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1 **Loss to follow-up among female sex workers in Zambia: findings from a 5-year HIV**
2 **incidence cohort**

3 Kalonde Malama^{1,2}, Luis Sagaon-Teyssier^{1,2}, Andréa Gosset¹, Rachel Parker³, Kristin M.
4 Wall^{3,4}, Amanda Tichacek³, Tyronza Sharkey⁵, William Kilembe⁵, Mubiana Inambao⁶, Matt A.
5 Price^{7, 8}, Bruno Spire^{1,2}, Susan Allen³

6 1) Aix Marseille Univ, INSERM, IRD, SESSTIM, Sciences Economiques & Sociales de la
7 Santé & Traitement de l'Information Médicale, Marseille, France

8 2) ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille,
9 France

10 3) Rwanda Zambia HIV Research Group, Emory University, Pathology & Laboratory
11 Medicine, School of Medicine, Atlanta, GA, United States

12 4) Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta,
13 GA, United States

14 5) Zambia Emory HIV Research Project, Rwanda Zambia HIV Research Group, Emory
15 University, Lusaka, Zambia

16 6) Zambia Emory HIV Research Project, Rwanda Zambia HIV Research Group, Emory
17 University, Ndola, Zambia

18 7) International AIDS Vaccine Initiative (IAVI) New York, New York, United States of
19 America

20 8) Department of Epidemiology and Biostatistics, University of California at San Francisco,
21 San Francisco, California, United States of America

22 Correspondance to : Kalonde Malama. Institut Hospitalo-Universitaire 19-21 Boulevard Jean
23 Moulin, 13005 Marseille, France (email: kalondemalama@gmail.com) Tel:+33413732296

24 The authors have no conflicts of interest to declare.

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1 **ABSTRACT**

2 HIV incidence studies are used to identify at-risk populations for HIV prevention trials
3 and interventions, but loss to follow-up (LTFU) can bias results if participants who
4 remain differ from those who drop out. We investigated the incidence and factors
5 associated with LTFU among Zambian female sex workers (FSWs) in an HIV
6 incidence cohort from 2012 to 2017. Enrolled participants returned at month 1, month
7 3 and quarterly thereafter. FSWs were considered LTFU if they missed six
8 consecutive months, or if their last visit was six months before the study end date. Of
9 420 FSWs, 139 (33%) were LTFU at a rate of 15.7 per 100 person-years. In
10 multivariable analysis, LTFU was greater for FSWs who: never used alcohol, began
11 sex work above the age of consent, and had a lower volume of new clients. Our
12 study appeared to retain FSWs in most need of HIV prevention services offered at
13 follow-up.

14 **Keywords:** Incidence studies; Risk behaviour; Prevention trials; Alcohol

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27 **INTRODUCTION**

1 With a prevalence of 11.5%, Zambia is a country with a generalised HIV epidemic
2 (UNAIDS, 2018). Female sex workers (FSWs) are at an even greater risk of acquiring
3 HIV, with a prevalence ranging from 46% to 73% in Zambia (Family Health
4 International & Tropical Disease Research Centre, 2015). High numbers of sexual
5 partners, inconsistent condom use, limited access to health care, stigma, and poverty
6 put Zambian FSWs at elevated risk of HIV infection (Family Health International &
7 Tropical Disease Research Centre, 2015; Scorgie et al., 2012; USAID, 2013; Prüss-
8 Ustün et al., 2013).

9 Prospective cohort studies are the benchmark for measuring HIV incidence as they
10 allow chronological measurement of risk factors and HIV infection (Mann, 2003).
11 Studies of this nature require special attention because the drop out or loss to follow-
12 up (LTFU) of participants may introduce selection bias. HIV incidence rates
13 calculated from cohort studies with large rates of LTFU may thus be imprecise.
14 Understanding the characteristics of participants who drop out of studies is crucial for
15 generalisability of results. For FSWs in HIV incidence cohorts, measuring factors
16 associated with LTFU could help determine if FSWs who drop out are more
17 vulnerable to HIV than those who remain. High risk cohorts are needed for HIV
18 prevention studies, whose participants must be willing to be followed over time
19 (Fishbein & Coutinho, 1997; Mills et al., 2006).

20 Many studies on LTFU among FSWs focus on HIV-positive sex workers enrolled in
21 antiretroviral treatment programmes (Eakle et al., 2017; Mountain et al., 2014). There
22 is limited data on the incidence and predictors of LTFU among HIV-negative FSWs.
23 Previous longitudinal studies among HIV-negative FSWs in Kenya and China found
24 that FSWs who display increased HIV risk behaviour were less likely to be lost to
25 follow-up (Graham et al., 2013; Su et al., 2016). To our knowledge, there have been
26 no studies published on LTFU among Zambian FSWs. Measuring the incidence of
27 LTFU for Zambian FSWs and its associated factors could have important implications
28 for retaining this hard-to-reach population. Retaining FSWs in sex worker-friendly
29 services is important because routine health services typically marginalise FSWs
30 (Lafort et al., 2017). Using data from a five-year HIV incidence cohort of FSWs at the
31 Zambia-Emory HIV Research Project (ZEHRP), we analysed the incidence and
32 factors associated with LTFU.

33 **METHODS**

1 **Study Setting**

2 The study took place in Zambia's capital city of Lusaka and the Copperbelt Province
3 city of Ndola, which are the two most populated and urbanised provinces in the
4 country. The HIV prevalences in Lusaka (15.7%) and the Copperbelt (13.8%) are well
5 above the national average (11.5%) (ZAMPHIA Collaborating Institutions, 2019).
6 FSWs in Zambia face difficulties accessing HIV testing services due to stigmatisation
7 from healthcare workers (Chanda, Perez-Brumer, et al., 2017). Recent efforts to
8 provide HIV testing services to FSWs in Zambia have employed self-tests that are yet
9 to be rolled out nationally (Chanda, Ortblad, et al., 2017).

10 **Study Design**

11 This analysis was part of a prospective cohort study to determine HIV incidence
12 among Zambian FSWs. Recruitment began in September 2012 and ended in March
13 2015. The end of the study follow up was the 15th of September 2017. Follow-up
14 lasted from the date of enrolment until LTFU or study end- whichever came first. The
15 maximum study duration for each participant was 60 months (five years). FSWs who
16 attended at least the baseline visit and completed all enrolment procedures were
17 eligible for analysis. Individuals who were duplicate-enrolled in this study were
18 excluded from our analysis (Figure 1).

19 **Study Population**

20 In the prospective HIV incidence cohort, we defined sex workers as women who offer
21 sex in exchange for money. Enrolment criteria included being HIV-negative,
22 unmarried, between the ages of 18 and 45, available for five-year follow-up and
23 willing to provide locator information.

24 **Study Procedures**

25 Community health workers and peer sex workers at ZEHRP recruited FSWs from
26 commercial sex work hotspots in Lusaka and Ndola, i.e. bars, restaurants, boarding
27 houses, lodges/hotels, and streets. Participants provided informed consent in
28 English, Nyanja or Bemba. At enrolment, FSWs completed a baseline questionnaire
29 administered face-to-face by nurses trained in risk reduction counselling. Information
30 was collected on sociodemographics, sexual history, risk factors for HIV acquisition,
31 and gynaecological and reproductive health status. Enrolled participants were invited

1 to attend a follow-up visit one month after enrolment, two months later, and quarterly
2 thereafter. Manual and electronic fingerprinting were used to verify the identity of
3 study participants at each visit as described in detail elsewhere (Wall et al., 2015). All
4 participants were reimbursed for travel to and from the study site, and were provided
5 food and beverages during study visits.

6 ***Ethical Approval***

7 The University of Zambia Research Ethics Committee (Lusaka, Zambia) and the
8 Emory University Institutional Review Board (Atlanta, USA) approved the study
9 protocol.

10 ***Sexual and Reproductive Health Services***

11 At each study visit, FSWs received HIV risk reduction and family planning
12 counselling, and testing for HIV (rapid antibody tests for screening and antigen test
13 for confirmation of positive test results). Women who tested HIV-positive were
14 referred for assessment and treatment to a government antiretroviral therapy clinic in
15 accordance with Zambian HIV testing guidelines. FSWs were also screened for
16 syphilis using rapid plasma reagin serology and for *trichomonas vaginalis* using
17 microscopy of vaginal swabs. Syphilis and *trichomonas vaginalis* were treated at the
18 study site. Participants were offered condoms and the choice of two long-acting
19 reversible contraceptives: an intrauterine device or a hormonal implant.

20 ***Outcome***

21 The event of interest for the main study was HIV-incidence, but the event of interest
22 for this analysis was LTFU. Participants were considered LTFU if they were absent
23 for more than six months or their last study visit occurred greater than six months
24 before the study end date. This six-month cut-off is, according to evidence from a
25 multinational study, the least likely to lead to misclassification bias in defining LTFU
26 (Chi et al., 2011).

27 ***Independent Variables***

28 Potential factors associated with LTFU were a combination of time-fixed and time-
29 varying covariates. Time-fixed covariates comprised variables collected at baseline
30 and included year of entry into the study, city of residence, age at enrolment, age at
31 initiation into sex work, level of education, number of children, lifetime number of

1 sexual partners and alcohol use. For our analysis, we combined the years 2012 and
2 2013 because only six FSWs (1.5% of the study sample) were enrolled in 2012.
3 Time-dependent variables considered for this analysis were syphilis and
4 trichomoniasis test results, number of non-repeat/new clients seen in the previous
5 month and consistent (100%)/inconsistent (less than 100%) condom use with clients
6 during vaginal, anal and oral sex in the previous month.

7 ***Statistical Analysis***

8 *LTFU rate*

9 The time each participant spent in the study between enrolment and final visit was
10 calculated in person-years (PY). The Kaplan-Meier method was used to estimate the
11 rate of LTFU in our cohort. The incidence rate of LTFU was calculated as the number
12 of LTFU cases divided by the total number of PY. Time-fixed covariates were used to
13 generate stratified Kaplan-Meier failure curves. The log-rank test for equality of
14 survival functions was computed to determine statistically significant ($p < 0.05$)
15 differences between curves.

16 *Cox model*

17 A univariate extended Cox model with time-varying covariates was used to estimate
18 the hazard ratios (HR) and 95% confidence intervals (CI) of potential factors
19 associated with LTFU. Variables found to be significantly associated with LTFU
20 ($p < 0.25$) in univariate analysis were tested in multivariable analysis through a forward
21 selection procedure (Bursac et al., 2008). Adjusted hazard ratios (AHR) were
22 computed for variables with a p-value < 0.05 .

23 *Sensitivity analysis*

24 The majority (85%) of LTFU in our study occurred during the first three years (Figure
25 2). We therefore performed a sensitivity analysis with a right truncated cohort (cut-off
26 point= visit month 36) to determine if our results would differ between the full and
27 truncated model.

28 All statistical analyses were performed using Stata version 14.2 (StataCorp, College
29 Station, Texas, USA).

30 **RESULTS**

1 In total, 420 of FSWs enrolled between 2012 and 2015 were eligible for this study
2 (Figure 1). Almost half of all recruitments (46.7%) took place in the year 2014 (Table
3 1). The majority of study participants (64.8%) were from Ndola and the rest (35.2%)
4 were Lusaka based (Table 1). The mean age at enrolment was 25 years with a
5 standard deviation (SD) of 6.3 years. Most FSWs were educated up to primary level
6 (56.9%) and had an average of 1.6 children (SD: 1.3, range: 0-7). The majority of
7 participants (65.5%) at baseline reported inconsistent condom use with their clients
8 (Table 1).

9 As shown in Table 1, 281 (67%) participants completed the study and had a median
10 follow-up time of 33.7 months (IQR: 30.6-42.9). For the 139 (33%) FSWs who were
11 LTFU, the median follow-up time was 27.6 months (IQR: 15.5-36.5). The overall
12 person-years of observation contributed by all 420 participants was 883.5 (Table 1).
13 During this period, LTFU occurred at a rate of 15.7 per 100 PY (95% CI: 13.32-
14 18.58). The Kaplan-Meier failure curve in Figure 2 illustrates the rate of LTFU across
15 all five years of the study.

16 There was not much variation in the LTFU rate between FSWs by age, number of
17 children and relationship status (Table 1). However, FSWs who had the fewest
18 number of lifetime sexual partners had a higher LTFU rate than those who reported
19 more lifetime partners ($p < 0.05$) (Table 1). FSWs who lived in Lusaka had a higher
20 LTFU rate than FSWs who lived in Ndola ($p < 0.05$). We also found that FSWs
21 enrolled later in the study had higher LTFU rates than those enrolled earlier ($p < 0.05$)
22 (Table 1).

23 In multivariate analysis (Table 2), LTFU was significantly associated with later year of
24 enrolment, no alcohol use, older age at initiation into sex work, and lower monthly
25 volume of new clients. In comparison to FSWs enrolled in 2012/2013, FSWs enrolled
26 in 2014 (AHR: 2.24, 95% CI: 1.12-4.47) and 2015 (AHR: 2.97, 95% CI: 1.44-6.14)
27 had a higher likelihood of being LTFU. FSWs who reported never using alcohol were
28 45% more likely to be LTFU than FSWs who reported ever using alcohol (AHR: 1.45,
29 95% CI: 1.04-2.12). FSWs who began sex work at 16 and above were two-times
30 likelier to be LTFU (AHR: 2.02, 95% CI: 1.25-3.28) than FSWs who began sex work
31 at 15 and below. FSWs who received 1-4 (AHR: 0.61, 95% CI: 0.39-0.95), 5 to 9
32 (AHR: 0.51, 95% CI: 0.29-0.88) or >10 (AHR: 0.46, 95% CI: 0.26-0.82) new clients in
33 the previous month were less likely to be LTFU than FSWs who received no new

1 clients in the preceding month. The results of our sensitivity analysis mirrored those
2 of our original model, thus we maintained all study visits for greater statistical power.

3 **DISCUSSION**

4 Over five years, a third (33%) of our study participants were LTFU at a rate of 15.7
5 per 100 PY. This is lower than the LTFU rates observed in HIV-negative FSWs
6 cohorts in Kenya and China, which were 23.4 to 26.7 per 100 PY respectively
7 (Graham et al., 2013; Su et al., 2016). Despite our relatively low LTFU rate, it is vital
8 that retention efforts be improved because participants who drop out may suffer poor
9 health outcomes. Retention in our cohort was associated with earlier year of
10 enrolment and increased HIV risk factors (alcohol use, younger age at entry into sex
11 work, and more monthly number of new clients).

12 Our study appeared to retain FSWs at higher HIV risk. Retained women may have
13 been motivated by their need for HIV prevention services offered routinely at follow-
14 up. It is possible that participants who dropped out of the cohort no longer practised
15 sex work, thus the services offered by the study were less pertinent to them. Previous
16 studies in Africa show that FSWs quit sex work because they had established steady
17 relationships (Graham et al., 2013; Tekola, 2005). If applicable, this implies that
18 FSWs who dropped out of our study were at reduced HIV risk by virtue of having
19 fewer multiple concurrent partnerships. It is unclear why FSWs enrolled later in the
20 study were more likely to be lost to follow-up than those enrolled earlier; other
21 longitudinal FSWs cohorts have observed this effect (Graham et al., 2013; Su et al.,
22 2016).

23 Primary behavioural indicators for HIV acquisition among FSWs include number of
24 sexual partners and condom use with these partners (Odek et al., 2008; Platt et al.,
25 2013). New clients may pose a risk to the sexual health of FSWs because their HIV
26 status is more likely to be unknown to the sex workers (Baral et al., 2014). Condom
27 use was not significantly associated with LTFU in our analysis. We did however note
28 that FSWs who reported more new clients per month were less likely to be lost to
29 follow-up than those who reported none. Our findings suggest that FSWs who
30 completed the study engaged in more behaviours considered to increase HIV risk
31 than FSWs who dropped out. Retained FSWs were therefore well suited for HIV risk
32 reduction counselling provided routinely at follow-up.

1 The Zambian National Registration Act defines a minor as any person under the age
2 of 16. In our cohort, a quarter of sex workers entered the profession as minors.
3 Underage FSWs in Southern Africa have poorer access to health facilities than their
4 adult counterparts (Busza et al., 2016; Inguane et al., 2015). In Mexico, underage
5 entry into sex work was associated with HIV risk factors such as fewer condom
6 negotiation attempts, drug use, and physical and sexual abuse (Goldenberg et al.,
7 2012; Servin et al., 2015). We found that beginning sex work as a minor had a
8 protective effect against LTFU. This result strengthens our supposition that FSWs
9 who remained in the study were motivated by a greater need of HIV prevention
10 services than FSWs who were lost to follow-up.

11 The literature in Zambia and around the globe shows that alcohol use is associated
12 with an increase in HIV infection (Coldiron et al., 2008; Rehm et al., 2012, 2017;
13 World Health Organization et al., 2018). Sex work often occurs in drinking venues
14 and in conjunction with alcohol consumption (Fritz et al., 2002; Li et al., 2010; Roth et
15 al., 2017). The consumption of alcohol among FSWs has been correlated with
16 condomless sex and, STI and HIV acquisition (M. F. Chersich et al., 2007; Matthew F
17 Chersich et al., 2014; Semple et al., 2016; Wang et al., 2010). We found that FSWs
18 who ever used alcohol were more likely to be retained in our study than FSWs who
19 never used alcohol. It is possible that FSWs who consumed alcohol associated this
20 behaviour with a greater risk of HIV acquisition. This would further reinforce that
21 FSWs retained in our cohort perceived a greater benefit of the HIV prevention
22 services offered at every visit.

23 This study has its limitations. Our findings lacked information on structural factors that
24 could have been associated with LTFU, such as previous incarceration and migration
25 for the purpose of sex work. Demographic and behavioural variables were self-
26 reported by FSWs during face-to-face interviews, which could have introduced social
27 desirability and/or recall bias. Biases of this nature were minimised by trained
28 counsellors who administered all questionnaires to study participants. Counsellors
29 received training in voluntary HIV counselling and testing, during which
30 negative/stigmatising attitudes towards participants engaging in high risk behaviours
31 were discouraged.

32 Despite its limitations, our study is—to the best of our knowledge—the first in Zambia
33 to examine the incidence and predictors of LTFU among FSWs. These findings add

1 to the limited evidence base in sub-Saharan Africa on factors associated with LTFU
2 among FSWs, who are a high-risk group for HIV infection. We recommend that future
3 studies build on our quantitative approach by interviewing FSWs who remain, and—if
4 they can be reached—FSWs who are lost to follow-up. Such studies would provide
5 vital information on the motivations for FSWs remaining in long-term studies and their
6 reasons for dropping out.

7 **CONCLUSIONS**

8 Taken together, our results demonstrate that LTFU among Zambian FSWs was
9 associated with no alcohol use, older age at entry into sex work, and lower volume of
10 new clients. FSWs who were not lost to follow-up appeared to be in greater need of
11 the HIV prevention services offered during the study and may have perceived
12 themselves to be at higher HIV risk. This suggests that we retained at-risk individuals
13 who are suitable candidates for future HIV prevention trials. However, more intensive
14 retention efforts –combining in-depth locator information, mapping, and adequate
15 backup measures such as next-of-kin information– will be required during these trials
16 to minimise LTFU and ensure the validity and generalisability of findings.

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29

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1 **Table 1:** Rate of LTFU among Zambian FSW by selected baseline characteristics (N=420)

	N (%)	Lost to Follow-Up			Log-rank test (p-value)
		n	PY	Rate per 100 PY (95% CI)	
All	420 (100)	139	883.5	15.7 (13.32-18.58)	
Year of enrolment					
2012/13*	78 (18.5)	18	244.6	7.4 (4.31-11.48)	0.0005
2014	196 (46.7)	66	415.5	15.9 (12.46-20.26)	
2015	146 (34.8)	55	223.4	24.6 (19.09-32.38)	
City					
Ndola	272 (64.8)	81	650.2	12.5 (10.02-15.49)	0.0007
Lusaka	148 (35.2)	58	233.3	24.9 (19.22-32.16)	
Age					
18-20	125 (29.8)	36	287.8	12.5 (9.02-17.34)	0.2689
21-25	142 (33.8)	49	294.2	16.7 (12.59-22.04)	
26+	153 (36.4)	54	301.5	17.91 (13.72-23.38)	
Education					
Primary or none	239 (56.9)	72	526.4	13.7 (10.86-17.23)	0.1027
Secondary or higher	158 (42.9)	66	356.9	18.5 (14.53-23.54)	
Number of children					
0	93 (22.1)	29	193.9	15.0 (10.97-22.44)	0.8290
1	135 (32.1)	47	275.2	17.1 (12.92-23.03)	
2+	192 (45.8)	63	414.4	15.2 (11.64-19.32)	
Have a non-paying partner					
Yes	166 (39.5)	55	349.0	15.8 (12.10-20.52)	0.9946
No	252 (60.0)	88	531.8	15.6 (12.59-19.35)	
Lifetime number of partners					
<=50	132 (31.4)	48	207.5	23.1 (17.43-30.69)	0.0122
51-200	111 (26.4)	34	281.8	12.1 (8.62-16.88)	

201-800	68 (16.2)	17	184.3	9.2 (5.73-14.84)	
800+	107 (25.5)	39	206.0	19.0 (13.83-25.91)	
Age at sex work initiation					
<=15	101 (24.1)	21	237.94	8.8 (5.75-13.54)	0.0029
16+	316 (75.2)	116	640.0	18.1 (15.11-21.74)	
Condom use with clients [#]					
Inconsistent	275 (65.5)	71	380.9	18.6 (14.77-23.52)	-
Consistent	77 (18.3)	50	338.9	14.8 (11.18-19.46)	
Number of new clients in previous month [#]					
None	19 (4.5)	32	140.4	21.8 (15.44-30.87)	-
1-4	118 (28.1)	55	372.8	14.6 (11.19-18.98)	
5-9	95 (22.6)	24	143.9	16.5 (11.06-24.62)	
10+	128 (30.5)	24	128.0	18.4 (12.31-27.39)	
Ever use alcohol					
Yes	325 (77.4)	98	698.7	14.0 (11.51-17.10)	0.0190
No	91 (21.7)	39	177.6	22.0 (16.05-30.06)	
Syphilis [#]					
Positive	50 (11.9)	118	77.4	25.8 (16.67-40.05)	-
Negative	370 (88.1)	18	806.1	14.8 (12.33-17.67)	
Trichomonas vaginalis [#]					
Positive	35 (8.3)	10	40.9	24.4 (13.15-45.42)	-
Negative	371 (88.4)	126	819.7	15.4 (12.91-18.30)	

-
- 1 *2012 and 2013 combined because only 6 FSW were enrolled in 2012
 - 2 FSW: female sex worker; PY: person-years; #: time-dependent variable (for which log-rank test was not applicable); CI: confidence interval
 - 3
 - 4 Ns don't
 - 5

1 **Table 2:** Factors associated with loss to follow-up in univariate and multivariable Cox regression
 2 models for Zambian FSW (N=420)

	Loss to Follow-up			
	Crude HR	95% CI	Adjusted HR	95% CI
Year of enrolment				
2012/2013	1	-	1	-
2014	2.08	1.21-3.58**	2.24	1.12-4.47*
2015	2.93	1.66-5.17***	2.97	1.44-6.14**
City of residence				
Ndola	1	-	-	-
Lusaka	1.77	1.26-2.50**	-	-
Age				
18-20	1	-	-	-
21-25	1.32	0.84-1.84	-	-
26+	1.40	0.92-2.14	-	-
Education				
Primary or none	1	-	-	-
Secondary or higher	1.32	0.94-1.84	-	-
Number of Children				
0	1	-	-	-
1	1.15	0.72-1.82	-	-
2+	1.05	0.68-1.64	-	-
Have a non-paying partner				
Yes	1	-	-	-
No	1.00	0.71-1.41	-	-
Lifetime sexual partners				
<=50	1	-	-	-
51-200	0.59	0.38-0.92*	-	-
201-800	0.46	0.26-0.80**	-	-
800+	0.86	0.56-1.31	-	-

Age at sex work initiation				
≤15	1	-	1	-
16+	1.99	1.25-3.17**	2.02	1.21-3.37**
Condom use with clients [#]				
Inconsistent	1	-	-	-
Consistent	1.12	0.78-1.63	-	-
Number of new clients in previous month [#]				
None	1	-	1	-
1-4	0.57	0.37-0.88*	0.52	0.30-0.91*
5-9	0.50	0.29-0.86*	0.50	0.26-0.95*
10+	0.46	0.27-0.81**	0.47	0.25-0.91*
Ever use alcohol				
Yes	1	-	1	-
No	1.55	1.07-2.24*	1.60	1.04-2.47*
Syphilis [#]				
Positive	1	-	-	-
Negative	0.66	0.41-1.07	-	-
Trichomonas vaginalis [#]				
Positive	1	-	-	-
Negative	0.78	0.41-1.49	-	-

1

2 *FSW: female sex worker; HR: hazard ratio; CI: confidence interval; #: time-varying covariate*

3 **p-value < 0.05, **p-value < 0.01, ***p-value < 0.001*

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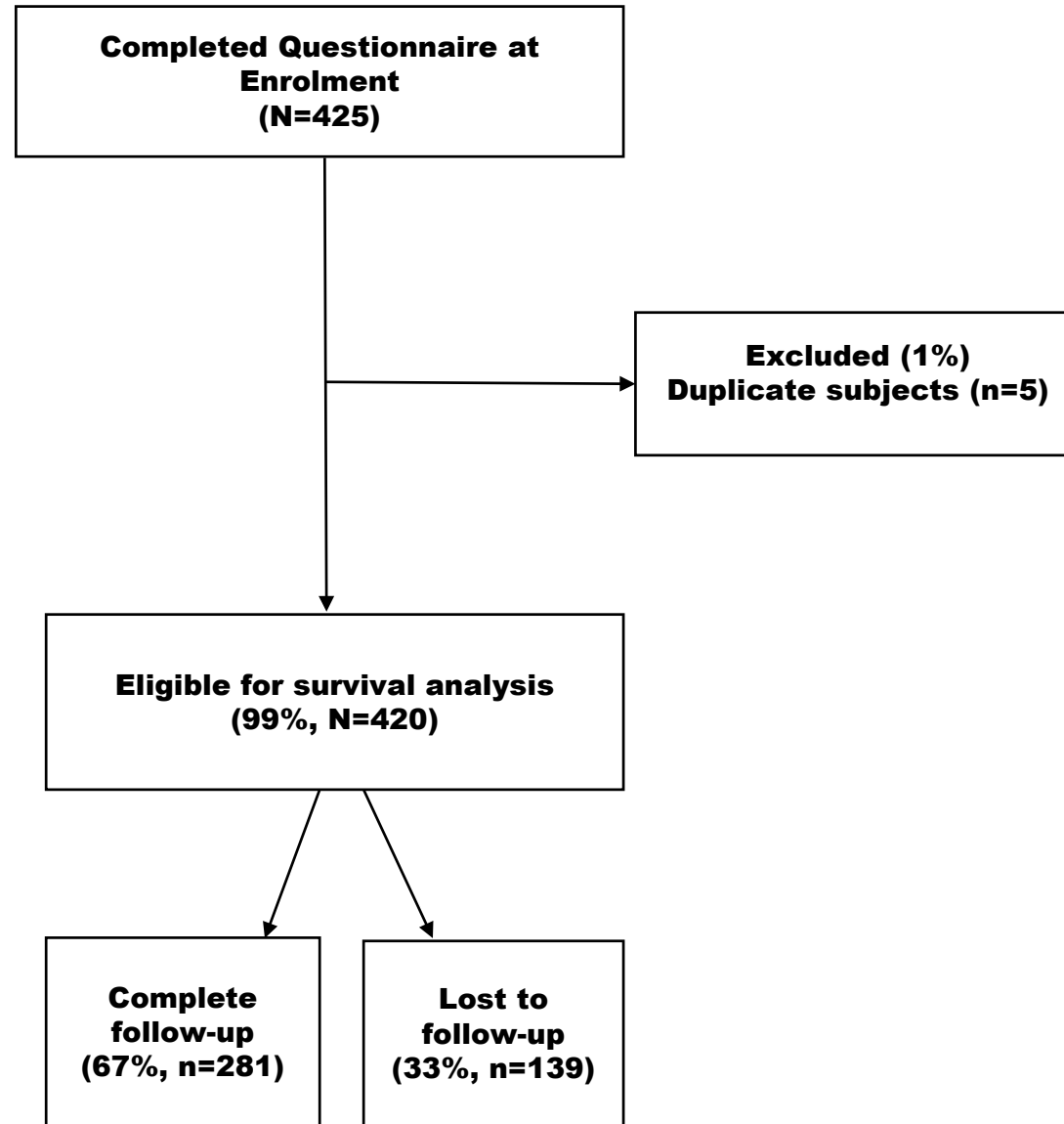
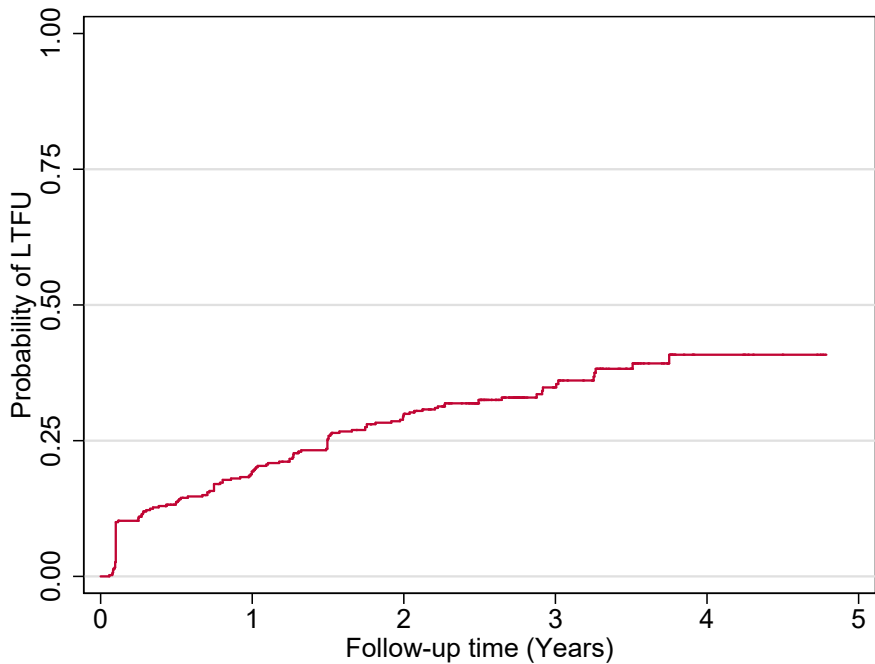


Figure 1: Flow chart showing Zambian FSW who were eligible for survival analysis, lost to follow-up, and completed the five-year prospective cohort study (N=420)



Number at risk

420 (79) 313 (39) 260 (14) 103 (7) 10 (0) 0