the presentation of data and/or analysis of the results.

Discussion
In the Discussion section, you should discuss your main findings and place these within the context of the current body of knowledge in the field. Limitations of the study, for example, selection bias, can also be discussed, and should address how these influence the results and conclusions. If statistically significant differences were found between men and women or between different racial or cultural groups in the effects of the studied intervention, the implications, if any, for clinical and/or public health should be adequately discussed.

Conclusions
In your Conclusions section, state your key messages from the study and explain their importance and relevance, as well as implications. Future studies and recommendations can be included in this section. The conclusions drawn must be strictly based on the data provided.

Conflict of Interest Statement
Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the **Editorial Policies and Ethical Considerations** section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Authorship
Please refer to the journal's Authorship policy in the **Editorial Policies and Ethical Considerations** section for details on author listing eligibility. The individual contributions of each author must be specified in the Authors' Contributions section. Please use authors' initials and state that all authors have read and approved the final manuscript. An example of a suitable statement is: "S.W., N.J., D.W. and S.S. performed the research. S.W., N.J., H.H. and T.L. designed the research study. H.H. and S.S. contributed essential reagents or tools. S.W., N.J. and D.W.

analysed the data. S.W. and N.J. wrote the paper." Please see the 'Authorship' section in the Editorial Policies and Ethical Considerations section below for what constitutes authorship.

Acknowledgments
Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

References
All external sources of information should be referenced within the text, the tables and figures, using consecutive numbering in square brackets, e.g. [1], [3-5], [3,4]. The references should be up to date and adequately reflect the current state of knowledge in the field. Citation bias, for example, by country or point of view must be avoided. Numbers of references are unlimited for all article categories and should be formatted in standard Vancouver style; **see Sample references from ICMJE** . Unpublished observations, personal communications and manuscripts currently under consideration should be cited in the text in round brackets and not in the reference list.

Tables
They should be supplied as editable files, not pasted as images. Tables should be inserted into the text. They should have the header: "Table 1. Title of table". All tables should be cited in the text in consecutive order. The tables should not contain colour or shading, and no vertical, visible lines. If tables are copied or adapted from another source, permission must be sought by the authors prior to publication and these should be clearly cited as such. If a table spans more than one page, authors may want to consider uploading the table as an additional file instead. Tables should be self-contained and complement, not duplicate, information contained in the text. A legend can be provided underneath the title, listing any abbreviations or meanings of symbols used. If several tables are included, please ensure that symbols are used consistently. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Guidance on the use of tables to display qualitative quotes:

Quotes placed in tables should be organized thoughtfully and strategically, to aid the clear presentation of results. Displaying quotes in tables may be particularly useful when comparing and contrasting subgroup variations in responses or showing temporal variation. Tables may also help visually organize themes when they correspond to the order or way in which findings are described in the text. Please note that tables that present quotes will contribute to the overall word count of the manuscript, with intention to avoid excessively long tables in which quotes are placed without sufficient context. Accordingly, the word limit for qualitative and mixed methods submissions is 5,000 words, inclusive of tables with quotes.

Figures
Figures should be cropped as closely as possible and have the header: "Figure 1. Title of figure". All figures need to be cited in the text in consecutive order.

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted. **Click here** for the basic

figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Figure legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement. If several figures are included, please ensure that symbols are used consistently.
Post-peer review, the manuscript should be submitted as a main text file including tables and figures. Tables and figures should also be submitted separately as high-resolution versions.

Additional Files

Appendices
Appendices will be published after the references. For submission, they should be supplied as separate files but referred to in the text.

Supporting Information
Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc. **Click here** for Wiley's FAQs on supporting information.

Note : if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

General Style Points
The following points provide general advice on formatting and style:

• **Abbreviations:** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only.
• **Acronyms:** Acronyms should be used sparingly, and not in headings or in the Abstract. Only commonly known acronyms may be used, and they should be spelt out at first use followed by the abbreviation in brackets. SI units should be used, with litre and molar being permitted.
• **Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website **here** for more information about SI units.
• **Numbers:** Numbers under 10 are spelt out, except for: measurements with a unit (8mmol/l); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
• **Trade Names:** Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.
• **Footnotes:** Footnotes are not allowed in the text, the information shall be included directly into the text, where it fits best, and if these are references, to include in the reference section at the end.
• **Language**: All submissions must be in UK English (International) and UN-accepted terminology should be followed. No capitalization should be used except for grammatically correct use, official names and titles, and abbreviations.
• **General recommendation:** Use line spacing of 1.5 and an easily readable font, for example,

Times New Roman, size 12. Your manuscript should contain line numbers to facilitate editors' and reviewers' comments

Wiley Author Resources
Manuscript Preparation Tips: Wiley has a range of resources for authors preparing manuscripts for submission available **here** . In particular, authors may benefit from referring to Wiley's best practice tips on **Writing for Search Engine Optimization** .

Editing, Translation, and Formatting Support: **Wiley Editing Services** can greatly improve the chances of a manuscript being accepted. Offering expert help in English language editing, translation, manuscript formatting, and figure preparation, Wiley Editing Services ensures that the manuscript is ready for submission.

5. EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS

Editorial Review and Acceptance
The acceptance criteria for all papers are the quality and originality of the research and its significance to journal readership. Except where otherwise stated, manuscripts are single-blind peer reviewed, meaning that reviewers remain anonymous to the authors, although the authors' identity is known to the reviewers. Papers will only be sent to review if the Editors-in-Chief determine that the paper meets the appropriate quality and relevance requirements.

All manuscripts are reviewed by at least two independent experts with experience in the subject area and selected by the Editors. Dedicated statistical reviewers may be used if needed. Reviewers have to declare any competing interests to the Editors. Authors can suggest peer reviewers during the submission step. Suggested peer reviewers should not have co-authored publications with any of the authors during the past five years, should not be current collaborators, and should not be members of the same institution. Suggested reviewers will be considered alongside potential reviewers identified by the Editorial team. Authors may also request exclusion of individuals as potential reviewers: those who have clear competing interests, are close collaborators, or have given input into the manuscript previously.

The Editors assess revised manuscripts based on whether the authors have adequately addressed all comments. Re-reviews are only requested when revisions fall out of the technical expertise of the Editors. Further rounds of major revisions are usually not allowed, and manuscripts that have not been satisfactorily revised will be rejected. Minor revisions though may be requested as needed.

Wiley's policy on the confidentiality of the review process is available **here**.

Data Storage and Documentation
The Journal of the International AIDS Society expects that data supporting the results in the paper will be archived in an appropriate public repository. Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. Exceptions may be granted at the discretion of the editor for sensitive information such as human subject data or the location of endangered species. Authors are expected to provide a data availability statement, including a link to the repository they have used, to accompany their paper. More details and templates of data availability statements can be found **here.**

Protein and nucleotide sequences
For nucleic acid sequences, protein sequences or atomic coordinates, which are cited in the

manuscript, and the accession number, together with the database where the information was deposited, should be cited in square brackets in the text, for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116]. Relevant databases are: EMBL Nucleotide Sequence Database (**EMBL**), DNA Data Bank of Japan (**DDBJ**), GenBank at the NCBI (GenBank), Protein Data Bank (**PDB**), Protein Information Resource (**PIR**) and the Swiss-Prot Protein Database (**Swiss-Prot**).

Mass spectrometry
Mass spectrometry data should be provided in the mzML format according to the **HUPO Protein Standards Initiative Mass Spectrometry Standards Working Group guidelines**. The data should also be deposited in the **ProteomeExchange** through the **PRIDE** website, and protein interaction data can be deposited through members of the IMEx consortium.

Structures
Protein structures can be submitted with one of the members of the **Worldwide Protein Data Bank**. Nucleic acid structures can be deposited with the **Nucleic Acid Database** at Rutgers. Crystal structures of organic compounds can be deposited with the **Cambridge Crystallographic Data Centre**.

Chemical structures and assays
Structures of chemical substances can be deposited with **PubChem Substance**. Bioactivity screens of chemical substances can be deposited with **PubChem BioAssay**.

Functional genomics data (such as microarray or CHIP-Seq data)
Please refer to standards proposed by the **Functional Genomics Data Society** and deposit your microarray data in MIAME-compliant format in one of the public repositories, for example, **ArrayExpress** or **Gene Expression Omnibus** (GEO). Deposition of high-throughput functional genomics sequencing data (such as RNA-Seq or ChIP-Seq data) with ArrayExpress or GEO in compliance with MINSEQE is also needed.

Computational modelling
Please prepare models of biochemical reaction networks using the **Systems Biology Markup Language** and submit your model to the **BioModels database**, as well as providing it as an additional file with your submission.

Plasmids
Please submit copies of your plasmids as DNA or bacterial stocks with **Addgene**, a non-profit repository, or **PlasmID**, the Plasmid Information Database at Harvard.

Ethical approval – Human and animal studies

Human Studies and Subjects
For manuscripts reporting medical studies that involve human participants, a statement identifying the ethics committee that approved the study and confirmation that the study conforms to recognized standards is required, for example: **Declaration of Helsinki**; **US Federal Policy for the Protection of Human Subjects**; or **European Medicines Agency Guidelines for Good Clinical Practice**.

A statement on the ethical aspects, including the consent procedure followed, must be included in the Methods section of the manuscript. The Editors may reject manuscripts where the research has not been carried out within an ethical framework. Images and information from

individual participants will only be published where the authors have obtained the individual's free prior informed consent. Confidentiality of study participants must be ensured at all stages of research and reporting. Authors do not need to provide a copy of the consent form to the publisher; however, in signing the author license to publish, authors are required to confirm that consent has been obtained. Wiley has a **standard patient consent form available for use**.

Animal Studies
A statement indicating that the protocol and procedures employed were ethically reviewed and approved, as well as the name of the body giving approval, must be included in the Methods section of the manuscript. Authors are encouraged to adhere to animal research reporting standards, for example the **The Gold Standard Publication Checklist from Hooijmans and colleagues** or the **ARRIVE reporting guidelines** for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

• US authors should cite compliance with the US National Research Council's **Guide for the Care and Use of Laboratory Animals**, the US Public Health Service's **Policy on Humane Care and Use of Laboratory Animals**, and **Guide for the Care and Use of Laboratory Animals**.
• UK authors should conform to UK legislation under the **Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039)**.
• European authors outside the UK should conform to **Directive 2010/63/EU**.

Clinical Trial Registration
The journal requires that clinical trials are prospectively registered in a publicly accessible database and clinical trial registration numbers should be included in all papers that report their results. Authors are asked to include the name of the trial register and the clinical trial registration number at the end of the abstract. If the trial is not registered, or was registered retrospectively, the reasons for this should be explained.

Research Reporting Guidelines

Standard of reporting
The *JIAS* endorses international standards of reporting. Please see the **Uniform Requirements for Manuscripts Submitted to Biomedical Journals** guidelines produced by ICMJE as a reference standard of reporting. Authors are also referred to the **EQUATOR** network website for further information on the available reporting guidelines for health research, and the **MIBBI** Portal for prescriptive checklists for reporting biological and biomedical research where applicable. A number of checklists are available for various study designs, including randomized controlled trials (**CONSORT**), interventional trials (**SPIRIT**), qualitative research (**COREQ**), systematic reviews (**PRISMA**), observational studies (**STROBE**), economic evaluations of health interventions (**CHEERS**), meta-analyses of observational studies (**MOOSE**) and diagnostic / prognostic studies (**STARD** and **TRIPOD**). For systematic reviews, an additional file should be provided by the authors listing all details concerning the search strategy. Please refer to the **Cochrane Reviewers' Handbook** for an example of how a search strategy should be presented.

Guidelines on mutation nomenclature are provided by the **Human Genome Variation Society** , and authors should use the recommended gene name by referring to the appropriate genetic nomenclature database, for example, HUGO for human genes, and the International Committee on Standardized Genetic Nomenclature for Mice. When describing human phenotypes, please

use standardized terms, such as those proposed by the Elements of Morphology working group (see **http://research.nhgri.nih.gov/morphology/index.cgi**).

Contributions from pharmaceutical companies or other commercial organizations should follow the **Good Publication Practice guidelinEs for pharmaceutical companies**, which also apply to any companies or individuals that work on industry-sponsored publications, such as freelance writers, contract research organizations and communications companies.

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Reporting by gender and race or ethnicity
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