The predictors and patterns of alcohol use in HIV positive women during their postpartum period, and how these impact viral load.

Thandeka Mazubane

MZBTHA014

Submitted to the University of Cape Town in partial fulfilment of the requirement for the degree Master of Public Health (Epidemiology and Biostatistics)

School of Public Health and Family Medicine

Faculty of Health Sciences, University of Cape Town

February 2022

Supervisor:

Professor Landon Myer

School of Public Health and Family Medicine

Faculty of Health Sciences

University of Cape Town

DECLARATION

I, …Thandeka Mazubane…, 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.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: ……

Date: …2022/02/13……

PREAMBLE

ACKNOWLEDGEMENTS

I would like to begin with sincere thanks to my supervisor Prof. Landon Myer who provided me with invaluable support, guidance, and encouragement throughout this dissertation process; and who has helped nurture my interests in maternal and child epidemiology.

I'd like to thank my friends and family for your constant motivation, re-focusing, and reminder of the goal post; without your presence and words of encouragement, I would not have continued to push through the tough days and completed this project.

ABSTRACT

Introduction

Although alcohol use among HIV-positive pregnant women in South Africa has been extensively researched, few studies have attempted to understand the longitudinal patterns and predictors of alcohol use when HIV-positive status intersects with the postpartum period. We examined the patterns of alcohol consumption during the postpartum period among HIV-positive women from Gugulethu, Cape Town; explored predictors associated with any alcohol consumption; and lastly, examined the association between alcohol use and HIV viral load.

Methods

Participants were recruited at the Gugulethu Community Health Centre while receiving prevention of mother to child transmission (PMTCT) services, obstetric, or postnatal care. The Alcohol Use Disorder Identification Test (AUDIT) screening tool was used to assess the alcohol use of eligible women. Participants were also assessed for hazardous alcohol use using the AUDIT-C scoring system during the analysis. The factors associated with patterns of alcohol use were then investigated using multiple logic regression and Generalised Mixed Effect Models. Using Generalised Mixed Effect Models, we also investigated the relationship between alcohol consumption and HIV viral load.

Results

Among 360 women (median age: 33 years), 10–28% reported alcohol use during the 24-month postpartum period, with alcohol use characterised by hazardous, binge drinking, and risky/dependency behaviour and associated with single relationship status and depression, whereas strong social support and patient-provider relationship was protective against risky/dependent alcohol use. We also found an association between hazardous, risky/dependent alcohol use and viral load, where hazardous drinkers and risky/dependent were 3 and 5 times more likely to have a suboptimal (>1000 copies/ml) viral load, respectively.

Conclusion

These unique data sheds light on the trajectories of alcohol use during the postpartum period in this vulnerable population, emphasizing the critical support and attention needed by this population and the strong focus public health needs to put on screening and interventions at a primary care level.

Contents

LIST OF TABLES

PART A

PART B

LIST OF FIGURES

PART A: RESEARCH PROTOCOL

Aim

The proposed study aims to examine the prevalence and the predictors associated with alcohol use during the postpartum period, and also understand the association between alcohol use and HIV Viral load in women undergoing routine ANC in Cape Town, South Africa.

Objectives

- Describe the prevalence of alcohol use in women living with HIV over the 24 months follow up period
- Examine which characteristics are associated with alcohol use during the postpartum period
- Examine if alcohol use is associated with HIV viral load in women living with HIV during their postpartum period

Protocol Synopsis

Alcohol use has been shown to have an impact on the viral load, HIV disease progression, HIV transmission, antiretroviral therapy (ART) adherence, (1–3) and could also lead to unfavourable consequences during the postpartum period (4–6). The existing literature reveals the effects of alcohol consumption on HIV alone, as well as on the postpartum period alone but there is limited literature on the effects of alcohol use when the HIV and the postpartum population overlap. This study aims to contribute to the literature on the effects of alcohol use on HIV-positive women during their postpartum period. It will do so by investigating the following aims: (1) describe the prevalence of alcohol use in women living with HIV over the 24 months follow up period, (2) Examine which characteristics are associated with alcohol use during the postpartum period, and (3) Examine if alcohol use is associated with HIV viral load in women living with HIV during their postpartum period.

It will achieve these aims by analysing secondary data from the parent study 'Delivery of antiretroviral therapy to HIV-infected women during the postpartum period: The Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) trial'. The parent study is a randomized controlled trial that enrolled HIV-positive women (18 years and older) who initiated antiretroviral therapy (ART) during the preceding antenatal period and were within 70 days post-delivery. The parent study collected data through various forms of methods, including questionnaires, laboratory measures, and clinical data abstractions for a follow-up period of 24 months. The data generated from the parent study will be used to understand the aims of this current study. The informed consent documents from the parent study included information explaining that the collected data would be used for further studies, this information was clearly explained to the participants.

This study provides no direct benefits to the participants. The study will contribute to the advancement of knowledge on the prevalence of alcohol consumption in HIV-positive women postpartum, the predictors of alcohol use, and the association between alcohol use and maternal HIV viral load. This study has the potential to provide information on the need for screening for alcohol use during the postpartum period and interventions at a primary care level.

A possible risk in the use of secondary data is the risk of loss of confidentiality, however, the PACART study used anonymous participant identification numbers on all the study documents and password encrypted files, therefore minimizing this particular risk.

1. LITERATURE REVIEW

1.1. Backgrounds

In South Africa the prevalence of people living with HIV (PLWHIV) is estimated to be 8,2 million. For adults between the ages of 15-49 years, 19,5% of the population is estimated to be HIV positive (7). The relatively high prevalence of alcohol use during pregnancy among South African women is well-documented. The estimated prevalence of alcohol use among pregnant women in South Africa is 3.2% (8) and 6.5% (9) up to 20.4% (10) and 42.8% (11), mostly based in local survey's. In a study conducted in Kwa Zulu Natal, 18% of women living with HIV reported drinking during pregnancy, of those, 67% usually binge drank when drinking (12). Maternal perinatal alcohol consumption programs have led to a reduction of alcohol consumption during pregnancy (13,14). However, after a year of delivery, a significant number of mothers continue to binge drink (14-16). Estimates of the prevalence of postpartum alcohol use range from 30-50% and between 6-20 percent for binge drinking (13).

Although alcohol consumption during the postpartum period is prevalent in the general population, HIV-infected women are a particularly vulnerable group (1). The high rates of hazardous drinking (13,17) and binge drinking (12,13) among HIV-positive non-pregnant women suggest the need for alcohol cessation interventions during the postpartum period. Presently, there is scant research on the patterns of alcohol use in HIV-positive women during the postpartum period.

Pregnant South African women living with HIV consume alcohol for a variety of intricate and multidimensional reasons. For instance, stress, social isolation, poverty, depression (12,18–20) have all been found to be major risk factors for alcohol consumption among HIV-positive pregnant women in South Africa. Furthermore, among South African women with, gender-based violence and trauma have been connected to alcohol use (21,22).

The postpartum period poses unique challenges to HIV-positive new mothers, including the need to adhere to antiretrovirals. In order to address alcohol consumption in HIV-positive postpartum women, it is important to understand the patterns and predictors of alcohol consumption during the postpartum period in HIV-positive women in order to develop screening tools and successful interventions.

In this paper, hazardous alcohol use is defined as an pattern of drinking that increases the risk of harmful outcomes (22), this was measured using AUDIT-C with a score of 3 or more . On the other hand, binge drinking is defined as a single drinking session leading to intoxication, it was assessed by only the third question of the AUDIT ("How often do you have 5 or more drinks on one occasion") (12,23). In addition, the period following childbirth and lasting up to two years is referred to as the postpartum period.

Alcohol use has been shown to have a negative impact on HIV treatment outcomes, including medication adherence and viral suppression. According to a study conducted in KwaZulu-Natal, South Africa, women with HIV who reported hazardous drinking had a lower chance of achieving viral suppression and were more likely to miss antiretroviral medicine doses. As viral suppression is essential for the long-term health of persons living with HIV and is linked to a lower risk of HIV transmission to sexual partners, this is very alarming.

1.2. Impact of alcohol use on HIV Viral Suppression

Despite the availability and advancement of HIV treatment, only 62% of people living with HIV are engaged in care and only 54% achieve viral suppression (<1000 copies/ml) (25). Lack of viral suppression is associated with enhanced disease progression and transmission of HIV within the community (26). A low percentage of HIV-infected women achieving viral suppression has made it urgent to identify and address modifiable factors that affect disease progression or survival, alcohol use among women is one such behaviour.

Alcohol consumption could affect HIV viral suppression via both behavioural (e.g ART adherence) and biological (e.g. immune dysfunction) mechanisms. Studies show the negative association between alcohol consumption and ART adherence, therefore impacting the patient's ability to attain optimum viral suppression (15,16,27).

1.3. Alcohol consumption and HIV positive people

Although HIV treatment is available, it is still a pandemic, particularly in low-income developing countries. Here, preventing HIV transmission and disease progression remains a challenge. A factor contributing to these challenges is alcohol consumption (binge drinking, heavy alcohol use, acute, and chronic alcohol use) among the population at risk of HIV infection and those currently infected with HIV (3).

The detrimental role of alcohol abuse in the HIV/AIDS epidemic is amplified by its correlation to lack of adherence to ART and decreased effectiveness of ART in control of the infection, which in turn directly affects their viral load and disease progression (2).

1.4. Alcohol consumption in postpartum women

Although alcohol consumption during postpartum is not considered as dangerous as during pregnancy, its effects on the child may still be significant. During the postpartum period, alcohol consumption has been linked to poorer family functioning, lower quality of parental intellectual stimulation, and increased likelihood of being reported for child abuse or neglect (4–6). Studies on postpartum period alcohol use show this period increases alcohol use compared to pregnancy, however, it is noted that postpartum drinking levels do not reach the level generally seen before pregnancy (15,28,29). In the United States, a report based on the National Survey on Drug Use and Health (NSDUH) found that in the third trimester to three months

postpartum period, women who reported alcohol use in the past month went from 6.2% to 31.9% three months postpartum, and 52% by 11 months postpartum (16).

1.5. Alcohol consumption among HIV positive postpartum women

HIV-infected women are a vulnerable group due to intersecting challenges of motherhood and managing their HIV status. Predictors and trends in this vulnerable population of HIV-positive women during the postpartum period have received less research attention.

Alcohol is a well-established risk factor for non-adherence among pregnant/postpartum women living with HIV. This makes it important to thoroughly understand the predictors of alcohol use during these periods in order to improve adherence and HIV treatment outcomes and consequently, both maternal and child health (30,31). Studies show adherence during the antepartum period reached 75%, however, adherence was significantly lower during the postpartum period with only 53% of women achieving optimal ART adherence (31). These studies suggest issues including alcohol use, postpartum depression, and the stress and responsibility of a newborn child make adherence difficult.

Retaining HIV-positive women in HIV care has proven to be a challenging task. In Sub-Saharan Africa, the mother-infant attrition rate from 72 hours post-delivery to five years, is 50% (32,33), with some studies finding attrition rates as high as 81% from prenatal enrolment to 6 months follow up period (34).

The absence of integrated care for HIV and alcohol use treatment may be a gap. Due to stigma, worry about discrimination, or a lack of access to specialized treatments, many HIV-positive women may choose not to seek treatment for alcohol consumption. Access to and acceptance of treatment for both conditions could be improved by integrating alcohol use treatment into already-available HIV care facilities.

1.6. Conclusion

The literature suggests that in the general HIV population, alcohol has an immense impact on the viral load, HIV disease progression, HIV transmission, adherence ART retention, retention to HIV care, and during the postpartum period. From this, we know the effects of alcohol on HIV alone, and on postpartum alone but there isn't a lot of literature on its effects when both these populations overlap. This study aims to understand the patterns of alcohol use among HIV-positive women during their postpartum period and the predictors of alcohol use in this population. It also aims to understand alcohol's impact on the viral load in this vulnerable population, therefore contributing important information which would be valuable when designing interventions aimed at alcohol use during the postpartum period in HIV-positive women. These interventions could help improve postnatal hospital visits and ART adherence.

2. METHODOLOGY

2.1. Study design

This analysis draws on longitudinal data from the Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) study (35). This was a two-arm randomised control trial conducted in Gugulethu, South Africa which aimed to compared maternal viral load suppression up to 24 months postpartum among HIV-infected women who received the current standard care ART services at local adult clinics, versus those who received ART care through the community-based adherence club system.

Women from both study arms were asked to attend 6 study measurement visits in total, at enrolment and approximately 3,6, 12, 18, and 24 months postpartum. Details of study measurements are provided in the Appendix. Among the collected measurements were alcohol use, psychological distress, and postnatal depression, maternal adherence, etc all of which are some of the measurements which will be included in this study.

The sample size for the PACART study was 388 participants, which provided the study with 90% power to detect a difference in HIV viral suppression between the two study arms (adherence clubs compared to standard care).

According to literature, there is an estimated 18% prevalence of alcohol use among HIV-positive pregnant women in Kwa Zulu Natal (12). There is no information on the prevalence of alcohol use among HIV-positive pregnant women in Cape Town during the postpartum period. With these parameters, a sample size of 288 participants would provide the study with enough power to detect a 25% prevalence, with 5% precision and 95% confidence intervals.

2.2. Characteristics of the study population

Inclusion criteria

- Age 18 years or older
- Documented HIV infection with ART initiation during the preceding antenatal period
- Within 70 days post-delivery
- Viral suppression documented in pregnancy with the most recent viral load <400 copies/mL within the last 3 months
- Able to provide informed consent for research

Exclusion criteria

- Any medical, psychiatric or social condition which in the opinion of the investigators would affect the ability to consent and/or participate in the study including:
- Refusal to take ART/antiretrovirals (ARVs)
- Denial of HIV status
- Loss of pregnancy/neonate at the time of eligibility determination
- Current co-morbidity requiring additional health care attention, including opportunistic infections such as tuberculosis (TB) disease or any chronic condition or other condition that is not controlled or stable.

2.3. Recruitment and enrolment

Recruitment into the PACART study (parent study) took place at the Gugulethu Maternal Obstetric Unit (MOU). Women who initiated antiretroviral therapy, who were within 70 days postpartum and who eligible as per the inclusion criteria, were approached to participate in the trial.

2.4. Research procedures and data collection methods

This analysis uses secondary data from the PACART study which was collected using various methods. This study includes information collected using questionnaires, bio-physiological methods, and clinical data abstraction forms. The information was collected throughout the 24-months period during specific visits. The information used in this study is detailed in table 1, while the schedule of study measurements of the parent study is included in the appendix.

The questionnaires included some of the following important tools:
The alcohol use disorders identification test (AUDIT) is a questionnaire, developed by the WHO to screen for excessive alcohol consumption and drinking behaviours (23). The AUDIT tool has been validated across various populations and genders, specifically, it has been validated for use in pregnant and HIV-positive women in South Africa (19).

The Kessler Psychological Distress Scale (K10) is a self-report questionnaire intended to measure a participant's psychological distress based on questions about anxiety and depressive symptoms experienced in the past 4 weeks (36).

Edinburgh Postnatal Depression Scale (EPDS) is a questionnaire used to identify patients at risk for postnatal depression (37). This screening tool has been validated across various populations.

Table 1: Detailed list of variables used in this study

Item for completion	Description	Variable type	Options

Questionnaires			
Demographics			
Age in years	Age in years	Continuous	
Education level	Education attainment	Binary	Primary/some secondary Completed secondary/any tertiary
Employment	Employment	Categorical	Employed (full time + part time) Informal job Student Tertiary student
Relationship status	Relationship status	Categorical	Married Single Divorced
HIV disclosure	HIV status disclosure	Binary	Yes No
Maternal & infant adherence			
Maternal_arvs	In the last 30 days, how often did the participant take ARV medicine in a way that they were supposed	Categorical	Never Rarely Sometimes Usually Almost always Always
Mat_miss_arvs (days)	In the last 30 days, on how many days did you miss at least 1 dose of any of your ARVs?	Discrete	
Patient-provider relationship scale			
PP_score	Patient-provider relationship score	Discrete	
Alcohol Use Disorders Identification Test (AUDIT)			
Audit_score	Total audit score	Categorical	Low-risk (0-7) Risky drinking (>6 – 20)

			Alcohol dependence (>20)
Alcohol_use	Audit scores categorized into two categories	Binary	No alcohol Alcohol use
Mental health			
EPDS	Edinburgh Postnatal Depression Survey score	Binary	Non-depressed (<12) Depressed (≥12/13)
K-10	Kessler-10 score	Categorical	Healthy (<20) Mild mental disorder (20-24) Moderate mental disorder (25-29) Severe mental disorder (>30)
Social support scale			
Aval_suppo	Perceived availability of social support	Discrete	
Study laboratory measures			
Mat_VL	Maternal HIV viral load	Continuous	

2.5. Data analysis

Alcohol behaviour was assessed using the self-report AUDIT and AUDIT C screening tool. Data from the PACART study will be grouped according to HIV-positive women who consumed alcohol during the follow-up postpartum period compared to women who consumed no alcohol during this period. Of those who consumed alcohol, they will be further categorized into low-risk drinking (score 0-5) compared to harmful/hazardous drinkers (score between >6) and alcohol-dependent (scores 20 or above).

All the data will be analysed using the R software (Gnu Project).

2.5.1. *Describe the pattern of alcohol use over the 24 months follow up period*

We will analyse the pattern of alcohol use by calculating the proportion of women who reported alcohol use at each study visit, except for study visit one since participants were not screened for alcohol use during this

visit (Appendix). Since this study will use longitudinal data, it is possible to analyse the prevalence, as well as the pattern of alcohol consumption in the 24-months, follow-up period.

Table 2: Proportion of women who drank at different follow-up time points

Months	Percentage of drinkers
3 (Visit 2)	
6 (Visit 3)	
12 (Visit 4)	
18 (Visit 5)	
24(Visit 6)	

In table 3, the alcohol drinkers will be categorized as low (AUDIT score ≤6), risky drinkers (AUDIT score >6 – 20), and alcohol-dependent (AUDIT score >20). Hazardous alcohol use is defined as the pattern of alcohol use that increases the risk of harmful consequences (13). Hazardous alcohol use is defined as consuming ≥ 7 drinks per week or > 3 drinks per occasion for women, and ≥ 14 drinks per week or > 4 drinks per occasion for men (38,39).

Table 3: Use the AUDIT tool to determine the percentage of women who have low risk, risky drinkers, or alcohol-dependent drinking patterns

Months	Of drinkers (%)		
	Low-risk alcohol use	Risky drinking	Alcohol dependent
3			
6			
12			
18			
24			

Table 4: Use the AUDIT-C tool to determine the percentage of women who have no hazardous or hazardous alcohol use drinking patterns.

Months	Below threshold	Hazardous alcohol use
3		
6		

12		
18		
24		

2.5.2. Examine which characteristics are associated with alcohol use

In order to examine the predictors of alcohol use in women living with HIV during the postpartum period, we will use the data presented in table 1. This information was collected using questionnaires and mainly details the demographic details, behavioural and/or clinical behaviour and social support of the participants.

At baseline or at visit 2, we will compare the alcohol categories hazardous and alcohol dependence to low risk (AUDIT score categories) as well as hazardous to non-hazardous drinkers (AUDIT-C scores), then use a student t-test to analyse a difference in means, and the chi-squared test to compared categorical variables. We will further run a comparison between hazardous drinking and alcohol dependence compared to low-risk alcohol use, comparing the various characteristics by analysing the mean difference and then using the one-way ANOVA to examine whether there is a statistically significant mean difference between the three groups. We hypothesize that there will be a mean difference between certain analysed characteristics.

Generalized estimating equations (logistic model) (GEE) will be used to analyse any factors which are associated with alcohol use, using the AUDIT and AUDIT-C categories as the outcome variable, and the abovementioned characteristics as independent variables, over the 24 months follow up period.

Table 5: Predictors of alcohol use at baseline

	Low-risk alcohol use		Risky drinking		Alcohol dependence	
	Mean	P value	Mean	P value	Mean	P-value
Age						
Relationship status						
Education						
HIV disclosure						
Etc.						

Table 6: Generalised linear models (Logistic regression) to determine the predictors of hazardous alcohol use in women living with HIV during their postpartum period.

Variable	Adjusted Odds ratio	95% Confidence interval	P-value
Age			
Relationship status			
Education			
HIV disclosure			
Etc.			

2.5.3. Examine if alcohol use is associated with HIV viral load

Using the variables from abstraction forms and lab tests (table 1) to examine this objective, the maternal HIV viral load will be used to conduct a 2x2 table where suppressed/unsuppressed HIV viral load is the dependant and alcohol use vs no-alcohol is the independent. Where "suppressed" is defined as a viral load <1000 and "unsuppressed" ≥ 1000.

The 2x2 table will then be used to calculate the odds ratio of the association between alcohol use and unsuppressed viral load.

This analysis will be repeated for all study visits to analyse the pattern and associations over time.

We will also conduct a conduct a generalised mixed effect models to the longitudinal Odds Radio association between sub-optimal (> 1000 copies/ml) viral load and AUDIT and AUDIT-C.

Table 7: Examine if alcohol use is associated with HIV viral load

	HL >1000 (unsuppressed)	HL <1000 (suppressed)	Total
Low risk			
Risky drinking			
Alcohol dependence			
Total			

	HL >1000	HL <1000	Total

Non-hazardous			
Hazardous			
Total			

3. DESCRIPTION OF RISKS AND BENEFITS

3.1. Risks

A possible risk in the use of secondary data is the risk of loss of confidentiality, however, the PACART study used anonymous participant identification numbers on all the study documents and password encrypted files, therefore minimizing this particular risk. See the Confidentiality section below for more information on methods which were set in place to minimize the risk of loss of confidentiality.

3.2. Benefits

This study provides no direct benefits to the participants. The study will contribute to the advancement of knowledge on the prevalence of alcohol consumption in women living with HIV postpartum, the predictors of alcohol use and the association between alcohol use and maternal HIV viral load. This study has the potential to provide information on the need for screening for alcohol use and interventions at a primary care level.

Since maternal HIV viral load can be a measurement of ART adherence, this study may also shed information on how alcohol use affects ART adherence postpartum. Being cognizant of this could result in better interventions which focus on this issue and this could help the country reach their 90:90:90 targets.

4. INFORMED CONSENT PROCESS

The informed consent documents did include the information explaining that the collected data would be used for further studies, this information was clearly explained to the participants.

This protocol will also be submitted to the Faculty of Health Faculty of Health Sciences Human Research Ethics Committee for ethics review.

5. PRIVACY AND CONFIDENTIALITY

Several steps were set in place by the parent study to minimize the loss of any confidentiality, only the following were relevant to this study as they focused the collected data:

The PACART study used anonymous participant identification numbers on all study documents, including the Case report form (CRF) which reflected the maternal and infant HIV status, HIV test results and information about ART use.

The study data will be kept in a password-protected file. All data storage at the University of Cape Town will be kept within a firewall-protected SQL server.

6. STUDY RESULTS

The data from the PACART cohort will only be used towards answering the proposed aims of this dissertation.

REFERENCE

1. Chander G, Lau B, Moore RD. Hazardous Alcohol Use: A Risk Factor for Non-Adherence and Lack of Suppression in HIV Infection. J Acquir Immune Defic Syndr 1999. 2006 Dec 1;43(4):411–7.

2. Samet JH, Cheng DM, Libman H, Nunes DP, Alperen JK, Saitz R. Alcohol Consumption and HIV Disease Progression. J Acquir Immune Defic Syndr 1999. 2007 Oct 1;46(2):194–9.

3. Bryant KJ, Nelson S, Braithwaite RS, Roach D. Integrating HIV/AIDS and Alcohol Research. Alcohol Res Health. 2010;33(3):167–78.

4. Olsen LJ, Allen D, Azzi-Lessing L. Assessing risk in families affected by substance abuse. Child Abuse Negl. 1996 Sep 1;20(9):833–42.

5. Jester JM, Jacobson SW, Sokol RJ, Tuttle BS, Jacobson JL. The Influence of Maternal Drinking and Drug Use on the Quality of the Home Environment of School-Aged Children. Alcohol Clin Exp Res. 2000;24(8):1187–97.

6. Miller BA, Smyth NJ, Mudar PJ. Mothers' alcohol and other drug problems and their punitiveness toward their children. J Stud Alcohol [Internet]. 2015 Jan 4 [cited 2020 Jun 14]; Available from: https://www.jsad.com/doi/abs/10.15288/jsa.1999.60.632

7. StatsSA. Statistical Release: Mid-year population estimates. 2021; Available from: https://www.statssa.gov.za/publications/P0302/P03022021.pdf

8. Erasmus I, Nicolaou E, van Gelderen CJ, Nicolaides KH. Cervical length at 23 weeks' gestation--relation to demographic characteristics and previous obstetric history in South African women. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 2005 Sep;95(9):691–5.

9. Matseke G, Peltzer K, Mlambo G. Partner violence and associated factors among pregnant women in Nkangala district, Mpumalanga. South Afr J Obstet Gynaecol [Internet]. 2012 [cited 2023 Apr 10];18(3). Available from: https://www.ajol.info/index.php/sajog/article/view/82940

10. Vythilingum B, Roos A, Faure SC, Geerts L, Stein DJ. Risk factors for substance use in pregnant women in South Africa. S Afr Med J. 2012;Vol 102(n 11):851–4.

11. Croxford J, Viljoen D. Alcohol consumption by pregnant women in the Western Cape. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 1999 Sep;89(9):962–5.

12. Desmond K, Milburn N, Richter L, Tomlinson M, Greco E, van Heerden A, et al. Alcohol consumption among HIV-positive pregnant women in KwaZulu-Natal, South Africa: prevalence and correlates. Drug Alcohol Depend. 2012 Jan 1;120(1–3):113–8.

13. Brittain K, Remien RH, Phillips T, Zerbe A, Abrams EJ, Myer L, et al. Factors associated with alcohol use prior to and during pregnancy among HIV-infected pregnant women in Cape Town, South Africa. Drug Alcohol Depend. 2017 Apr;173:69–77.

14. Substance Abuse and Mental Health Services Adminstration. Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings. NSDUH Series H-41,. 2011;4658(11):156.

15. Jagodzinski TD, Fleming MF. Correlates of postpartum alcohol use. WMJ Off Publ State Med Soc Wis. 2007 Sep;106(6):319–25.

16. Laborde ND, Mair C. Alcohol Use Patterns Among Postpartum Women. Matern Child Health J. 2012 Dec;16(9):1810–9.

17. Cook RL, Zhu F, Belnap BH, Weber K, Cook JA, Vlahov D, et al. Longitudinal Trends in Hazardous Alcohol Consumption Among Women With Human Immunodeficiency Virus Infection, 1995–2006. Am J Epidemiol. 2009 Apr 15;169(8):1025–32.

18. Brittain K, Mellins CA, Phillips T, Zerbe A, Abrams EJ, Myer L, et al. Social Support, Stigma and Antenatal Depression Among HIV-Infected Pregnant Women in South Africa. AIDS Behav. 2017 Jan;21(1):274–82.

19. Myer L, Smit J, Roux LL, Parker S, Stein DJ, Seedat S. Common Mental Disorders among HIV-Infected Individuals in South Africa: Prevalence, Predictors, and Validation of Brief Psychiatric Rating Scales. AIDS Patient Care STDs. 2008 Feb 1;22(2):147–58.

20. Kalichman SC, Simbayi LC, Kaufman M, Cain D, Jooste S. Alcohol use and sexual risks for HIV/AIDS in sub-Saharan Africa: systematic review of empirical findings. Prev Sci Off J Soc Prev Res. 2007 Jun;8(2):141–51.

21. Miller AP, Fatch R, Lodi S, Marson K, Emenyonu N, Kekibiina A, et al. Unhealthy alcohol use and intimate partner violence among men and women living with HIV in Uganda. BMC Public Health. 2022 Oct 10;22(1):1886.

22. Shi CF, Kouyoumdjian FG, Dushoff J. Intimate partner violence is associated with HIV infection in women in Kenya: A cross-sectional analysis. BMC Public Health. 2013 May 28;13(1):512.

23. Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. The Alcohol Use Disorders Identification Test Guidelines for Use in Primary Care. World Health Organization. 2001;41.

24. Herring R, Berridge V, Thom B. Binge drinking: an exploration of a confused concept. J Epidemiol Community Health. 2008 Jun 1;62(6):476–9.

25. UNAIDS. South Africa HIV Factsheet. 2018;

26. Cook RL, Zhou Z, Kelso-Chichetto NE, Janelle J, Morano JP, Somboonwit C, et al. Alcohol consumption patterns and HIV viral suppression among persons receiving HIV care in Florida: an observational study. Addict Sci Clin Pract. 2017 Dec;12(1):22.

27. Jagodzinski TD, Fleming MF. Postpartum and Alcohol-Related Factors Associated with the Relapse of Risky Drinking. J Stud Alcohol Drugs. 2007 Nov;68(6):879–85.

28. McLeod D, Pullon S, Cookson T, Cornford E. Factors influencing alcohol consumption during pregnancy and after giving birth. N Z Med J. 2002 Jul 2;115(1157):U29.

29. Muhuri PK, Gfroerer JC. Substance use among women: associations with pregnancy, parenting, and race/ethnicity. Matern Child Health J. 2009 May;13(3):376–85.

30. Mellins CA, Chu C, Malee K, Allison S, Smith R, Harris L, et al. Adherence to antiretroviral treatment among pregnant and postpartum HIV-infected women. AIDS Care. 2008 Sep 1;20(8):958–68.

31. Nachega JB, Uthman OA, Anderson J, Peltzer K, Wampold S, Cotton MF, et al. Adherence to Antiretroviral Therapy During and After Pregnancy in Low-, Middle and High Income Countries: A Systematic Review and Meta-Analysis. AIDS Lond Engl. 2012 Oct 23;26(16):2039–52.

32. Kurewa EN, Kandawasvika GQ, Mhlanga F, Munjoma M, Mapingure MP, Chandiwana P, et al. Realities and Challenges of a Five Year Follow Up of Mother and Child Pairs on a PMTCT Program in Zimbabwe. Open AIDS J. 2011 Jun 14;5:51–8.

33. Rawizza HE, Chang CA, Chaplin B, Ahmed IA, Meloni ST, Oyebode T, et al. Loss to Follow-up within the Prevention of Mother-to-Child Transmission Care Cascade in a Large ART Program in Nigeria. Curr HIV Res. 2015;13(3):201–9.

34. Manzi M, Zachariah R, Teck R, Buhendwa L, Kazima J, Bakali E, et al. High acceptability of voluntary counselling and HIV-testing but unacceptable loss to follow up in a prevention of mother-to-child HIV transmission programme in rural Malawi: scaling-up requires a different way of acting. Trop Med Int Health TM IH. 2005 Dec;10(12):1242–50.

35. Odayar J, Malaba TR, Allerton J, Lesosky M, Myer L. Delivery of antiretroviral therapy to HIV-infected women during the postpartum period: The Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) trial. Contemp Clin Trials Commun. 2019 Dec 1;16:100442.

36. Kessler RC, Andrews G, Colpe LJ, Hiripi E, Mroczek DK, Normand SLT, et al. Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychol Med. 2002 Aug;32(6):959–76.

37. Cox JL, Holden JM, Sagovsky R. Detection of Postnatal Depression: Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987 Jun;150(6):782–6.

38. Kachadourian LK, Pilver CE, Potenza MN. Trauma, PTSD, and Binge and Hazardous Drinking Among Women and Men: Findings from a National Study. J Psychiatr Res. 2014 Aug;55:35–43.

39. McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011. Adv Nutr Bethesda Md. 2011 May;2(3):293–4.

40. Meyer-Leu Y, Lemola S, Daeppen JB, Deriaz O, Gerber S. Association of Moderate Alcohol Use and Binge Drinking During Pregnancy with Neonatal Health. Alcohol Clin Exp Res. 2011;35(9):1669–77.

41. Ethen MK, Ramadhani TA, Scheuerle AE, Canfield MA, Wyszynski DF, Druschel CM, et al. Alcohol Consumption by Women Before and During Pregnancy. Matern Child Health J. 2009 Mar;13(2):274–85.

42. Liu W, Mumford EA, Petras H. Maternal patterns of postpartum alcohol consumption by age: A longitudinal analysis of adult urban mothers. Prev Sci Off J Soc Prev Res. 2015 Apr;16(3):353–63.

43. Cole G, Tucker L, Friedman GM. Relationships among Measures of Alcohol Drinking Behavior, Life-Events and Perceived Stress. Psychol Rep. 1990 Oct 1;67(2):587–91.

44. Dawson DA, Grant BF, Ruan WJ. The association between stress and drinking: modifying effects of gender and vulnerability. Alcohol Alcohol Oxf Oxfs. 2005 Oct;40(5):453–60.

45. Myer PL. Postpartum Adherence Clubs for Antiretroviral Therapy: a Randomised Controlled Trial [Internet]. clinicaltrials.gov; 2019 Aug [cited 2021 Aug 5]. Report No.: NCT03200054. Available from: https://clinicaltrials.gov/ct2/show/NCT03200054

46. Myer LMbc, Phillips TK, Zerbe A, Ronan A, Hsiao NYMbbc, Mellins CA, et al. Optimizing Antiretroviral Therapy (ART) for Maternal and Child Health (MCH): Rationale and Design of the MCH-ART Study. J Acquir Immune Defic Syndr. 2016 Aug;

47. Barrera M. A method for the assessment of social support networks in community survey research. Connections. 1980;3(3):8–13.

48. Arnsten JHM, Li XM, Mizuno Y, Knowlton ARM, Gourevitch MNM, Handley K, et al. Factors Associated With Antiretroviral Therapy Adherence and Medication Errors Among HIV-Infected Injection Drug Users. J Acquir Immune Defic Syndr. 2007 Nov;

49. Barry OM, Bergh AM, Makin JD, Etsane E, Kershaw TS, Forsyth BWC. Development of a measure of the patient–provider relationship in antenatal care and its importance in PMTCT. AIDS Care. 2012 Jun 1;24(6):680–6.

50. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. Addict Abingdon Engl. 1993 Jun;88(6):791–804.

51. Burns E, Gray R, Smith LA. Brief screening questionnaires to identify problem drinking during pregnancy: a systematic review. Addiction. 2010;105(4):601–14.

52. Vythilingum B, Roos A, Faure SC, Geerts L, Stein DJ. Risk factors for substance use in pregnant women in South Africa. S Afr Med J. 2012 Sep 14;102(11):851–4.

53. Peltzer K, Davids A, Njuho P. Alcohol use and problem drinking in South Africa: Findings from a national population-based survey. Afr J Psychiatry. 2011 Mar 1;14:30–7.

54. Strategic Development Information and GIS Department. City of Cape Town – 2011 Census Suburb Gugulethu. 2013; Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://resource.capetown.gov.za/documentcentre/Documents/Maps%20and%20statistics/2011_Census_CT_Suburb_Gugulethu_Profile.pdf

55. Davis EC, Rotheram-Borus MJ, Weichle TW, Rezai R, Tomlinson M. Patterns of Alcohol Abuse, Depression, and Intimate Partner Violence Among Township Mothers in South Africa Over 5 Years. AIDS Behav. 2017 Nov;21(S2):174–82.

56. Kotzé M, Visser M, Makin J, Sikkema K, Forsyth B. Psychosocial variables associated with coping of HIV-positive women diagnosed during pregnancy. AIDS Behav. 2013 Feb;17(2):498–507.

57. Peltzer K, Ramlagan S. Perceived stigma among patients receiving antiretroviral therapy: a prospective study in KwaZulu-Natal, South Africa. AIDS Care. 2011 Jan;23(1):60–8.

58. Hiarlaithe MO, Grede N, de Pee S, Bloem M. Economic and Social Factors are Some of the Most Common Barriers Preventing Women from Accessing Maternal and Newborn Child Health (MNCH) and Prevention of Mother-to-Child Transmission (PMTCT) Services: A Literature Review. AIDS Behav. 2014 Oct;18:S516-30.

59. Hodgson I, Plummer ML, Konopka SN, Colvin CJ, Jonas E, Albertini J, et al. A Systematic Review of Individual and Contextual Factors Affecting ART Initiation, Adherence, and Retention for HIV-Infected Pregnant and Postpartum Women. PLoS One [Internet]. 2014 [cited 2023 Apr 14]; Available from: https://open.uct.ac.za/handle/11427/15002

60. Kapetanovic S, Dass-Brailsford P, Nora D, Talisman N. Mental health of HIV-seropositive women during pregnancy and postpartum period: a comprehensive literature review. AIDS Behav. 2014 Jun;18(6):1152–73.

61. Eastwood J, Jalaludin B, Kemp L. Relationship of postnatal depressive symptoms to infant temperament, maternal expectations, social support and other potential risk factors: findings from a large Australian cross-sectional. BMC Pregnancy Childbirth [Internet]. 2012 [cited 2023 Apr 14];12(148). Available from: https://link.springer.com/article/10.1186/1471-2393-12-148

62. Rotheram-Borus MJ, Weichle TW, Wynn A, Almirol E, Davis E, Stewart J, et al. Alcohol, But Not Depression or IPV, Reduces HIV Adherence Among South African Mothers Living with HIV Over 5 Years. AIDS Behav. 2019 Dec;23(12):3247–56.

63. Myers B, Lombard C, Joska JA, Abdullah F, Naledi T, Lund C, et al. Associations Between Patterns of Alcohol Use and Viral Load Suppression Amongst Women Living with HIV in South Africa. AIDS Behav [Internet]. 2021 Apr 19 [cited 2021 Nov 1]; Available from: https://link.springer.com/10.1007/s10461-021-03263-3

64. Woolf-King SE, Sheinfil AZ, Ramos J, Foley JD, Moskal D, Firkey M, et al. A Conceptual Model of Alcohol Use and Adherence to Antiretroviral Therapy: Systematic Review and Theoretical Implications for Mechanisms of Action. Health Psychol Rev. 2022 Mar;16(1):104–33.

PART B: JOURNAL MANUSCRIPT

The predictors and patterns of alcohol use during the postpartum period in HIV positive women, and how these affect the HIV viral load

Thandeka Mazubane [1]*

Author affiliations

[1] University of Cape Town, Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, South Africa

***Corresponding author**

Thandeka Mazubane

Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, South Africa

Faculty of Health Sciences, University of Cape Town,

Anzio Road, Observatory, Cape Town,

7925, South Africa

Email: mzbtha014@myuct.ac.za

---

[1] The target peer reviewed journal chosen to guide the formatting of this manuscript is the **PLOS One**. The journal submission guidelines for authors are included in **Part C** of the thesis in **appendix 3**. For the purposes of this thesis the student is the sole and first author, the co-authors contributions are recognised in the acknowledgements section of the thesis.

ABSTRACT

Introduction

Although alcohol use among HIV positive pregnant women in South Africa has been extensively researched, few studies have attempted to understand the longitudinal patterns and predictors of alcohol use when HIV positive status intersects with the postpartum period. We examined the patterns of alcohol consumption during the postpartum period among women living with HIV from Gugulethu, Cape Town; explored predictors associated with any alcohol consumption; and lastly, examined the association between alcohol use and HIV viral load.

Methods

Participants were recruited at the Gugulethu Community Health Centre, which is predominantly attended by people from the surrounding communities who are Xhosa speaking. Participants were recruited while receiving prevention of mother to child transmission (PMTCT) services, obstetric, or postnatal care. The Alcohol Use Disorder Identification Test (AUDIT) screening tool was used to assess the alcohol use of eligible women. Participants were also assessed for hazardous alcohol use using the AUDIT-C scoring system during the analysis. The factors associated with patterns of alcohol use were then investigated using multiple logic regression and Generalised Mixed Effect Models. Using Generalised Mixed Effect Models, we also investigated the relationship between alcohol consumption and HIV viral load.

Results

Among 360 women (median age: 33 years), 10–28% reported alcohol use during the 24-month postpartum period, with alcohol use characterised by hazardous, binge drinking, and risky/dependency behaviour and associated with single relationship status and depression, whereas strong social support and patient-provider relationship was protective against risky/dependent alcohol use. We also found an association between hazardous, risky/dependent alcohol use and viral load, where hazardous drinkers and risky/dependent were 3 and 5 times more likely to have a suboptimal (>1000 copies/ml) viral load, respectively.

Conclusion

These unique data sheds light on the trajectories of alcohol use during the postpartum period in this vulnerable population, emphasizing the critical support and attention needed by this population and the strong focus public health needs to put on screening and interventions at a primary care level.

1. INTRODUCTION

It is well documented that there is a high prevalence of alcohol use during pregnancy among South African women, particularly in the Western Cape. Alcohol consumption during pregnancy is associated with various adverse birth outcomes such as stillbirth and neuro behavioural problems such as foetal alcohol syndrome (FAS) (40). To help prevent the adverse birth outcomes which result from substance abuse, there has been a growing body of research examining the patterns and risk factors of maternal perinatal alcohol consumption (41,42), ultimately leading to several programs which aim to decrease alcohol consumption in these women.

Introducing these programs has since been suggested to have successfully achieved the intended goal and led to a reduction in alcohol consumption during pregnancy (13,14). However, there is still a significant proportion of mothers who engage in binge drinking within a year postdelivery (14,16,27). Prevalence estimates of any alcohol use postpartum range from 30 - 50 %, and binge drinking range from 6-20 % (13).

The postpartum period could either result in a decrease in alcohol consumption due to the new responsibilities, expectations and changing norms, or the increased pressures and expectations associated with caring for a child may lead to stressful challenges (16). Since there is a well-established link between stress and excessive alcohol use, this could lead to increased alcohol consumption (43,44).

Although alcohol consumption during the postpartum period is prevalent in the general population, HIV infected women may be a particularly vulnerable group (13). The well-documented high rates of hazardous drinking (13,17) and binge drinking (12,13) among HIV positive non-pregnant and pregnant suggest the need for alcohol cessation interventions during pregnancy and the postpartum period. To our knowledge, there is no information on the patterns of alcohol use in women living with HIV during the postpartum period.

Additional to the challenges faced by a new mother, the postpartum period poses unique challenges to HIV positive new mothers, including stigma and the need to adhere to antiretroviral treatment. In order to address alcohol consumption in HIV positive postpartum women, it is important to understand the patterns and risk factors of alcohol consumption during the postpartum period in women living with HIV in order to develop screening tools and successful interventions.

We thus examined the patterns of alcohol consumption during the postpartum period among women living with HIV from Gugulethu, Cape Town; explored predictors associated with any alcohol consumption; and lastly, examined the association between alcohol use and HIV viral load.

2. METHODS

2.1. Study design

This analysis draws on a prospective cohort study from the Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) parent study, a randomized control trial (RCT) conducted in Gugulethu, Cape Town, evaluating the success of community-based adherence club (AC) system to deliver long-term HIV care to antenatal women initiating on antiretroviral therapy (ART). This two-arm RCT, primarily aimed to compare maternal viral load suppression (defined as viral load [VL] < 1000 copies/ml) up to 24 months postpartum among HIV-infected women who received the current standard care ART services at local adult clinics, versus those who received ART care through the ACs (35,45).

There was a total of six study visits: at enrolment and approximately three, six, twelve, eighteen and twenty-four months postpartum. Among the measurements collected were phlebotomy for viral load and questionnaires assessing alcohol use, psychological distress and postnatal depression, maternal adherence, and so on, all of which will be included in this current study (35).

The current study will be a longitudinal secondary data analysis study that will investigate the proposed aims and objectives using the data collected from the parent study.

2.2. Participants

Eligible participants were HIV-infected women who were 18 years or older and within 70 days postpartum. Participants had to have a documented HIV infection with ART initiation during the preceding antenatal period, as well as a viral suppression documented in pregnancy with a most recent viral load of <400 copies/mL within the previous 3 months. Participants were excluded if they had any medical, psychiatric, or social condition that, in the investigators' opinion, would impair their ability to consent and/or participate in the study, including refusal to take ART/ARVs and denial of HIV status. Mothers were also excluded if they had lost their foetus or infant or had current co-morbidities requiring additional medical attention, including opportunistic infections such as tuberculosis (TB) disease or any chronic condition or other condition that is not controlled or stable (35,45).

2.3. Study procedures and measures

2.3.1. Recruitment, enrolment, and randomisation

Participants were enrolled from Gugulethu, Cape Town, at the Gugulethu Community Health Centre (CHC) and the associated Midwife Obstetric Unit (MOU) and MOU ART clinic, where women were provided with antenatal care (ANC), PMTCT services and obstetric services and postnatal care

(19,46). Eligible women were provided with basic information, and if interested, were screened and referred to study counsellors for the informed consent process (35). Those who provided consent went through an enrolment interview and viral testing, and they were then randomised to either the general adult ART clinic management's strategy or the AC strategy. The study coordinator accessed the opaque sealed envelopes containing the randomisation allocations and communicated them to the study staff (35).

Following enrolment, women in both arms were seen at the MOU ART clinic, where they were counselled, prescribed ART, and given a referral letter; those referred to the general adult ART clinic were advised to return within a month, while those referred to the AC were escorted by a counsellor to the AC club office at the CHC for a club appointment.

All who participated provided written informed consent, and the parent study, as well as the current study, were both approved by the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee and the Institutional Review Board of the Columbia University Medical Center.

2.3.2. Measurements

Quantitative assessments include questionnaires were administered at every visit, including demographic information, medical history, range of measures of ART adherence (appendix 1). At the participant's first study visit, which coincided with their first routine antenatal visit, basic demographic characteristics, maternal adherence, psychosocial measures, and availability of social support were assessed. These included age, education, marital status and primigravida, income category and a composite poverty score was calculated to categorise participants based on their relative level of disadvantage (18). The poverty score was calculated by combining several socio-demographic factors including assets and employment status, and then divided into tertile scores (13,18). Income was collected as seven categories in the parent study, with category 7 being > R R25000; however, because this is a predominantly economically disadvantaged population, income was reduced to just two categories (<R999 and R1000 – R 15000). Viral load (VL) measurement was conducted at each study visit, through batch testing by the South African National Health Laboratory Services using the Abbott Realtime HIV-1 assay (Abbott Laboratories, Waltham, MA).

The psychosocial measures included a 12-item measure to assess the perceived availability of social support (47,48), the Edinburgh Postnatal Depression Scale (37) to measure postnatal depression, as well the Kessler Psychological Distress Scale (K10) (36) developed to measure recent depressive symptoms. The development and scoring of these measures have been previously described (13,18).

In addition, a 19-item Patient Healthcare Provider Relationship Scale was developed and validated in the context of antenatal care in South Africa (49).

The 10-item Alcohol Use Disorders Identification Test [AUDIT (50)] was conducted on participants during their second antenatal study visits in order to assess alcohol use during the previous 30 days. The AUDIT is a screening tool for excessive drinking that was developed as part of a World Health Organization collaboration study at primary care facilities in six countries (13,51). The tool has been tested and approved for use during pregnancy (51) and, more specifically, in a sample of HIV-infected people in South Africa (19). For the purposes of this research, the cut-off score for risky drinking was an AUDIT score of >6, while alcohol dependency was described with a score of >20, this was derived from the original scoring system (50) as well as previously determined cut-off scores specifically suitable for the South African demographic (52).

The AUDIT-C (AUDIT-Consumption) scoring system was used to measure hazardous drinking, this test focused on the frequency and quantity of alcohol consumption, as well as the frequency of binge drinking. Here, hazardous drinking is defined as a pattern of alcohol consumption that increases the risk of harmful consequences, and binge drinking as a single drinking session that usually results in intoxication (13). We used the AUDIT tool's third question, "How many times have you had six standard drinks or more at a time since your last visit up until today?" as a marker for binge drinking. To determine whether a person was hazardously drinking or not, the first three questions of the AUDIT scale (each scored between 0 and 4) were summed for a maximum score of 12, and those scoring 3 and above were classified as hazardous drinkers (1,51). A standard drink in South Africa contains approximately 12 g of alcohol and typically includes one beer or glass of wine (13).

2.4. Data analysis

Data were analysed using R studio R version 4.0.5 (52). To describe the pattern of alcohol use over the 24 months follow up period, the percentage of those who self-reported alcohol use within the last 30 days versus no alcohol use, low-risk alcohol consumption compared to risky/alcohol dependence (AUDIT) and finally hazardous alcohol use versus non-hazardous alcohol use (AUDIT-C scores) was analysed. To examine which characteristics are associated with alcohol use we compared baseline visit characteristics. Initially, AUDIT scores were categorised as low-risk, risky-drinking and dependency drinking, however, during analyses, only 2 (0.5%) participants were alcohol dependant therefore, risky and dependency were combined into one category called risky/dependent drinkers.

Similarly, the original income categories were <R999, R1000-R4999, and R5000-R15000, however, the low numbers in the R5000-R15000 when cross tabulated with AUDIT made it difficult to produce accurate models. Therefore we merged R1000-R4999 and R5000-R15000.

We used the Fisher exact test to compare categorical variables and the Wilcoxon rank-sum test (Mann-Whitney test) to compare means of non-normally distributed continuous variables. Next, we used multivariable logistic regression to assess the effect of sociodemographic variables which were only collected at baseline and therefore were not longitudinal. To analyse the association between AUDIT and AUDIT-C categorise to longitudinal variables we used the Generalised Mixed Effect Models, allowing participants to contribute data from each time point, with adjustment for repeated measures within subjects. To determine the association viral load to AUDIT and AUDIT-C similarly used the logistic regression and the Generalised Mixed Effect Model for the longitudinal variables.

3. RESULTS

3.1.1. Demographic, clinical, and psychosocial characteristics

A total of 412 women were enrolled into the PACART study, however, AUDIT assessments were only conducted from visit 2 (3 months postpartum), during this visit 360 participants provided a response to the AUDIT assessments, this was the total number of participants analysed at visit 2 (3 months pp). The total number of participants varied at each visit due to some participants naturally missing some postnatal visits/study visits. Detailed demographic, clinical and psychosocial characteristics of this sample are presented in Table 1. Most participants reported that they were not married or co-habiting, were classified as moderate disadvantaged according to their poverty score and the majority of participants achieved optimal adherence. The majority (89%) of participants were categorised as healthy on the Kessler-10 mental health score at 3 months postpartum, and on average participants scored 45/55 when questioned about their Perceived Social Support.

3.1.2. Describe the pattern of alcohol use over the 24 month follow up period

In this sample, at 3 months postpartum (visit 2), 10% self-reported any alcohol consumption in the prior 30 days, among those, 7% reported as hazardous drinkers according to the AUDIT-C tool and 4% displayed risky/dependency alcohol behaviour according to the AUDIT tool (Figure 1). We see a gradual increase in overall alcohol consumption, as well as hazardous and risky/dependency behaviours over the 24 month, follow up period. By 18 months postpartum, 28% consumed alcohol, of those, 20% were hazardous drinkers and 5% indicated risky/dependency alcohol behaviour. In the final study visit, at 24 months, there is a sudden decrease in alcohol consumption. However, it is still higher than the baseline consumption at 3 months.

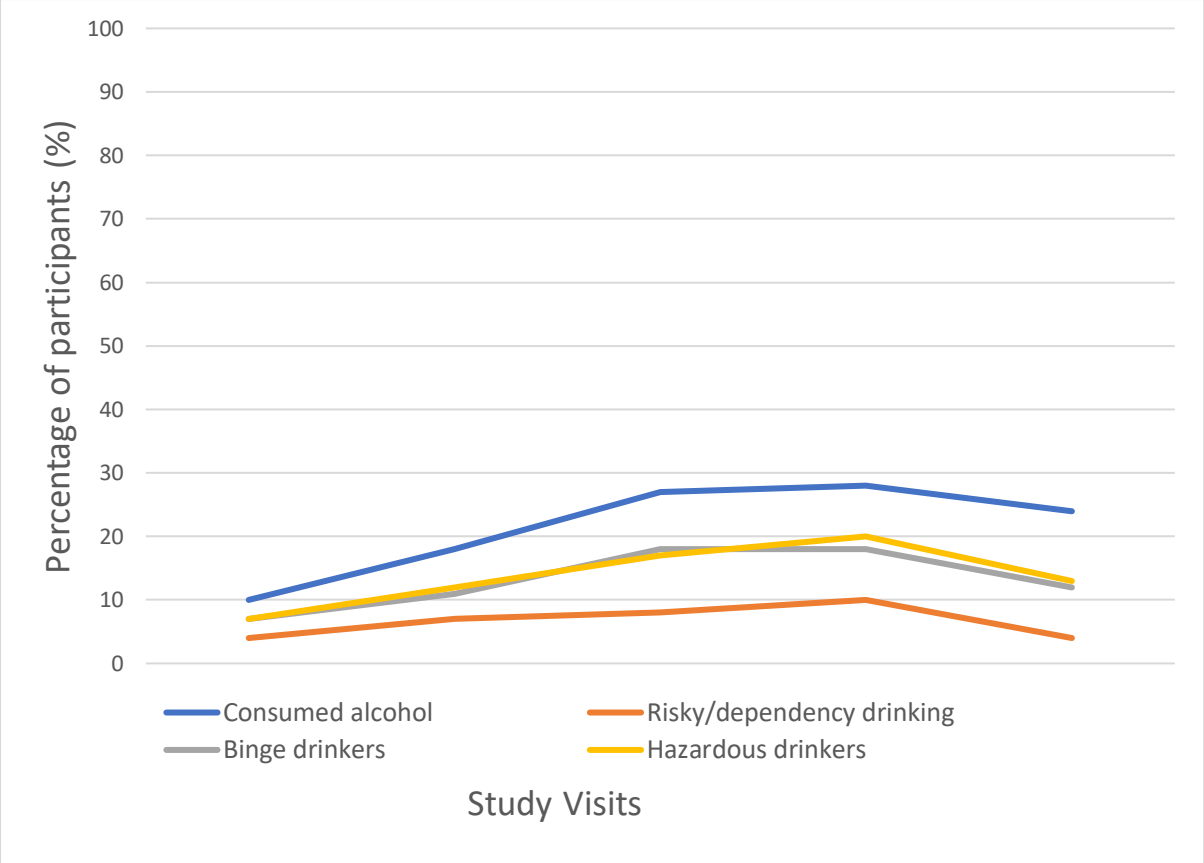

Figure 1: Alcohol prevalence in HIV-infected women during a 24-month postpartum follow-up period. The graph shows the percentage of participants who self-reported alcohol consumption in the past 30 days, of those, the percentage of those who displayed risky/dependency patterns according to the AUDIT tool and those who displayed hazardous alcohol consumption behaviour according to the AUDIT-C tool. A. Is completed using all participants in the sample, while B. only participants with data from all 5 study visits.

3.1.3. Factors associated with AUDIT and AUDIT-C during the postpartum period in women living with HIV

We used logistic regression to analyse which cross-sectional demographic factors were associated with hazardous alcohol consumption (Table 2). Table 2 shows that women who were single were 5 times were more likely to display hazardous alcohol consumption. Those who have had primary/some secondary education and in the R1000 – R15000 income category were less likely to be hazardous drinkers, although these results were statistically insignificant. When analysing longitudinal factors associated with hazardous use, patient-provider scores relationship scores and perceived social support (Table 3) were both associated with hazardous alcohol use, where high scores showed participants to be slightly protected from hazardous alcohol use.

Similarly, those who were single were more likely to be risky/dependent alcohol drinkers compared to the married/co-habiting women (Table 2). Patient-provider score and perceived social support

were associated with AUDIT risky/dependent alcohol use (Table 3). And those who were depressed were 1.4 times more likely to display risky/dependent alcohol use compared to those who were not depressed. K10 mental disorder measurement tool was statistically insignificant for both hazardous drinkers and risky/dependant alcohol drinkers.

3.1.4. Examine if alcohol use is associated with HIV viral load

In Table 4, hazardous alcohol use was statistically associated with viral load, where hazardous drinkers had been 3 times more likely to have sub-optimal (>1000 copies/ml) viral load. This analysis was after adjusting for age, marital status and poverty tertile score. Similarly, those who were risky/dependant drinkers were 5 times more likely to have sub-optimal viral load compared to low-risky drinkers.

Table 8: Baseline description of the study sample of HIV-infected Postpartum women from Gugulethu, South Africa, by AUDIT and AUDIT-C.

Variables	Total sample	AUDIT			AUDIT-C		
		Low risk	Risky/dependency drinking	p-value	Non-hazardous	Hazardous	p-value
Total alcohol prevalence at baseline	360	345 (96)	15 (4)	-	336 (93)	24 (7)	-
Age	33 [30;37]	34 [30;37]	31[29.5;34.5]	0.100	34 [30;37]	31[29;34]	0.075
Education				0.390			0.367
Completed secondary/tertiary	105 (29)	99 (29)	6 (40.0)		96 (29)	9 (38)	
Primary/some secondary	255 (71)	246 (71)	9 (60)		240 (71)	15 (62)	
Employment				0.255			0.366
Employed	108 (30)	106 (31)	2 (13)		103 (31)	5 (21)	
Unemployed	252 (70)	239 (69)	13 (87)		233 (69)	19 (79)	
Marital status				0.033			0.015
Married/Co-habiting	154 (43)	152 (44)	2 (13)		150 (45)	4 (17)	
Single	206 (57)	193 (56)	13 (87)		186 (55)	20 (83)	
Income category				0.454			0.767
<R999	49 (14)	46 (14)	3 (20)		45 (14)	4 (17)	
R1000 – R 15000	301 (86)	289 (86)	12 (80)		281 (86)	20 (83)	
Poverty Score							0.950
Least disadvantaged	128 (36)	121 (35)	7 (47)	0.694	120 (36)	8 (33)	
Moderate disadvantaged	190 (53)	183 (53)	7 (47)		177 (53)	13 (54)	
Most disadvantaged	41 (11)	40 (12)	1 (6)		38 (11)	3 (13)	
Primigravida				0.748			0.601
No	71 (20)	69 (20)	2 (13)		68 (20)	3 (13)	
Yes	288 (80)	275 (80)	13 (87)		268 (80)	20 (87)	
Adherence							
Optimal	331 (92)	320 (93)	11 (73)	0.020	315 (94)	16 (67)	<0.001
Sub-optimal	29 (8)	25 (7)	4 (27)		21 (6)	8 (33)	
Kessler-10 score (K-10) severe mental disorder							0.074
Healthy (<20)	335 (93)	322 (94)	13 (87)	0.325	298 (89)	21 (91)	
	21 (6.0)	19 (5)	2 (13)		34 (10)	2 (9)	

Mild-moderate mental disorder (20-29) Severe mental disorder (>30)	3 (0.9)	3 (1)	0		4 (1)	0	
Edinburgh Postnatal Depression Survey score (EPDS) Non-depressed Depressed	 307 (86) 51 (14)	 295 (86) 48 (14)	 12 (80) 3 (20)	0.455	 295 (86) 48 (14)	 12 (80) 3 (20)	0.466
Patient-Provider Relationship score median (IQR; max score = 76)	359	59 [54;62]	56 [52.5;59]	0.340	59 (54;62)	58 [52.75;59]	0.410
Perceived social support score. median (IQR; max score = 55)	360	45 [34;52]	34 [33;44]	0.700	45 [34;52.25]	40 [33;46.5]	0.503

Table 9: Multiple logistic regression model analysing factors associated to AUDIT and AUDIT-C alcohol consumption in HIV-infected women.

	AUDIT				AUDIT-C			
Variables	Unadjusted Odds Ratio [95% CI]	P – value	Adjusted Odds Ratio [95% CI]	P – value	Unadjusted Odds Ratio [95% CI]	P – value	Adjusted Odds Ratio [95% CI]	P – value
Age	0.921 [0.82;1.03]	0.150	0.91 [0.79;1.04]	0.115	1.083 [0.85;1.01]	0.095	0.92 [0.82;1.01]	0.115
Education Completed secondary/tertiary Primary/some secondary	0.608 [0.21;1.84]	0.350	0.39 [0.12;1.31]	0.112	0.677 [0.29;1.64]	0.352	0.53 [0.20;1.39]	0.194
Employment Employed Unemployed	2.883 [0.78;18.6]	0.161	2.75 [0.57;23]	0.345	1.689 [0.65;5.18]	0.325	2.11 [0.65;8.6]	0.273
Marital status Married/Co-habiting Single	5.127 [1.39;33.1]	0.035	5.52 [1.37;37.7]	0.044	4.035 [1.49;14.1]	0.013	3.50 [1.22;12.6]	0.038
Income category <R999 R1000 – R 15000	0.634 [0.19;2.87]	0.492	0.7 [0.19;3.37]	0.573	0.801 [0.29;2.85]	0.700	0.78 [0.25;2.92]	0.674
Poverty Score (asset + employment) tertiles Least disadvantaged Moderate disadvantaged Most disadvantaged	 2.313 [0.26;29] 1.485 [0.38;43]	 0.696 0.433	 0.28[0.11;22.7] 1.86 [0.39;44]	 0.968 0.747	 0.846 [0.28;4.00] 0.933 [0.23;4.00]	 0.910 0.810	 0.56 [0.10;3.61] 0.68[0.14;3.70]	 0.525 0.626
Primigravida No Yes	1.630 [0.44;10.6]	0.500	2.80 [0.63;20.1]	0.194	1.700 [0.55;7.33]	0.400	3.13 [0.92;14.7]	0.097

Table 10: Generalised mixed-effects models analysis of longitudinal factors associated with AUDIT and AUDIT-C adjusted for poverty status and age

	AUDIT		AUDIT-C	
Variables	Adjusted OR [95% CI]	P-value	Adjusted OR [95% CI]	P-value
Edinburgh Postnatal Depression Survey score (EPDS) Non-depressed Depressed	1.480[0.74; 2.95]	0.032	1.481 [0.74; 2.95]	0.258
Kessler-10 score (K-10) severe mental disorder Healthy (<20) Mild-moderate mental disorder (20-29) Severe mental disorder (>30)	1.192[0.49;2.9] 2.391 [0.206;13]	0.773 0.487	1.190 [0.49;2.9] 2.392 [0.206;13]	0.691 0.492
Patient-provider score	0.942 [0.10;1.00]	0.030	0.961 [0.9;1.00]	0.072
Perceived social support	0.852 [0.76;0.94]	0.002	0.900 [0.86;0.97]	0.007

Table 11: Generalised mixed-effects models analysing associations between sub-optimal (>1000 copies/ml) Viral load and AUDIT and AUDIT-C in HIV-positive Postpartum women adjusted for age, marital status, and poverty

Predictors	Adjusted Odds Ratio [95% Cl]	P – value
Audit-c Non-hazardous Hazardous	3.200 [1.67;6.1]	<0.001
Audit Low Risk Risky/dependence	5.625 [2.74;13.4]	<0.001

4. DISCUSSION

This was a longitudinal secondary study that aimed to analyse the prevalence and predictors of alcohol use among women living with HIV during their postpartum period and to further understand the relationship between alcohol use and viral load.

Over the 24-month follow up period, we found the prevalence of alcohol use to range between 10% – 28%, rising from 3-months and peaking at 18-months postpartum. Among those who consumed alcohol ranged by time point from 7-20% were hazardous drinkers, 7-18% were binge drinkers, and 4-9% were risky/dependant alcohol drinkers, all showing an increasing trend from baseline and peaking at 18 months.

Compared to the overall Western Cape (WC) alcohol consumption among women, this population had a lower percentage of people who consumed alcohol (33.1% vs 28% respectively) (53). However, the women from this study group had much higher levels of binge drinking, and hazardous alcohol use compared to the general WC population. At 18-months, women from this population had almost 3 times the levels of binge drinkers (18% vs 6.8%) and 3.5 times the prevalence of hazardous drinkers compared to the WC women (20% vs 5.6%) (53). More importantly, since this Gugulethu is a Black Xhosa-speaking women (54) , the prevalence of alcohol use among black women in South African general public was 10%, this was the same prevalence found at 3-months post-partum, however, by 18 months it was almost 3 times higher (10 vs 28% respectively). The nationwide binge drinking among black women was 2.9% and hazardous was 2.4%, these were significantly lower compared to these women living with HIV (53).

These percentages of hazardous alcohol use among women living with HIV are comparable to those found in other studies. Over an 11-year study period, Cooks found that between 14% - 24% of women living with HIV from the United States reported hazardous alcohol use in the past year (17). Alcohol use among postpartum women from Cape Town Township mothers increase from about 10.5% at 6 months postpartum to on average 24.7% 5 years postpartum, similarly 11% - 29.4% among HIV-infected mothers (55).

These alcohol patterns are opposite to those seen in Laborde et al. where postpartum drinking was protective against any drinking behaviours, including alcohol status, binge drinking and frequency of drinking (16). In this study, women living with HIV were more likely to be hazardous and binge drinkers during their postpartum period compared to non-HIV-infected and non-postnatal black women.

A strong Patient-provider score and perceived social support was seen as protective against risky/dependency behaviours. Lack of social support in has been previously linked to have negative effects on factors such as coping (56) and quality of life (57) in general population. In addition, it has also been identified as one of the barriers of accessing PMTCT services (58) and update of antiretrovirals for PMTCT (18,59) in the general HIV population in Africa. It has also been recognised to have an important protective effect against postnatal depression in women living with HIV assisting with managing HIV specific challenges (60,61).

Table 3 showed that those with depression were 1.4 times more likely to be hazardous drinkers. These findings are consistent with Davis et al who found depression to be a risk factor to alcohol use at baseline and 6 months postpartum, and a positive HIV status throughout the 5 years (62). Another study found, mothers living with HIV are more likely to face challenges from alcohol, depression and having seropositive partners than mothers without HIV over a 5-year study period (56). They also found that it was alcohol use, and not depression or intimate partner violence (IPV) which was significantly associated with the reduced uptake of HIV care and adherence to ARV over the 5 years (62).

Lastly, after adjusting for age, marital status and poverty score, sub-optimal viral load (>1000 copies/ml) was significantly associated with increased odds among both hazardous and risky drinking behaviours (OR 3.2, 95% CI 1.67–6.1 and OR 5.6, 95% CI 2.74–13.4). This was similar to those seen by Cook et al, where heavy drinking was associated with increased odds of failing to achieve durable viral suppression (OR 2.16, 95% CI 1.08–4.32) whereas binge drinking alone was not significantly associated with this outcome (OR 1.04, 95% CI 0.64–1.70) (26).

A strength of the present study is the longitudinal assessment of alcohol use, given that previous research had assessed alcohol use at only one point in time after pregnancy. In addition, it is a study that aimed to understand the alcohol use in women living with HIV during their postpartum period, analysing an important intersection between people living with HIV and the vulnerable period after giving birth. It was important to specifically focus on this target group since previous studies have mostly focused on alcohol use prior to and during pregnancy.

These results suggest that there may be a normative environment for alcohol consumption among women living with HIV in the Western Cape, which should be countered by increased dissemination of information about the dangers of drinking, as well as social and environmental supports for abstinence or moderation. Binge drinking is a serious issue that must be addressed.

The findings highlight the urgent need for specific interventions to reduce alcohol consumption among women during the postpartum period. This could significantly help reduce the percentage of mothers with a sub-optimal viral load and help these women better deal with the compounded challenges arising from this period and their HIV status. Understanding the factors which are associated with alcohol use also can help health care providers know which people are at risk of hazardous alcohol use and therefore give them the necessary support to help deal with any distresses which may result in excessive alcohol use.

A limitation of this analysis is the use of only self-reported measures of alcohol use, given the potential impact of recall and social desirability bias of this measurement. However, the AUDIT has been validated for use during pregnancy (19,63) and in South Africa specifically (51). A major limitation was that the inclusion criteria for the primary study were women who already in HIV viral suppression, this creates a somewhat biased sample. One might have seen a stronger association had the we had a sample more representative of the general population, however, we were limited since this was a secondary analysis. Another limitation was we do not know the HIV diagnosis whether before or after pregnancy, it could have been used as a copying mechanism when diagnosed.

Furthermore, this study included women from the townships of Cape Town; it therefore, may not be generalizable to the other populations in South Africa. This study needs to be repeated using the general population of HIV-positive women in South Africa in order to confirm these patterns.

5. CONCLUSION

Despite some limitations, these results are notable. Although the high prevalence of alcohol use during pregnancy in South Africa is widely documented, there are only a few longitudinal studies that document the alcohol use trajectories 24-months post-delivery. These results highlight the significantly high levels of binge, risky and hazardous drinking among women living with HIV . Single women reported a greater risk of hazardous alcohol use, and those single and depressed were mostly risky/dependant alcohol drinkers. Developing strong social support and patient-provider relationship was protective against risky/dependent alcohol use. Furthermore, these results also highlight the importance of controlling hazardous ad risky alcohol use in order to manage viral load suppression. Given the lack of interventions for alcohol use at the primary care level, these data suggest a possible area of interventions in this high-risk population and indicate that screening tools such as AUDIT need to be routinely implemented in primary care postnatal services in this region.

REFERENCE

1. Chander G, Lau B, Moore RD. Hazardous Alcohol Use: A Risk Factor for Non-Adherence and Lack of Suppression in HIV Infection. J Acquir Immune Defic Syndr 1999. 2006 Dec 1;43(4):411–7.

2. Samet JH, Cheng DM, Libman H, Nunes DP, Alperen JK, Saitz R. Alcohol Consumption and HIV Disease Progression. J Acquir Immune Defic Syndr 1999. 2007 Oct 1;46(2):194–9.

3. Bryant KJ, Nelson S, Braithwaite RS, Roach D. Integrating HIV/AIDS and Alcohol Research. Alcohol Res Health. 2010;33(3):167–78.

4. Olsen LJ, Allen D, Azzi-Lessing L. Assessing risk in families affected by substance abuse. Child Abuse Negl. 1996 Sep 1;20(9):833–42.

5. Jester JM, Jacobson SW, Sokol RJ, Tuttle BS, Jacobson JL. The Influence of Maternal Drinking and Drug Use on the Quality of the Home Environment of School-Aged Children. Alcohol Clin Exp Res. 2000;24(8):1187–97.

6. Miller BA, Smyth NJ, Mudar PJ. Mothers' alcohol and other drug problems and their punitiveness toward their children. J Stud Alcohol [Internet]. 2015 Jan 4 [cited 2020 Jun 14]; Available from: https://www.jsad.com/doi/abs/10.15288/jsa.1999.60.632

7. StatsSA. Statistical Release: Mid-year population estimates. 2021; Available from: https://www.statssa.gov.za/publications/P0302/P03022021.pdf

8. Erasmus I, Nicolaou E, van Gelderen CJ, Nicolaides KH. Cervical length at 23 weeks' gestation--relation to demographic characteristics and previous obstetric history in South African women. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 2005 Sep;95(9):691–5.

9. Matseke G, Peltzer K, Mlambo G. Partner violence and associated factors among pregnant women in Nkangala district, Mpumalanga. South Afr J Obstet Gynaecol [Internet]. 2012 [cited 2023 Apr 10];18(3). Available from: https://www.ajol.info/index.php/sajog/article/view/82940

10. Vythilingum B, Roos A, Faure SC, Geerts L, Stein DJ. Risk factors for substance use in pregnant women in South Africa. S Afr Med J. 2012;Vol 102(n 11):851–4.

11. Croxford J, Viljoen D. Alcohol consumption by pregnant women in the Western Cape. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 1999 Sep;89(9):962–5.

12. Desmond K, Milburn N, Richter L, Tomlinson M, Greco E, van Heerden A, et al. Alcohol consumption among HIV-positive pregnant women in KwaZulu-Natal, South Africa: prevalence and correlates. Drug Alcohol Depend. 2012 Jan 1;120(1–3):113–8.

13. Brittain K, Remien RH, Phillips T, Zerbe A, Abrams EJ, Myer L, et al. Factors associated with alcohol use prior to and during pregnancy among HIV-infected pregnant women in Cape Town, South Africa. Drug Alcohol Depend. 2017 Apr;173:69–77.

14. Substance Abuse and Mental Health Services Adminstration. Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings. NSDUH Series H-41,. 2011;4658(11):156.

15. Jagodzinski TD, Fleming MF. Correlates of postpartum alcohol use. WMJ Off Publ State Med Soc Wis. 2007 Sep;106(6):319–25.

16. Laborde ND, Mair C. Alcohol Use Patterns Among Postpartum Women. Matern Child Health J. 2012 Dec;16(9):1810–9.

17. Cook RL, Zhu F, Belnap BH, Weber K, Cook JA, Vlahov D, et al. Longitudinal Trends in Hazardous Alcohol Consumption Among Women With Human Immunodeficiency Virus Infection, 1995–2006. Am J Epidemiol. 2009 Apr 15;169(8):1025–32.

18. Brittain K, Mellins CA, Phillips T, Zerbe A, Abrams EJ, Myer L, et al. Social Support, Stigma and Antenatal Depression Among HIV-Infected Pregnant Women in South Africa. AIDS Behav. 2017 Jan;21(1):274–82.

19. Myer L, Smit J, Roux LL, Parker S, Stein DJ, Seedat S. Common Mental Disorders among HIV-Infected Individuals in South Africa: Prevalence, Predictors, and Validation of Brief Psychiatric Rating Scales. AIDS Patient Care STDs. 2008 Feb 1;22(2):147–58.

20. Kalichman SC, Simbayi LC, Kaufman M, Cain D, Jooste S. Alcohol use and sexual risks for HIV/AIDS in sub-Saharan Africa: systematic review of empirical findings. Prev Sci Off J Soc Prev Res. 2007 Jun;8(2):141–51.

21. Miller AP, Fatch R, Lodi S, Marson K, Emenyonu N, Kekibiina A, et al. Unhealthy alcohol use and intimate partner violence among men and women living with HIV in Uganda. BMC Public Health. 2022 Oct 10;22(1):1886.

22. Shi CF, Kouyoumdjian FG, Dushoff J. Intimate partner violence is associated with HIV infection in women in Kenya: A cross-sectional analysis. BMC Public Health. 2013 May 28;13(1):512.

23. Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. The Alcohol Use Disorders Identification Test Guidelines for Use in Primary Care. World Health Organization. 2001;41.

24. Herring R, Berridge V, Thom B. Binge drinking: an exploration of a confused concept. J Epidemiol Community Health. 2008 Jun 1;62(6):476–9.

25. UNAIDS. South Africa HIV Factsheet. 2018;

26. Cook RL, Zhou Z, Kelso-Chichetto NE, Janelle J, Morano JP, Somboonwit C, et al. Alcohol consumption patterns and HIV viral suppression among persons receiving HIV care in Florida: an observational study. Addict Sci Clin Pract. 2017 Dec;12(1):22.

27. Jagodzinski TD, Fleming MF. Postpartum and Alcohol-Related Factors Associated with the Relapse of Risky Drinking. J Stud Alcohol Drugs. 2007 Nov;68(6):879–85.

28. McLeod D, Pullon S, Cookson T, Cornford E. Factors influencing alcohol consumption during pregnancy and after giving birth. N Z Med J. 2002 Jul 2;115(1157):U29.

29. Muhuri PK, Gfroerer JC. Substance use among women: associations with pregnancy, parenting, and race/ethnicity. Matern Child Health J. 2009 May;13(3):376–85.

30. Mellins CA, Chu C, Malee K, Allison S, Smith R, Harris L, et al. Adherence to antiretroviral treatment among pregnant and postpartum HIV-infected women. AIDS Care. 2008 Sep 1;20(8):958–68.

31. Nachega JB, Uthman OA, Anderson J, Peltzer K, Wampold S, Cotton MF, et al. Adherence to Antiretroviral Therapy During and After Pregnancy in Low-, Middle and High Income Countries: A Systematic Review and Meta-Analysis. AIDS Lond Engl. 2012 Oct 23;26(16):2039–52.

32. Kurewa EN, Kandawasvika GQ, Mhlanga F, Munjoma M, Mapingure MP, Chandiwana P, et al. Realities and Challenges of a Five Year Follow Up of Mother and Child Pairs on a PMTCT Program in Zimbabwe. Open AIDS J. 2011 Jun 14;5:51–8.

33. Rawizza HE, Chang CA, Chaplin B, Ahmed IA, Meloni ST, Oyebode T, et al. Loss to Follow-up within the Prevention of Mother-to-Child Transmission Care Cascade in a Large ART Program in Nigeria. Curr HIV Res. 2015;13(3):201–9.

34. Manzi M, Zachariah R, Teck R, Buhendwa L, Kazima J, Bakali E, et al. High acceptability of voluntary counselling and HIV-testing but unacceptable loss to follow up in a prevention of mother-to-child HIV transmission programme in rural Malawi: scaling-up requires a different way of acting. Trop Med Int Health TM IH. 2005 Dec;10(12):1242–50.

35. Odayar J, Malaba TR, Allerton J, Lesosky M, Myer L. Delivery of antiretroviral therapy to HIV-infected women during the postpartum period: The Postpartum Adherence Clubs for Antiretroviral Therapy (PACART) trial. Contemp Clin Trials Commun. 2019 Dec 1;16:100442.

36. Kessler RC, Andrews G, Colpe LJ, Hiripi E, Mroczek DK, Normand SLT, et al. Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychol Med. 2002 Aug;32(6):959–76.

37. Cox JL, Holden JM, Sagovsky R. Detection of Postnatal Depression: Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987 Jun;150(6):782–6.

38. Kachadourian LK, Pilver CE, Potenza MN. Trauma, PTSD, and Binge and Hazardous Drinking Among Women and Men: Findings from a National Study. J Psychiatr Res. 2014 Aug;55:35–43.

39. McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, January 2011. Adv Nutr Bethesda Md. 2011 May;2(3):293–4.

40. Meyer-Leu Y, Lemola S, Daeppen JB, Deriaz O, Gerber S. Association of Moderate Alcohol Use and Binge Drinking During Pregnancy with Neonatal Health. Alcohol Clin Exp Res. 2011;35(9):1669–77.

41. Ethen MK, Ramadhani TA, Scheuerle AE, Canfield MA, Wyszynski DF, Druschel CM, et al. Alcohol Consumption by Women Before and During Pregnancy. Matern Child Health J. 2009 Mar;13(2):274–85.

42. Liu W, Mumford EA, Petras H. Maternal patterns of postpartum alcohol consumption by age: A longitudinal analysis of adult urban mothers. Prev Sci Off J Soc Prev Res. 2015 Apr;16(3):353–63.

43. Cole G, Tucker L, Friedman GM. Relationships among Measures of Alcohol Drinking Behavior, Life-Events and Perceived Stress. Psychol Rep. 1990 Oct 1;67(2):587–91.

44. Dawson DA, Grant BF, Ruan WJ. The association between stress and drinking: modifying effects of gender and vulnerability. Alcohol Alcohol Oxf Oxfs. 2005 Oct;40(5):453–60.

45. Myer PL. Postpartum Adherence Clubs for Antiretroviral Therapy: a Randomised Controlled Trial [Internet]. clinicaltrials.gov; 2019 Aug [cited 2021 Aug 5]. Report No.: NCT03200054. Available from: https://clinicaltrials.gov/ct2/show/NCT03200054

46. Myer LMbc, Phillips TK, Zerbe A, Ronan A, Hsiao NYMbbc, Mellins CA, et al. Optimizing Antiretroviral Therapy (ART) for Maternal and Child Health (MCH): Rationale and Design of the MCH-ART Study. J Acquir Immune Defic Syndr. 2016 Aug;

47. Barrera M. A method for the assessment of social support networks in community survey research. Connections. 1980;3(3):8–13.

48. Arnsten JHM, Li XM, Mizuno Y, Knowlton ARM, Gourevitch MNM, Handley K, et al. Factors Associated With Antiretroviral Therapy Adherence and Medication Errors Among HIV-Infected Injection Drug Users. J Acquir Immune Defic Syndr. 2007 Nov;

49. Barry OM, Bergh AM, Makin JD, Etsane E, Kershaw TS, Forsyth BWC. Development of a measure of the patient–provider relationship in antenatal care and its importance in PMTCT. AIDS Care. 2012 Jun 1;24(6):680–6.

50. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. Addict Abingdon Engl. 1993 Jun;88(6):791–804.

51. Burns E, Gray R, Smith LA. Brief screening questionnaires to identify problem drinking during pregnancy: a systematic review. Addiction. 2010;105(4):601–14.

52. Vythilingum B, Roos A, Faure SC, Geerts L, Stein DJ. Risk factors for substance use in pregnant women in South Africa. S Afr Med J. 2012 Sep 14;102(11):851–4.

53. Peltzer K, Davids A, Njuho P. Alcohol use and problem drinking in South Africa: Findings from a national population-based survey. Afr J Psychiatry. 2011 Mar 1;14:30–7.

54. Strategic Development Information and GIS Department. City of Cape Town – 2011 Census Suburb Gugulethu. 2013; Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://resource.capetown.gov.za/documentcentre/Documents/Maps%20and%20statistics/2011_Census_CT_Suburb_Gugulethu_Profile.pdf

55. Davis EC, Rotheram-Borus MJ, Weichle TW, Rezai R, Tomlinson M. Patterns of Alcohol Abuse, Depression, and Intimate Partner Violence Among Township Mothers in South Africa Over 5 Years. AIDS Behav. 2017 Nov;21(S2):174–82.

56. Kotzé M, Visser M, Makin J, Sikkema K, Forsyth B. Psychosocial variables associated with coping of HIV-positive women diagnosed during pregnancy. AIDS Behav. 2013 Feb;17(2):498–507.

57. Peltzer K, Ramlagan S. Perceived stigma among patients receiving antiretroviral therapy: a prospective study in KwaZulu-Natal, South Africa. AIDS Care. 2011 Jan;23(1):60–8.

58. Hiarlaithe MO, Grede N, de Pee S, Bloem M. Economic and Social Factors are Some of the Most Common Barriers Preventing Women from Accessing Maternal and Newborn Child Health (MNCH) and Prevention of Mother-to-Child Transmission (PMTCT) Services: A Literature Review. AIDS Behav. 2014 Oct;18:S516-30.

59. Hodgson I, Plummer ML, Konopka SN, Colvin CJ, Jonas E, Albertini J, et al. A Systematic Review of Individual and Contextual Factors Affecting ART Initiation, Adherence, and Retention for HIV-Infected Pregnant and Postpartum Women. PLoS One [Internet]. 2014 [cited 2023 Apr 14]; Available from: https://open.uct.ac.za/handle/11427/15002

60. Kapetanovic S, Dass-Brailsford P, Nora D, Talisman N. Mental health of HIV-seropositive women during pregnancy and postpartum period: a comprehensive literature review. AIDS Behav. 2014 Jun;18(6):1152–73.

61. Eastwood J, Jalaludin B, Kemp L. Relationship of postnatal depressive symptoms to infant temperament, maternal expectations, social support and other potential risk factors: findings from a large Australian cross-sectional. BMC Pregnancy Childbirth [Internet]. 2012 [cited 2023 Apr 14];12(148). Available from: https://link.springer.com/article/10.1186/1471-2393-12-148

62. Rotheram-Borus MJ, Weichle TW, Wynn A, Almirol E, Davis E, Stewart J, et al. Alcohol, But Not Depression or IPV, Reduces HIV Adherence Among South African Mothers Living with HIV Over 5 Years. AIDS Behav. 2019 Dec;23(12):3247–56.

63. Myers B, Lombard C, Joska JA, Abdullah F, Naledi T, Lund C, et al. Associations Between Patterns of Alcohol Use and Viral Load Suppression Amongst Women Living with HIV in South Africa. AIDS Behav [Internet]. 2021 Apr 19 [cited 2021 Nov 1]; Available from: https://link.springer.com/10.1007/s10461-021-03263-3

64. Woolf-King SE, Sheinfil AZ, Ramos J, Foley JD, Moskal D, Firkey M, et al. A Conceptual Model of Alcohol Use and Adherence to Antiretroviral Therapy: Systematic Review and Theoretical Implications for Mechanisms of Action. Health Psychol Rev. 2022 Mar;16(1):104–33.

PART C: APPENDICES

Appendix 1: Supplementary figures

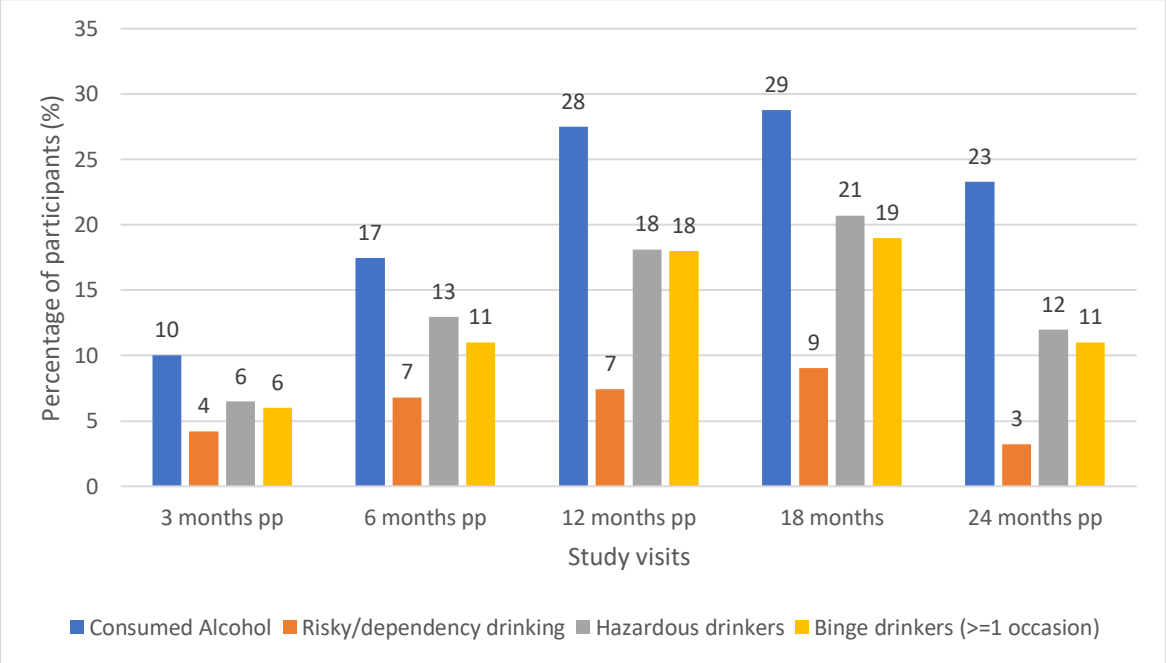

Figure 2: Alcohol prevalence in HIV-infected women during a 24-month postpartum follow-up period of only the participants with data from all 5 study visits.

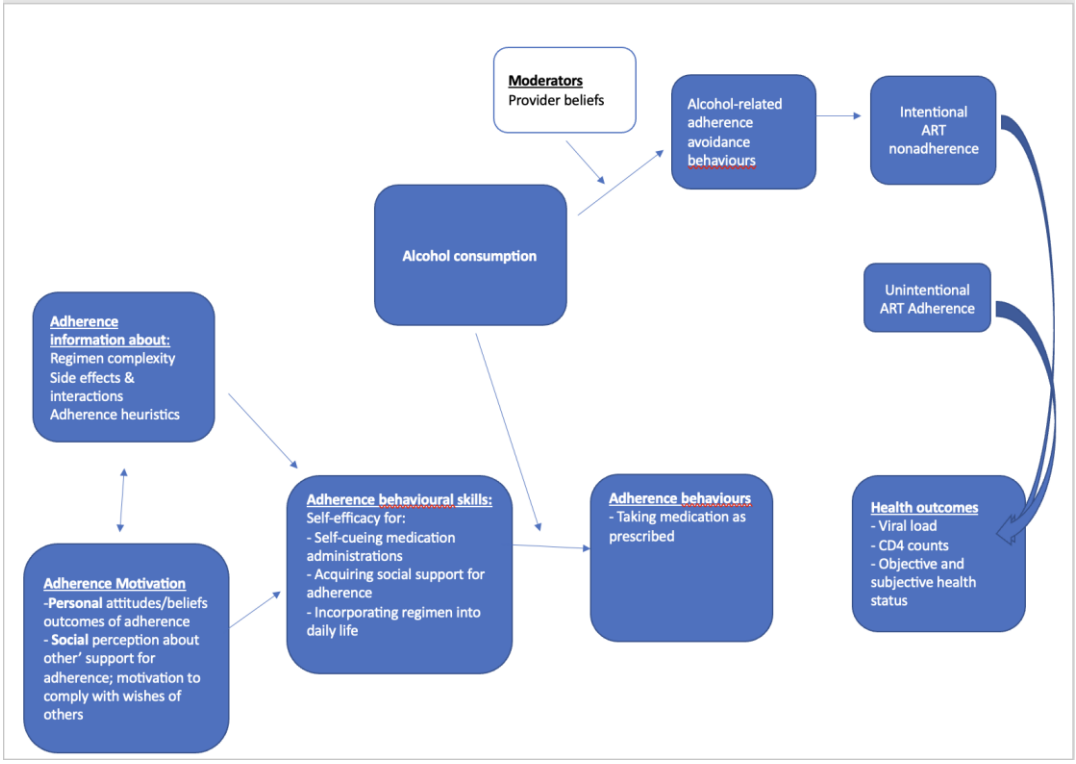

Figure 3: Conceptual model of alcohol use and adherence to antiretroviral therapy. Adapted from Woolf-King et al (2022) (64)

Appendix 2: Schedule of study measurements

Item for completion	**1st postnatal or PMTCT visit within 70 days pp (study visit 1)**	**3 months pp (study visit 2)**	**6 months pp (study visit 3)**	**12 months pp (study visit 4)**	**18 months pp (study visit 5)**	**24 months pp (study visit 6)**
Procedural forms						
Eligibility Checklist	X					
Informed Consent	X					
Locator Information	X	X[1]	X[1]	X[1]	X[1]	X[1]
Randomization Log	X					
Questionnaires						
Demographics & medical history *	X	X[2]	X[2]	X[2]	X[2]	X[2]
K-10 & EPDS [3]*	X	X	X	X	X	X
Alcohol screen [4]*		X	X	X	X	X
Social impact scale*		X		X		X
Availability of social support scale*	X		X		X	X
Infant feeding intentions/practices	X	X	X	X	X	X
Family planning use/ pregnancy intentions*	X	X	X	X	X	X
Patient-Provider relationship scale*	X	X	X	X	X	X
Maternal adherence [5]*	X	X	X	X	X	X
Infant adherence[5]*	X	X	X	X	X	X
Infant demographics & medical history	X	X	X	X	X	X
Resource Interview			X			X
Study laboratory measures						
Phlebotomy for maternal HIV viral load testing and dried blood spot testing for tenofovir diphosphate levels*	X	X	X	X	X	X
Qualitative interviews						
Acceptability interviews				X		X
Clinical data abstraction [6]						
ART follow-up	X	X	X	X	X	X
Antenatal & PMTCT information	X					
Pharmacy ART dispensing records	X	X	X	X	X	X
Maternal laboratory test results [7]*	X		X	X		X

Infant growth and well-being	X	X	X	X	X	X
Infant HIV PCR result [7]*			X		X	

* Measurements used in this study

A subset of locator information will be asked at every visit.

A subset of demographic questions will be asked at all every visit.

Kessler-10 (screening questionnaire for non-specific psychological distress) and Edinburgh Postnatal Depression Survey.

Alcohol use disorders identification test (AUDIT).

Adherence assessments will include maternal ART adherence and questions regarding infant adherence to nevirapine prophylaxis and cotrimoxazole

Abstraction of routine clinical data and laboratory data will take place at the end of the study.

Results of routine laboratory testing on mothers (including pre-ART and on-ART CD4 cell count, pre-ART serum creatinine, alanine aminotransferase and full blood count) and infants (HIV testing at 6 weeks, 2 weeks after cessation of breastfeeding (here marked for the 6 month visit) and 18 months) will come from record review.

Appendix 3: Ethics approval documents

(63)

Related information for authors

- PLOS Writing Center
- Submission system
- Journal scope and publication criteria
- Getting started guide
- Guidelines for revisions
- Publication fees
- APC Support

Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.

Abbreviations

Define abbreviations upon first appearance in the text.

Do not use non-standard abbreviations unless they appear at least three times in the text.

Keep abbreviations to a minimum.

Reference style

PLOS uses "Vancouver" style, as outlined in the ICMJE sample references.

See reference formatting examples and additional instructions below.

Equations

We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable.

Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values.

Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.

Nomenclature

Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Name (rINN).
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HGNC for human genes; we strongly recommend using this tool to check against previously approved names). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior to manuscript publication. Examples of the systematic allergen

nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

Copyediting manuscripts

Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like “scientific editing service” or “manuscript editing service.”

Submissions are not copyedited before publication.

Submissions that do not meet the *PLOS ONE* publication criterion for language standards may be rejected.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* • Title page: List title, authors, and affiliations as first page of the manuscript • Abstract • Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* • Materials and Methods • Results • Discussion • Conclusions (optional)
Ending section	*The following elements are required, in order:* • Acknowledgments • References • Supporting information captions (if applicable)
Other elements	• Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. • Tables are inserted immediately after the first paragraph in which they are cited. • Supporting information files are uploaded separately.

Refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

- Download sample title, author list, and affiliations page (PDF)
- Download sample manuscript body (PDF)

Viewing Figures and Supporting Information in the compiled submission PDF

The compiled submission PDF includes low-resolution preview images of the figures after the

reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

Corresponding author

The submitting author is automatically designated as the corresponding author in the submission system. The corresponding author is the primary contact for the journal office and the only author able to view or change the manuscript while it is under editorial consideration.

The corresponding author role may be transferred to another coauthor. However, note that transferring the corresponding author role also transfers access to the manuscript. (To designate a new corresponding author while the manuscript is still under consideration, watch the video tutorial below.)

Only one corresponding author can be designated in the submission system, but this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication. Include an email address for each corresponding author listed on the title page of the manuscript.

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include its name in the manuscript byline. Do not add it to the author list in the submission system. You may include the full list of members in the Acknowledgments or in a supporting information file.

PubMed only indexes individual consortium or group author members listed in the article byline. If included, these individuals must qualify for authorship according to our criteria.

Read the group authorship policy.

Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

- Summarize the study's contribution to the scientific literature
- Relate the study to previously published work
- Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)
- Describe any prior interactions with PLOS regarding the submitted manuscript
- Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)
- List any opposed reviewers

IMPORTANT: Do not include requests to reduce or waive publication fees in the cover letter. This information will be entered separately in the online submission system.

Read about publication fee assistance.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Download our sample title, author list, and affiliations page (PDF)

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Supporting reproducibility with protocols

To enhance the reproducibility of your results, we recommend and encourage you to make your protocols public. There are several options:

Protocols associated with Research Articles

Protocol documents may be uploaded as Supporting Information or linked from the Methods section of the article. For laboratory protocols, we recommend protocols.io. Include the DOI link in the Methods section of your manuscript using the following format: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]. This allows editors and reviewers to consult the detailed step-by-step protocol when evaluating your manuscript. You can choose to keep the protocol private on the protocols.io platform until your article is published—at which time it will be published automatically.

Protocols published in their own right

PLOS ONE offers two options for publishing stand-alone protocol articles: Lab Protocols that describe reusable methodologies and Study Protocols that describe detailed plans and proposals for research projects. Specific guidelines apply to the submission of Lab Protocol and Study Protocol manuscripts. Read the detailed instructions for submitting Lab Protocols and Study Protocols.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled “Results and Discussion”) or a mixed Discussion/Conclusions section (commonly labeled “Discussion”). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
- Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article's retracted status in your article's text and reference list.

Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article's submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute “Forthcoming” for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.

Source	Format
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an “S” and number. For example, “S1 Appendix” and “S2 Appendix,” “S1 Table” and “S2 Table,” and so forth.

Supporting information files are published exactly as provided,and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

 Read the guidelines for figures and requirements for reporting blot and gel results.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and “Figure” abbreviated to “Fig” (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of “Fig 1” must refer to a figure file named “Fig1.tif”).
- A concise, descriptive title

The caption may also include a legend as needed.\

 Read more about figure captions.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., “Table 1”) and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

 Read the guidelines for tables.

Statistical reporting

Manuscripts submitted to *PLOS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- SAMPL guidelines, for general guidance on statistical reporting
- *PLOS ONE* guidelines, for clinical trials requirements
- *PLOS ONE* guidelines, for systematic review and meta-analysis requirements
- EQUATOR, for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
- Describe technical details or procedures required to reproduce the analysis
- Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
- For pre-processed data:
    - Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).
    - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
- Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).
- For step-wise multiple regression analyses:
    - Report the alpha level used
    - Discuss whether the variables were assessed for collinearity and interaction
    - Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). See SAMPL guidelines.
- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

- **Units of measurement.** Clearly define measurement units in all tables and figures.
- **Properties of distribution.** It should be clear from the text which measures of variance (standard deviation, standard error of the mean, confidence intervals) and central tendency (mean, median) are being presented.
- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.

- **Reporting parameters.** Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.
- **P-values.** Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.
- **Displaying data in plots.** Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.
- **Open data.** As explained in PLOS's Data Policy, be sure to make individual data points, underlying graphs and summary statistics available at the time of publication. Data can be deposited in a repository or included within the Supporting Information files.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

See instructions on providing underlying data to support blot and gel results.

 Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

 See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a “Striking Image” that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

- Specific grant numbers
- Initials of authors who received each award

- Full names of commercial companies that funded the study or authors
- Initials of authors who received salary or other funding from commercial companies
- URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

- Study design
- Data collection and analysis
- Decision to publish
- Preparation of the manuscript

If they had no role in the research, include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."

If the study was unfunded, include this sentence as the Financial Disclosure statement: "The author(s) received no specific funding for this work."

 Read our policy on disclosure of funding sources.

Competing interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

 Read our policy on competing interests.

Guidelines for Specific Study Types

Study design, reporting, and analyses are assessed against all relevant research and methodological technique standards held by the community. Guidelines for specific study types are outlined below.

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the PLOS Data policy
- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form here. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s)**. If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

> *A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.*

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- The name of the registry and the registration number must be included in the Abstract.

- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

Lab Protocols consist of two interlinked components: a protocol hosted on the protocols.io platform and a peer-reviewed article on *PLOS ONE* that contextualises the protocol.

protocols.io is a secure open access platform that specializes in laboratory protocols. It allows scientists to share, discover and reuse up-to-date protocol knowledge. The platform provides specialist tools and guidance on how to add each element of the protocol, including the title, abstract, steps, files, links, reagents, measurements, formulae, videos, charts and more.

The *PLOS ONE* article component must comply with the general submission guidelines (detailed above in this article).

The *PLOS ONE* article component must also comply with the general *PLOS ONE* criteria for publication and in addition it should:

- Present a step-by-step protocol that adds value to the published literature.
- Provide evidence that the protocol works, by:
    - Linking, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.
      or
    - Providing supporting validation or benchmarking data which demonstrates that the underlying method achieves its intended purpose.
- Link, in the Materials and Methods section, to the protocol.io component, using the digital object identifier (DOI) and format provided by protocols.io, for example *https://dx.doi.org/10.17504/protocols.io[....]*.
- Describe the appropriate controls, sample sizes and replication needed to ensure that the data are robust and reproducible.
- Provide the protocol as a supporting information (S1) file for printing purposes. You can download a PDF from protocols.io for this purpose.

Download a sample Lab Protocol template

Lab Protocols describing routine methods, or extensions or modifications of routine methods, add little or no value to the published literature and will not be considered for publication.

Lab Protocols are subject to the same editorial and peer review process as all other articles, except that the peer review process may be expedited and carried out by one internal Academic Editor and one external reviewer.

Lab Protocols are eligible for both signed and published peer review.

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to *PLOS ONE*, or at the latest, before the editorial and peer review process. This approach is optional, but beneficial, because:

- Your DOI is assigned on the protocols.io platform. You need this identifier to link out from the Material and Methods section of your manuscript.
- You can keep your protocol private on the protocols.io platform (until you are satisfied that it is ready for publication), but still assign a DOI.
- The protocol will be accessible to editors and reviewers during the editorial and peer review process.

If you prefer to submit your manuscript to *PLOS ONE* before uploading your protocol to protocols.io, please provide your protocol as a supporting information (S1) file. You can use protocols.io's editorial service at no cost: they will check and publish your protocol for you. As part of *PLOS ONE*'s partnership with protocols.io, your waiver code for this purpose will be provided in the first decision letter.

Lab Protocols are eligible for both signed and published peer review.

Preprint posting is not available for Lab Protocols and bioRxiv does not accept them.

Study Protocols

Study Protocols describe plans for conducting research projects and consist of a single article on *PLOS ONE*.

Study Protocols must comply with the *PLOS ONE* general submission guidelines (detailed above in this article) and any guidelines specific to the related research study type. In addition, the protocol must:

- Relate to a research study that has not yet generated results.
- Be submitted before recruitment of participants or collection of data for the study is complete.
- Meet the same standards for ethics of experimentation and research integrity as the research study. If it involves human or animal subjects, cell lines or field sampling, or has potential biosafety implications, prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

- Clinical trials:
    - The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors).
    - The name of the registry and the trial or study registration number must be included in the Abstract.
    - A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an addtional English translation if the original document is not in English.
    - A SPIRIT schedule of enrollment, interventions, and assessments must be included as the manuscript's Figure 1, and a completed SPIRIT checklist must be uploaded as Supporting Information file S1.
- Systematic reviews and meta-analyses:
    - A completed PRISMA-P checklist must be provided as a supporting information (SI) file. See PRISMA-P Explanation and Elaboration for more information on completing your checklist.

Study Protocols must also comply with general *PLOS ONE* criteria for publication and in addition you should:

- include the word “Protocol” in your Title.
- include a detailed description of the planned study in the Materials and Methods section.This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:
    - the aim, design, and settling
    - the sample size calculation
    - how data saturation will be determined (for qualitative studies)
    - the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained
    - how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured
    - the data management plan
    - safety considerations
    - the type of data and statistical analyses to be used
    - the status and timeline of the study, including whether participant recruitment or data collection has begun
    - where and when the data will be made available. See our Data Availability policy for more.
- Include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.
- We encourage authors you to register with OSF and provide the your registration number in the Materials and Methods section. This is optional.
- Optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the supporting information guidelines for more details about adding SI files.

Download our sample Study Protocol template or an OSF discipline or study-specific template.

Study Protocols are subject to the same editorial and peer review process as all other articles, and are eligible for both signed and published peer review.

You can expedite the review process by providing:

- Proof of external funding. This is typically your funding approval letter and a list of the names and credentials of the funders who conducted the external peer review of the protocol. Include an English translation if needed.
- Proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved by this body.

These documents are used for internal purposes and do not form part of the published Study Protocol. Expedited review is conducted by an internal Staff Editor only and bypasses the external review process.

If the Study Protocol describes a replication study or involves re-analysis of published work, we will invite the author of the initial or replicated study to provide a signed review.

We encourage you to share your Study Protocol with other researchers, either before or after submission. You can publish it on your website or protocols.io, or submit it for posting on medRxiv or another preprint server.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

See our reporting guidelines for human subjects research.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility
The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation
Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability
If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

Read the PLOS policy on sharing materials, software and code.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist or Standards for reporting qualitative research

(SRQR) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.