Impact of HIV on the burden and severity of influenza illness in Malawian adults: a prospective cohort and parallel case-control study

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Summary:

- This paper highlights HIV-related immunosuppression as a major risk factor for influenza illness and severity in Malawian adults.
- Household crowding, food insecurity and poor sanitation are additional risk factors.
- Influenza preventative strategies should be targeted at HIV-infected adults in Africa.

Short title: HIV and influenza in Malawian adults

ABSTRACT

Background. The impact of HIV infection on influenza incidence and severity in adults in sub-Saharan Africa is unclear. Seasonal influenza vaccination is recommended for HIV-infected persons in developed settings, but is rarely implemented in Africa.

Methods. We conducted a prospective cohort study to compare the incidence of laboratory-confirmed influenza illness between HIV-infected and HIV-uninfected adults in Blantyre, Malawi. In a parallel case-control study, we explored risk factors for severe influenza presentation of severe (hospitalized lower respiratory tract infection (LRTI)), and mild influenza (influenza-like illness (ILI)).

Results. The cohort study enrolled 608 adults (360 (59%) HIV-infected). Between April 2013 and March 2015, 24/229 (10.5%) ILI episodes in HIV-infected and 5/119 (4.2%) in HIV-uninfected adults were influenza PCR positive (incidence rates 46.0 vs. 14.5 per 1000 person years, incidence rate ratio 2.75; 95% confidence interval [CI] 1.02-7.44; p=0.03, adjusted for age, gender, household crowding and food security). In the case control study, influenza was identified in 56/518 (10.8%) patients with hospitalized LRTI, and 88/642 (13.7%) with ILI. HIV prevalence among influenza-positive cases and controls were 69.6% and 29.6% respectively. HIV was a significant risk factor for severe influenza (odds ratio 4.98, 95%CI 2.09-11.88, p<0.001; population attributable fraction 57%, adjusted for season, sanitation facility and food security).

Conclusions. HIV is an important risk factor for influenza-associated ILI and severe presentation in this high HIV prevalence African setting. Targeted influenza vaccination of HIV-infected African adults should be re-evaluated and the optimal mechanism for vaccine introduction in overstretched health systems needs to be determined.

Keywords: HIV; influenza; Malawi

INTRODUCTION

Influenza and its complications are leading causes of morbidity and mortality worldwide [1]. Vaccination of patient groups at increased risk of influenza-related complications is key to minimizing the impact of disease. Influenza vaccines are currently unavailable in the public sector in most sub-Saharan African countries [2]. The World Health Organization (WHO) has therefore called for more data on influenza disease burden in the region to guide influenza prevention and control programmes [3].

HIV-infected persons are designated a priority for immunization in many well-resourced countries [4, 5], but data to support this recommendation are inconsistent. HIV does not significantly increase influenza burden or severity in developed settings [6, 7]. Conversely, in low-resource, high HIV prevalence settings there is a higher incidence of influenza illness [8-10], and a greater risk of hospitalization [11-13] and death among HIV-infected persons [9, 14, 15]. Current studies however are limited by incomplete ascertainment of HIV status, CD4+ cell counts and antiretroviral treatment and few have studied the impact of environmental factors, such as household crowding and sanitation.

We therefore aimed to determine the impact of HIV on the frequency and severity of adult laboratory-confirmed influenza illness in an urban, high HIV prevalence African setting, and identify additional risk factors associated with influenza illness and severity.

METHODS

Study setting and design

Malawi is a low-income Southern African country, with an HIV prevalence of 10.6% [16]. Influenza predominantly circulates between January and April [17]. There is no national influenza immunization program.

We performed two prospective observational studies at the Queen Elizabeth Central Hospital (QECH), the only inpatient facility providing free health care to 1.3 million residents in Blantyre district, and a primary care center adjacent to QECH.

We conducted a cohort study of HIV-infected and HIV-uninfected adults over two years; the primary endpoint was laboratory-confirmed influenza illness. We also did a case-control study of adults presenting with mild and severe influenza, to establish risk factors for severe influenza (including HIV infection).

Procedures

Cohort study

We enrolled adults aged ≥18 years from the antiretroviral treatment (ART), and voluntary counselling and testing (VCT) clinics at QECH from April 1, 2013 (eligibility criteria, Figure 1). Active follow-up comprised bi-monthly routine clinic reviews. Additionally, participants were instructed to attend the study clinic when they developed an influenza-like illness (ILI), defined as reported or documented fever (≥38°C) and ≥2 of: cough, rhinorrhea, sore throat, myalgia, headache and vomiting/diarrhea. The study clinician assessed ill participants and instituted appropriate management. Paired nasopharyngeal and oropharyngeal swabs (FLOQswabs™, Copan Diagnostics, Brescia, Italy) were obtained at routine and ILI visits [18].

We compared the incidence of laboratory-confirmed influenza-associated ILI between HIV-infected, and HIV-uninfected participants. At-risk period was calculated from enrolment to the study end date (March 31, 2015), death or loss to follow-up. In cases of the latter, follow-up was censored on the

date of relocation, withdrawal of consent, or the last recorded clinic visit.

Case-control study

Between May 15, 2013 and Feb 28, 2015, we recruited adults admitted to QECH with acute lower respiratory tract infection (LRTI) (severe cases), and adults attending the primary care center with ILI (non-severe disease) (eligibility criteria, Figure 2). Nasopharyngeal aspirates were obtained at enrolment. Influenza-positive hospitalized LRTI and outpatient-managed ILI formed cases and controls respectively.

Laboratory procedures

Laboratory testing was performed at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme laboratory. HIV status was established by sequential rapid HIV tests (Alere Determine™, Japan, and Uni-Gold™, Trinity Biotech, Ireland) [19]. CD4+ cell count was performed on a FACScount™ flow cytometer (Becton Dickinson, BD Biosciences, USA). Nasopharyngeal specimens were tested for influenza A and B viruses using the CDC Human Influenza qRT-PCR diagnostic panel, and influenza A subtyping kit [20].

Statistical analysis

Analysis was performed with Stata 12.1. We tested differences in categorical variables using χ^2 or Fisher's exact test, and continuous variables by *t*-test or Wilcoxon rank-sum test as appropriate.

The cohort study was powered to detect an incidence rate ratio (IRR) of 3.0 or greater (alpha 0.05, two-tailed beta 0.2), for influenza-associated ILI by HIV status requiring 608 recruits allowing for 20% loss-to follow-up, based on an estimated cumulative incidence of 40 per 1000 person years in the HIV-uninfected cohort and HIV+: HIV- ratio of 60:40.

Incidence rates of influenza-associated ILI were calculated by dividing the number of events by the number of person-years (PYs) follow-up. Poisson regression models estimated IRRs and 95% confidence intervals (CI) for the effect of HIV and other risk factors on influenza. Age, sex and HIV

were included as potential confounders in the multivariable models. Population average Poisson regression models using generalized estimating equations were constructed for recurrent events.

In both studies, stepwise backwards elimination of covariates with a p-value <0.20 was used to rationalize the multivariable models. We limited the number of covariates in a multivariable model to maintain events per variable at 10 [21]. Two-way interactions were evaluated in all final models. All available case information was used in each univariable analysis. In the multivariable models, we excluded patients with missing data for included variables (data were >95% complete for all variables). The impact of ART and CD4+ cell count was assessed in subgroup analyses of HIV-infected individuals.

For the case-control study, a sample size of 57 cases and 114 unmatched controls provided 80% power to detect an odds ratio (OR) of 2.5 or greater. Based on observed influenza prevalence of 11% and 16.4% in adults presenting to QECH with severe and mild acute respiratory illness respectively in our sentinel surveillance (Ho *et al*. unpublished), we estimated recruitment of approximately 518 adults with hospitalized LRTI and 695 adults with ILI.

We estimated the OR of having HIV infection and other potential risk factors for severe influenza in cases and controls, controlling for confounders, using unconditional logistic regression models. HIV and recruitment season were included *a priori* in the multivariable model.

Population attributable fractions (PAF) of modifiable risk factors for severe influenza were estimated from the prevalence of exposure in cases and adjusted OR from the multivariable logistic regression model [22].

Ethical approval was provided by the University of Malawi College of Medicine Research Ethics Committee, Blantyre, Malawi (P.11/12/1310), and the Research Ethics Committee of the University of Liverpool, Liverpool, UK (12.43).

RESULTS

Cohort study - participant characteristics

In total, 608 adults were enrolled; 360 (59%) had HIV infection (Figure 3 and Table 1). Compared to HIV-uninfected participants, HIV-infected participants were older (median age 37 vs. 31 years, p<0.001) and a higher proportion were female (69% vs. 55%, p=0.001). Chronic lung disease and smoking were uncommon in both groups. A significantly higher proportion of HIV-infected participants reported previous tuberculosis (25% vs. 2%, p<0.001) and pneumonia in the past 5 years (16% vs. 5%, p<0.001).

HIV-uninfected participants had larger households, but crowding was similar between the two groups. HIV-uninfected participants had better sanitation facilities, water supply and food security. No significant differences were observed in education level, employment or asset ownership.

234 of 360 (65.0%) HIV-infected participants were on ART at enrolment; the majority (82%) for over 12 months. Median CD4+ cell count at enrolment was 390 cells/μl (interquartile range (IQR) 244-547).

Eleven participants died during the study (HIV-infected, n=10); none had reported respiratory symptoms prior to death. 61 (10%) participants migrated out of Blantyre, 24 (3.9%) withdrew consent, and 25 (4.1%) were lost to follow-up (Figure 3). There was no differential loss-to-follow-up by HIV status. Total person-time follow-up were 520 and 348 PYs in the HIV-infected and HIV-uninfected cohorts respectively.

The impact of HIV infection on influenza-associated ILI incidence

We recorded 348 ILI episodes in 208 participants (HIV-infected, n=130) (clinical characteristics, Supplementary Table 1). The incidences of ILI presentation were 442 and 341 per 1000 PYs in the HIV-infected and HIV-uninfected participants, respectively (IRR 1.21; 95%CI 0.99-1.48). 29 (8.3%) ILI episodes were influenza PCR positive (influenza A(H3N2) (n=11), influenza B (n=18)); 24 of 229

(10.5%) in HIV-infected participants, and 5 of 119 (4.2%) in HIV-uninfected participants. The incidence of laboratory-confirmed influenza-associated ILI in HIV-infected adults per 1000 PYs was 46.0 (95%CI 30.8-68.6), and 14.5 (95%CI 6.0-34.7) in HIV-uninfected adults (IRR 3.21; 95% CI 1.22-8.41) (Table 2).

In the univariable analysis, previous history of pneumonia, household crowding, lower education level, unemployment, and food insecurity were associated with influenza-related ILI (Table 2). After adjusting for age, gender, household crowding and food security, HIV-infected adults had an approximately three-fold increased rate of influenza-associated ILI compared to HIV-uninfected adults (adjusted incidence rate ratio (aIRR) 2.75; 95%CI 1.02-7.44; Pearson's goodness-of-fit test, p=0.12).

Among HIV-infected participants, individuals with enrolment CD4+ count <200 cells/µl had higher incidence of influenza-associated ILI than those with CD4 >200 cells/µl, but the association was non-significant (79.1 vs. 40.5 per 1000 PYs; IRR 1.95; 95%CI 0.78-4.92). The effect of HIV on influenza did not differ by ART status.

Other risk factors for influenza infection

A household crowding index of 1.5-2.4 was associated with a three-fold increased risk of influenza-associated ILI, compared to households with <1.5 persons/sleeping room (aIRR 3.41, 95%CI 1.12-10.36). The increased risk was not observed in participants who lived in household with crowding index >2.5 (aIRR 2.06, 95%CI 0.62-6.83) (Table 2). Participants who reported difficulties accessing food >2 times/month had a three-fold increased risk of influenza-associated ILI, compared to those with no food access difficulties (aIRR 3.09, 95%CI 1.30-7.36). Food insecurity was also a possible effect modifier of the impact of HIV on influenza (p=0.01). Among those with frequent difficulty obtaining food, HIV was associated with 10-fold increased incidence of influenza-associated ILI (IRR 9.50, 95%CI 1.27-70.99), but no association was evident in those with little or no food insecurity (IRR 1.18, 95%CI 0.32-4.39). No interaction was demonstrated with the other covariates.

Case-control study – patient characteristics

1645 patients were assessed for eligibility for hospitalized LRTI and 846 for ILI, with subsequent recruitment of 518 and 642 patients, respectively (Figure 4). Patients with severe respiratory presentation were older (median age 35 vs. 32 years, p<0.001), predominately male (62% vs. 43%, p<0.001), and had a substantially higher prevalence of HIV (77% vs. 30%, p<0.001) (Table 3). They also had a higher prevalence of previous pulmonary tuberculosis (18% vs. 7%, p<0.001), pneumonia within the past five years (22% vs. 7%, <0.001), smoking (11% vs. 6%, p<0.001) and regular alcohol intake (26% vs. 12%, p<0.001). Sanitation facility, water supply, cooking fuel, asset ownership, education level, and food security were also worse among patients with hospitalized LRTI.

Contribution of influenza to mild and severe respiratory infection

Influenza was identified in 56 (10.8%) patients with hospitalized LRTI (cases) and 88 (13.7%) patients with ILI (controls) (Table 4). Influenza A virus was detected in 30 (53.6%) cases and 35 (39.8%) controls; all were A(H3N2) except one control (un-subtyped). Two controls were positive for both influenza A(H3N2) and B. Influenza A(H1N1)pdm09 was not detected during the study period.

Risk factors for severe influenza presentation

Among the 56 cases and 88 controls, no difference in age and sex was observed (Table 4). 39 (69.6%) cases and 26 (29.6%) controls were HIV-infected (p<0.001). Compared to HIV-infected controls, HIV-infected cases had more advanced immunosuppression (median CD4+ count 140 vs. 265 cells/µl, p=0.03) and were more likely to be receiving ART (35.7 vs. 9.1%, p<0.001). Cases were more likely to have a low body mass index (BMI), report prior history of tuberculosis and pneumonia. Controls had better sanitation, education, and food security. However, household exposure to children <5 years or crowding did not differ by case-control status.

In the univariable analysis, HIV, reported history of tuberculosis, pneumonia within past five years, low BMI (<18.5 kg/m^2), month of recruitment, type of water supply, sanitation facility, lower

education level, and food insecurity were associated with being a case (Table 4). Month of recruitment was included in the multivariable model *a priori* due to seasonal discrepancy in the recruitment of hospitalized LRTI and ILI patients. In the multivariable model, HIV was strongly associated with severe influenza (adjusted OR (aOR) 4.98, 95%CI 2.09-11.88; p<0.001). Additionally, reported pneumonia within 5 years (aOR 6.49, 95%CI 2.00-21.07; p<0.001), poor sanitation facility (aOR 3.14, 95%CI 1.25-7.84, p=0.01); and frequent difficulty accessing food (aOR 20.85, 95%CI 1.97-221.16; p=0.01) were independent risk factors for severe influenza.

In a subgroup analysis of HIV-infected cases and controls, there was a trend towards lower CD4+ counts in cases (OR 2.72; 95%CI 0.97-7.60, CD4+ count <200 vs. >200 cells/µl). No association was found between ART status and influenza severity.

Population attributable fraction

The highest proportions of hospitalized influenza were attributed to HIV (aPAF 56.7%, 95%CI 42.3-67.4) and poor sanitation (aPAF 40.9%, 95%CI 20.5-56.0) (Table 5). Frequent difficulty accessing food accounted for 12% (95%CI 10.6-13.6) of cases.

DISCUSSION

In this urban African adult population, HIV is an important risk factor for both symptomatic influenza and severe illness. Compared to HIV-uninfected adults, HIV-infected adults had a three times higher incidence of influenza-associated ILI, and a five-fold greater odds of severe influenza disease. Furthermore, nearly 60% of influenza-related hospitalized LRTI were attributable to HIV. Although neither study was powered to examine the effect of HIV at different levels of immunosuppression, our data suggest higher incidence and greater disease severity among those with CD4+ cell count of <200 cells/µl.

Previous population-level surveillance, and retrospective studies in South Africa and Kenya that have examined the association between HIV and influenza found a higher disease burden [8-10], and an

increased risk of influenza-associated hospitalization in HIV-infected persons [11-13], particular among those with severe immunosuppression [13]. The current studies provide robust evidence to support these findings having prospectively ascertained HIV status, CD4+ cell count and information on ART, as well as other exposures including household and socio-economic characteristics that may confound the association between HIV and influenza.

We found a higher incidence of influenza-associated ILI among HIV-infected, compared to HIV-uninfected individuals. While this could indicate an increased susceptibility to infection, it may instead, or additionally, reflect a greater propensity of HIV-infected individuals to develop symptomatic illness following influenza infection, which has been previously described [23]. This is a pertinent finding in high HIV prevalence settings, since HIV-infected individuals may play an important role in the community transmission of influenza.

Taken together, data from the two studies present a persuasive argument for a strong association between HIV and influenza. The finding that over half of hospitalized influenza presentations in Malawian adults were attributable to HIV further emphasizes its critical role in severe influenza in this population. Since pneumonia is the commonest cause of adult medical admissions at QECH [24], effective influenza preventative strategies could substantially reduce the burden of acute respiratory infections in Malawi and other similar resource-limited settings.

Inactivated influenza vaccines have demonstrated safety and efficacy in HIV-infected persons [25]. Clinical efficacy of 75.5% has been reported in South African HIV-infected adults, but the trial excluded patients with co-morbidities and ART-naïve patients with CD4+ count <100 cells/μl [26]. Influenza vaccines are not widely deployed in most African countries [2, 27]. Prior to consideration of HIV-infected persons as a target group for immunization, policymakers will require evidence of vaccine efficacy in the context of advanced immunosuppression and/or co-morbidities, as well as potential public health impact and cost effectiveness of vaccinating HIV-infected individuals, compared to other target groups (e.g. pregnant women, young children). Acceptability of annual vaccination, feasibility of vaccine administration at ART clinics, and optimal timing of vaccination in

the absence of clear seasonality will require elucidation. Numerous regulatory, logistical, and financial obstacles will need to be overcome if targeted influenza vaccination policies were to be successfully and sustainably implemented in the region.

The introduction of ART was associated with a dramatic decline in influenza-attributable hospitalizations in the US [28], and improved survival following pandemic influenza A(H1N1) infection in Mexico [29]. However, this beneficial effect has not been observed in Africa. Cohen *et al.* found no difference in case fatality ratio among HIV-infected individuals with influenza-positive severe acute respiratory illness by ART status [30]. A Malawian study demonstrated poor reconstitution of influenza-specific CD4+ T-cell response in HIV-infected adults following 12 months of HAART, despite a rise in CD4+ count [31]. Hence the impact of ART on the relationship between HIV and influenza severity requires further evaluation.

Identification of household crowding, poor sanitation and food insecurity as risk factors for influenza highlight the importance of current public health interventions to alleviate hunger and poverty, and improve access to clean water and sanitation [32]. Food insecurity emerged as a previously unrecognized risk factor for both influenza illness and severity. More in-depth evaluation of food insecurity and its association with influenza and other respiratory infections are warranted [33].

Our results, along with other data from the region, contrast with those from developed setting [6]. This highlights the limitations of extrapolating findings from developed setting to inform influenza control policies in Africa. Differences in observed impact of HIV on influenza burden and severity may be due to more advanced immunosuppression, poorer access to ART, higher prevalence of co-morbid conditions, as well as poverty-related factors identified in this study, compared to other regions.

Our study has several limitations. First, it was conducted in a single urban center, which may limit the generalizability of our findings to rural populations. Second, this study was not powered to assess the impact of CD4+ count or ART on influenza incidence and severity. CD4+ count was

measured during acute illness in the case-control study; the degree of immunosuppression may not be accurately represented since CD4+ cell depression can occur during acute illness. Third, passive surveillance was used for case ascertainment in both studies; under-ascertainment of ILI episodes, and therefore influenza cases are conceivable. Biased estimates away from the null could have resulted if HIV-infected cohort participants were more likely to present with ILI compared to uninfected individuals. Similar bias could have arisen if HIV-infected individuals had a higher propensity to attend hospital with severe respiratory symptoms in the case-control study. However, there was no significant difference in ILI incidence between HIV-infected and uninfected cohorts, and a greater proportion of HIV-related ILI had severe clinical signs (Supplementary Table 1). Furthermore, the majority of case-control study participants were unaware of their HIV status at enrolment. Lastly, we were unable to control for bacterial co-infection in the case-control study as diagnostic tests for bacteria were undertaken in cases but not controls.

We have comprehensively evaluated the association between HIV and influenza, identifying HIV-infected persons at particular risk of symptomatic influenza and severe disease. Influenza preventative strategies should be an important aspect of the management of HIV-infected adults in sub-Saharan Africa. Further studies are needed in Malawi and other high HIV prevalence settings to determine influenza vaccine efficacy in persons with advanced immunosuppression and evaluate potential public health impact ahead of operational research to address the logistical barriers to implementing large-scale vaccination programs.

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CONFLICT OF INTEREST

NF has received grant support from GlaxoSmithKine outside the submitted work. AH, SA, HJ, TM, MA, MM, JM, MN, DE and RH declare no competing interests.

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Table 1. Demographic, clinical, household and socioeconomic characteristics of the cohort participants

	HIV-infected	HIV-uninfected	P for difference[a]
	N=360	N=248	
Sex			
Male	113/360 (31)	111/248 (45)	0.001
Age (years)			
Median (IQR)	37 (31-45)	31 (25-39)	<0.001[b]
Medical history			
Asthma	18/360 (5)	8/248 (3)	0.29
Chronic lung disease	1/360 (0.3)	0/248 (0)	-
Chronic cardiac disease	2/360 (0.6)	1/248 (0.4)	0.80
Chronic kidney disease	0/360 (0)	0/248 (0)	-
Chronic liver disease	2/359 (0.6)	0/248 (0)	0.24
Pregnant (at enrolment)	4/359 (1)	4/248 (2)	0.84
Previous pulmonary tuberculosis	90/359 (25)	6/248 (2)	<0.001
Pneumonia in past 5 years	59/358 (16)	13//248 (5)	<0.001
Smoking - current	11/360 (3)	4/248 (2)	0.14[c]
Drinks alcohol	44/359 (12)	35/248 (14)	0.47
Body mass index <18.5 (kg/m^2)	38/346 (11)	17/242 (7)	0.18
Household and socioeconomic factors			
Children <5 years in household (n)			
0	219/358 (61)	132/247 (54)	
1	114/358 (32)	83/247 (33)	
$\geq$ 2	25/358 (7)	32/247 (13)	0.03
Individuals >5 years in household (n)			
0-2	89/358 (25)	56/247 (23)	
3-4	161/358 (45)	91/247 (37)	
$\geq$ 5	108/358 (30)	100/247 (41)	0.03

Crowding index[d]			
<1.5	110/360 (30)	66/248 (27)	
1.5-2.4	144/360 (40)	95/248 (38)	
>2.5	106/360 (30)	87/248 (35)	0.33
Sanitation facility			
None/non-VIP toilet	327/359 (91)	205/248 (83)	
VIP toilet/ flush toilet	32/359 (9)	43/248 (17)	0.002
Water supply			
River/stream/borehole	75/354 (21)	44/246 (18)	
Public tap/standpipe	212/354 (60)	131/246 (53)	
Piped to dwelling	67/354 (19)	71/246 (29)	0.02
Principal cooking fuel			
Firewood	77/357 (22)	44/246 (18)	
Charcoal	244/357 (68)	131246 (53)	
Electricity	36/357 (10)	71/246 (29)	0.66
Highest level of education			
Never attended	18/359 (5)	10/248 (4)	
Primary	152/359 (42)	89/248 (36)	
Secondary/Tertiary	189/359 (53)	149/248 (60)	0.19
Employment			
Unemployed	87/359 (24)	47/248 (19)	0.13
Assets owned[e]			
1-2	141/360 (39)	83/248 (33)	
3	151/360 (42)	113/248 (46)	
4-5	68/360 (19)	52/248 (21)	0.36
Difficulties obtaining food			
Never	137/356 (38)	133/247 (54)	
1-2 times/month	149/356 (42)	86/247 (35)	

> 2 times/month	70/356 (20)	28/247 (11)	<0.001

Data are n/N (%) unless otherwise indicated. VIP=ventilated improved pit.

[a]Mantel-Haenszel χ^2 test unless stated otherwise.

[b]Wilcoxon rank sum test.

[c]Fisher's exact test.

[d]Total household size/ number of living rooms.

[e]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

Table 2. Risk factors for laboratory-confirmed influenza illness among cohort participants

Characteristic	Incidence rate per 1000 PYFU (95%CI)	Univariable[a]		Multivariable (model C)[a,b]	
		IRR* (95% CI)	P-value	IRR* (95% CI)	P-value
Sex					
Male	31.4 (16.9-58.3)	1		1	
Female	34.6 (22.1-54.2)	1.21 (0.52-2.84)	0.66	0.88 (0.40-1.93)	0.74
Age group (years)					
18-29	20.9 (8.7-50.2)	1		1	
30-39	39.9 (23.2-68.7)	1.73 (0.60-4.97)		1.55 (0.54-4.43)	
≥ 40	36.3 (20.1-65.6)	1.33 (0.43-4.05)	0.30	1.42 (0.47-4.28)	0.70
HIV status					
Negative	14.5 (6.0-34.7)	1		1	
Positive	46.0 (30.8-68.6)	3.21 (1.22-8.41)	0.02	2.75 (1.02-7.44)	0.03
Medical history					
Previous pulmonary tuberculosis	28.2 (23.3-51.1)	0.82 (0.28-2.43)	0.87		
Pneumonia in past 5 years	54.3 (24.4-120.9)	1.86 (0.75-4.57)	0.14		
Body mass index < 18.5	40.3 (13.0-125.0)	0.81 (0.25-2.68)	0.73		

Housing characteristics					
Children <5 years in household (n)					
0	36.2 (22.8-57.5)	1			
1	27.8 (13.9-55.6)	0.77 (0.33-1.77)			
≥ 2	37.3 (12.0-115.5)	1.03 (0.30-3.50)	0.80		
Individuals ≥5 years in household (n)					
0-2	25.5 (10.6-61.3)	1			
3-4	28.1 (15.1-52.3)	1.10 (0.38-3.22)			
≥ 5	44.7 (26.5-75.4)	1.76 (0.63-4.87)	0.41		
Crowding index[c]					
<1.5	16.3 (6.12-43.5)	1		1	
1.5-2.4	47.1 (28.8-76.8)	2.88 (0.96-8.62)		3.41 (1.12-10.36)	
≥ 2.5	32.0 (16.6-61.5)	1.95 (0.60-6.35)	0.11	2.06 (0.62-6.83)	0.056
Socio-economic characteristics					
Highest level of education					
Never attended/ Primary	47.6 (30.0-75.6)	1			
Secondary/ Tertiary	22.5 (12.5-40.7)	0.47 (0.22-1.00)	0.05		
Employment					

No	49.6 (28.8-85.4)	1			
Yes	26.4 (16.2-43.1)	0.53 (0.26-1.11)	0.09		
Food security – difficulty obtaining food					
Never	25.9 (13.9-48.2)	1		1	
1-2 times/month	20.7 (9.9-43.5)	0.80 (0.30-2.10)		0.71 (0.27-1.88)	
> 2 times/month	87.2 (49.5-153.6)	3.35 (1.45-7.76)	0.005	3.09 (1.30-7.36)	0.006

VIP=ventilated improved pit.

[a]IRRs estimated for the incidence of laboratory-confirmed influenza using Poisson regression.

[b]Adjusted for sex, age group, HIV status, crowding index and food security.

[c]Total household size/ number of living rooms.

Table 3. Demographic, clinical, household and socioeconomic characteristics of adults enrolled with hospitalised LRTI and mild ILI

	Hospitalised LRTI N=518	ILI N=642	P for difference[a]
Sex			
Male	323/518 (62)	273/642 (43)	<0.001
Age (years)			
Median (IQR)	35 (30-42)	32 (25-43)	<0.001[b]
Pre-hospital/clinic attendance & treatment			
Attended another health facility[c]	312/513 (61)	62/635 (10)	<0.001
Antibiotics within 2 weeks[d]	309/509 (61)	70/630 (11)	<0.001
Antimalarials within 2 weeks	83/511 (16)	13/630 (2)	<0.001
HIV status			
Positive	396/517 (77)	192/640 (30)	<0.001
CD4+ count (cells/µl)	101 (46-196)	313 (167-450)	<0.001[b]
On antiretroviral treatment at enrolment[e]	188/233 (81)	68/87 (78)	0.62
Medical history			
Chronic lung disease	18/503 (4)	15/635 (2)	0.23
Chronic cardiac disease	2/503 (0.4)	1/635 (0.2)	0.59[f]
Hypertension	9/503 (2)	19/635 (3)	0.19
Chronic kidney disease	1/503 (0.2)	0/503 (0)	0.44[f]
Chronic liver disease	1/503 (0.2)	0/503 (0)	0.44[f]
Pregnant	2/195 (0)	10/369 (3)	0.19[f]
Previous pulmonary tuberculosis	90/514 (18)	45/638 (7)	<0.001
Pneumonia in past 5 years	113/512 (22)	46/638 (7)	<0.001

Body mass index <18.5 (kg/m^2)	147/495 (30)	67/638 (11)	<0.001
Smoking - current	58/513 (11)	39/638 (6)	<0.001
Drinks alcohol	135/512 (26)	78/638 (12)	<0.001
Household and socioeconomic factors			
Children <5 years in household (n)			
0	296/513 (58)	381/638 (60)	
1	156/513 (30)	195/638 (30)	
≥ 2	61/513 (12)	62/638 (10)	0.48
Crowding index			
<1.5	174/497 (35)	219/612 (36)	
1.5-2.4	203/497 (41)	258/612 (42)	
≥ 2.5	120/497 (24)	135/612 (22)	0.71
Sanitation facility			
None/non-VIP toilet	284/514 (55)	260/637 (41)	
VIP toilet	188/514 (37)	252/637 (39)	
Flush toilet	42/514 (8)	125/637 (20)	<0.001
Water supply			
River/stream/borehole	150/514 (29)	134/638 (21)	
Public tap/standpipe	272/514 (53)	308/638 (48)	
Piped to dwelling	92/514 (18)	196/638 (31)	<0.001
Principal cooking fuel			
Firewood	140/514 (27)	129/637 (20)	
Charcoal	325/514 (63)	421/637 (66)	
Electricity	49/514 (10)	87/637 (14)	0.006
Highest level of education			
Never attended school	49/508 (10)	52/638 (8)	
Primary	274/508 (54)	266/638 (42)	
Secondary / Tertiary	185/508 (36)	320/638 (50)	<0.001

Employment			
Unemployed	68/514 (13)	91/638 (14)	0.61
Assets owned[g]			
0	84/515 (16)	75/638 (12)	
1	106/515 (20)	112/638 (18)	
2	122/515 (24)	165/638 (26)	
3	158/515 (31)	206/638 (32)	
4-5	35/515 (9)	80/638 (12)	0.04
Difficulties obtaining food			
Never	247/514 (48)	367/638 (58)	
1-2 times/month	226/514 (44)	250/638 (39)	
> 2 times/month	41/514 (8)	31/638 (3)	<0.001
Season of recruitment			
Jan-Mar	99/518 (19)	204/642 (32)	
Apr-Jun	100/518 (19)	180/642 (28)	
Jul-Sep	161/518 (31)	145/642 (24)	
Oct-Dec	15/518 (31)	102/642 (16)	<0.001

Data are n/N (%) unless otherwise indicated. VIP=ventilated improved pit latrine.

[a]Mantel-Haenszel χ^2 test unless stated otherwise.

[b]Wilcoxon rank sum test.

[c] Included attendance to other hospital, health center, private clinic, traditional healer or pharmacy.

[d]Excluded co-trimoxazole prophylaxis in HIV-infected individuals.

[e]Denominator is the number of patients with known HIV infection at enrolment.

[f]Fisher's exact test.

[g]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

Table 4. Risk factors for severe influenza presentation in influenza PCR positive cases and controls

Characteristic	Cases[a] n=56	Controls[b] n=88	Univariable[c]		Multivariable[c,d]	
			OR (95% CI)	P-value	OR (95% CI)	P-value
Sex						
Male	28/56 (50)	43/88 (49)	0.96 (0.49-1.88)	0.89		
Age group (years)						
18-29	17/56 (30)	36/88 (41)	0.75 (0.32-1.73)	0.32		
30-39	22/56 (39)	25/88 (28)	1.40 (0.61-3.22)			
≥ 40	17/56 (30)	27/88 (31)	1			
HIV status					1	
Positive	39/56 (70)	26/88 (30)	5.47 (2.63-11.36)	<0.001	4.98 (2.09-11.88)	<0.001
Medical history						
Previous pulmonary tuberculosis	10/56 (18)	5/88 (6)	3.61 (1.16-11.20)	0.03		
Pneumonia in past 5 years	17/55 (31)	7/88 (8)	5.18(1.98-13.53)	0.001	6.49 (1.95-21.25)	0.001
Body mass index <18.5 (kg/m^2)	12/53 (23)	9/88 (10)	2.57 (1.00-6.60)	0.05		
Housing and socioeconomic characteristics						
Sanitation facility						
None/non-VIP toilet	34/55 (61)	37/88 (42)	2.12 (1.08-4.22)	0.03	3.14 (1.25-7.84)	0.01
VIP toilet/ flush toilet	22/55 (40)	51/88 (58)	1		1	

Water supply						
River/stream/borehole	15/56 (27)	19/88 (22)	2.54 (0.93-6.98)	0.07		
Public tap/standpipe	32/56 (57)	40/88 (45)	2.58 (1.07-6.22)			
Piped to dwelling	9/56 (16)	29/88 (33)	1			
Highest level of education						
Never attended	5/55 (9)	15/88 (17)	1			
Primary	34/55 (62)	28/88 (32)	3.64 (1.18-11.27)			
Secondary/ Tertiary	16/55 (29)	45/88 (51)	1.07 (0.33-3.41)	0.002		
Difficulties obtaining food						
Never	28/56 (50)	58/88 (66)	1		1	
1-2 times/month	21/56 (38)	29/88 (33)	1.50 (0.73-3.08)		1.15 (0.47-2.84)	
> 2 times/month	7/56 (12)	1/88 (1)	14.4 (1.70-123.65)	0.007	20.85 (1.97-221.16)	0.01
Month of recruitment						
Jan-Mar	16/56 (29)	49/88 (56)	1		1	
Apr-Jun	15/56 (27)	18/88 (20)	2.55 (1.05-5.20)		3.36 (1.13-9.95)	
Jul-Sep	12/56 (21)	9/88 (10)	4.08 (1.45-11.46)		6.34 (1.69-23.80)	
Oct-Dec	13/56 (23)	12/88 (14)	3.32 (1.25-8.72)	0.01	3.60 (1.07-12.10)	0.01

Data are n/N (%).

VIP=ventilated improved pit latrine.

[a]Influenza-PCR positive hospitalised lower respiratory tract infection.

[b]Influenza-PCR positive influenza-like illness.

[c]Unconditional logistic regression.

[d]Adjusted for HIV status, history of pneumonia within 5 years, month of recruitment, sanitation facility, and food security.

Table 5. Population attributable fraction of modifiable risk factors for severe influenza

	% exposed among cases	Adjusted OR (95% CI)	p-value	Adjusted PAF (95%CI)
HIV infection	70	4.98 (2.09-11.88)	<0.001	56.7% (42.3-67.4)
Sanitation facility				
None/non-VIP toilet	39	3.14 (1.25-7.84)	0.01	40.9% (20.5-56.0)
VIP/ flush toilet	61	1		
Difficulties obtaining food				
Never	50	1		-
1-2 times/week	38	1.15 (0.47-2.84)		6% (-40.9-38.0)
> 2 time/ week	12	20.85 (1.97-221.16)	0.01	12.0% (10.3-13.7)

VIP=ventilated improved pit latrine.

Figure legends

Figure 1. Eligibility criteria for cohort study

Figure 2. Eligibility criteria for case control study

Figure 3. Recruitment and progress of cohort participants

Flow diagram for recruitment, loss to follow-up and influenza-like illness events among cohort participants.

[a]Reasons for ineligibility: intention to relocate out of Blantyre (n=10); unable to attend regular study visits (n=6); unable to give written informed consent (n=1); evidence of active acute respiratory disease at enrolment (n=17); and another household member enrolled in study (n=2).

[b]Reasons for declining consent: no time (n=3); not interested (n=2); fear of participation (n=4); and, wished to seek spouse's consent (n=2).

Figure 4. Case control study recruitment

Figure 1.

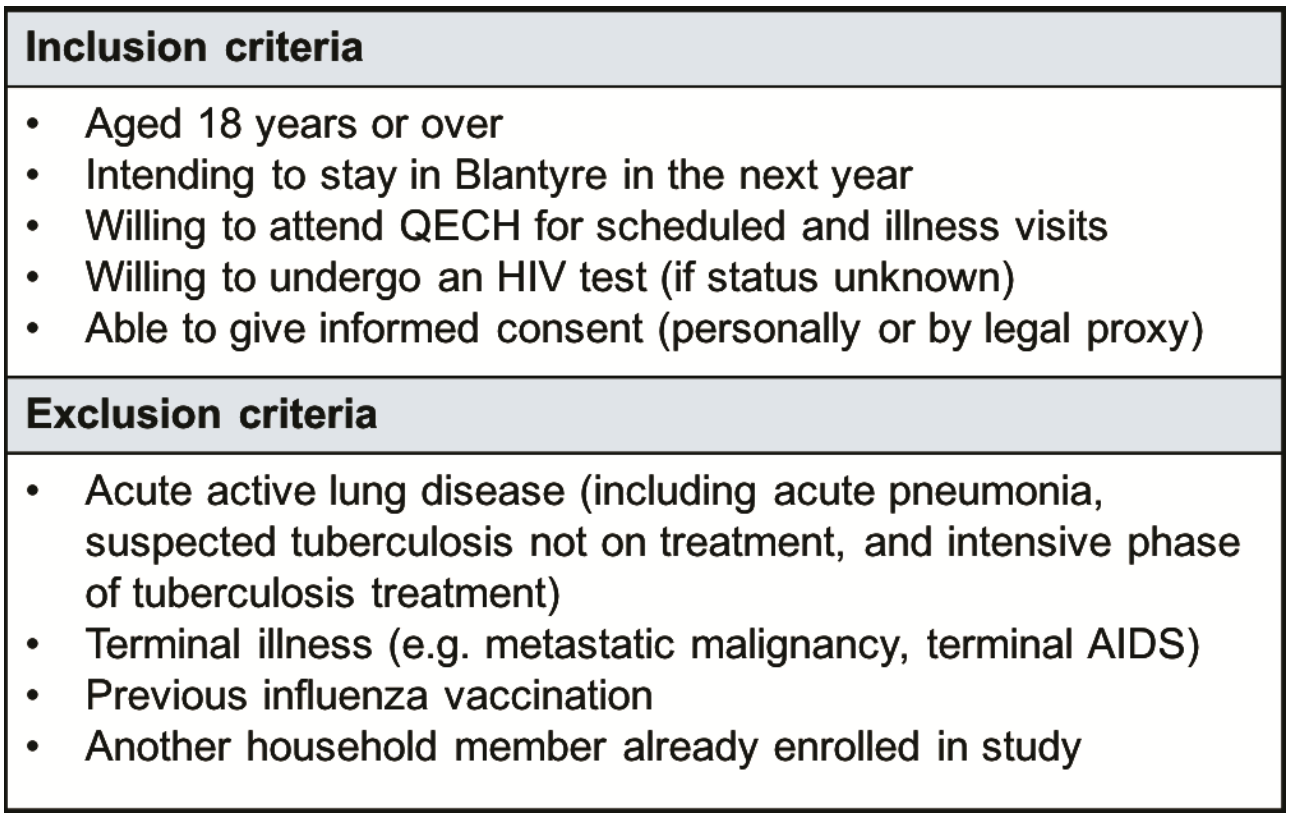

Figure 2.

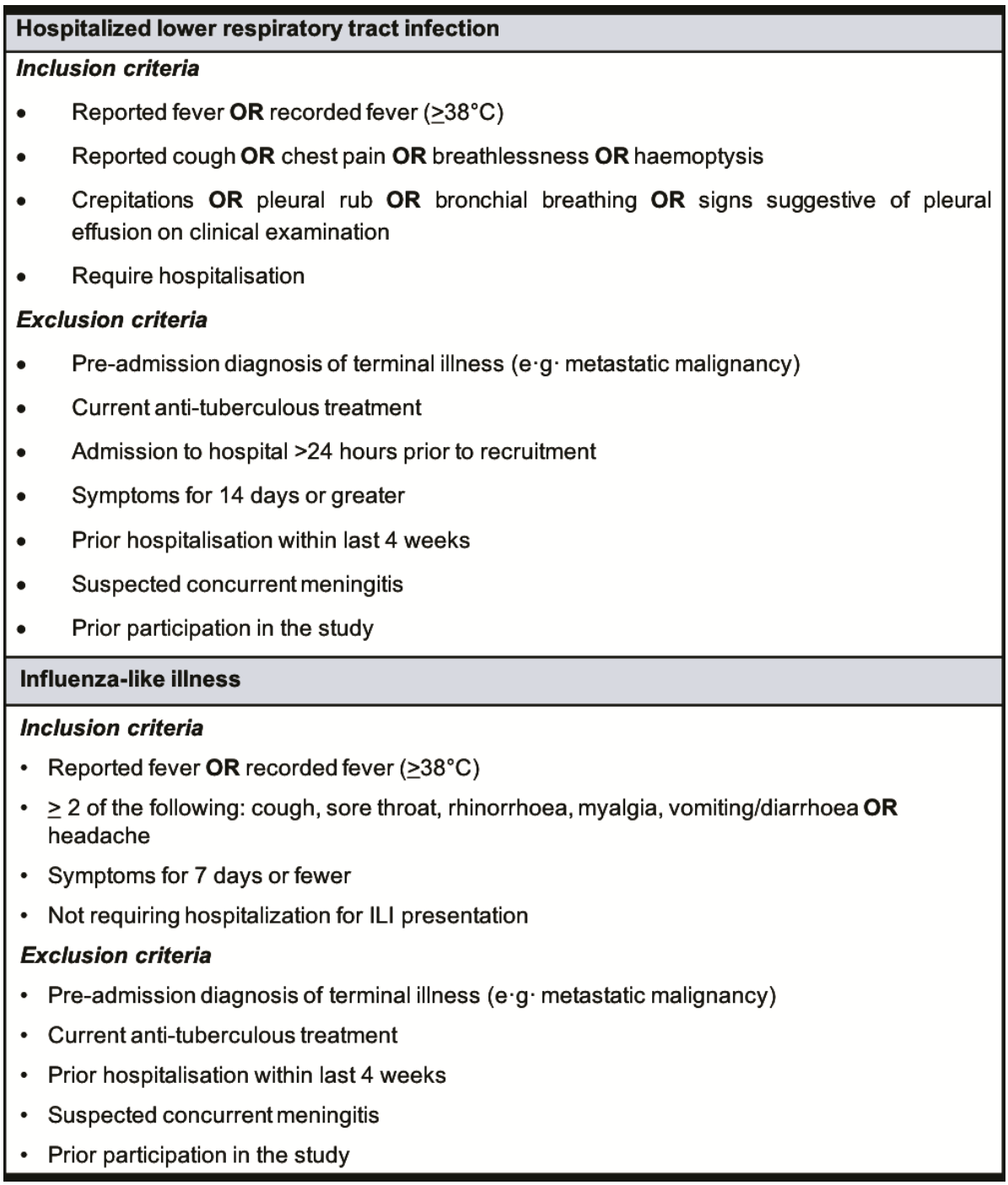

Figure 3.

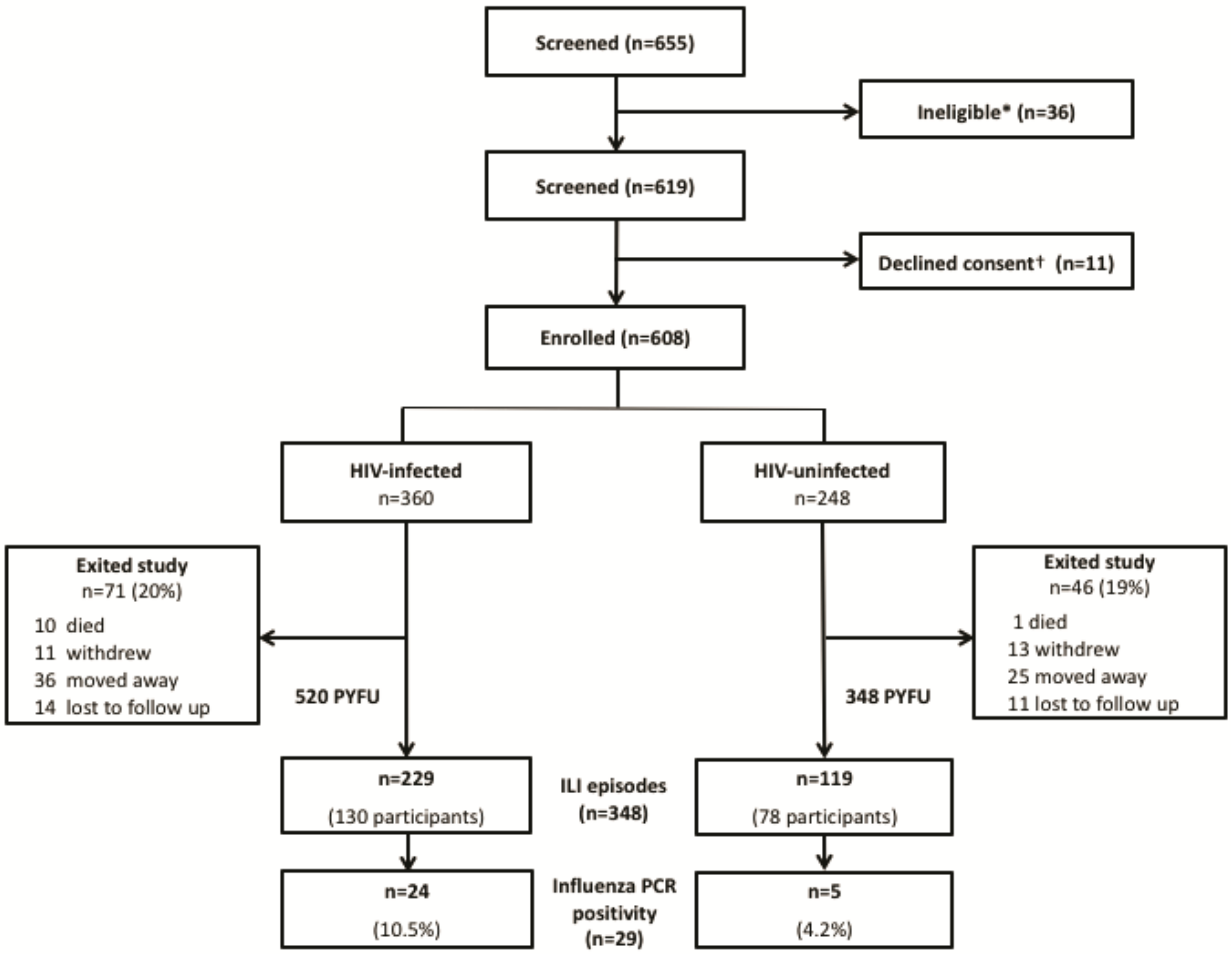

Figure 4.